STUDIES ON NOVEL APPROACHES FOR BETTER OCULAR DELIVERY OF FLURBIPROFEN

THESIS

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By

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Under the Supervision of

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CERTIFICATE

This is to certify that the thesis entitled "**STUDIES ON NOVEL APPROACHES FOR BETTER OCULAR DELIVERY OF FLURBIPROFEN**" and submitted by **SAJEEV CHANDRAN,** ID No. **1998PHXF008** for award of Ph. D. Degree of the Institute, embodies original work done by him under my supervision.

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SUMMARY

The eye presents with very unique challenges to topical drug delivery, particularly to the anterior chamber, due to its anatomical isolation and multiplicity of protective barriers. Because of this reason topical administration, though the most preferred route of administration of ophthalmic drugs, fails to maintain the therapeutic drug concentration for the desired length of time within the ocular tissues and fluids. This drug disposition pattern is characterized by a transient overdosing, followed by a relatively short period of acceptable dosing, in turn followed by a prolonged period of under dosing. In conventional dosage forms, drug instillation in the precorneal space provide unusually high concentration of the drug and preservatives at the corneal epithelial surface leading to various ocular cytopathologies and systemic side effects due to conjunctival and naso-lachrymal absorption.

Though corticosteroids are the drugs of choice for treating ocular inflammatory disorders, but their long-term use results in increased intra ocular pressure (mostly leading to cataract), exacerbation of ocular infections and diminished corneal and stromal wound healing. Non-steroidal anti-inflammatory drugs are devoid of such side effects and are extensively used in the treatment of intraocular inflammatory disorders on long term basis. The objective of this work was to study various novel approaches for the design of better ocular delivery of flurbiprofen, a potent cyclooxygensae inhibitor commonly used for ocular inflammation as an alternative to corticosteroids. Extended or controlled release drug delivery systems for flurbiprofen were envisaged to be designed, developed and evaluated based on following approaches.

(a) Prolonging its contact with the corneal surface
(b) Controlling the release of the drug

Various studies like, pure drug analysis, assay of formulations, *in-vitro* dissolution, stability and permeability studies, and *in-vivo* studies involving analysis in blood serum and aqueous humor require the support of rapid, accurate, sensitive and validated analytical method. In the present study, three simple, sensitive, accurate and reproducible analytical methods with better detection range for estimation of flurbiprofen in pure form and in its pharmaceutical dosage forms were developed. These methods include UV spectrophotometric method, spectroflourimetric method and liquid chromatographic method. The developed methods were

later used to estimate the flurbiprofen in pure form, in designed formulations, stability samples, *in-vitro* dissolution study samples and *in-vitro* permeability study samples. A new reversed phase liquid chromatographic method, with UV detection, for the quantitative estimation of flurbiprofen in blood serum and aqueous humor, was also developed in the present study. The developed method was used for the estimation of flurbiprofen in serum and aqueous humor samples obtained in *in-vivo* studies. The results of the analysis were validated by statistical methods as recommended by USP 2000, ICH guidelines 1996 and by recovery studies.

It was also considered important to study the stability of flurbiprofen under various storage conditions in the presence or absence of prospective formulation additives and also, some of the preformulation physico-chemical properties of flurbiprofen relevant to ocular formulation development work envisaged. Stability of the drug was studied *per se* and in the presence of common formulation and manufacturing additives in both solid state and in aqueous solutions under various temperature and humidity conditions and the drug was found to be stable. Flurbiprofen was found to exhibit pH dependent stability with stability increasing with increase in pH. Investigations on the solubility study of flurbiprofen revealed that solubility increases with increase in pH from acidic to basic side. Flurbiprofen was found to possess very high solubility in most of the organic solvents investigated. Nature of the vegetable oil was also found to influence the solubility of the drug. Studies on the effect of buffered and unbuffered pH on the log P value indicated that lower pH of the aqueous phase favours increased partitioning of the drug into the oily phase.

An attempt was made to develop different types of extended or controlled release ophthalmic formulations of flurbiprofen. Extended release formulations were prepared using various mucoadhesive polymers, parentral grade vegetable oils and combination of polymer and vegetable oils each containing 750 μg/ml of the drug. Formulations of flurbiprofen were also prepared in the form of matrix embedded discs and polymer coated matrix embedded discs with 15 mg drug per disc.

The developed liquid formulations were found to possess good physical properties like, appearance, clarity, odour, pH, drug content uniformity, flow property, spreadability and mucoadhesiveness. Various parameters, including proportion and type of the retardant mucoadhesive polymer, type of oil and polymer-oil combination, affected the prolongation of

the release character of flurbiprofen to varying degree. The release was found to follow first order kinetics and the release was extended from 30 mins to 40 hours in the developed formulations.

Studies involving development and evaluation of controlled release ocular disc type formulations of flurbiprofen prepared by the matrix-embedding technique alone or in combination with membrane barrier technique has also been presented. Drug impregnated small polymeric discs of flurbiprofen were prepared using varying proportion (10 to 50 % w/w of the drug content) of polymers. Proportion and type of the retardant polymer were varied and were found to influence the duration and rate of drug release from designed formulations. The release from polymeric matrix discs was found to follow non-Fickian kinetics in most cases and Fickian kinetics in few. The duration of release was extended from 2 to 36 hours. Ethyl cellulose coating on matrix-embedded polymeric discs extended the flurbiprofen release for several days to weeks. The result demonstrated the efficiency of dual mechanism controlled release systems in providing zero order release patterns for several weeks. Fabricated discs possessed good physical properties like, hardness, low friability, minimum weight variation and uniform drug content.

Effect of sterilization on stability of the drug and its release from the designed formulations were also investigated and the sterilization methods employed did not affect the drug stability and release kinetics. The manufacturing processes employed for the preparation of the developed formulations were found to be reliable as indicated by high degree of batch reproducibility. All the developed formulations possessed commercially viable predicted shelf life.

From each category of formulations investigated, selected formulations were subjected to trans-corneal permeability studies using modified Franz diffusion cell on excised goat cornea. The *in-vitro* transcorneal permeability of flurbiprofen was also carried out to demonstrate the effect of buffer (phosphate, citrate-phosphate and citrate) and pH (both buffered and unbuffered vehicle) on the permeability of flurbiprofen. Effect of formulation excipients like, preservatives and chelating agents on the transcorneal permeability was also investigated. It was observed that with increase in solubility of the drug in various buffers, the partitioning into the corneal membrane decreased thereby decreasing the permeability. In case of buffered and unbuffered vehicles of varying pH, the permeability decreased with increase in pH of the

vehicle. Studies on the effect of formulation additives on the transcorneal permeability revealed that the compounds like, preservatives and chelating agents increased the rate of drug corneal permeation as well as corneal hydration significantly. The results confirmed the adverse effect of these agents on the corneal cell structure and its integrity, thereby increasing the drug permeability. Transcorneal permeability studies involving mucoadhesive polymeric gels revealed an increased corneal permeability of flurbiprofen probably due to increased adherence of the formulation to the corneal epithelium, thereby facilitating increased partitioning and permeation of the drug. Studies with selected vegetable oil based formulations showed that the permeability results correlated well with the solubility data of flurbiprofen with permeability decreasing with increase in solubility of the drug in the oil. Permeability rate of flurbiprofen from selected polymeric discs and from polymer coated discs showed statistically insignificant difference in the permeation characteristics compared to aqueous drops, except in case of ethyl cellulose matrix and matrix coated discs, where it was significantly low.

Ocular safety studies by Draize's test protocol revealed that most of the formulations were non-irritating and few were practically non-irritating to the rabbit eye. *In-vivo* ocular bioavailability studies revealed that *in-vitro* extension of the release of the drug from the designed formulations manifested in the form of enhanced area under the aqueous humor drug concentration time profile and prolonged mean residence time (MRT) of the drug in the aqueous humor.

A good inverse correlation was obtained between time for 90 % of the drug released *in-vitro* ($t_{90\%}$) and $AUC_{0\text{-}24hr}$ *in-vivo* for developed liquid topical preparations, but in case of polymeric discs type formulations only a moderate correlation was seen. Good correlation was obtained between $t_{90\%}$ *in-vitro* and MRT *in-vivo*. A very good correlation with high 'r' value was obtained between time for maximum aqueous humor concentration (t_{max}) and time for 50 % of the drug released *in-vitro* and between t_{max} and time for 70 % of the drug released *in-vitro*.

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

The eye is a specialized sensory organ that is relatively secluded from systemic access by the blood-retinal, blood-aqueous, and blood-vitreous barriers. Because of this anatomical isolation, the eye presents some unique opportunities as well as challenges for drug delivery. From drug delivery point of view, it is very difficult to study ocular drug disposition due to non availability of representative specimens of human eye tissues containing drug. As a result, majority of the information on ocular disposition of ophthalmic drugs used on humans are incomplete or totally unknown and mostly based on empirical models developed on the basis of animal studies. Another critical issue in ocular drug delivery is its ability to circumvent the protective barrier of eye without causing irreversible tissue damage and at the same time to ensure that the drug entity reaches the bio-phase in sufficient concentration. Barring a few, most ocular treatment calls for topical administration of ophthalmically active drugs to the tissues around the ocular cavity. But because of an efficient drainage mechanism (that of tears and tear drainage and nasolachrymal drainage), only a very small fraction of the applied dose (normally less than 10 %) is absorbed into the target tissues. Thus, a relatively concentrated solution is required for instillation into the eye cavity to achieve an adequate level of therapeutic effect. The frequent periodic instillations of ocular formulations become a necessity to maintain a continuous minimum level of medication. This gives the eye a massive and unpredictable dose of medication. Higher the drug concentration in the formulations, the greater is the amount of drug lost through nasolachrymal and other drainage systems. Subsequently higher dose is potentially absorbed into the systemic circulation resulting in undesirable systemic side effects. To overcome above noted problems, continuous maintenance of a constant and effective level is desired. This makes delivery of drugs to the eye somewhat different from most other areas of the body. Moreover, the ocular disposition and elimination of a therapeutic agent depends on its physicochemical properties and relevant ocular anatomy and physiology (Gibaldi and Perrier, 1982). The successful design of an ocular drug delivery system therefore requires an integrated knowledge of the drug entity, anatomy of eye and the constraints to delivery offered by the ocular route of administration.

This chapter mainly contains: (i) Anatomy and physiology of eye pertaining to ocular drug delivery with emphasis on ocular drug transport and disposition; (ii) Ocular drug delivery: traditional approach vs. novel therapeutic systems; (iii) An overview of ocular inflammatory disorders and treatment regimens. Lastly based on these three subjects, objective of the present

research and development endeavor to design better ocular drug delivery systems has been presented.

1.1 Anatomy and physiology of eye pertaining to ocular drug delivery

Human eye is a highly protected and isolated organ that maintains its connection with other part of the body through a specialized vascular network and nerve and muscular attachments. Any exogenous substance administered need to cross numerous physiological and protective barriers. Thus, in the pursuit of developing effective ocular drug delivery system it is imperative to understand the relevant anatomical and physiological constraints that restrict or alter the drug and vehicle disposition.

The eye, with a circumference of about 75 mm, is a combination of two spheres, one set in the other and commonly referred to as globe (Figure 1.1), occupying a volume of 6.5 ml and a combined weight of 6.7-7.5 g (Duke-Elder, 1961). The front sphere is smaller of the two and is bordered anteriorly by the cornea, whereas the larger posterior sphere is an opaque fibrous shell encased by the sclera (Warwick, 1976). The eye is approximately 80 % of its adult size at birth. Various parts of the eye are described in the following sections.

1.1.1 Extraocular structures

The extraocular structures of the eye mainly consist of the orbit, eyebrows, eyelids and extraocular muscles. Each one of these structures is explained below briefly.

(a) Orbit: The eye rests in a bony cavity of the skull located on either side of the nose called orbit. Each orbit has multiple fissures and foramina that conduct nerve, muscles and vessels (Riordan-Eva and Tabbara, 1992). Seven bones make up the orbit, namely: the maxilla, the palatine, the frontal, the sphenoid, the zygoma, the ethmoid, and the lacrimal bones. The globe occupies approximately 20 % of the cavity, lying slightly nearer the upper and lateral sides but never in contact with the orbital bones. The optic foramen located at the apex of the orbit acts as the conduit for the optic nerve, ophthalmic artery and sympathetic nerves. In the orbit, connective and adipose tissues and six extra ocular muscles support and align the eye for vision.

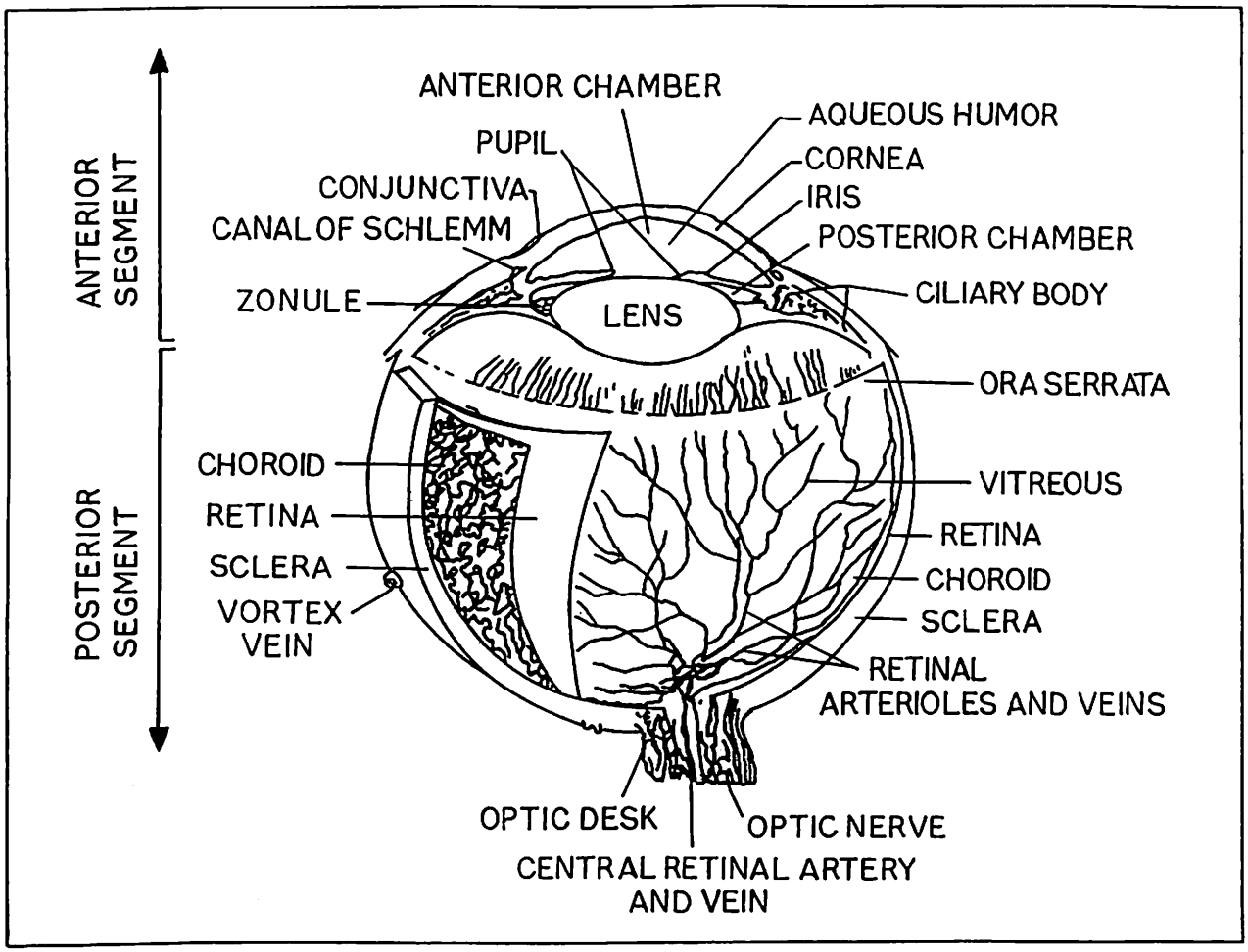


Figure 1.1: Anatomy of the eye showing the anterior and the posterior segments (Riordan-Eva and Tabbara, 1992).

(b) Eyebrows and eyelids: Eyebrows are positioned transversely above eyes along the superior orbital ridges of the skull. Owing to their position and curvature, they prevent perspiration or particles from running into the eyes and thus aids in the protection of the eye. Eyelids are two movable folds, upper and lower, which form the anterior protection for the eye. These mobile folds along with their dense sensory innervations and eyelashes protect the eye from mechanical or chemical injury, provide a barrier against excessive light, aid in blinking, and retard evaporation from the surface of the eye. Blinking, a coordinated movement of the orbicularis oculi, levator palpebrae, and Muller's muscle, serves to assist in spreading of the secreted tear

film over the cornea and conjunctiva. In human beings average blink rate is 15 to 20 times per minute, with a single blink averaging from 0.29 to 0.75 sec (Records, 1989) and the average pressure exerted on the globe by the lids during blinking is 10 mm Hg (Miller, 1967). The lid margins also contain tiny openings that lead to the sweat and oil glands. Secretions from these glands, apart from providing lubrication and barrier to evaporation of tears, also have an anti-microbial role.

(c) Conjunctiva: The internal surface of the eye is lined with a highly vascularized mucus membranous structure called conjunctiva. At the reflection of the palpebral conjunctiva (lining the interiors of upper eyelid) and bulbar conjunctiva (lining the interiors of the lower eyelid) is a space called fornix or cul-de-sac. The inferior or lower cul-de-sac is the site of administration of topical medication in the eye.

(d) Extraocular muscles: The coordinated movement of six extraocular muscles ensures the rotation of the eye about different axes. These muscles include two oblique (superior and inferior) and four rectus (superior, inferior, lateralis and medialis) muscles.

1.1.2 Anterior segment

The knowledge of anterior segment of the eye is very important for topical drug administration to the eye. The major components of this segment include cornea, limbus, trabecular meshwork and Schlemm's canal, anterior uvea (iris, pupil and ciliary body), and lens.

(a) Cornea: The cornea is a transparent and avascular tissue organized into five layers: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. Description and composition of each of these layers is presented in Table 1.1. The diameter of the cornea is about 11.5 mm and the anterior corneal surface radius of curvature is about 7.8 mm. Total corneal and conjunctival surface area in normal healthy adult is 16 cm^2, with a thickness of 0.5 mm (at the center) and 0.7 mm (towards the limbus) (von Bahr, 1948; Martola and Baum, 1968).

(b) Limbus and associated structures: It includes the transitional zone between the cornea and sclera and conjunctiva, which is about 1-2 mm in width. The limbic structures are: corneal and anterior conjunctival epithelium (externally), and internally trabecular meshwork and Schlemm's canal. The limbic blood supply and tears are the major source of nutrients and defense mechanism for the corneal membrane. Trabecular meshwork and Schlemm's canal are located just above the apex of the peripheral anterior chamber angle (formed by the cornea and the iris

root). The anterior chamber holds approximately 250 µl of aqueous humor. The trabecular meshwork and Schlemm's canal forms the conduit for the outflow of aqueous humor from the anterior segment of the eye. The posterior chamber holds approximately 50 µl of aqueous humor. The rate of aqueous humor production and its rate of outflow control the intraocular pressure and prevent the eye from collapsing. Ideally to prevent such a collapse the intraocular pressure is maintained higher than the normal atmospheric pressure, the usual value being 13 to 19 mm Hg (Moses, 1981). An increase in intraocular pressure leads to glaucoma.

Table 1.1: Various cell layers of the corneal membrane

Layer	**Thickness**	**Composition**	**Function**
Epithelium	50-60 µm	5-6 layers of epithelial cells, highly hydrophobic in nature	Outer most layer of the cornea and thus, barrier to invasion by foreign substances; holds tear to anterior surface of the eye
Bowman's membrane	8-14 µm	Homogenous acellular sheet consisting of fine fibrils and lacks elastin	Connective tissue between the basement membrane and stroma
Stroma	400-500 µm	200-250 alternating lamellae of collagenous tissue, highly hydrophilic in nature	Gives physical strength and optical transparency
Descemet's membrane	10-15µm	Modified basement membrane of the epithelial cells	Imparts elasticity and resistance to proteolytic enzymes
Endothelium	5-6 µm	Lowest layer of the cornea with single layer of flattened epithelial cells with substantial intercellular space	Active fluid transport through mitochondria, vesicles and ion pumps

(c) Anterior uvea: It consists of ciliary body, iris, and pupil. The ciliary body comprises of the ciliary muscles and ciliary processes. It secretes aqueous humor, nourishes the lens, provides the muscle power for accommodation and may secrete the unique zonular fibres. The ciliary

processes are highly vascularized folding of the ciliary body which originates from the anterior ciliary and protrude into the posterior chamber and the ciliary body is attached posteriorly to the retina at the Ora Serrata. The iris is a thin disc suspended in the aqueous humor between the cornea and the lens. The central circular aperture of the iris is called pupil and is composed of pigmented epithelial cell layer, the iridial sphincter, radial dilator muscles and the stroma. The pupil has a diameter of 2 to 9 mm depending upon the extent of illumination, emotional state and fatigue. The pupillary diameter directly depends on the relative activity of sphincter and dilatory muscles. The amount of melanin in the stroma determines the color of the iris.

(d) Lens: The lens is a biconvex, transparent epithelial body located behind the pupil between the iris and the vitreous body. The lens is approximately 10 mm in diameter and is enclosed in a capsule. The bulk of the lens is composed of fibres derived from the proliferating lens epithelial cells located under the anterior portion of the lens capsule. These lens fibres are continuously produced throughout the life.

1.1.3 Posterior segment

Sclera, retina and optic nerve together with vitreous constitute the posterior segment of the eye. Due to its vascular and anatomical barrier, drug delivery to the posterior lobe of the eye is very difficult.

(a) Sclera: It covers the posterior portion of the eye and constitutes the outermost layer of the eye. The sclera is about 0.6-1 mm in thickness and has a diameter of 22 mm and a radius of curvature of 12 mm (Hogan et al, 1971). It consists of dense bundles of collagen fibres, which vary in width, with the width decreasing from exterior to interior (Spitznas, 1971). The sclera protects the interior of eye, aids in maintaining its shape and serves as a site for the attachment for the extrinsic muscles of the eye.

(b) Choroid: Choroid is a vascularized tissue between the retina and the sclera. It consists of the vessel layer, the choriocapillary layer and Bruch's membrane.

(c) Retina: The retina is a thin, transparent, highly organized structure of neurons, glial cells, and blood vessels. The retina primarily consists of nine layers: internal limiting membrane, nerve fiber layer, ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, outer nuclear layer, external limiting membrane, and the inner and outer segments of rods and cones. The retina is approximately 0.11 to 0.18 mm thick. The neurosensory retina, with its

unique composition of photoreceptors, has been subject of investigation in transductional mechanisms (Stryer, 1987), protein and gene structure of rhodopsin (Khorana, 1992), and for targeted gene therapy for hereditary retinal diseases.

(d) Optic nerve: The optic nerve is the means thorough which the retinal output travels to the optic chiasm (Anderson and Hoyt, 1969). It is a bundle of myelinated fibres and comprises of intraocular portion, intraorbital portion, intracanalicular portion, and intracranial portion. The optic nerve is sheathed by the meninges, which are continuous with those of the brain, and it is the ophthalmic artery that is the primary source of all arterial branches to the optic nerve (Anderson, 1970).

1.1.4 Eye fluids

Various fluids in the eye include the lachrymal system (including tear, secretory and drainage mechanism), aqueous humor and vitreous humor (Moroi and Lichter, 2001).

(a) Lachrymal system: It has glandular secretory elements and excretory ductal elements as collection portion (Moroi and Lichter, 2001) (Figure 1.2). The tears are complex mixtures of salts, proteins, lipids, phospholipids, and enzymes in a water base. Tear covers the bulbar and palpebral conjunctiva and the cornea. It moistens, lubricates and flushes the anterior surface of the eye. The tear is a trilaminar film with each layer having different composition. The anterior layer is made of lipid along with small amount of mucin and proteins. The middle layer, that comprises 98 % of the tear film, is predominantly aqueous in nature containing electrolytes, water and various proteins. The posterior layer of the tear is mucin (a glycoprotein containing protein to carbohydrate in the ratio 1:3) (Nichols et al, 1983). The cul-de-sac normally holds 10 µl of tears and up to 25 % of the tear fluid is lost due to evaporation. Various physical properties and composition of human tear are tabulated in Table 1.2 and 1.3 respectively. The tear drainage system starts through small puncta located on the medial aspects of both the upper and lower eyelids. Tears enter the puncta with each blinking and from the puncta move into the nose through canaliculi, lachrymal sac and nasolachrymal duct.

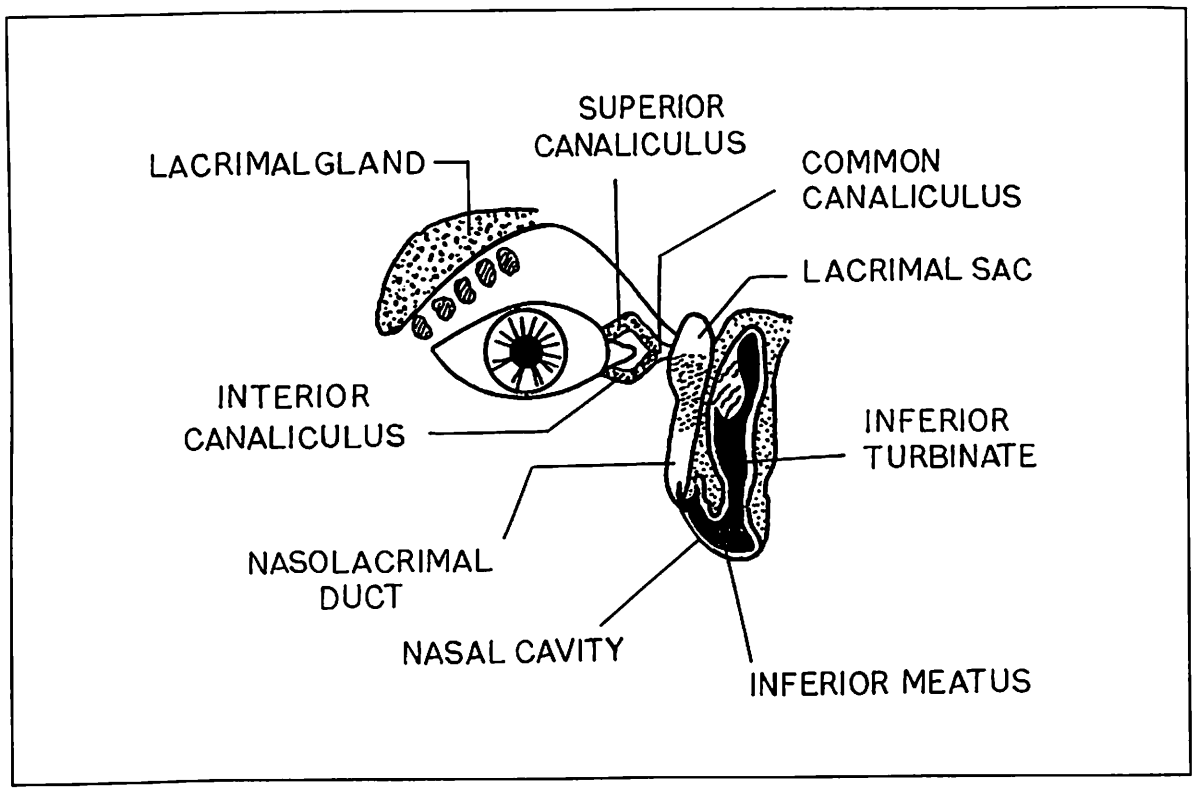


Figure 1.2: Anatomy of lachrymal system (Riordan-Eva and Tabbara, 1992).

Table 1.2: Physical property of human tear

Physical property	**Values**
Osmotic pressure	311-350 mOsm/l
pH	7.4 (7.3-7.7)
Refractive index	1.357
Volume	7.0-30.0 µl
Oxygen tension (precorneal tear film)	140-160 mm Hg
Flow rate	0.5-2.2 µl/min
Buffer capacity	3.6×10^{-5}

Table 1.3: Composition of human tear

Category	Contents	Composition
Electrolytes	Bicarbonates	26 mEq/l
	Chlorides	120-135 mEq/l
	Potassium	15-29 mEq/l
	Sodium	142 mEq/l
	Calcium	2.29 mg/100 ml
Nitrogenous substances	Total protein	0.669-0.800 g/100 ml
	Albumin	0.394 g/100 ml
	Globulin	0.275 g/100 ml
	Ammonia	0.005 g/100 ml
	Urea	0.04 mg/100 ml
	Total nitrogen	158 mg/100 ml
	Nonprotein nitrogen	51 mg/100 ml
Carbohydrates and steroids	Glucose	2-3 mg/100 ml
	Cholesterol and its esters	8-32 mg/100 ml
Miscellaneous organic acids, vitamins and enzymes	Citric acid	0.6 mg/100 ml
	Ascorbic acid	0.14 mg/100 ml
	Lysozyme	800-2500 units/100 ml
	Lactoferrin	1.6-2.1 mg/100 ml

(b) Aqueous humor: The non-pigmented epithelial cells of the ciliary processes secrete aqueous humor into the posterior chamber of the anterior segment of the eye. The approximate volume of aqueous humor held in this chamber is 250 µl with a turnover rate of ~ 1 %/min. The non-pigmented cells mainly contain two enzymes; sodium-potassium activated adenosine triphosphatase and carbonic anhydrase. These cells actively transport sodium ions, chloride or bicarbonates into the enfolding of the cells. This creates an osmotic pressure that attracts water. From the posterior chamber, aqueous humor flows into the anterior chamber through the pupil. The ion composition of aqueous humor is almost same as that of plasma and is devoid of any cell or protein. The blood-aqueous barrier prevents large molecules like, colloids or proteins to

diffuse into the aqueous humor. The aqueous humor supplies nutrition and oxygen to the avascularized tissues of the eye and removes the metabolites from them.

(c) Vitreous humor: Approximately 80 % of the eye's volume is a clear hydrogel medium. It contains water bound with collagen type II (99 %), hyaluronic acid, and proteoglycans. It also contains glucose, ascorbic acid, amino acids, and a number of inorganic salts. It acts as a metabolic pathway for the nutrients of the lens and the retina.

1.2 Ocular drug delivery: Traditional approach vs. novel therapeutic systems

Earliest accounts of ophthalmic treatments date back to the Mesopotamian era (circa 3000-4000 B.C.) and the classical Greek era (460–375 B.C.) when Hippocrates revolutionized the therapeutics of disease and several hundred remedies for eye problems were described. Galen and Susruta categorized eye diseases on an anatomical basis and applied medicinal as well as surgical remedies advocated by Hippocrates. Various texts of Ayurveda also bear testimony to the use of crude drugs of plant, animal and mineral origin for the treatment of various ocular cytopathologies (Duke-Elder, 1962a, 1975). In the nineteenth century numerous organic compounds were isolated from plants and used in the treatment of ocular disorders. The belladonna and hyoscyamous were used to treat iritis in the early 1800s. Atropine was isolated and used therapeutically in eye in 1832. In 1875, pilocarpine was isolated and the therapeutic effect of lowering intraocular pressure was recognized in 1877, providing the basis for a safe and effective treatment for glaucoma that is still efficacious.

Drug molecules intended for therapeutic action in various eye diseases/ pathologies often have specific sites of action within the globe of the eye. An ideal ocular drug delivery system should deliver the drug to the intended site of action in the eye at an appropriate therapeutic level for a desired period of time to achieve a desired pharmacological effect and at the same time avoid untoward side effects. An underlying assumption here is that a correlation exists between the concentration of a drug at the intended site of action and the resulting pharmacological effect.

Topical preparations have been the most preferred means of delivering a therapeutic entity to treat ocular diseases over the years. The conventional preparations administered through this route include solutions, suspensions, semisolids, etc. But most of these drug delivery systems are

considered inefficient and fail to ensure optimum efficacy and bioavailability (Shell, 1982; Shofner et al, 1989). In most of the cases, the anatomy and physiology of eye is the major culprit. In this section, issues related to ocular drug delivery with respect to eye, factors affecting ocular drug delivery and ocular pharmacokinetics have been covered in brief. Also described are the traditional ocular drug delivery systems and their inherent disadvantages, approaches for better ocular delivery, novelty investigated in ocular drug delivery and empirical models for ocular drug transport, availability and disposition.

1.2.1 Issues related to ocular drug delivery pertaining to eye

It is now imperative that the basic anatomy and physiology of eye pertaining to ocular drug delivery can markedly influence the availability of the drug from its formulations. Here, an attempt has been made to summarize various issues pertaining to ocular drug delivery with respect to anatomy and physiology of eye. Each of the anatomical portions of the eye, namely, extraocular structures, anterior and posterior segments, and eye fluids are important areas for consideration in developing ocular drug delivery system (Robinson, 1993; Lee, 1993).

(a) Extraocular structure: The upper eyelid covers around 1 mm of the cornea in Caucasians and even more in Orientals. This can influence the comfort of ocular products as placement of the formulation deeper into the lower cul-de-sac will improve patient comfort as well as increase residence time. Blinking or the coordinated movement of the eyelids can markedly influence the residence time of the liquid ophthalmic formulations. With increase in patient discomfort and with increase in grittiness, the rate of blinking increases. Perhaps this becomes even more important in case of ocular inserts that should be small, thin and carefully shaped. Movement of the lids is also responsible for the movement of the tears into the collecting portion of the lachrymal apparatus as well as the distribution of new tear over the ocular surface (Brieman and Snell, 1969; Brown and Dervichian, 1969). This drainage of tear and instilled solution can account for the removal of approximately 25-50 µl of the instilled volume in human eye in a matter of 90 sec, and thus has a major role in nasolachrymal absorption of the instilled dose (Lee and Robinson, 1986) and decrease in ocular bioavailability of the drug from topical solutions (Chrai et al, 1973; Chrai et al, 1974). The smallest instilled drop (5 µl) will minimize the systemic load through drainage. For effective local treatment, good mixing of the topical formulation with tear and rapid spreading over the intended surface area of the eye is necessary.

Precorneal metabolism of the drug is another issue that needs to be addressed during the design and development of better ocular drug delivery. But unfortunately, very little is known about this aspect due to poor analytical method back up. Also the issue of precorneal metabolism is more critical in case of prolonged release formulations.

(b) Anterior segment: Effectiveness of the topically applied dose depends on how well the liquid delivery system coats the cornea or the conjunctival surface. Physically immobile dosage forms like, inserts and gels when swept across the corneal surface, cause certain degree of discomfort. As mentioned earlier, a system designed for Caucasians will be more uncomfortable when placed in the eyes of Orientals. The epithelial and stromal layer of the cornea plays an important role as barrier to trans-corneal drug delivery. As corneal epithelium is reasonably lipophilic, it acts as a barrier to water-soluble drugs with low oil/ water partition coefficient (typically less than one) and stroma, as it is hydrophilic in nature, acts as a barrier to lipophilic drugs resulting in low bioavailability, unless there is prolonged residence time. The preferable oil/ water partition coefficient is between 10-100 for a favorable trans-corneal permeation. Except for contact lens, it is very difficult to place the drug delivery system in contact with the cornea. So, most of the formulations are placed in the other tissues in front of the eye and as a result, there is significant loss to these tissues leading to increased systemic load of the drug. Conjunctival absorption is significant because of the large surface area (approximately 17 times the surface area of the human cornea) (Walsky et al, 1988; Robinson, 1993).

(c) Posterior segment: Placement of drug into the posterior portion of the eye by route other than injection is difficult. But injection of drug delivery systems based on liposomes, microcapsules and nanoparticles must not interfere with the visual pathway and their components. Also the physical properties of the drug and the delivery mechanism should be compatible with the posterior ocular tissues. Periphery of the posterior segment of the eye is highly vascularized and the vitreous humor presents a low barrier to drug diffusion resulting in a massive and rapid loss of drug upon administration from the posterior segment. The only way this can be curbed is by limiting the rate of release of the drug from the delivery system.

(d) Eye fluids: Excessive formation and secretion of the lachrymal fluid occur when any foreign body or other irritants or drug formulations are instilled into the eye. These in turn contribute to nasolachrymal drainage and subsequent systemic absorption of the drug from the nasal mucosa. Topical application of ophthalmic drug is further made inefficient by tear turnover, which is

about 16 %/min in human. The lower cul-de-sac can hold 7-9 µl of tear under normal rate of blinking but if care is taken to control the rate of blinking it can accommodate approximately 20-30 µl of tear without over-flowing. But instillation of drops in the eye results in increased rate of blinking and at the same time causes increased tear drainage and turnover. Due to these factors, typically less than 10 % of the administered dose reaches the aqueous humor. The low absorbed fraction of the applied dose further undergoes rapid elimination from the intraocular tissues and loss through the canal of Schlemn or via absorption through the ciliary body or superachoroid into episcleral space (Robinson, 1993).

1.2.2 Factors affecting ocular bioavailability

There are several factors which affect the optimal ocular bioavailability from the topically applied dose like, precorneal fluid dynamics, conjunctival and systemic drug absorption, mechanism of corneal drug penetration, physicochemical properties of the drug, protein-drug interaction, drug metabolism and formulation related factors. For reference, general scheme of ocular drug penetration is depicted in Figure 1.3.

(a) Precorneal fluid dynamics: One of the most critical precorneal factors influencing bioavailability is the dynamics of fluid in the precorneal spaces. All forms of topical liquid dosage forms (aqueous solutions, oily solutions, suspensions and liposomes) are rapidly drained into the nasolachrymal passage with residence time varying from 4-23 mins (Lee and Robinson, 1979; Sieg and Triplett, 1980; Stratford et al, 1983a; Lee and Carson, 1985; Lee and Carson, 1986). The irritancy of the formulation contributes to the increase in the drainage rate and thus decrease in the residence time, an example being that of positively charged liposomes (highly irritant) with residence time of 4.3 min (Lee and Carson, 1986) as compared to that of neutral liposomes and solutions with residence time of 6.5 and 7.3 mins (Stratford et al, 1983b; Taniguchi et al, 1988) respectively.

(b) Conjunctival drug absorption: As mentioned earlier, conjunctiva is capable of competing with cornea for the drug absorption due to its larger surface area (approximately 9 times in rabbit and 17 times in human) (Walsky et al, 1988) and 2 to 30 times greater permeability to the drugs (Wang et al, 1991). The conjunctiva also differs from cornea in terms of its metabolic activity (Nicholas et al, 1983), length and density of microvilli (Nicholas et al, 1985) and permeability of water-soluble compounds such as mannitol, dextran (Huang et al, 1989). Thus, conjunctival

absorption becomes equally effective in reducing the availability of the drug for corneal absorption as tear drainage (Lee and Robinson, 1986).

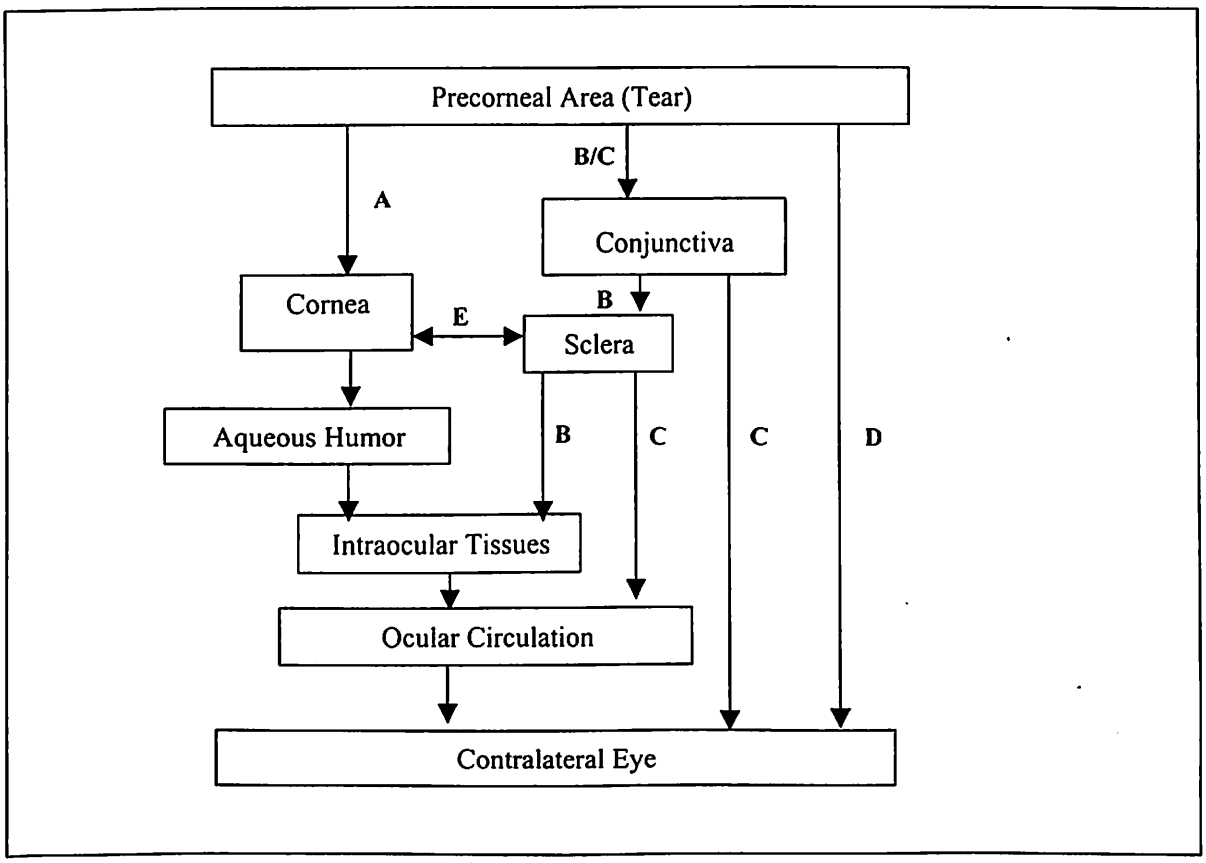


Figure 1.3: Various routes for drug penetration from topical ophthalmic applications

(A = Corneal route, B = Non-corneal route, C = Systemic, D = Naso-lachrymal absorption route, E = Lateral diffusion)

A study suggested that an attempt to change the lipophilicity of the beta-blockers caused more change in corneal permeability than conjunctival permeability (Wang et al, 1991). In this study, change in lipophilicity of the molecule showed to increase the conjunctival permeability by 12 times as compared to 48 fold increase in case of corneal permeability. Conjunctival permeability coefficient is also less sensitive to formulation changes than the corneal permeability coefficient (Ashton et al, 1991). Conjunctival absorption does not necessarily lead to systemic accumulation of the absorbed drug and rather has shown to facilitate drug entry into uveal tract called noncorneal route of drug absorption (Doane et al, 1978; Ahmed and Patton, 1985, 1986). Drug

disposition through this route is increased whenever there is inefficient mixing, instillation of viscous solution, use of dispersed systems like liposomes and nanoparticles (Stratford et al, 1983a, b; Ahmed and Patton, 1987; Li and Robinson, 1989; Diepold et al, 1989).

(c) Systemic drug absorption: A large percentage of topically applied drug get absorbed into the systemic circulation from the nasal mucosa following drainage, leading to serious systemic side effects. For example, cortisol 30-35 % (Janes and Stiles, 1964), dipivalylepinepherine 65 % and epinephrine 55 % (Anderson, 1980), flurbiprofen 74 % (Tang et al, 1984a, b), levobunolol 46 % (Tang et al, 1987) and timolol 80 % (Chang and Lee, 1987). The extent of systemic availability can only be decreased by preventing drainage of the ophthalmic dose into the nasal cavity. This can be achieved by-

(i) Nasolachrymal occlusion for 5 mins (Zimmerman et al, 1984; Kaila et al, 1986),

(ii) Use of viscosity enhancing polymers in the vehicle (Kyyronen and Arto, 1990),

(iii) Change in solution pH, tonicity and preservative concentration (Urtti et al, 1990),

(iv) Co-administration with vaso-constrictors like, phenyl epinephrine and epinephrine (Kyyronen and Arto, 1990),

(v) Designing ophthalmic drugs that are poorly absorbed into the systemic circulation (Sasaki et al, 1989) or that get rapidly metabolized into inactive forms in the systemic circulation (soft drug approach) (Bodor et al, 1984), and

(vi) Selecting dosing time that ensures minimum systemic absorption (Ohdo et al, 1991).

Since most of the above approaches are based on preventing absorption from nasal mucosa rather than the conjunctival sac, decrease in systemic absorption does not necessarily ensure increased corneal absorption.

(d) Mechanism of corneal drug penetration: There are two major pathways for the entry of the compounds through the corneal membrane - transcellular and paracellular. Paracellular transport involves diffusive and convective transport occurring through intercellular spaces and tight junctions. On the other hand, transcellular pathway involves cell/ tissue partitioning and/ or diffusion, channel diffusion and carrier mediated transport. It has been observed from studies on a series of compounds that the fraction absorbed through corneal route varies from less than 1 % for hydrophilic drugs to 7 % for lipophilic drugs (Patton and Robinson, 1976; Tang et al, 1984a,

b; Chiang and Schoenwald, 1986). Corneal epithelium and stroma exert resistance to penetration with varying degree depending on the nature of the drug as shown in Table 1.4 (Schoenwald and Huang, 1983).

Table 1.4: Degree of corneal epithelial and stromal resistance to drug penetration for various type of drugs

Nature of the drug	**Percent contribution of the corneal resistance**	
	Corneal epithelial layer	**Stromal layer**
Hydrophilic (log P < 0)	90	5
Moderately lipophilic (log P = 0.1-1.6)	50	30
Lipophilic (log P = 1.6-2.5)	10	50

General mechanisms (Grass and Robinson, 1988a, b) of drug movement through the cornea are following.

(i) At the organ level, corneal epithelium acts as a barrier to drug penetration as well as a reservoir for the drug. The top two cell layers of the epithelium act as rate limiting barriers for highly lipophilic drugs. Studies using corneal membrane, denuded of the top layer, have shown remarkable increase in the permeability of hydrophilic drugs like, 10 to 30-fold increase for 5-floururacil [log partition coefficient (log P) = –0.96] (Wang et al, 1991) and 60-fold increase in case of inulin (log P = –2.90) (Lee et al, 1983). Stroma is the rate-limiting layer for lipid-soluble drugs.

(ii) At the cellular level, small molecules like, water, methanol, ethanol, propanol and butanol readily traverse the cornea through assumed aqueous pores. Their permeability constants are very high. Usually a permeability coefficient of the order of 0.1- 4.0 × 10^{-5} cm/ sec is suitable for efficient permeation (Wang et al, 1991). Water-soluble compounds, peptides, ions and other charged compounds travel across the cornea through paracellular route (Huang et al, 1989). Substances with biphasic solubility traverse across the cornea much easily. Changes in the corneal epithelial permeability during ocular inflammation cause increased permeability of drugs

like, cyclosporine (BenEzra and Maftzir, 1990) and dexamethasone phosphate (Kupferman et al, 1974). Baum et al, 1974, found that the type and degree of injury to the cornea affect the extent of improvement in corneal drug absorption. Corneal permeability increases when the corneal integrity is compromised by the usage of high concentration of certain formulation excipients like, preservatives and chelating agents. The details are given under formulation related factors. Another important aspect of the cornea is its lipophilicity gradient across five-six cell layers. The lipophilicity decreases from the tear side to the intra ocular tissue side (Shih and Lee, 1990). A parabolic relationship has been predicted between corneal penetration and lipophilicity for wide range of compounds like steroids (Schoenwald and Ward, 1981), n-alkyl-p-amino-benzoate esters (Mosher and Mikkelson, 1979), substituted anilines (Kishida and Otori, 1980), timolol ester prodrugs (Chein et al, 1988, 1991) and beta-blockers (Schoenwald and Huang, 1983). However deviation from the parabolic relationship has been observed when compounds of diverse chemical structure and molecular size are considered (Grass and Robinson, 1984).

(e) Physicochemical properties of the drug: The physicochemical properties like, molecular weight, partition coefficient, pKa, and solubility of a drug entity play a critical role in its effective absorption through the corneal route. The change in molecular weight (MW) or size shows an inverse relationship to permeability. In case of small molecular weight (< 500) compounds diffusional coefficient is inversely proportional to the molecular weight whereas, in larger compounds permeability coefficient is inversely proportional to molecular weight. The transcorneal permeability of a hydrophobic/ biphasic-soluble substance is not governed significantly by its molecular weight but its lipid solubility, while the permeability of a hydrophilic substance is governed by its molecular weight (Kishida and Otori, 1980). Compounds with high molecular weight like, bacitracin, colistin sulphate, polymixin, cyclosporine, trifluridine, and dexamethasone phosphate penetrate the eye only in diseased state models and not the non-inflamed eye (Cox et al, 1972; Kupferman et al, 1974; Pavan-Langston and Nelson, 1979; BenEzra and Maftzir, 1990). Obviously, an injury to the corneal membrane enhances the penetration of otherwise non-permeable high molecular weight compounds (Baum et al, 1974).

The solubility of the drug in the precorneal tear film limits its rate of absorption due to low concentration gradient. A high concentration of drug, having reasonable solubility, permits a

greater penetration rate as compared to a drug with limited solubility. This is observed by the mathematical equation given below (Eller et al, 1985).

$$MPR = CPC \times TS \qquad (1)$$

Where, MPR = maximum attainable permeation rate across the corneal barrier; CPC= corneal permeability coefficient and TS = tear solubility, the solubility of the drug in tear under physiological condition. Also CPC is calculated as,

$$CPC = Flux / (360 \times C_0 \times A) \qquad (2)$$

In the above equation, C_0 = initial concentration of the drug placed on the epithelial side of the cornea; A = surface area exposed to drug.

For drugs that have a very low permeability coefficient (< 10 $\times 10^{-6}$ cm/ sec), a high concentration needs to be applied into the eye to overcome the permeability barrier (Schoenwald, 1993). Examples include phenylephrine, epinephrine and sulfacetamide. These drugs, being highly water soluble and less potent, are applied in the concentration range of 2-10 %. But for soluble and potent drugs like, timolol, chloramphenicol, predinisolone acetate, flouromethalone, dexamethalone, or tobramycin, it is not necessary to apply high concentration. The equation 1 also gives explanation for the low penetration rate of nonsteroidal anti-inflammatory drugs (NSAID's). These drugs, being weakly acidic in nature, are predominantly ionized at the tear pH of 7.4 and thus, are less bioavailable. This postulate has been used in the design of poorly water-soluble prodrugs of NSAID's which are expected to penetrate more rapidly than the parent compound and at the same time being metabolized by the esterase enzymes in the corneal epithelium (Schoenwald, 1993).

Because of lipophilic nature of the epithelium and also its low porosity and high tortuosity, a rapidly penetrating drug must possess a log P (Where, P = octanol/ buffer (pH 7.65) partition coefficient) greater than 1 to attain optimum permeability. A log-log plot of CPC and partition coefficient (P, determined at pH = 7.65) for a series of beta-blockers have shown an increase in permeability with increase in P which approaches an upper-limit or plateau region between log P value of 1.5 and 2.5. Similar results were found with steroids (Schoenwald and Ward, 1981), n-alkyl-p-amino-benzoate esters (Mosher and Mikkelson, 1979), substituted anilines (Kishida and Otori, 1980), and timolol ester prodrugs (Chein et al, 1991) and the following relationship (Huang et al, 1983; Schoenwald and Huang, 1983) has been established:

$$\log CPC = \log (D.b/h) + (a \times \log P) \qquad (3)$$

In the above equation, 'a' is the slope and 'log (D.b/h)' is the intercept, Where, D = diffusion coefficient, h = effective thickness and b = constant. The values of 'a' and 'b' can be obtained from the linear regression by substituting biological permeability coefficient with P. The upper plateau region in the plot occurs due to the permeability barrier to highly lipophilic drug across the aqueous stroma. At a very low log P values (–1 to –2) a limiting plateau is also evident, which is due to the parallel pore pathways through the cellular epithelium and endothelium. These pore pathways allow very slow passage of very hydrophilic drugs through the cornea. For beta-blockers, Wang et al, 1991, established that a sigmoid relationship between log CPC and log P explains the influence of lipophilicity on corneal drug penetration better than the earlier proposed parabolic relationship (Schoenwald and Huang, 1983).

(f) Drug binding: Binding of the topically applied drug to tear protein and melanin has been another constraint in ocular drug delivery. Total protein content in tear is around 0.7 % w/v in human and around 0.5 % w/v in rabbit (Mikkelson et al, 1973a, b), of which three types of proteins (albumin, globulin and lysozyme) have high potential for binding with topically applied drug and thus decreasing the availability of free drug for absorption (Mikkelson et al, 1973a; Chrai and Robinson, 1976). The problem is further exacerbated when there is an increase in tear protein in certain disease states, like, corneal inflammation (Woodward and Ledgard, 1985), herpes infection and allergic conjunctivitis (Anderson and Leopold, 1981). In post corneal region, binding of the drug to melanin can increase or decrease the bioavailability of topically applied drugs. Zane et al, 1990, concluded from a study of 27 acidic and basic compounds that strong basic functional groups (like, piperidine or piperazine or other amines) and increased lipophilicity are the main factors contributing to accumulation of the drug in melanin tissues. A slow releasing formulation will result in higher fraction to become melanin bound as compared to a fast releasing formulation, since the binding to the melanin is saturable.

(g) Drug metabolism: In ocular drug pharmacokinetics, the drug metabolism in ocular tissues has not been fully explored (Shichi and Nebert, 1980). It has been reported that the human or rabbit cornea, iris-ciliary body and sclera does not possess any enzyme that typically detoxify a compound by hydrogenation or oxidation (both ω and β types) (Cheng-Bennett et al, 1990). But certain other enzymes, like esterase (Peterson et al, 1965; Lee, 1983), monoamine oxidase, and

catecholamines ortho-methyl transferase (Wattman and Sears, 1964; Abraham et al, 1987) have been identified in these tissues. Thus, pilocarpine, a cyclic ester, is hydrolyzed to the extent of 30 % post instillation (Lee et al, 1980), whereas cycolphosphamide (Schoenwald and Houseman, 1982) and timolol (Putterman et al, 1985), which degrade by oxidation pathway, remain intact in isolated pigmented rabbit cornea. Some other enzymes that have been implicated to exist in the eye are NADPH cytochrome p-450c reductase (Abraham et al, 1987), N-demethylase (Duffel et al, 1986), deethylase (Aimoto et al, 1985), 11-β-oxidoreductase (Weinstein et al, 1991), N-acyltransferase (Campbell et al, 1991), sulfatase (Ono et al, 1972), ketone reductase (Lee et al, 1988a), and glucuronidase (Ono et al, 1972). Apart from levobunolol (Lee et al, 1988a) and aminozolamide (Putnam et al, 1987), the concentration of the metabolites is usually very low when compared to parent drugs. Of all the enzymes discussed above, ketone reductase and esterase are the most important ones in drug delivery owing to their application in biotransformation of prodrugs and soft drugs.

(h) Formulation related factors: Some of the formulation related factors that can influence the drug bioavailability are particle size and shape, instilled volume and concentration, viscosity, pH, tonicity, preservatives and presence of permeation enhancers. Hui and Robinson, 1985a, have reported an increase in aqueous humor concentration of flouromethalone from a 0.1 % w/v solution when particle size is decreased from 10.4 to 2.0 μm. However, there was no increase in aqueous humor drug concentration seen when the particle size was reduced from 2.0 to 1.0 μm. Similar findings have been reported for dexamethasone in the particle size range of 5.75 to 22.0 μm (Schoenwald and Stewart, 1980). But varying the particle size did not affect the permeability coefficient across the cornea since it is lipophilicity, which is the dominant and rate-limiting factor to permeation across the epithelial layer of the cornea (Bisrat et al, 1990).

The human eye can withhold approximately 30 μl of the fluid without spillage or overflow, provided there is no blinking (Mishima et al, 1966) but this volume reduces to 10 μl if blinking is allowed (Wright and Merger, 1962). Table 1.5 presents the results of work done using gamma scintigraphy on the relationship between time taken for 90 % of instilled volume cleared (Chrai et al, 1973; Chrai et al, 1974).

Table 1.5: Relationship between time of 90 % of the instilled solution drainage and instilled volume

Time taken (mins)	Instilled volume (µl)
2.0	50
4.0	25
6.0	10
7.5	05

This volume dependency relationship has also been extended to percentage of drug absorbed and the pharmacological effect (Chang et al, 1988; Podder et al, 1992). Reducing the instilled volume to approximately 5 µl affects the ocular bioavailability of only low permeability drugs (by 4 times) and not that of highly permeable drugs (Keister et al, 1991). Thus, a reduction in instilled volume and simultaneous increase in instilled drug concentration would result in substantial dose reduction and at the same time would provide the drug at a therapeutically effective level in the ocular tissues (Patton, 1977). Such results have been observed in case of suspensions (3 µm in size) (Sieg and Triplett, 1980), but not in case of liposomes (Lee et al, 1984)

Increasing the viscosity of the instilled solution is another popular method for prolonging the residence time of the instilled dose in the conjunctival sac. Various polymers that have been used to increase viscosity include polyvinyl alcohol (PVA), polyvinylpyrolidone (PVP), carboxymethylcellulose (CMC), hydroxypropylmethylcellulose (HPMC), polycarbophil (PCB-934P), cyanoacrylate block copolymer, hyaluronic acid and its derivatives (Saettone et al, 1989a; Camber et al, 1987; Davies et al, 1991).

Formulation pH other than 7.4 stimulate the production of tear because of ocular surface reflexes caused due to the irritation produced by the non-compatible formulation pH in the eye surface. A three-time increase in the tear turnover rate from the normal value has been observed in rabbit due to ocular surface reflexes (Dartt et al, 1988). A simultaneous increase in tear protein level decreases the availability of free drug for absorption. Alkaline pH induces greater irritation and thus higher lachrymation than the acidic pH (Conrad et al, 1978), as the tear has higher buffering capacity in acidic region than in the basic pH (Ahmed and Choudhuri, 1988; Carney et al, 1989). Keeping the tonicity of the formulation low (Mitra and Mikkelson, 1982) could be a solution to

overcome the poor buffering capacity of the tear and thus prevent irritation-induced lachrymation.

Corneal integrity can be severely hampered by the presence of certain preservatives like, benzalkonium chloride, organomercurials (phenyl mercuric acetate, phenyl mercuric nitrate, thiomersal), chlorbutol and chelating agents like ethylene diamine tetra acetic acid (EDTA). Studies on most surfactants show corneal permeability enhancement with nonionic, cationic and anionic surfactants suggesting a transient change in the functional structure of the epithelium (Burstein and Klyce, 1977; Marsh and Maurice, 1971; Green and Tonjum, 1971; Green, 1976). These agents act as permeation enhancers and have been found to increase the corneal permeability of various drugs of different lipophilicity and molecular size. Some of the drugs investigated are pilocarpine (Mikkelson et al, 1973a), carbachol (Smolen et al, 1973), prednisolone (Green and Downs, 1974), homatropine (Kassem et al, 1983), inulin (Keller et al, 1980), and horseradish peroxidase (Tonjum, 1974). EDTA at 0.5 % level altered the permeability of the corneal epithelium at the intercellular junction level. It served to enhance the corneal permeability of water-soluble drugs but the same results were not obtained with oil-soluble drugs (Grass et al, 1985).

1.2.3 Ocular disposition of ophthalmic drugs

Physiological barriers to diffusion and productive absorption of topically applied drug exist in the precorneal and corneal spaces with bioavailability from topically applied dose ranging from 1-10 % (Lee, 1985). Two factors, which play an important role in determining drugs effectiveness from the topical formulations, are rate of dissolution and rate of absorption. The type of formulation, especially in case of suspension or ointment formulations which contain insoluble or poorly soluble drugs, affects the rate of dissolution. Thus rate of dissolution (as per equation proposed by Noyes and Whitney, 1897) become the controlling factor in any drug absorption process (Kaplan, 1972). The rate and extent of ophthalmic drug absorption is restricted largely by the non-corneal absorption in the precorneal area and the drainage. Most of the drugs have a corneal contact time of 3-6 min due to the rapidity of elimination by tear drainage. The physicochemical properties of drug and its formulation restrict the rate and extent of corneal penetration of the drug (Maurice and Mishima, 1984; Schoenwald, 1985; Lee and Robinson, 1986; Lewis et al, 1986; Chien et al, 1990). Since most drugs are absorbed by passive

diffusion, various physicochemical laws and the inherent property of the corneal membrane control the rate of ocular absorption of the drugs (Maurice and Mishima, 1984).

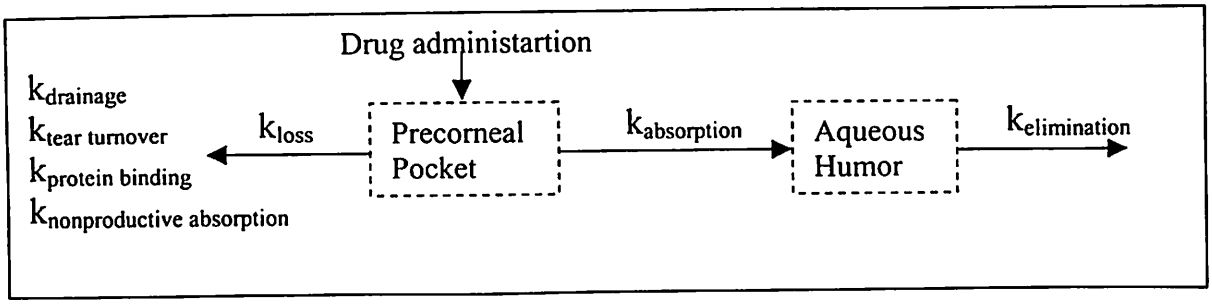
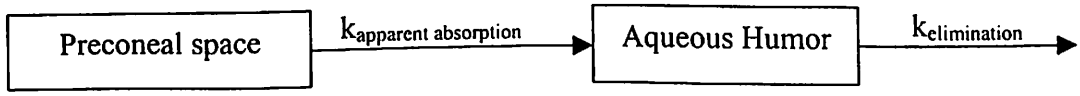


Figure 1.4: Elementary kinetic model for drug movement in the precorneal area

The drug disposition pattern in the ocular space can be explained as interplay of the three factors namely: precorneal factor, drug and its formulations and eye related factors. It is clear from the compartment model depicted for disposition of ophthalmic drug in Figure 1.4 that if there were no loss from the precorneal space then the aqueous humor drug concentration vs. time profile will be identical in shape to that of blood serum vs. time profile with ascending and descending loop showing flip-flop profile. Magnitude of $k_{absorption}$ and $k_{elimination}$ will determine the slope of the ascending and descending part of the curve. If we take the example of pilocarine, the true $k_{absorption}$= 0.003 min^{-1} and $k_{elimination}$ = 0.02 min^{-1}. Since $K_{absorption} << K_{elimination}$ a flip-flop model can be expected (Liaw and Robinson, 1993). But this assumption fails due to the fact that there is enormous amount of drug lost from the precorneal space (k_{loss} is of the order of 0.45-0.65 min^{-1}) and this gives rise to parallel elimination kinetic scheme. This represent a process in which the apparent $k_{absorption}$ is actually the sum of the true $k_{absorption}$ in the aqueous humor and the k_{loss} in the precorneal pocket as shown in equation 4.

$$\text{apparent } k_{absorption} = \text{true } k_{absorption} + k_{loss} = 0.003 + 0.55 = 0.553\ min^{-1} \qquad (4)$$

Thus the new scheme will be



which do not follow flip-flop profile because of fast elimination is very fast and there is appreciable gap between two successive administered dose. Now to improve ocular

bioavailability from topically applied drugs, it is imperative to reduce k_{loss} significantly through formulation modification or substantially increase of true $k_{absorption}$ by increasing corneal permeability through the use of permeation enhancers or prodrugs. The over all transcorneal permeation and disposition of topically applied pilocarpine in the lower cul-de-sac can be represent by the model given in Figure 1.5.

In Figure 1.5, the notations have the following meaning: q_T= normal tear fluid production rate; k_{nl} = composite first order elimination rate constant of nasolachrymal drainage; k_c = apparent rate constant for conjunctival uptake of pilocarpine; k_{aE} = apparent absorption rate constant into epithelium; k_{eE} = apparent elimination rate constant from epithelium; k_{aS} = apparent absorption rate constant into stroma-endothelium; k_{eS} = apparent elimination rate constant from stroma-endothelium; k_{aAH} = apparent absorption rate constant into aqueous humor; k_{eAH} = apparent elimination rate constant from aqueous humor; and = apparent metabolism rate constant from the epithelium. A simplified pharmacokinetics model can be established to analyze transcorneal permeation of ophthalmic drugs if it is assumed that eye consists of two major compartments (as mentioned earlier), the precorneal area and the aqueous humor (Chien, 1992).

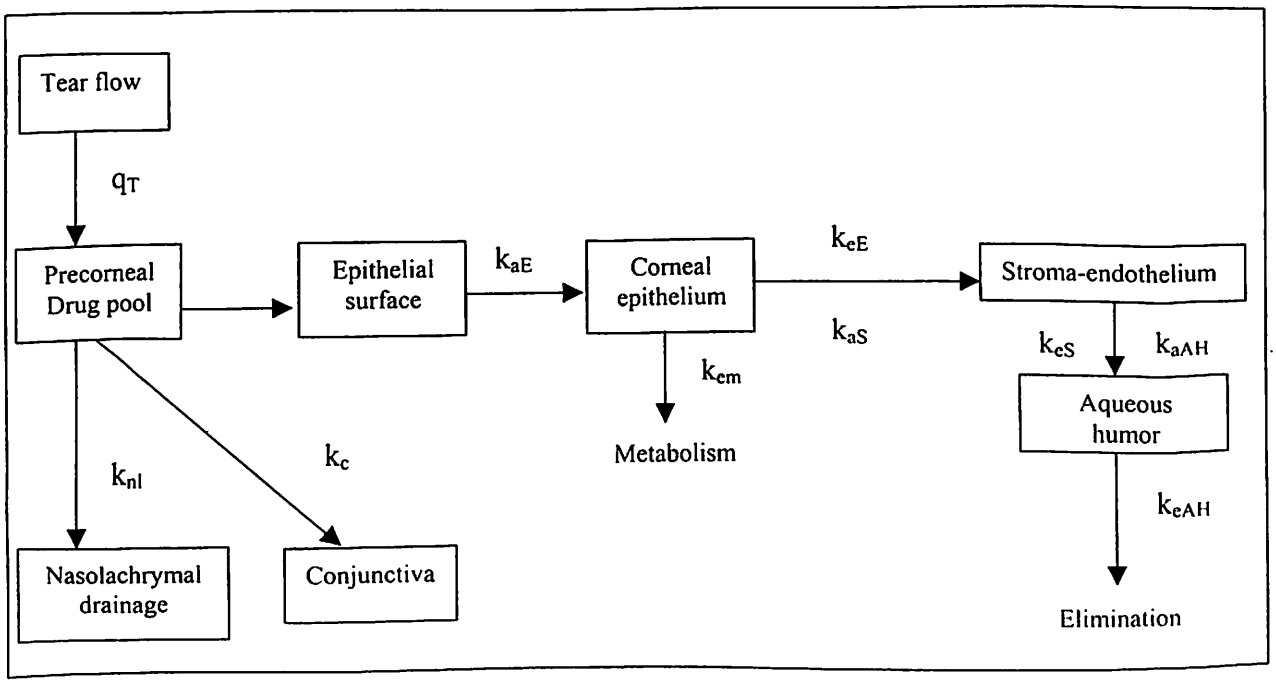


Figure 1.5: Proposed overall dispositions of ocular drugs (here pilocarpine is taken as a representative drug) topically applied in the lower cul-de-sac (Liaw and Robinson, 1993).

By this model the rate of disappearance of the drug from the precorneal area can be mathematically expressed by equation 5.

$$dC_T/dt = [-q_T C_T - (k_p S_c/ h_c)(C_T - C_{AH})] / (V_D e^{-k_{nl} t} + V_0) \quad (5)$$

The rate of appearance of the drug in the aqueous humor can be expressed by equation 6 below.

$$dC_{AH}/dt = (k_p S_c/V_{pc} h_c)(C_T - C_{AH}) - [k_{cAH} (C_{AH}/V_{AH})] \quad (6)$$

In equation 5 and 6 above the notations have the following meaning: C_T = drug concentration in the tear fluid; q_T = normal tear fluid production rate (0.66 µl/ min); k_p = specific transcorneal permeability rate of the drug; S_c = surface area of the cornea (2 cm^2); h_c = thickness of the cornea (0.035 cm); C_{AH} = drug concentration in the aqueous humor; V_D = drop size of the drug solution instilled; k_{nl} = composite first order elimination rate constant of nasolachrymal drainage; V_0 = normal resident tear volume (7.5 µl); V_{pc} = volume of drug pool in the precorneal area after instillation of the dose; k_{aAH} = apparent absorption rate constant into aqueous humor; and V_{AH} = volume of aqueous humor. The above two mathematical expressions have been developed using pilocarpine as the model drug (Chien, 1992).

1.2.4 Traditional ocular drug delivery and their inherent disadvantages

Most of the topically applied drugs from conventional ocular formulations follow a pulse entry pattern as shown in Figure 1.6. The tear concentration of the medication surges to a peak every time eye drops are instilled followed by a rapid exponential decay in drug level as the time passes. A plot of intraocular concentration of the drug vs. time yields a series of peaks of drug level separated by extended troughs. This pattern is characterized by a transient overdosing, followed by a relatively short period of acceptable dosing, in turn followed by a prolonged period of underdosing. Frequent dosing, most often than not, leads to surpassing the drug level beyond the toxic threshold limits of the drug and in the trough portions the drug level could be below the critical level required for achieving the desired therapeutic efficacy. Frequent local instillation of anti-inflammatory agents, antiglaucoma agents, antibiotics, antivirals, and sulfonamides provide unusually high concentration of the drug and preservatives at the corneal epithelial surface leading to various ocular cytopathologies.

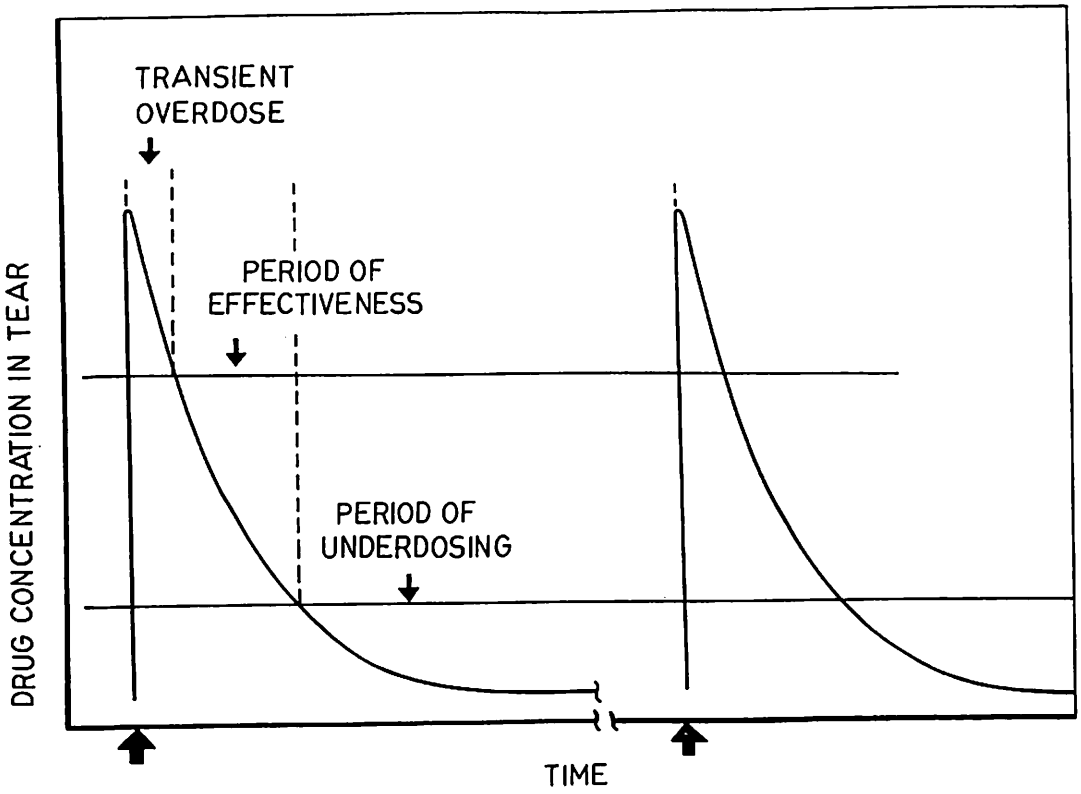


Figure 1.6: A typical tear drug concentration-time profile for topically applied drug in the form of aqueous drop

Topical administration is by far the most common route of drug delivery to the eye, but from biopharmaceutical standpoint, it has drawbacks. The conventional preparations like, solutions, suspensions and semisolids, fail to establish the therapeutic drug level for the desired length of time within the ocular tissues and fluids. There is a limitation with respect to the maximum volume of the formulation that can be accommodated in the lower cul-de-sac (Chrai et al, 1974) and also the maximum time period (usually 1-2 min) for which the applied dose remain in direct contact with the absorptive surface of the eye (Chrai et al, 1973; Blanksma et al, 1977). While these mode of delivery can be suitable for treating external conditions of the eye but most of the ailments of the anterior and posterior segment are badly managed by the pulsatile topical drug instillation. The extent of drug penetration into the anterior segment is greatly influenced by the precorneal area fluid dynamics. This often leads to patient-to-patient variability and the difference in results from animal (rabbit) models (Sugaya and Nagtaki, 1978; Saettone et al, 1982a). Figure 1.7 below highlights some of the routes of drug loss from conventional topical ocular formulations (Adapted from Olejnik, 1993). Tear turnover (Mishima et al, 1966) and tear evaporation from the corneal surface (Mishima, 1965) contribute to decrease in the rate of transport of drugs into the various ocular chambers.

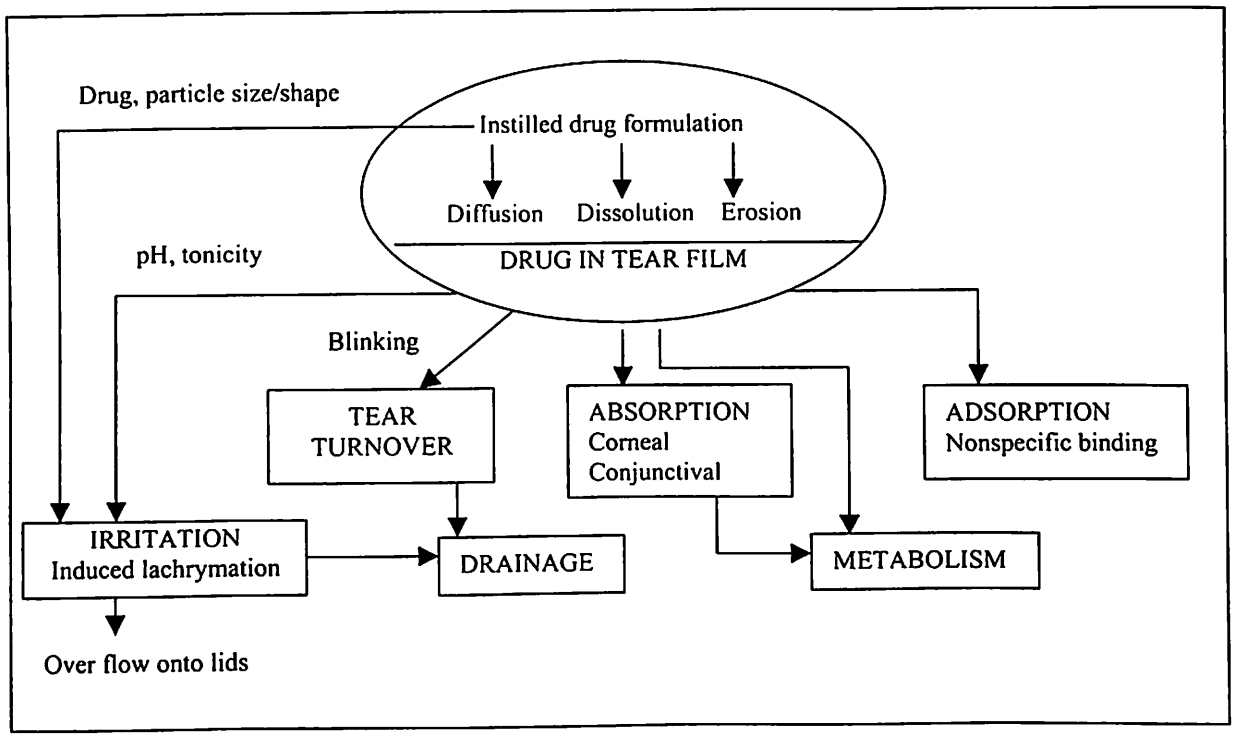


Figure 1.7: Routes of drug loss from conventional topical ocular formulations (Olejnik, 1993)

Factors such as pH, tonicity, particle size and irritation caused by the drug can stimulate and accelerate drug loss (Conrad et al, 1978; Holly and Lamberts, 1981) and, depending upon the delivery system, can cause loss of the dosage form itself. Included in this realm are losses by metabolic degradation (Lee et al, 1980; Schoenwald and Houseman, 1982), protein binding (Mikkelson et al, 1973a, b; Lyons and Krohn, 1975; Salazar et al, 1976), and other non-productive loss mechanisms (Makoid et al, 1976; Patton and Robinson, 1976; Ahmed and Patton, 1985) discussed earlier in this chapter.

Most of the ophthalmic drugs can be formulated as aqueous solutions using their water-soluble salts. But the ocular absorbability of drug from these formulations depends on the molecular weight, solubility, partition coefficient, and pK_a. Solutions are, normally, preferred mode of delivery as they cause less blurring of vision, easy administration and frequent instillation of concentrated dose can achieve high intraocular concentrations. But the major disadvantages of this type of formulation include decreased corneal residence time due to rapid drainage because

of very less viscosity, imprecise and inconsistent dosing, and chances of contamination. Frequent instillation of relatively high concentration of the drug leads to increased conjunctival and nasolachrymal absorption leading to severe systemic side effects.

Ophthalmically active drugs that are sparingly soluble in water are often formulated as suspensions, with micronised drug (< 10μm in diameter) suspended in suitable aqueous vehicle. The duration of action of drug from suspension exceeds that of the solution due to the ability of the drug particle to persist in the conjunctival sac giving rise to sustained release effect (Leibowitz and Kupferman, 1975). But other studies (Greens and Downs, 1974; Kupferman et al, 1974) on in vivo performance of ocular suspensions did not provide any data on the residence time profile of the suspended particles. In addition to particle drug disposition, suspensions also need to be evaluated for drug potency, intrinsic solubility, dissolution rate and particle size (Sieg and Triplett, 1980; Schoenwald and Stewart, 1980; Roberts and Leibowitz, 1984). Increase in drug particle size has been shown to decrease the bioavailability from suspensions (Schoenwald and Stewart, 1980). A particle size of approximately 10 μm can significantly minimize the drug-induced irritation and related discomfort and reflex tear production and drainage. Other properties of the drug particle such as concentration, density, and shape may contribute to the patient comfort threshold. Also the problem of precipitation and sedimentation need to be addressed. Like solutions, ocular suspension also utilizes quite a few inactive formulation additives. A brief summary of the formulation additives used in ophthalmic solutions and suspensions is presented below.

(a) Tonicity adjusting agents: An ophthalmic solution need to be isotonic to tear fluid, which is approximately equal to 0.9 % NaCl (with a tolerance range equivalent to 0.5 % to 1.8 % NaCl).

(b) Buffers: All ophthalmic drops, barring few exceptions, are buffered at pH 7.4. Buffering at extreme acidic or basic pH would lead to increased lachrymation, blinking and corneal damage. Sometimes drug solutions or suspensions are formulated at pH very different from 7.4 due to stability and/or solubility consideration of the drug. The most commonly used buffer is that of phosphates.

(c) Stabilizers: Anti-oxidants like, sodium bisulphite or metabisulphite are used at concentration less than 0.3 % w/v to prevent the degradation of the active ingredient. Sometimes, EDTA, thiourea, and sodium thiosulphate are also used.

(d) Surfactants: Non-ionic surfactants are used in extremely low concentrations to aid dispersing of steroidal or other drugs in suspension and to achieve solution clarity. The surfactants mainly used for this purpose are polysorbate 20 and 80, and polyoxyl 40 stearate.

(e) Viscosity enhancers: Viscosity enhancers can significantly reduce the surface tension and thus increase ocular contact time, decrease the drainage rate, and increase drug bioavailability. Main disadvantage of these polymers is their tendency to dry to a film on the eyelids. Polymers mainly used for this purpose include PVA, methyl cellulose (MC), HPMC, dextran-70, ethyl cellulose (EC) and polyvinyl pyrrolidone (PVP).

(f) Vehicles: Purified water obtained by distillation, deionization or reverse osmosis is the universal vehicle for most of the ophthalmic solutions and suspensions. Vegetable oils of highest purity are sometimes used for certain drugs, to be formulated as ophthalmic drops, which are extremely sensitive to moisture. Vegetable oils commonly used are olive oil, castor oil, peanut oil, and sesame oil.

(g) Preservatives: Most pharmacoepia require antimicrobial preservatives at appropriate concentration if the formulation is supplied in multidose container unless otherwise the drug itself has sufficient antimicrobial activity. At high concentrations preservatives exert microbicidal activity but at the same time are potentially irritating. Also these preservatives are capable of disrupting the corneal integrity and thus increasing the corneal permeability of many drugs. Commonly used preservatives are benzalkonium chloride (0.004-0.02 % w/v), chlorbutanol (0.5 % w/v), phenyl mercuric nitrate (0.002- 0.004 % w/v), thiomersal (0.005 – 0.02 % w/v), methyl-paraben (0.1- 0.2 % w/v) and propylparaben. EDTA (chelating agent) is sometimes used to increase anti-pseudomonas activity along with benzalkonium chloride.

Semisolid preparation mainly used in ophthalmology is an anhydrous ointment in petrolatum base. The ointment vehicle is a mixture of mineral oil and white petrolatum. Ointments offer the advantage of longer contact time and better drug bioavailability. The major disadvantage is its greasy nature and blurring of vision.

1.2.5 Approaches for better ocular drug delivery

The therapeutic efficacy of an ophthalmic drug can be greatly improved by- (a) prolonging its contact with the corneal surface; (b) enhancing corneal permeability; (c) controlled release; and (d) enhancing site specific action (oculoselective action). Conventional ocular delivery systems

like, suspension and ointments are able to provide some sort of sustaining effect. But the rate of release from the suspension and ointment type preparations depend upon the rate of dissolution of the drug particles in the medium, which varies constantly in its composition with the constant inflow and outflow of lachrymal fluid. The newer approaches employed in developing a truly continuous controlled delivery of ophthalmically active drugs to the eye are: (a) using viscosity enhancers and permeation enhancers; (b) muco-adhesive polymeric systems; (c) polymeric matrix and membrane coated system; and (d) drug modification through prodrug or development of oculoselective drugs. Other approaches are based on liposomal and particulate based drug delivery.

The drug delivery systems based on above approaches have succeeded in significantly reducing the frequency of dosing and also in remarkably improving the therapeutic efficacy of ophthalmic drugs.

(a) Viscosity enhancers and permeation enhancers: Viscosity enhancers and permeation enhancers in ophthalmic formulation have been used extensively in an attempt to over come precorneal (using viscosity enhancers) and corneal (using permeation enhancers) barrier to ocular drug delivery.

The most commonly used viscolyzing agents include PVA and cellulose derivatives (mainly MC and HPMC). These agents increase ocular bioavailability due to prolonged residence time of the instilled dose in the conjunctival sac. Use of PVA and MC has shown to decrease the clearance rate from the precorneal space when compared to saline (Hardberger, et al, 1975; Zaki, et al, 1986). An increase in pharamcodynamic activity of pilocarpine was seen in glaucoma patients when administered as viscous 3.5 % PVA drop (Davies, et al, 1977) with duration of efficacy increasing from 3 to 7 hrs. Viscous solutions using different polymers were tried for 0.2 % tropicamide as the drug and tested for mydriatic activity in both human and rabbits (Saettone, et al, 1982b). Rheological properties of the polymeric solution play an important role in the retention pattern on the ocular surface (Patton and Robinson, 1975). Pseudoplastic, plastic and thixotropic systems will undergo thinning as the shear force, experienced as a result of blinking, will exceed the yield value of the system, thereby causing drainage from the eye. On the other hand in case of dilatant systems the shear produced by blinking thickens the system and thereby increasing the corneal retention (Saettone, et al, 1982a; Olejink, O, 1993). Any benefits from rheological behavior are likely to involve other contributing factors relating to surface chemistry

and biological interactions (Benedetto, et al, 1975; Versura et al, 1989; Saettone, et al, 1989). Also the chemical nature of the polymer plays an important role in improving corneal drug absorption and also attenuated corneal membrane disruption (Saettone et al, 1985). For most of the viscous solutions the optimum viscosity has been found to range between 12 to 15 cps (1.2 to 1.5 mPa) (Patton and Robinson, 1975, 1976). A viscosity beyond 15 cps has been shown to cause poor product-tear intermixing accompanied by distortion of the optical surface, producing visual disturbances for patient (Eriksen, 1980). In case of highly lipophilic drugs, viscosity considerations have to be meticulously calculated since such drugs readily partition into the corneal epithelium thus any increase in residence time of a solution would not increase the total amount of drug absorbed (Lee and Carson, 1985).

Permeation enhancers and the mechanisms by which they improve corneal drug absorption is being extensively investigated. It has been proposed that penetration enhancers improve corneal drug transport by two methods. The first method is an expanded paracellular pathway by compounds that change the cell cytoskeleton structure, which participates in the regulation of the corneal permeability (Frederiksen and Leyssac, 1977), by disrupting the actin microfilaments at the tight junction of the epithelial cells (Craig and Prado, 1979). Promoting glucose or amino acid transport with sodium cotransport can alter tight junction permeability. Phorbol esters (Mullin and O'Brien, 1986), glucose, alanine and leucine (Pappanheimer and Reiss, 1987), lysine (Martinez-Palomo, 1975) has been found to induce opening of the tight junctions by this mechanism. Charge type and charge density also affects the transcorneal absorption. Rojanasakul and Robinson, 1989, showed that cornea contain both positive and negative charged groups at its isoelectric pH of 3.2. At pH above this, cornea carries a net negative charge and is thus selective for positively charged molecules and vice versa at pH below 3.2. A two to three fold increase was seen in the *in-vitro* permeability of lysine (molecular weight 146, positive charge) and glutamic acid (molecular weight 147, negatively charged) at pH above and below 3.2 respectively (Grass and Robinson, 1988a, b).

The second mechanism by which penetration enhancers act by affecting membrane lipids and protein components of transcellular pathway. Oleic acid has been indicated to lower the membrane lipid transition temperature in addition to increasing the conformational freedom and flexibility of the endogenous lipid alkyl chain above their transition temperature. Medium chain monoglycerides like glyceryl-1-monooctanoate, glyceryl-1-monodecanoate, glyceryl-1-

monododecanoate have been postulated to increase permeation by extracting cholesterol from the epithelial membrane. Nonprotein thiols are yet another membrane component where enhancers like diethyl maleate, diethyl ethoxy methylene malnoate, ethanol or salicylates act to increase permeability (Liaw and Robinson, 1993).

(b) Mucoadhesive polymeric system: As discussed earlier, increasing vehicular viscosity is a popular method to prolong ocular residence time of the drug but with minimal (2-3 folds) improvement of bioavailability (Lemp and Szymanksi, 1975; Saettone et al, 1982a). As mentioned earlier, vehicle with viscosity above 10 cps cause blurring of vision and thus, patient acceptability is low. Muco-adhesives for ocular delivery have been used to provide intimate contact of the drug with the mucin layer of the outer cornea providing high drug concentration in localized area for high drug flux through absorbing tissues. This procedure can improve the availability of high molecular weight compounds like peptides and proteins (Harris and Robinson, 1990). Mucus layer in the eye is a 50 to 450 μm thick gel layer adherent to the corneal epithelium containing water (95 %) and high molecular weight glycoporteins capable of forming slimy viscoelastic gels (Allen, 1981; Marriot and Gregory, 1990). Sometimes lipids and covalently bound fatty acids are also found in mucin layer. Mucin is secreted mainly from the goblet cells or specialized exocrine glands in various portions of the body (Schater and Williams, 1982).

The mechanism of adhesion of bioadhesive polymeric vehicles to mucin layer has been explained on the basis of electron transfer (Derjaguin et al, 1977), wetting (Baier et al, 1968; Helfand and Tagami, 1971, 1972), diffusion, adsorption (Reinhart and Peppas, 1984; Peppas and Buri, 1985; Peppas and Lustig, 1985), fracture (Ponchel et al, 1987; Mikos and Peppas, 1988), and mechanical interlocking (Wake, 1976) theories. The entire process can be viewed as a process of physical entanglement. The polymer undergoes swelling in water which permits this entanglement with mucin on the epithelial surface (Mikos and Peppas, 1986) with the unionized polar carboxylic acid, amide or sulfate residues of the polymer forming hydrogen bonds with the mucin molecule. This results in the inter-penetration of the polymeric chains. Other attractive forces of interaction can be van der Waals forces, electrostatic forces and hydrophobic forces. Repulsive forces of interaction include electrostatic and steric repulsion. In some polymers, at pH equal to or less than 3.0, the protonated polar residues of the polymer form the hydrogen bond with mucin thus getting maximum bioadhesion but with complete loss of mucoadhesive

property at pH 5.0. Degree of hydration also affects the mucoadhesive properties (Park and Robinson, 1985). At physiological pH of 7.4 mucin is negatively charged due the sialic acid group at the terminal end of the mucoploysaccharide chain (Gottschalk, 1960). At this pH hydration of the cross-linked polymer in the precorneal fluid is maximum but number of hydrogen bonds is low. Inspite of the decrease in mucoadhesive property it is sufficient to provide firm attachment of the delivery system to the corneal surface.

Use of bioadhesive polymers for enhancing the bioavailability was first demonstrated by Hui and Robinson (1985b) for progesterone. After that, several natural and synthetic polymers have been screened for this purpose. Saettone et al, 1989b, evaluated several polymers of mucoadhesive delivery of tropicamide and pilocarpine and found hyaluronic acid to be the most suitable candidate. Some of the other natural mucoadhesive polymers investigated in ocular drug delivery are collagen, fibrin and fibronectin. In case of synthetic polymers 1.4 % PVA, 0.5 % HPMC, and 2.0 % hydroxy ethyl cellulose vehicles have been shown to have comparable corneal adhesion to that of mucin (Krishnamoorthy and Mitra, 1993). Cross-linked polyacrylic acid polymers, polyacrylamide and copolymer of acrylamide (N-vinyl pyrrolidone and ethyl acrylate) have also shown promising results in significantly enhancing ocular bioavailability in various studies (Krishnamoorthy and Mitra, 1993).

(c) Polymeric matrix and membrane coated system: The rate of removal of instilled dose from the cul-de-sac increases linearly as the volume of instillation is increased. If the drug is placed in the precorneal space in the form of solid layer or film, tear drainage can be kept to minimum. Further, Lerman, 1970, have demonstrated the clinical advantage of administering pilocarpine micro drops in a continuos manner simulating controlled release systems. So, ocular drug delivery devices consisting of a central reservoir of drug, enclosed between specially tailored membranes or drug embedded or solubilized in the matrix base will allow the drug to diffuse from the reservoir at a precisely predetermined rate. Such delivery systems have been designed and developed using bioerodible and/or non-bioerodible polymeric system with varying degree of hydrophilicity. These systems will also improve patients compliance by drastically reducing the dosing frequency. The desired criteria for a controlled release ocular insert are; comfort, ease of manufacturing, handling and insertion, reproducible release kinetics, non-interference with vision and oxygen permeability, and the sterility and stability virtues.

Controlled release non-bioerodible systems give more reproducibility in drug release kinetics but it need to be removed from the body after desired duration. On the other hand, bioerodible inserts need not be removed after the lapse of duration of release but owing to variation in rate of tear generation and the composition of metabolic enzymes in the tear, patient to patient variability is seen in release characteristics.

Many workers have considered eye to be an ideal organ for implantable drug delivery system because of ease of implantation and removal (Langer, 1986). Various solid, hydrophilic ocular inserts have been attempted at for better ophthalmic delivery of some of the drugs. Alza Corporation, Palo Alto, USA, introduced the most promising formulation of this type in 1974. The ocusert systems – Ocusert pilo-20® and ocusert pilo-40®, delivered pilocarpine at a constant rate of 20μg/hr and 40μg/hr respectively for 7 days. The non-compliance issues, the low intraocular drug bioavailability and the potential systemic side effects associated with pilocarpine eye drops were significantly improved with development of pilocarpine releasing ocusert system (Shell and Baker, 1974; Friederich, 1974; Urquhart, 1980). Several bio-erodible drug inserts have been developed and evaluated for ocular use such as, pilocarpine containing CMC wafers (Haddad, 1974; Haddad and Lucas, 1975), PVA disc (Maichuk, 1975; Grass et al, 1984), PVA rod (Bondi and Harwood, 1988), collagen waffers of gentamycin sulfate (Bloomfield et al, 1978; Slatter et al, 1982; Punch et al, 1985) and erodible ocular inserts containing hydrophobic polycarboxylic acid (Heller and Baker, 1974). Despite the efforts only few devices have been successfully marketed till date.

(i) Ocusert pilo-20® and ocusert pilo-40® (Alza Corporation, USA; 1974)- Ocuserts are flat, flexible, elliptical, device with larger and smaller diameters as 13.4 mm and 5.7 mm respectively. It consists of two outer layer of ethylene vinyl acetate that encloses the inner core of pilocarpine gelled with alginate. On the circumference a ring of titanium dioxide impregnated with ethylene vinyl acetate is placed for visibility. Ocuserts come in two sizes namely Ocusert pilo-20 ® and Ocusert pilo-40 ®, that deliver pilocarpine at a constant rate of 20μg/hr and 40μg/hr respectively for 7 days. In ocusert pilo-40 the rate of drug release is increased by decreasing the thickness of the outer membrane and by adding a flux enhancer di (2-ethylhexyl) phthalate. The size and shape of these devices influence the retention of these devices in the eye. It has been observed that smaller and rod shaped system is better retained than larger and oval ones.

(ii) Lacriserts® (Merck, Sharp & Dohme, USA; 1981) (LaMotte et al, 1985)- It is s sterile rod shaped device made of hydroxy propyl cellulose without any preservative used for dry eye syndrome (keratoconjunctivitis sicca). The device can give relief from dry eyes for approximately 24 hrs per insertion. It is 5 mg in weight with a length of 3.5 mm and diameter of 1.27 mm. In a cross over study, 78 % patients (out of total of 32 patients) who were suffering from keratoconjunctivitis sicca preferred insert to liquid artificial tear.

(iii) Soluble ocular drug inserts (SODI$^{\phi}$)- It was first developed by soviet scientists for cosmonauts who could not use eye drops. SODI is a small oval wafer of polyacrylamide impregnated with drug with dimensions of 9.0 mm x 4.5 mm and a thickness of 0.35 mm (Bawa, 1993). SODI impregnated with pilocarpine and tetracycline has demonstrated very high efficiency in the treatment of glaucoma and trachoma respectively when compared to conventional drops.

(iv) Ocular therapeutic system (OTS) or mini disc (Bawa et al, 1988)- OTS is a miniature contact lens like contoured disc of 4-5 mm in diameter with a convex front and concave back in contact with the eye ball. It is made of silicone based prepolymer-α-ω-bis-(4-methaacryloxy)-butylpolydimethyl siloxane. OTS can be designed as hydrophilic or hydrophobic matrix base for the extended release of water insoluble or water soluble drugs respectively. An extended release of 170 hrs has been achieved for poorly water soluble drug like sulfisoxazole when incorporated in hydrophilic matrix whereas hydrophobic OTS released gentamycin (relatively water soluble) for 320 hrs. Gamma irradiation and heat treatment decrease the rate of release due to further crosslinking (Bawa and Nandu, 1990).

(v) New ophthalmic delivery system (NODS) - NODS is a method of presenting water soluble drug within loaded film devoid of any preservative. The drug is incorporated into a water soluble PVA film. Each NODS contains a drug loaded film (called flag) attached to a handle film by means of a thin membrane. The flag, which is semicircular in shape, is approximately 4 mm in length, 6 mm in width and has an area of 21 mm^2 and a thickness of 20 μm. It is attached to a handle, which is 0.7 mm in length and 30 μm thick. Each NODS weighs approximately 500 μg out of which 40 % is drug. In contact with tear the outer handle membrane quickly dissolves

$^{\phi}$ SODI-Soluble polymeric drug delivery system for ophthalmic applications, company brochure, Diversified Tech Inc., Salt Lake City, Utah, USA

releasing the flag into the tear film. After which flag hydrates and disperses allowing diffusion and absorption of the drug.

(vi) Medicated lenses- Drug loaded, by presoaking, contact lenses have been extensively studied (Podos et al, 1972; Mizutani and Miwa, 1975; Ramer and Gasset, 1974; Matoba and McCulley, 1985; Friedman et al, 1985; Reccia et al, 1985; Massimo and Spitznas, 1988; Jain, 1988) for variety of drugs like anti glaucoma agents, antibiotics, and polypeptides. But the rate of release of the medicament from these lenses is very rapid (Shell and Baker, 1974), usually around 30 min. Therapeutic soft lenses aid corneal wound healing in patients suffering from infections and corneal ulcers which is characterized by thinning of the cornea (Matoba and McCulley, 1985). Hillman, 1975, have demonstrated the deleterious effect of benzalkonium chloride used in presoaked soft lenses on the corneal epithelium. Improperly fitted contact lenses can also impede oxygen supply to the cornea and also lead to build-up of toxic metabolite like carbon dioxide in the eye. Some of these problems can be overcome by incorporating the drug solution or suspension of solid particles in the monomer mix prior to polymerization of the lenses (Bawa and Ruscio, 1990). The duration of release in such systems has been enhanced to 180 hrs as compared to 30 min of presoaked lenses. Also the corneal membrane disrupting activity is abolished since it does not uses any preservative.

(vii) Corneal collagen shields- It has been demonstrated that use of collagen shields promote corneal healing after radical keratotomy and provide lubricity to the eye (Aquavella et al, 1988). Corneal shields are made up of porcine collagen with a 14.5 mm in diameter, a 9.0 mm back curve, and about a 0.1 mm thickness at the centre. Such corneal collagen shields have been extensively investigated by several workers for improved ocular delivery of various medicaments (Peiffer et al, 1983; Ellis et al, 1984; O'Brien et al, 1988; Poland and Kaufman, 1988; Phinney et al, 1988; Unterman et al, 1988; Sawusch et al, 1989; Hwang et al, 1989;) Exposure to ultraviolet radiation for different time period results in collagens with varying degree of crosslinking and thus variable dissolution rates. Finally the collagen shields are subjected to sterilization by gamma irradiation.

(d) Prodrug approach: Of all the enzymes present in ocular tissues, ketone reductase and esterase are the most important ones in ocular drug delivery. This is due to their application in biotransformation of prodrugs and soft drugs for better oculoselective action (Lee, 1992). Prodrugs for ocular purpose are chemical modification of the parent drug that are chemically or

enzymatically liable for conversion to active parent drugs in the eye. Prodrug design of ophthalmic drugs has been either with an objective to change physicochemical properties of the drug like solubility, pK_a and lipophilicity to aid in corneal drug penetration or to produce prodrug that are systemically inactive but highly active in the ocular tissues. Another approach is to use prodrugs to reduce the systemic absorption of ocularly applied drugs as exemplified in timolol. Reduced systemic absorption is achieved by improved corneal permeability of the prodrug because of increased lipophilicity.

In eye, the major clinical advantage of prodrug is reduction in dose with concomitant decrease in side effects potentially due to decreased nasolachrymal absorption (Goldberg et al, 1980; Chang et al, 1987). It was demonstrated that O-butyryl timolol, an ester prodrug of timolol, was ocularly absorbed 5.5 times better than timolol, while the systemic absorption was comparable (Chang et al, 1987). Conversion of epinephrine to its prodrug dipivalyl ester improved epinephrine permeability by 10 fold, with drastic reduction in ocular side effect (allergic conjunctivitis) when used clinically (Karback et al, 1976). Other ophthalmic drugs that have shown increased permeability upon conversion to prodrug are phenylepinephrine (Schoenwald et al, 1987; Chien and Schoenwald, 1986), pilocarpine (Bundgaard et al, 1986,1996), and idoxuridine (Narukar and Mitra, 1989). Several other β-blocker's like, betoxolol, esmolol, butoxamine and their prodrugs are being investigated for ocular selectivity (Shell, 1987; Chein, 1992). The concept of double prodrug (prodrug of a prodrug) has been applied to (dibenzyl) bispilocarpate, double prodrugs of pilocarpine (Jarvinen et al, 1992).

(e) Liposomes: Smolin et al, 1981, were the first to report the advantage of using liposomes in ocular delivery of idoxuridine in the treatment of herpetic keratitis in rabbits. Detailed method of preparation and characterization of liposomal vesicles has been reviewed by Sajeev et al, 1997. Later appreciable enhancement of corneal permeation of idoxuridine was shown when entrapped in liposomal vesicles (Dharma et al, 1986). Corneal penetration of penicillin-G, a water soluble compound, and indoxole, a lipophilic compound, was increased when liposomal vesicle formulation was compared with that of solution of each drug (Schaeffer and Krohn, 1982). Increased ocular bioavailability of atropine and atropine sulfate has been shown with liposomal entrapment (Meisner et al, 1989). Singh and Mezei investigated the ocular delivery of both a lipophilic drug, triamcinolone acetonide (Singh and Mezei, 1983), and a hydrophilic drug, dihydrostreptomycin sulfate (Singh and Mezei, 1984), and reported that the drug levels in the

ocular tissues were increased for triamcinolone acetonide by more than two fold but aqueous humor level decreased 15-20 times for dihydrostreptomycin sulfate. In a similar study, Stratford et al, 1983a, b, found that liposomal entrapment of inulin increased its concentration in eye by 3-15 folds but decreased for epinephrine by 1.5-3.0 fold. Several mechanisms have been proposed to explain the liposomal interaction with cells though the precise mechanism by which it interacts with the cell is not fully understood (New et al, 1990). Some of the proposed mechanisms include: (i) Intermembrane transfer, (ii) Contact release, (iii) Adsorption of the liposomes to the cell surface via nonspecific means or specific ligands such as antibodies, hormones, and lectins, (iv) Fusion of liposomes with the cell membranes, and (v) Endocytosis of the liposomes by the cell.

(f) Particulate based drug delivery: Particle size reduction methods in aqueous suspensions of water insoluble drugs have shown improved ocular bioavailability. However, such an approach is not possible with water soluble drugs. Nanoparticles are particulate drug delivery systems with 10-1000 nm particle size in which the drug may be dispersed, encapsulated or adsorbed. Nanoparticles for ophthalmic delivery is mainly produced by emulsion polymerization, in which poorly soluble monomer is dispersed in continuos aqueous phase. Polymerization is initiated by irradiation method at pH below 3.0, which can be adjusted to desired value at post polymerization. Non-ionic emulsifying agents are used to stabilize the resulting polymeric particles. The drug can be added before, during or after polymerization. Most commonly used material for ophtahlmic use is polyalkylcyanoacrylates.

Pilocarpine nanoparticles has shown enhanced mitotic response by 22-23 %. The miosis time was prolonged from 180 to 240 min in comparison to pilocarpine solution (Harmia et al, 1986a, 1986b, Harmia, 1989). High loading of progesterone in polybutylcyanoacrylates was found to hinder its effective delivery due to faster precorneal elimination rate of the nanoparticles than the rate of release of the drug from the particles (Li et al, 1986). In 1989, Diepold et al, demonstrated the beneficial aspects of nanoparticles in targeting drugs to inflamed tissues of the eye. In this study tissue concentration of polyhexylcyanoacrylate loaded pilocarpine concentration was found to be 3-5 times more in inflamed eyes than in normal eyes.

(g) Ocular iontophoresis: Iontophoresis or the technique of increasing the flux of charged particles into tissues by the application of electric current was first reported by Veratti in the year 1947 (Duke-Elder, 1962b). Since then iontophoresis has been adapted for use in a variety of

therapy in the area of diagnostics of cystic fibrosis by iontophoretic application of pilocarpine (Carter et al, 1984), in dermatology in the treatment of hyperhidrosis (Sloan and Soltani, 1986), in dentistry for treatment of hypersensitive teeth and oral ulcers (Gangarosa Sr., 1983) and in ophthalmology. The earliest account of iontophoresis in ophthalmology dates back to 1908 when Wirtz performed iontophoresis of zinc salt for the treatment of corneal ulcers (reported by Duke-Elder, 1962b).

Cathodal iontophoresis techniques employed using flourescein for study of aqueous humor dynamics or fluid dynamics of the anterior chamber at different potential and duration (Jones and Maurice, 1966; Starr, 1966; Holm, 1968; Tonjum and Green, 1971: Brubaker, 1982). Ocular iontophoresis has been attempted by several workers for variety of drugs with varying degree of success in ensuring high drug concentration in the site of action like, adrenergic agents (6-hydroxydopamine and α-methylparatyrosine) for the treatment of glaucoma (Kitazawa et al, 1975; Colsanti and Trotter, 1977), 5-flourouracil for the control of cellular proliferation after glaucoma surgery (Kondo and Araie, 1989), 4 % lidocaine for anesthesia of eye-lid (Meyer et al, 1990). Several antibiotics like gentamycin (Hughes and Maurice, 1984; Barza et al, 1987), tobramycin (Hodbden et al, 1988), cefazolin and tiracilllin (Barza et al, 1986), vancomycin (Choi and Lee, 1988), ketoconazole (Grossman and Lee, 1989) were attempted by transcorneal or transscleral iontophoresis to increase the aqueous or vitreous humor concentration of the drugs. Iontophoretic techniques were also used for the delivery of hydrocortisone acetate (Lachaud, 1965) and dexamethasone (Lam et al, 1989) in rabbit eye model. Antivirals like, idoxuridine, phosphonoacetic acid and vidarabine monophosphate for the treatment of epithelial keratitis were iontophoresed in unaffected and herpes simplex virus (HSV) keratitis rabbit model by Hill et al (1978, 1979) and in HSV stromal infection (1982). In all the above studies involving ocular iontophoresis a common deduction was that this technique offers a fast, painless and high concentration of drug to the specific site usually in aqueous or in vitreous humor.

(h) Non-corneal delivery: The main pharmacokinetic problem of low vitreous drug concentration by topical or systemic administration can either be overcome by subconjunctival or by intravitreal injection. While subconjuctival injection of steroids like, hydrocortisone (Maurice and Mishima, 1984) and antibiotics like, gentamycin (Hillman, 1979), oxacillin (Barza et al, 1980), ceftriaxone (Jay et al, 1984), netilmicin (Orr et al, 1985) and cefotaxime (Rubinstein et al, 1987) suspensions were able to produce prolonged drug concentration in the eye due to slow

dissolution but were unable to produce sufficient vitreous concentration for being effective against many common causatives of endophthalmitis. Also the non-productive systemic drug absorption was one of the main limiting factor with such prolonged release delivery systems. Intravitreal injections was first attempted by von sallmann and his co-workers (1945) and found that Intravitreal administration of penicillin in rabbits favorably influenced the course of experimental endophthalmitis. Peyman and co-workers (1974) demonstrated the clinical benefits of intravitreal injections of antibiotics in treating endophthalmitis. A comparative summary of systemic, topical, subconjunctival and intravitreal dose of various ocular anti-infectives have been presented by Schulman and Peyman (1993) along with few pharmacokinetic parameters.

(i) Peptide and protein delivery through eye: Ocular route for systemic delivery of peptides and proteins have been considered as an viable alternative to several other routes like, nasal, buccal, transdermal etc. There are several advantages of systemic delivery of protein and peptide through ocular route. First of all ocular drops present a convenient mode of delivery and systemic absorption is really rapid from the precorneal spaces. Since this route of systemic absorption circumvents first-pass metabolism the dose of the drug required will be low and therapeutic concentration can be achieved through low doses. Prolonged action can be achieved by formulation modification. But permeation enhancers need to be added for compounds of molecular weight greater than 10,000. Also the absolute amount of polypeptide that can be instilled is 2.5 mg (10 % w/v solution and a maximum volume of 25 µl). Systemic bioavailability of insulin following ocular delivery has been related to lowered blood sugar levels in rabbits (Christie and Hanzal, 1931). Small polypetides like thyrotropin releasing hormone (MW-300), enkephalins (MW-600), luteinizing hormone releasing hormone (MW-1200) and glucagons (MW- 3500) have been shown to be 99 % systemically bioavailable through ocular delivery (Chiou and Chuang, 1988). High molecular weight polypeptides like insulin (MW-6000) can be systemically delivered by ocular route using suitable permeation enhancers (Chiou and Ching, 1975).

1.3 An overview of ocular inflammatory reactions and role of NSAID's in its therapeutics

1.3.1 Ocular inflammatory reactions

Due to anatomical isolation and exposure to environment, the eye presents with variety of primary and secondary inflammatory and infectious disorders (Duke-Elder, 1962a). These disorders have been extensively reviewed by various authors (Howest, Jr., 1985; Lucas, 1989; Turner, 1992; Owen, 1977; Willoughby, 1973; Wiggins and Cochrane, 1979; Spencer and Zimmerman 1985; O'Connor Davies et al, 1989; Moroi and Lichter, 2001). It is important to have understanding of the various inflammatory and infectious disorders of the eye for designing better drug delivery systems to cure or manage them.

(a) Conjunctivitis: The conjunctiva, because of being exposed and having anatomical proximity to the lid, orbit and other ocular structures, is prone to primary and secondary inflammations. Conjunctivitis is characterized by the rapid onset of vascular dilation and congestion (hyperemia), accompanied by watering, lymphatic dilatation and transudation of fluid and cells into the perivascular tissues (edema) and finally, secretion of mucus, degenerating epithelial cells and inflammatory cells into the conjunctival sac (exudation). During acute inflammation, there is an increase in mucus secretion accompanied by an outpouring of inflammatory cells and serous exudates containing varying amounts of protein and fibrin. The accumulated material that adheres loosely to the epithelial surface (pseudomembrane) forms a coagulum. With more intense inflammation, the conjunctival epithelial cells become necrotic and a firm fibrovascular adhesion forms between the coagulum and the stroma (true membrane).

Contents of the acute inflammatory secretion can vary depending upon the cause as well as the intensity of the inflammation. For example, bacterial infections and some toxic agents (e.g. corrosive chemicals) provoke a polymorphonuclear leukocytic reaction (i.e. neutrophilic cellular reaction), while viral infections usually elicit a mononuclear cellular response with a predominance of lymphocytes and monocytes and allergic stimuli cause an increase in the number of eosinophils or basophils.

(b) Bacterial infection: Staphylococcus aureus, Streptococcus pneumoniae, and Haemophilus aegyptius are the main causative organisms for this type of inflammatory reponse. In infants, ophthalmia neonatorum is caused by Neisseria gonorrhoeae. Bacterial infections usually spreads to the lids and is referred to as blepharoconjunctivitis. The majority are exogenous and are

caused either by the introduction of virulent bacteria capable of initiating breakdown of conjunctival epithelial integrity or by depletion of host resistance (immunodeficiency, avitaminosis A), enabling proliferation and infection by opportunistic endogenous or exogenous flora. Certain factors that predispose conjunctiva to infection include: dry eyes, conjunctival exposure or naso lacrimal duct obstruction causing tear stagnation. Chronic infection is often accompanied by inflammatory granulation of tissue.

(c) Viral infections: Most viral infections of the conjunctiva are bilateral and develop, primarily, in association with systemic infection. Adenovirus infection being the commonest cause of acute follicular conjunctivitis in children and young adults. Ten of the 31 serotypes of adenoviruses have been implicated in causing eye infections. Presentation of infection is with acute onset of watering, redness, photophobia, and discomfort. Both eyes are affected in 60 % cases. The serotypes commonly identified are 1, 3, 4, 7, 8 & 19. The serotypes 3 & 7 cause pharyngoconjunctival fever and serotypes 8 & 19 cause epidemic keratoconjunctivitis. Unilateral, occasionally bilateral, conjunctivitis also occurs in association with ocular infections produced by Herpes simplex virus (serotypes 1 and 2) and varicella zoster virus.

(d) Chlamydial infections: Chlamydiae are obligate intracellular parasites. They possess both DNA and RNA, have cell wall and ribosomes similar to those of gram-negative bacteria and are inhibited by antibiotics such as, tetracycline. Chlamydiae are classified as bacteria belonging to their own order (chlamydiales). The genus chlamydia contains three species that infect humans: chlamydia psittaei, chlamydia trachomatis and chlamydia pneumoniae. Trachoma is caused by serotypes A, B, Ba and C of Chlamydia trachomatis while serotypes D, E, F, G, H, I, J, K are the agents causing inclusion conjunctivitis (paratrachoma). Agents of trachoma inclusion conjunctivitis are transmitted to the eye by insect vectors and by venereal or other forms of direct and indirect contact. Ocular discharge can also transmit trachoma from eye to eye. As inflammation continues, conjunctival scarring eventually distorts the eyelids, causing them to turn inward so that the inturned lashes constantly abrade the eyeball (trichiasis and entropion). Eventually the corneal epithelium is abraded and may ulcerate with subsequent corneal scarring and blindness. Destruction of conjunctival goblet cells, lacrimal ducts and lacrimal gland may produce a dry eye syndrome, with resultant corneal opacity due to drying (xerosis) or secondary bacterial corneal ulcers.

Inclusion conjunctivitis (paratrachoma) occurs either as an acute purulent infection in newborn infants (inclusion blennorrhea or ophthalmia neonatorum) or as a subacute follicular conjunctivitis in adults (inclusion conjunctivitis). The infection in infants is characterized by an acute onset with edema and mucopurulent discharge, usually in both eyes, 5 to 10 days after birth. The adult form of inclusion conjunctivitis is less acute at its onset but follows similar stages.

(e) Mycotic infections: Aspergillus sp. and Candida sp. may cause indolent and intractable corneal ulceration or endophthalmitis. But they are uncommon in temperate climates, but patients on prolonged immunosuppressive and steroid therapy are susceptible to be infected by Candida species.

(f) Uveitis: The term uveitis is used to describe various diseases in which the inflammation is largely localized to the uveal tract. The inflammation in anterior uveitis is usually of a non-specific character and localized in the anterior segment. If the process is not controlled, serious sequel ensues, such as secondary glaucoma due to interference with aqueous drainage by adhesions. The onset is often acute and the condition tends to recur.

(g) Keratitis: Keratitis is the inflammation of cornea. If the inflammation is in the epithelium and Bowman's membrane, it is called " superficial keratitis". If the inflammation is superficial, but only occurs in certain, small discrete patches of the cornea, it is called "superficial punctate keratitis". Inflammation of stroma is called "deep", "stromal" or " interstitial" keratitis. Inflammation with a significant loss of corneal epithelium is called corneal ulcer.

1.3.2 The non steroidal anti-inflammatory drugs

The use of medicinal substances to relieve pain and fever dates back to ancient Egypt (Vane, 1971), where a decoction of dried leaves of myrtle, applied to the back and abdomen of patients, was used to relieve pain from the womb. Later, in Greece, the bitter extracts from the bark of the poplar tree were used in patients with eye diseases. Extracts of the willow bark were chewed to relieve the pain of childbirth and to reduce fever. These beneficial effects remained largely unknown until the first published report of willow bark came from England by Reverend Edward Stone in 1763 (Vane, 1971). Later on the active component of willow bark was identified as salicin, which is metabolized to salicylate. Salicylic acid was synthesized in Germany in 1860 by

Kolbe and Lautemann, leading to an extended use as an external antiseptic, an antipyretic, and an antirheumatic drug, which caused dyspepsia and tasted bitter when given orally.

In 1875, Felix Hoffman from the Bayer Company developed a more palatable form of salicylate, acetylsalicylic acid (Vane, 1971). In 1899, Heinrich Dreser named the compound 'Aspirin', the "a" referring to the acetyl grouping and the 'spirin' recalling the botanical genus spiraea, from which salicylates could be extracted. After the development of aspirin in the late 1800's, numerous other drugs were discovered and used as antipyretics, analgesics and anti-inflammatories. These drugs were regarded as a group and became known as 'aspirin-like drugs'. The term nonsteroidal anti-inflammatory drug (NSAID) was first applied to phenylbutazone after its introduction into clinical practice in 1949, three years after the demonstration of the anti-inflammatory properties of glucocorticoids. Development of Indomethacin, in 1963, inspired pharmacologists and biochemists to search for a common mode of action. Little was known except that they produced an anti-inflammatory effect both quantitatively and qualitatively different from the more potent glucocorticoids. The original of all these investigations started in 1933 with the studies in bovine seminal vesicles by Goldblatt, who discovered prostaglandin activity (Vane, 1971).

During the 1960's, a British pharmacologist, Harry Collier, suggested that aspirin and related drugs inhibit some underlying cellular mechanism that takes part to different extents in different responses and was mediated by different endogenous substances (Collier, 1969).

In 1969 Piper and Vane demonstrated the first association between prostaglandin production and the actions of aspirin like drugs (Higgs and Vane, 1983). They presented evidence showing that aspirin inhibited the production of a substance named rabbit aorta contracting substance (RCS) (Higgs and Vane, 1983). In 1971, Vane, using cell-free homogenates of lung tissue, showed that aspirin, Indomethacin and Sodium Salicylate caused a dose dependent reduction in prostaglandin synthesis, proposing that this was the basis for the action of aspirin-like drugs. Vane published his paper in Nature (Vane, 1971) at the same time that another paper in the same issue (Smith and Willis, 1971) demonstrated that aspirin reduced prostaglandin release from aggregating platelets and from the perfused isolated spleen of dogs.

Vane's hypothesis was based on the critical role that the local production of prostaglandins exerted in the inflammatory process. This was substantiated by showing that prostaglandins

reproduce some of the cardinal signs of inflammation; their presence in the inflammatory focus; and that all NSAIDs despite differences in their chemical structures, inhibit the enzyme cyclooxygenase and thus the production of prostaglandins.

Not all the mechanisms of action of NSAIDs are completely understood, especially in the treatment of inflammatory disorders (Abramson and Weissman, 1989; Numo and Lapadul, 1987). A purified and enzymatically active cyclooxygenase (COX) or prostaglandin endoperoxide synthase was isolated in 1976. COX-1 was presumed to be the major target for NSAIDs acting in their analgesic and anti-inflammatory capacities. COX-1 was originally purified from ovine and bovine vesicular glands in 1976. In 1990, the existence of two different cyclooxygenases was hypothesized, based on the evidence that steroids inhibited the increase in COX activity induced by bacterial lipopolysaccharides in macrophages, without any effects on the basal production of prostaglandins or leukotrienes. This led to the isolation of the COX-2 isozyme in 1991 both from human and animal sources. In 1992 this new enzyme was cloned from human (Hla and Nelson, 1992) and animal sources (Herschman, 1996). It was called cyclooxygenase-2 (COX-2) or prostaglandin H-2 synthase-2 (PGH-2 S-2) and its expression was shown to be increased by conditions stimulating cellular proliferation as well as by inflammatory cytokines, and decreased by glucocorticoids.

Tissue localization studies under physiological conditions found a constitutive expression of COX-1 in virtually all tissues whereas COX-2 appeared to be 'constitutively' restricted to the brain, kidney, bones, testicles, ovaries, uterus, tracheal epithelial cells and small intestine (Smith and DeWitt, 1995) in very low levels. COX-1 is responsible for the production of 'housekeeping' prostaglandins critical to the maintenance of normal cell function, gastric mucosal integrity, vascular hemostasis and the autocrine response to circulating hormones. COX-2 on the other hand was an inducible enzyme, upregulated 20-fold in macrophages, monocytes, synoviocytes, chondrocytes, fibroblasts, osteoblasts and endothelial cells by various inflammatory stimuli: interlukin (IL1), tumor necrosis factor (TNF), lipopolysaccharides (LPS), mitogens (phorbolesters), growth factors (GF), platelet derived growth factor (PDGF), transforming growth factor-b (TGFb), epidermal growth factor (EGF), fibroblast growth factor (FGF), reactive oxygen intermediates and chorionic gonadotropin in ovarian follicles (Smith and DeWitt, 1995; Spangler, 1996).

An upregulation in the expression of COX-2 has been noted in colorectal adenomas and carcinomas with an increase of COX-2 mRNA in 86 % of carcinomas compared with normal mucosa. Transcription is also likely increased in breast and head and neck cancers (crofford, 1997). After these discoveries, it was suggested that the anti-inflammatory actions of NSAIDs were due to COX-2 inhibition and the unwanted side effects due to inhibition of COX-I (Smith and DeWitt, 1995; Lipsky and Isakson, 1997; De Brum-Fernandez, 1997).

After isolation in the early 1990's of the COX-2 isozyme its genetic structure and regulation of expression were characterized and compared with COX-1. Separate genes on different chromosomes encode both enzymes: COX-1 is on Chromosome 9 and COX-2 is on chromosome 1. The COX-2 gene contains regions characteristic of early response genes, allowing a rapid upregulation in response to inflammatory stimuli as well as rapid turnover and diminished expression in the absence of continued stimulation. Meanwhile, the COX-1 gene is expressed in almost all normal tissues and is not upregulated by inflammatory stimuli (constitutive expression). Although both isozymes are 60 % homologous, there are small differences in the amino acid sequence lining the COX active sites. The COX-2 inhibitor binding site is 25 % larger than the COX-1 due to the substitution of a single amino acid (valine for an isoleucine at position 523), and has a secondary internal pocket off the inhibitor binding site not seen in COX-1. All these differences lead to the distinct inhibition profiles between traditional NSAID's and COX-2 inhibitors (Crofford, 1997; De Brum-Fernandez, 1997).

NSAIDs are among the most widely prescribed and used drugs in the community for rheumatologic as well as nonrheumatologic conditions which include acute and chronic pain, biliary and ureteric colic, dysmenorrhea, fever, closure of patent ductus arteriosus in infants, and other ocular applications that derive from their suppression of prostaglandin synthesis (Brooks, 1998). These drugs reduce the signs and symptoms of established inflammation without eliminating the underlying causes of it; they have no effect on the clinical course of the disease process and don't protect against tissue or joint injury. Despite the many available NSAIDs, both by prescription and over the counter, their efficacy seems to be relatively equivalent among patients though individual responses as well as tolerability profiles may vary.

The therapeutic effectiveness of NSAIDs as analgesics, antipyretics, anti-inflammatories and anti-thrombogenics is due to their inhibition of prostanoid synthesis, which also accounts for their side effect profile. There is an individual variability in the response to NSAIDs among

patients, which isn't fully understood. Several pharmacokinetics factors might play a role such as protein binding, metabolic profile of the drug, percentage of the drug available as the active compound, plasma half-life, urinary excretion and dose response. Traditionally NSAIDs can be grouped into nine classes based on their chemical structure as given in Table 1.6.

Table 1.6 Classification of NSAIDs on the basis of chemical structure

S. No.	Chemical Structure	Examples
1.	Salicylic acids	Aspirin, Diflunisal, Disalcid, Trilisate
2.	Phenylacetic acid	Diclofenac, Alclofenac, Fenclofenac, Tolmetin
3.	Carbo-and heterocyclic acid	Etodolac, Indomethacin, Sulindac, Oxaprozin
4.	Propionic acids	Flurbiprofen, Ketoprofen, Suprofen, Ibuprofen, Naproxen, Fenoprofen, Carprofen
5.	Fenamic acids	Mefenamic, Flufenamic, Meclofenamic
6.	Pyrazolones	Phenylbutazone, Oxyphenbutazone
7.	Oxicams	Piroxicam, Tenoxicam, Isoxicam, Meloxicam
8.	Nonacidic compunds	Nabumetone
9.	Pyrazole benzene suphonamides	Celecoxib, Rofecoxib

1.3.3 Role of NSAIDs in ocular inflammation

Increased levels of prostaglandin's (E and F) were found in the aqueous humor of inflamed eyes and inhibitors of arachidonic acid metabolism significantly reduced their levels and thus inflammatory response. Later on, the existence of PG E2 and PG F2 receptors were proved in bovine iris-ciliary body. (Csukas et al, 1993; Bhattacherjee and Paterson, 1994).

Prednisolone acetate, flurometholone, and dexamethasone are some of the corticosteroids currently in clinical use as anti-inflammatory agent in ophthalmic medicine. Corticosteroids have multiple pathway for inhibiting the inflammatory response to noxious stimuli such as radiation, mechanical, chemical, infectious or immunological, affecting the inflammatory process in many ways.

(i) They reduce the vasodilatation and stabilize mast cells thereby reducing the release of histamine.

(ii) They maintain the normal permeability of blood and prevent the development of edema.

Their mechanism of action involves the induction of specific protein syntheis that inhibits phospholipase A2, an enzyme responsible for the release of arachidonic acid. But, their continued use may result in severe side effects such as the development of ocular hypertension, cataract, immunosuppresion and susceptibility to Herpes infection. Corneal/stromal wound healing is inhibited by corticosteroids due to their effect on fibroblastic and keratocytic activity impairment. Therefore, it would be therapeutically beneficial to develop and use nonsteroidal anti-inflammatory drugs that have similar or greater efficacy than steroids but not their ocular side effects.

NSAIDs have found many useful roles in ophthalmology. NSAIDs are approved by the Food and drug administration, USA (US FDA) to prevent intraoperative miosis during cataract surgery, reduce postoperative inflammation following cataract surgery, and control symptoms of allergic conjunctivitis and pain following refractive surgery. In addition, they have been shown to be effective in preventing cystoid macular edema following cataract surgery or treating cystoid macular edema once it occurs (Nichols and Snyder, 1998). Currently, four topical NSAID preparations are approved worldwide: flurbiprofen and suprofen for the prevention of miosis during surgery; diclofenac for postoperative inflammation following cataract extraction, and ketorolac for the treatment of itching associated with seasonal allergic conjunctivitis (Abelson and Sloan, 1994). Caution should be exercised, however, as topical and systemic adverse effects may occur including stinging, photophobia, gastric sensitivity, and increased bleeding time.

Cycloplegics, corticosteroids, and nonsteroidal anti-inflammatory drugs have been applied in the treatment of postoperative inflammation following cataract extraction. Of these, topical preparations of nonsteroidal anti-inflammatory drugs, such as ketorolac tromethamine 0.5 % and diclofenac sodium 0.1 %, offer comparable efficacy to corticosteroids in the reduction of postoperative inflammation, and offer lower risks of adverse events in most patients (Simone and Whitacre, 2001). In a study intended to compare diclofenac sodium and flurbiprofen, commonly used prior to cataract surgery, for inhibition of surgically induced miosis (Roberts, 1996) fifty-one patients having phacoemulsification were randomly assigned to receive topical treatment with either diclofenac sodium 0.1 % or flurbiprofen 0.03 % every 15 minutes interval for four doses along with the dilating drops beginning 1 hour before surgery. There was no statistically significant difference between the two treatment groups in baseline pupil dilation; however,

regardless of the drug received, the light irides were, on average, more dilated at baseline than the dark ones. After surgery began, there were no statistically significant differences between the two groups at any time or surgical interval except at the start of phacoemulsification, at which point the flurbiprofen-treated eyes were more dilated than the diclofenac-treated eyes. Thus, flurbiprofen is also found to be an effective post-surgical anti-inflammatory agent following cataract extraction by phacoemulsification. In another study to compare anti-inflammatory effect of ophthalmic solution of diclofenac sodium 0.1 %, flurbiprofen 0.03 %, and indomethacin 1.0 % (Diestelhorst et al, 1996), diclofenac sodium appeared to be more potent than flurbiprofen in controlling intraocular inflammation after cataract surgery and appeared to be locally tolerated better than flurbiprofen and indomethacin. A comparative study to find the effect of diclofenac and ketrolac on patient discomfort and corneal sensitivity (Narvaez et al, 2002) found that neither diclofenac nor ketorolac decreased corneal sensation compared to control. There was no significant difference in burning sensation reported by patients upon instillation of the drugs in all the study groups.

NSAIDs are routinely administered in phacoemulsification surgery along with the dialating drops to inhibit intraoperative miosis. After surgery, these drugs can control inflammation and inhibit the development of cystoid macular edema. Brown and Roberts, 1996, compared the preoperative and postoperative use of nonsteroidal anti-inflammatory drugs in cataract surgery. In the first study, diclofenac sodium was compared with prednisolone acetate for control of postoperative inflammation. In the second, diclofenac sodium was compared with flurbiprofen for inhibition of intraoperative miosis. Diclofenac sodium was found to be as effective as prednisolone acetate for control of postoperative inflammation and as effective as flurbiprofen for inhibition of intraoperative miosis. Thus, in the past, NSAIDs were used before surgery for inhibition of intraoperative miosis and a steroid drop in the postoperative period to control postsurgical inflammation, but now, NSAIDs can be used with equal efficacy in perioperative period.

0.1 % indomethacin and 0.1 % diclofenac ophthalmic solutions may help to control the pain induced by excimer laser photorefractive keratectomy without any deleterious effect on corneal wound healing (Goes et al, 1997). Topical diclofenac was found to be more potent than topical enolicam in inhibiting the inflammatory polymorphonuclear leukocyte response following partial corneal injury. Both drugs did not affect the rate of reepithelialization following complete

(limbal to limbal) corneal deepithelialization (Kulkarni and Srinivasan, 1986). In 2000, Barba et al, found that limbal incisions heal faster than clear corneal incisions. It is steroids and not NSAIDs that inhibit wound healing. Cataract surgery using limbal incisions and postoperative topical NSAIDs may result in faster wound healing and provide a reduced risk of related postoperative complications. In another study no difference was found in the ability of diclofenac or ketorolac ophthalmic solutions to prevent posterior capsular opacification following cataract extraction and implantation of an intraocular lens. Both treatment regimens were equally well tolerated (Flach and Dolan, 2000).

Hessemer and co workers, in 1996, found that after extracapsular cataract surgery, the anti-inflammatory potency of topical indomethacin 1 % is superior to that of flurbiprofen 0.03 % and diclofenac 0.1 %, as demonstrated by the laser flare-cell meter. The rate of postoperative fibrin exudation is lower under treatment with topical indomethacin or flurbiprofen compared to diclofenac. Using the commercially available formulations of different drug concentrations and vehicles, indomethacin exhibits a higher aqueous humour concentration than flurbiprofen, and diclofenac (which shows the lowest concentration).

Gaynes and Fiscella , 2002, have reviewed the safety issues of topical NSAIDs for ophthalmic use. Topical ophthalmic NSAIDs are limited to the relatively water soluble phenylacetic and phenylalkanoic acids as well as indole derivatives, which are more suitable for ophthalmic use. Absorption of topical ophthalmic NSAIDs through the nasal mucosa results in systemic exposure and the occurrence of adverse systemic events, including exacerbation of bronchial asthma. Local irritant effects of topical ophthalmic NSAIDs include conjunctival hyperaemia, burning, stinging and corneal anaesthesia. A more serious complication involves the association of topical ophthalmic NSAIDs with indolent corneal ulceration and full-thickness corneal melts. Analysis of NSAID-associated corneal events implicates the now defunct generic diclofenac product, diclofenac sodium ophthalmic solution, as the agent primarily responsible. However, these events generated a renewed interest in the safety of ophthalmic NSAIDs and a scrutiny of the pharmacology regarding NSAID action in the eye. An elucidation of possible pharmacodynamic explanations of NSAID-induced corneal injury includes the role of epithelial hypoxia, which not only appears to aid in determining the metabolic destination of arachidonate, it may play a key role in orchestrating a novel inflammatory response unrelated to prostanoid formation. The use of NSAIDs under conditions of corneal hypoxia may therefore not only result in a disappointing

therapeutic response, it may result in a paradoxical inflammatory exacerbation. Other potential mechanisms include the relationship between NSAIDs and corneal matrix metalloproteinase and direct toxicity due to cytotoxic excipients such as surfactants, solubilisers and preservatives found in topical NSAID ophthalmic preparations. In general, ophthalmic NSAIDs may be used safely with other ophthalmic pharmaceuticals. However, concurrent use of agents known to adversely affect the corneal epithelium, such as gentamicin, may lead to increased corneal penetration of the NSAID. The concurrent use of NSAIDs with topical corticosteorids in the face of significant pre-existing corneal inflammation has been identified as a risk factor in precipitating corneal erosions and melts and should be undertaken with caution. Until clinical evidence dictates otherwise, data supporting theories of potential pharmacodynamic mechanisms of NSAID injury do not alter the favorable benefit-risk ratio of ophthalmic NSAID use when employed in an appropriate and judicious manner.

A brief insight of the inflammatory processes in eye tissue suggests prompt immunomodulatory therapy for controlling inflammation and preserving the vision. The mechanism of inflammatory reactions in the eye and the implication of prostaglandin and COX-2 in the process points to major role for NSAIDs in the management of ocular inflammatory malignancies and pre and post operatives eye surgeries.

1.4 Objective of the present research and development endeavor

NSAIDs are extensively used in the treatment of intraocular inflammatory disorders on long term basis. The basic goal of such treatment regimen is to achieve the desired therapeutic effect with lesser dosing and fewer or no side effects. The decrease in dosing frequency greatly improves patient compliance and assures round the clock medication. The objective of this work was to study various aspects of design of better ocular delivery of flurbiprofen, a potent COX inhibitor commonly used for ocular inflammation. Better ocular drug delivery systems for flurbiprofen were envisaged to be designed, developed and evaluated based on following principles.

(a) Prolonging its contact with the corneal surface

(b) Controlled drug release

The methodologies employed for designing better ocular delivery of flurbiprofen are:

(i) Viscosity enhancement: Additions of soluble mucoadhesive polymers to enhance viscosity. Viscosity enhancement approach was also attempted using various vegetable oils compatible with ocular tissues. Formulation containing combination of mucoadhesive polymers and oils were also designed in an attempt to prolong the corneal contact time of the formulation and thereby increasing the corneal flux of the drug for better ocular availability and to minimize nasolachrymal drainage and systemic side effects.

(ii) Polymeric matrix system and drug impregnated discs: Such delivery systems were designed and developed using polymers with varying degree of hydrophilicity. In such systems drug was embedded or solubilized in the matrix base.

(iii) Membrane controlled devices: These drug delivery systems were designed utilizing membrane barrier of fixed thickness over matrix embedded formulations. Such a system consisted of a central reservoir of drug enclosed between specially tailored membranes that allow the drug to diffuse from the reservoir at a precisely predetermined rate.

In all the methodologies based on viscosity enhancement, polymeric matrix systems and membrane controlled devices effect of nature of polymer or oil and their proportion on the release rate of the drug were studied. Also studied were various other factors controlling the rate of release of the drug from the designed formulations through *in-vitro* dissolution and corneal permeability studies. Effect of sterilization method on the drug release characteristics from the formulations and drug stability was investigated. Long-term stability of the drug in the designed formulations and reproducibility of the manufacturing process employed were also investigated.

As part of the broader approach for formulation development, preformulation studies like drug-additive admixture stability, drug stability in different vehicles, solubility and log P of flurbiprofen were studied. To support various studies like, pure drug analysis, assay of formulations, *in-vitro* dissolution and permeability studies, several rapid, accurate and validated UV-visible, spectroflourimetric and liquid chromatographic methods of analysis for flurbiprofen were developed. To support i*n-vivo* studies simple, rapid and validated liquid chromatographic method for the estimation of the drug in aqueous humor and serum were also developed. Effect of common ophthalmic additives and media on the corneal permeability of flurbiprofen was also studied. Eye irritation/ sensitivity profile of the formulations was tested as per Draize's test protocol. *In-vivo* ocular bioavailability studies were carried out on rabbit model to assess the

enhancement of aqueous humor drug bioavilability through the developed cont
drug delivery systems. All the animal experiments were carried out with the appro
Institutional Animal Ethics Committee of B.I.T.S., Pilani.

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CHAPTER 2

DRUG PROFILE

2. DRUG PROFILE

Flurbiprofen

Flurbiprofen, a phenyl alkanoic acid derivative, is a potent nonsteroidal anti-inflammatory drug (NSAID) having analgesic, anti-inflammatory and antipyretic properties (Marsh et al, 1986). It is official in various pharmacopoeias (BP, 1998; IP, 1996; USP, 2000).

2.1 Chemistry

Name : Flurbiprofen

Chemical name : (±)-2-fluoro-α-methyl-[1,1'-Biphenyl]-4-acetic acid,
(±)-2-(2-fluoro-4-biphenylyl) propionic acid

Molecular formula : $C_{15}H_{13}FO_2$

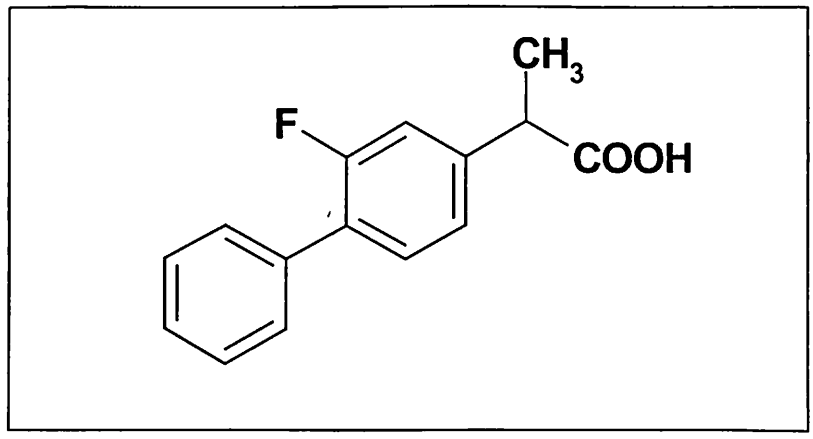

Figure 2.1: Chemical structure of flurbiprofen

Molecular weight : 244.27

Class : Non-steroidal anti-inflammatory drug (NSAID).

Category : Analgesic, anti-inflammatory and antipyretic.

Description : White or almost white crystalline powder.

Solubility : Freely soluble in ethanol (95 %), in chloroform and in ether; practically insoluble in water. It dissolves in aqueous solutions of alkali hydroxides and carbonates (IP, 1996).

pK_a : 4.27 (Craig, 1990).

log P : 3.75 (calculated using Clog P software) (Craig, 1990).

Identification (USP, 2000) :

(a) Infrared absorption: The infrared spectrum is concordant with the reference spectrum of flurbiprofen or with the spectrum obtained from flurbiprofen RS (reference standard).

(b) UV absorption: A 10 µg/ml solution (prepared in 0.1 N sodium hydroxide) shows a maximum absorbance of 0.8 at a wavelength of 247 nm.

(c) On heating 0.5 ml of a saturated solution of chromium trioxide in sulphuric acid in a water bath for 5 minutes, the solution wets the sides of the tube and there is no greasiness. On adding 2 to 3 mg of flurbiprofen to the above solution and heating in a water bath for 5 minutes, the solution does not wet the sides of the tube and does not pour easily.

(d) Melting range: Between 114 and 117 °C.

(e) Loss on drying: When dried at 60 °C in vacuum to constant weight, it loses not more than 0.5 % of its weight.

(e) Residue on ignition: Not more than 0.1 %.

(f) Heavy metals: 0.001 %.

2.2 Official methods of analysis

Pharmacopoeias (BP, 1998; IP, 1996; USP, 2000) have reported titrimetric and liquid chromatographic methods for the analysis of flurbiprofen in pure form and in pharmaceutical formulations.

(a) Titrimetric method (IP, 1996; USP, 2000): This involves dissolving about 0.5 g of accurately weighed flurbiprofen in 100 ml of alcohol (previously neutralized with 0.1 N sodium hydroxide VS to the phenolphthalein end point) and then, titrating the same (after adding phenolphthalein TS) with 0.1 N sodium hydroxide VS till the first appearance of faint pink colour that persists for not less than 30 seconds. Each ml of 0.1 N sodium hydroxide is equivalent to 24.43 mg of $C_{15}H_{13}FO_2$.

(b) Liquid chromatographic method: BP 1998 and USP 2000, both, have recommended liquid chromatographic (LC) method for analysis of related substances in pure flurbiprofen and assay of flurbiprofen in pharmaceutical dosage form (tablet and ophthalmic drop). Both the methods recommended use of a mobile phase of water: acetonitrile: glacial acetic acid (60:35:05) at a flow rate of 1 ml/min, using 2-(4-Biphenyl) propionic acid as the internal standard, on a 15 × 3.9 cm C-18 column (stainless steel) with 4μm particles. IP 1996 has also suggested a similar LC method using C-18 column with 5 μm particles.

Few other methods for the analysis of flurbiprofen in pure form and in pharmaceutical formulations, reported in peer-reviewed journals, have been presented in Chapter 3 of this thesis. Also presented in Chapter 3 are several liquid chromatographic methods reported for the estimation of flurbiprofen in biological samples like, serum, plasma, urine and aqueous humor using UV detection or fluorescence detection.

However, in the present thesis, in-house developed and validated UV-visible spectrophotometric and liquid chromatographic method (Sajeev et al, 2002a) and spectroflourimetric method (Sajeev et al, 2001) were used for the analysis of flurbiprofen in pure form, in designed formulations and in various *in-vitro* studies. For detection of flurbiprofen in aqueous humor and serum for *in-vivo* studies, in-house developed liquid chromatographic method with UV detection (Sajeev et al, 2002b) was employed. These developed methods have been described in detail in Chapter 3.

2.3 Pharmacology

Flurbiprofen is a nonselective inhibitor of cyclo-oxygenase enzyme and inhibits prostaglandin biosynthesis, thereby reducing hormones that cause inflammation and pain in the body. This drug is likely to have greater effect on platelet aggregation than most other NSAIDs (Adams, 1977; Thebault et al, 1977) and was found to be highly potent in diminishing leukocyte chemotaxis to areas of inflammation (Adams et al, 1977). It demonstrates comparable efficacy to other NSAIDs, e.g. aspirin (Barraclough et al, 1974), indomethacin (Brewis, 1977), ibuprofen (Mena et al, 1977), naproxen (Cherie-Lingniere et al, 1983), and diclofenac (Famsey and Ginsberg, 1983), in the treatment of rheumatoid arthritis. Flurbiprofen is also indicated for the management of vernal keratoconjunctivitis (Sud et al, 1995), postoperative ocular inflammation

(Diestelhorst et al, 1996), herpetic stromal keratitis (Vajpayee et al, 1996), excimer laser photorefractive keratectomy (Appiotti et al, 1998) and ocular gingivitis (Jones et al, 1999). Recent reports suggested potential topical and systemic use of flurbiprofen in radio-protection (Hofer et al, 1996), inhibition of colon tumor (McCracken et al, 1996), protection of post irradiation myelosuppression (Juchelkova et al, 1998), in pain management after foot surgery (Soulier et al, 1997) and peridontal surgery (Bragger et al, 1997).

2.4 Pharmacokinetics

Flurbiprofen is well absorbed on oral administartion, with peak serum levels occurring within 1.5-3.0 hr after administration of dose (Adams et al, 1977; Cardoe et al, 1977). The elimination half-life is 3.0-3.9 hr (Capell and Konetachnil, 1977) and is not dose dependent. The apparent volume of distribution is about 7.0 liters with very high protein binding (99 %) (Capell and Konetachnil, 1977). There is no apparent correlation between serum levels and therapeutic response (Capell and Konetachnil, 1977). Flurbiprofen is extensively metabolized and excreted primarily in the urine. The extent of biotransformation is around 75-80 % (Capell and Konetachnil, 1977; Risdall, et al, 1978). The major metabolite, 4'-hydroxy-flurbiprofen, has been detected in human plasma. Extent of drug excreted unchanged in urine is around 20- 25 %, remaining being excreted primarily as metabolites. Around 60-70 % of these metabolites are excreted as glucuronide or sulfate conjugates. In animal models these metabolites exhibit little activity (Adams et al, 1975).

In a study conducted to evaluate the ocular bioavailability of ophthalmic flurbiprofen in rabbit model (Tang et al, 1984), the decline in flurbiprofen concentration in aqueous humor following intracameral injection was found to be biexponential. Its ocular distribution half-life was about 15 mins and ocular elimination half-life was about 93 mins. Other related ocular pharmacokinetic parameters were: ocular volume of distribution at steady state- 0.62 ml and ocular clearance- 0.0144 ml/min. A pronounced absorption phase was observed in all animals after administration of topical doses, but the extent of absorption was not proportional to the dose applied. A 150 μg dose showed 30 % less absorption than the expected proportional absorption shown by a 75 μg dose (Tang et al, 1984).

2.5 Clinical studies

Table 2.1 and 2.2 summarizes the clinical trials using flurbiprofen in case of osteoarthritis and rheumatoid arthritis respectively. In most studies, the drug was as effective as aspirin, indomethacin, ibuprofen and sulindac.

2.6 Adverse effects

Side effects were reported in 52.5 % patients in a collective review of 1220 patients treated with flurbiprofen at dosages ranging from 75-400 mg daily for up to 48 months (Sheldrake et al, 1977). Nineteen percent of all flurbiprofen treated patients had to withdraw from the treatment because of adverse effects: gastrointestinal toxicity- 37 %, central nervous system (CNS) toxicity- 16.2 %, skin rash- 6.1 %, renal toxicity- 3.6 %, hematological effects- 2.4 %, pulmonary symptoms- 3.7 % and cardiovascular toxicity- 2.3 %. Headache was the most common CNS side effect noted. Toxicity increased when flurbiprofen was combined with other NSAIDs. Age had no influence on the occurrence of toxicity. More CNS toxicity was recorded with indomethacin as compared to flurbiprofen (Viara et al, 1983). Flurbiprofen is likely to increase blood urea nitrogen and uric acid levels (Dequeker et al, 1977). Statistically insignificant (when compared to baseline values) increase in mean fecal blood loss of 1.95 ml/day was observed in case of flurbiprofen (Vakil et al, 1977). In another study, initial endoscopic examination of nine patients treated with flurbiprofen, 300 mg daily, for 2-4 weeks showed at least some evidence of gastritis (Teixeira et al, 1981). However, there were no improved endoscopic findings in all the patients treated with flurbiprofen.

Ophthalmic use of flurbiprofen, in conjunction with ocular surgery, is likely to increase bleeding of ocular tissues when used. Delayed wound healing, decreased/ blurred vision, severe eye irritation, swelling, redness, or pain are some of the other ocular side effects reported with the ophthalmic use of flurbiprofen. In some rare instances, thrombocytopenia, agranulocytosis and toxic amblyopia (diminished vision without detectible lesion or disease of the eye) has also been recorded Gaynes and Fiscella, 2002).

Table 2.1: Comparative studies of flurbiprofen in osteoarthritis

Study design	No. of patients	Duration (wash out/treatment)	Dose (mg/day)	Side effects	Results	Reference
DBP	195	6 months	FL 80, IB 1600	FL = IB	FL = IB	Mena et al, 1976
DBCO	21	4 weeks	FL 75, FL 150, FL 300	75 = 150 = 300	75 = 150 = 300	Cardoe, 1977
DBCO	30	(3 days)[a]/ 2 weeks (1 week)[b]/ 2 weeks	FL 150, IN 75	IN > FL	FL = IN	Frank, 1977
DBCO	40	(3 days)[a]/ 4weeks (1 week)[b]/ 4 weeks	FL 200, DI 100	NR	FL = DI	Famsey and Ginsberg, 1977
OP	40	2 weeks	FL 300, DI 200	DI > FL	FL = DI	Benvenuti et al, 1977

[a]Pretreatment. [b]Between treatments. Key: DBCO = Double blind cross-over; DBP = double blind placebo; OP = Open parallel; FL = Flurbiprofen; IB = Ibuprofen; IN = Indomethacin; DI = Diclofenac; NR = Not reported

Table 2.2: Comparative studies of flurbiprofen in rheumatoid arthritis

Study design	No. of patients	Duration (wash out/treatment)	Dose (mg/day)	Side effects	Results	Reference
DBCO	21	(3 days)[a]/ 2 weeks	FL 100, ASA 4000	ASA > FL	FL = ASA	Barraclough et al, 1974
DBCO	30	(3 days)[a]/ 2 weeks (1 week)[b]/ 2 weeks	FL 200, IN 100	IN > FL	FL = IN[c]	Siegmeth, 1977
DBP	208	6 weeks	FL 120, IB 2400	FL = IB	FL = IB	Mena et al, 1977
DBCO	30	(2 weeks)[a]/ 2 weeks (2 weeks)[b]/ 2 weeks	FL 300, IN 150, PL	FL = IN	FL = IN	Kruger, 1977
DBP	23	6 months	FL 150-300, IN 75-150	FL = IN	FL = IN	Hazieman and Bulgan, 1977
DBCO	15	2 weeks	FL 150, FL 150 + ASA 3000	FL = ASA + FL	FL = ASA + FL	Brooks and Khong, 1977
DBCO	30	(3 days)[a]/ 2 weeks (1 week)[b]/ 2 weeks	FL 200, IN 100	FL > IN	FL > IN	Brewis, 1977
DBCO	24	2 weeks	PL, ME 500, FL 150, SU 150	NR	ME = SU = FL > PL	Stephens et al, 1977
DBP	40	3 months	FL 200, ASA 2000	ASA > FL	FL = ASA	Dequekar and Mardjuardi, 1977
DBP	30	(3 days)[a]/ 9 weeks	FL 300, IN 150	IN > FL	FL = IN	Viara et al, 1983
OP	118	4 weeks	FL 300, NA 150	FL = NA	FL > NA	Cherie-Ligniere et al, 1983

[a]Pretreatment. [b]Between treatments. [c]Pateint preference: FL > IN
Key: DBCO = Double blind cross-over; DBP = double blind placebo; OP = Open parallel; FL = Flurbiprofen; ASA = Aspirin; IN = Indomethacin; IB = Ibuprofen; PL = Placebo; ME = Meclofenamate; SU = Sulindac; NR = Not reported.

In animal studies (80-week study in mice at doses of 2, 5, and 12 mg/kg/day and a 2-year study in rats at doses of 0.5, 2, and 4 mg/kg/day), flurbiprofen did not show evidence of carcinogenicity at maximum tolerated doses. Flurbiprofen did not impair the fertility of male or female rats treated orally (before mating) with 2.25 mg/kg/day for 65 days and 16 days, respectively. Teratogenic effects were also not observed in animal models. But because there are no adequate and well-controlled studies in pregnant women, and animal teratology studies do not always predict human response, flurbiprofen is not recommended for use in pregnancy. Safety and effectiveness in children have not been established (US FDA, 1994).

2.7 Drug interactions

(a) Antacids: Administration of flurbiprofen tablets to volunteers under fasting conditions, or with antacid suspension, yielded similar serum flurbiprofen-time profiles in young subjects (n_s = 12). In geriatric subjects (number of subjects (n_s) = 7), there was a reduction in the rate but not the extent of flurbiprofen absorption (US FDA, 1994).

(b) Anticoagulants: There was no interaction between flurbiprofen and oral anticoagulant phenprocoumon (Marbet et al, 1977).

(c) Analgesics: Concurrent administration of aspirin and flurbiprofen resulted in 50 % lower serum flurbiprofen concentrations. However, there was no change in the plasma elimination half-life (Stephens et al, 1977). This effect of aspirin (which also lowers serum concentrations of other nonsteroidal anti-inflammatory drugs given with it) has been demonstrated in patients with rheumatoid arthritis (n_s = 15) as well as normal volunteers (n_s =16). Concurrent use of flurbiprofen and aspirin is therefore not recommended.

(d) Beta-adrenergic blockers: The effect of flurbiprofen on blood pressure response to propranolol and atenolol was evaluated in men with mild uncomplicated hypertension (n_s = 10). Flurbiprofen pretreatment attenuated the hypotensive effect of a single dose of propranolol but not atenolol. Flurbiprofen did not appear to affect the beta-blocker-mediated reduction in heart rate. Flurbiprofen did not affect the pharmacokinetic profile of either drug, and the mechanism underlying the interference with propranolol's hypotensive effect is unknown. Patients taking both flurbiprofen and a beta-blocker should be monitored to ensure that a satisfactory hypotensive effect is achieved (US FDA, 1994).

(e) H1 antagonists: In normal volunteers (n_s = 9), pretreatment with cimetidine or ranitidine did not affect flurbiprofen pharmacokinetics, except that a small (13 %) but statistically significant increase in the area under the serum concentration curve of flurbiprofen resulted with cimetidine (US FDA, 1994).

(f) Digoxin: Studies of concomitant administration of flurbiprofen and digoxin to healthy men (n_s = 14) did not show a change in the steady state serum levels of either drug (US FDA, 1994).

(g) Diuretics: Studies in normal volunteers have shown that flurbiprofen, like other nonsteroidal anti-inflammatory drugs, can interfere with the effects of furosemide. Although results have varied from study to study, effects have been shown on furosemide-stimulated diuresis, natriuresis, and kaliuresis. Other nonsteroidal anti-inflammatory drugs that inhibit prostaglandin synthesis have been shown to interfere with thiazide diuretics in some studies, and with potassium-sparing diuretics. Patients receiving flurbiprofen and furosemide or other diuretics should be observed closely to determine if the desired effect is obtained (US FDA, 1994).

(h) Oral hypoglycemic agents: In one study, flurbiprofen was given to adult diabetics who were already receiving glyburide (n_s = 4), metformin (n_s = 2), chlorpropamide with phenformin (n_s = 3), or glyburide with phenformin (n_s = 6). Although, there was a slight reduction in blood sugar concentrations during concomitant administration of flurbiprofen and hypoglycemic agents, there were no signs or symptoms of hypoglycemia (US FDA, 1994).

Flurbiprofen is contraindicated in patients who have previously demonstrated hypersensitivity to the product. Flurbiprofen should not be given to patients in whom flurbiprofen, aspirin, or other nonsteroidal anti-inflammatory drugs induce asthma, urticaria, or other allergic-type reactions. Fatal asthmatic reactions have been reported in such patients receiving this type of drug (US FDA, 1994).

2.8 Dosage

(a) Tablets- Recommended adult dose for the treatment of rheumatoid arthritis, osteoarthritis is: initial 200-300 mg/day in divided doses b.i.d. to q.i.d. and then the dose is adjusted according to patients response. Doses greater than 300 mg/day are not recommended. In case of

dysmenorrhea the recommended dose is 50 mg q.i.d (Martindale The Extra Pharmacopoeia, 1993).

(b) Ophthalmic drops- Flurbiprofen is used in the form of sodium salt as 0.03 % solution to inhibit intraoperative miosis during ocular surgery. Recommended dosage is 1 drop of a 0.03 % solution in the eye(s) undergoing surgery beginning 2 hours before the surgery and repeated thereafter at approximately 30-minute intervals for a total of 4 drops per affected eye. Flurbiprofen sodium eye drops have also been used in the treatment of cystoid macular oedema, inflammation following cataract surgery, and uveitis syndrome (Martindale The Extra Pharmacopoeia, 1993; Sabiston and Robinson, 1987).

2.9 References

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CHAPTER 3

DEVELOPMENT OF ANALYTICAL METHODS

3. DEVELOPMENT OF ANALYTICAL METHODS

3.1 Introduction

Analysis is an important component for any formulation development. Formulation Pharmacist should have a suitable validated method of analysis for the drug for which the drug delivery system is to be designed. If a suitable method, for specific need, is not available then it is imperative to develop suitable, rapid and sensitive method for the selected drug. On extensive survey of literature, no ultra-violet (UV) spectrophotometric method and simple spectroflourimetric method came to our notice for the determination of flurbiprofen in pure form and in pharmaceutical dosage form. However survey of literature revealed only two liquid chromatographic (LC) methods for the estimation of flurbiprofen in pharmaceutical formulations and several in plasma, serum and other biological fluids. One LC method for resolution of 21 related compounds of flurbiprofen using phenyl column and mobile phase of 60:40 acetonitrile: 0.1 % acetic acid has been reported (Beaulieu et al, 1991) and a stability indicating LC method for quantitation of the drug has also been reported (Mathew and Gupta, 1993). Also florescence detection has been employed in conjunction with liquid chromatographic methods for estimation of flurbiprofen in biological fluids (Albert et al, 1984; Knadler and Hall, 1989; Geisslinger et al, 1992; Hutzler et al, 2000). Fluorescence detection has been preferred in the above mentioned methods due to the interference evident in the chromatograms from UV detection. But some papers described use of UV detection (Beaulieu et al, 1991; Mathew and Gupta, 1993; Giagoudiakis and Markantonis, 1998), stereoselective assays utilizing either UV (Berry and Jamali, 1988; Geisslinger et al, 1992; Hutzler et al, 2000; Pe'hourcq et al, 2001) or fluorescence detector (Knadler and Hall, 1989). US Pharmacopoeia (USP, 2000a) have recommended LC method for analysis of related substances in pure flurbiprofen and assay of flurbiprofen in pharmaceutical dosage form (tablet and ophthalmic drop). The method recommended use of a mobile phase of water: acetonitrile: glacial acetic acid (60:35:05) at a flow rate of 1 ml/min, using 2-(4-Biphenyl) propionic acid as the internal standard, on a stainless steel column (15 × 3.9 cm) that contains 4µm, C-18 packing. Indian Pharmacopoeia (IP, 1996) has also suggested a similar LC method using columns with 5 µm, C-18 packing.

Over the last 20 years several LC methods using UV or fluorescence detection techniques, have been reported for the estimation of flurbiprofen, either alone or together with their metabolites in

plasma or serum (Sinder et al, 1981; Albert at al, 1984; Askholt and Nielsen-Kudsk, 1986; Johnson and Wilson, 1986; Giagoudiakis and Markantonis, 1998; Hutzler at al, 2000), and its enantiomeric form (Berry, and Jamali, 1988; Knadler and Hall, 1989; Geisslinger, at al, 1992; Pe'hourcq at al, 2001) in urine (Berry and Jamali, 1988; Knadler and Hall, 1989; Geisslinger et al, 1992; Hirai at al, 1997; Hutzler at al, 2000; Pe'hourcq at al, 2001) and in ocular fluids (Riegel and Ellis, 1994). In all these methods the analyte must be extracted from the respective biological matrices and the composition of the extracting solvent, column type, mobile phase and wavelength of detection differed in each assay. Most of the methods also used an internal standard.

None of the reported methods, on detailed study, was found to be suitable for estimation of flurbiprofen for routine analysis like, drug content estimation, dissolution, stability or similar studies for the intended project work. A UV spectrophotometric method or spectroflourimetric method would offer greater advantage in such situations as being simple and rapid for routine analysis. Also the reported LC method for the analysis of flurbiprofen in bio-matrices required complicated sample preparation steps and use of internal standards for analysis, thus increasing the time required and error in recovery. Also robustness and ruggedness of the earlier reported methods were very low. As a result, it was considered necessary to develop suitable and sensitive methods for the estimation of the drug from bio-samples, like aqueous humor and/ or serum, collected during *in-vivo* studies. The methods thus developed to be employed for the estimation of flurbiprofen in serum and aqueous humor should be accurate, precise, and easy. The limit of detection (LOQ) and limit of quantitation (LOD) of the proposed methods must be lower than the earlier reported works without interference of biological sample matrices. Less complicated sample preparation procedure and analysis of samples without internal standards can be additional advantages of the method. Though in LC method estimation can be done at nanogram level but in UV method sensitivity of the method is restricted to micro gram level. Therefore, a simple and rapid spectroflourimetric method with comparable sensitivity (at nanogram level) to LC method but without the complications and cost of LC method would be highly useful.

Thus, to support various studies like, pure drug analysis, assay of formulations, *in-vitro* dissolution and permeability studies, and *in-vivo* studies involving blood serum and aqueous humor rapid, accurate and validated UV spectrophotometric, spectroflourimetric and liquid chromatographic methods of analysis for flurbiprofen were developed.

In the present study, three simple, sensitive, accurate and reproducible analytical methods with better detection range for estimation of flurbiprofen in pure form and in its pharmaceutical dosage forms were developed. A validated rapid UV spectrophotometric method for estimation of flurbiprofen in phosphate buffer (pH 6.4) at 248 nm, a spectroflourimetric method in 1:1 mixture of methanol and 0.1N H_2SO_4 at the λ_{ex} and λ_{em} of 250 nm and 314 nm respectively and a LC method using reverse phase C-18 column in 40:20:40 mixture of methanol-acetonitrile-phosphate buffer (pH 5.6) with UV detection at 248 nm were also developed. In all the methods no extraction step is utilized thus reducing the time and error involved in the estimation. The developed methods were used to estimate the total drug content in two commercially available ophthalmic drops of flurbiprofen. The results of the analysis were validated by statistical methods (USP, 2000b and ICH guidelines 1996) and by recovery studies. The developed methods were later used to estimate the flurbiprofen in designed formulations, stability samples and in samples obtained *in-vitro* dissolution studies, *in-vitro* permeability studies.

A new reversed phase liquid chromatographic method, with UV detection, for the quantitative estimation of flurbiprofen in blood serum and aqueous humor fluid were also developed using reverse phase C-18 column in 40:20:40 mixture of methanol-acetonitrile-phosphate buffer (pH 5.6) with UV detection at 248 nm. Acetonitrile was used to precipitate protein content of the bio-matrices during sample preparation. The mobile phase and other chromatographic conditions were optimized to minimize interference from sample matrix and at the same time to provide sufficient sensitivity for the method to be adopted for *in-vivo* studies of developed ophthalmic formulations of flurbiprofen. In all the methods no extraction step is utilized thus reducing the time and error involved in the estimation. The developed methods were used for estimation of flurbiprofen in serum and aqueous humor samples obtained in *in-vivo* studies. The developed methods were validated by statistical methods (USP, 2000b and ICH guidelines 1996) and by recovery studies.

3.2 Analytical method development for the quantitative estimation of flurbiprofen in pure form and in ophthalmic formulations

Materials

Flurbiprofen was obtained as a gift sample from Optho Remedies Pvt. Ltd., Allahabad, India. Chromatographic grade methanol and acetonitrile were purchased from Merck, India. Analytical grade sodium dihydrogen phosphate, disodium hydrogen phosphate and sulphuric acid were purchased from Merck, Mumbai, India. High quality pure water was prepared using Millipore purification system (Millipore, Molsheim, France, model Elix SA 67120). Two commercially available ophthalmic drops of flurbiprofen [FLUR^{TM}, Nicholas Piramal India Ltd., Dhar, India and OCUFLUR, FDC Ltd., Aurangabad, India] were selected from the local market on random basis. FLUR^{TM} contained flurbiprofen sodium USP- 0.03% w/v, phenyl mercuric nitrate IP- 0.002% w/v, hydroxy propyl methyl cellulose IP- 0.25% w/v and aqueous buffered vehicle - q.s., whereas OCUFLUR contained flurbiprofen sodium USP- 0.03% w/v, phenyl mercuric nitrate I.P. – 0.001% w/v and water for injection IP- q.s.

Equipments

A UV-visible-NIR spectrophotometer (Jasco, Tokyo, Japan, model V-570) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells with Jasco spectra manager software was used for all absorbance measurements in the UV method. For spectroflourimetric method, a scanning spectrofluorimeter (Jasco, Tokyo, Japan, model FP-777) with built-in compatible software, link search mode, multiple PMT gain mode, automatic wavelength accuracy of 1.5 nm, range 220-750 nm and 10 mm quartz cells was used for fluorescence intensity measurement. And for LC estimations, a Jasco model liquid chromatograph equipped with two-pump gradient system (PU-1580), Rheodyne injector (7725i) fitted with a 20 µl loop, UV detector (UV-1575) and BORWIN-I software was used.

Method development

Different solvent systems were used to develop rugged, quick and suitable methods for the quantitative determination of flurbiprofen in pure form and in pharmaceutical formulations using different instrumental methods of analysis. The final decision on the suitability of a solvent system for method development using the three techniques were based on- cost, sensitivity, solvent noise, quenching effect of the solvent (only in case of spectroflourimetric method),

sample preparation time and steps involved, adaptability of the method for estimation of the drugs in their pharmaceutical dosage form and minimization of interference from commonly employed excipients in pharmaceutical formulations.

3.2.1 UV spectrophotometric method development

3.2.1.1 Experimental

Preparation of standard curve

A stock solution of flurbiprofen was prepared by dissolving 10 mg of drug in 100 ml of phosphate buffer [pH 6.4; 0.1M $NaH_2PO_4.H_2O$ (73.5 parts) and 0.1M $Na_2HPO_4.7H_2O$ (26.5 parts)] to get a final concentration of 100 µg/ml. The λ_{max} of flurbiprofen in the above media was determined by scanning a suitable dilution of the stock using the UV-visible spectrophotometer (Figure 3.1). From the stock solution, various standard dilutions were made to obtain solutions of 1, 5, 10, 15 and 20 µg/ml, and their respective absorbance was measured. The results are listed in Table 3.1 and the results of regression analysis and one-way ANOVA test for linearity (Duncan et al, 1983; Bolton, 1997) are presented in Table 3.2 and 3.3 respectively. The absorbance characteristics, accuracy, precision and other validation parameters of the proposed methods are given in Table 3.4. The stability of flurbiprofen solutions during analysis was also investigated by analyzing samples at different time intervals on the same day and the subsequent day by storing at room temperature.

Method validation

Following procedures were employed to determine various validation parameters of the developed UV spectrophotometric method (the results are presented in Table 3.4).

Accuracy and precision: To determine accuracy and precision of the proposed method two different (5 in each case) concentrations of flurbiprofen (5 and 15 µg/ml) standard and test solution were analyzed as per the procedure enlisted in the previous section (preparation of standard curve). To determine intra- and inter-day precision of the assay, replicate sets (number replicate samples = 5 at each concentration) of calibration solutions were analyzed. The percentage relative standard deviation (% RSD) for the assay results was determined.

Linearity: Five separate series of solutions of the drug, 1-20 µg/ml were prepared from the stock solution and analyzed.

Specificity: Series of five solutions of 10 µg/ml were prepared from the stock solution meant for method validation and analyzed.

LOQ and LOD: LOQ and LOD were calculated on the basis of standard error of estimate and slope of the regression equation based on replicate determinations. It was also felt necessary to investigate the lowest concentration that can be accurately quantified. Therefore experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the proposed method.

Ruggedness and robustness: Ruggedness was determined for the developed method by varying the analyst for analyzing standard and test solution the drug (5 and 15 µg/ml) in triplicate and by varying the instrument by using Jasco UV spectrophotometer model- 7800. Robustness was determined by varying the pH of phosphate buffer between 6.0 – 6.8 for analysis.

Estimation of flurbiprofen from two commercial ophthalmic formulations by the proposed method

Two commercially available ophthalmic drops of flurbiprofen (FLUR™ and OCUFLUR) from Indian market were selected randomly for estimation of total drug content per ml of the ophthalmic drops by the proposed method. For each brand, contents of ten containers were mixed and an aliquot volume (equivalent to 1 mg of flurbiprofen) was transferred to a series of 25 ml volumetric flasks (five in each case) and final volume was made using sodium phosphate buffer (pH 6.4). The resulting solution was filtered through Whatman filter paper no.1 and suitably diluted to get final concentration within the limits of linearity for the proposed method. The drug content per ml of different brands of flurbiprofen ophthalmic drops was calculated (on an average concentration basis) from the absorbance value. The results are tabulated in Table 3.5.

Recovery Studies

To keep an additional check on the accuracy of the developed assay methods and to study the interference of formulation additives, analytical recovery experiments were performed by adding known amount of pure drug to pre-analyzed samples of commercial dosage forms. The percent

analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are also listed in Table 3.5.

3.2.1.2 Results and discussion

Method development

To develop a rugged and sensitive UV spectrophotometric method various solvent systems were tried such as, water, methanol, acetonitrile, buffers (phosphate pH 5.6-8.0, acetate pH 3.5-5.6 and citrate pH 3.0-7.0) alone or in combinations of different proportions. The final decision of using phosphate buffer pH 6.4 was based on sensitivity, interference, ease of preparation, need for pH adjustment, tolerance for pH variation, suitability for drug content estimation and stability, analysis time and cost in that order. The maximum variation of pH of the selected media was ± 0.05, thus contributing to robustness of the method. Also phosphate buffer, during the period of studies did not show any microbial growth and therefore use of sodium azide (0.1 %) was not deemed to be necessary. Effect of various formulation additives on the absorbance of flurbiprofen has been studied and no interference was observed.

Calibration curve

The λ_{max} of flurbiprofen in sodium phosphate buffer (pH 6.4) was found to be 248 nm and the corresponding UV spectra is shown in Figure 3.1. Though there was another peak found at around 220 nm, but that is not used for estimation due to interference from the solvent used. The drug concentration in the selected solvent system of sodium phosphate buffer (pH 6.4) showed a linear relationship with the absorbance at 248 nm in the concentration range of 1-20 µg/ml. The statistical analysis (Duncan et al, 1983; ICH guidelines, 1996; Bolton, 1997; USP, 2000b) of data obtained for the estimation of flurbiprofen in pure solution indicated high level of precision for the proposed method as evidenced by the low standard deviation values (Table 3.1). The low values of coefficient of variation (Table 3.1) further established the precision of the proposed method. In phosphate buffer pH 6.4 flurbiprofen was found to be stable for a period of more than 48 hours thus suggesting the use of proposed method as stability indicating assay for flurbiprofen.

The linear regression equation was obtained as Y = 0.0759.X+ (–) 0.0462, where Y is the absorbance and X is the concentration (in µg/ml) of pure flurbiprofen solution. Linearity of the

regression equation and negligible scatter of points were demonstrated from the correlation coefficient (Table 3.2). The reported slope values without intercept on the ordinate, at 95 % confidence limits, suggested that the calibration lines of flurbiprofen solutions in sodium phosphate buffer (pH 6.4) did not deviate from the origin as the above obtained values lied within the confidence limits (Table 3.2). The precision of the fit was further confirmed from the standard error values of the intercept, slope and the estimate. A one-way ANOVA test (Duncan et al, 1983; Bolton, 1997) was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions. In selected solvent system flurbiprofen was found to be stable for about 48 hours. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significance levels (Table 3.3).

Validation of the developed method

The developed method was validated according to the standard procedures (ICH Guidelines, 1996; USP, 2000b) and the results obtained are tabulated in Table 3.4. The linearity range of flurbiprofen solution was found to be 1-20 µg/ml at a λ_{max} of 248 nm. Since the reported slope values without intercept fell within 95 % confidence limits the linearity characteristics of the proposed method could be practically considered as 0-20 µg/ml. The lowest limit of detection was obtained as 0.34 µg/ml and limit of quantitation was determined as 1.02 µg/ml. In the validation table (Table 3.4), the accuracy is reported in terms of percentage relative error and precision in terms of % RSD. The low values of these parameters reflected the excellent measurement accuracy and precision of the proposed method for estimation of flurbiprofen. The ruggedness (%) in the estimation of flurbiprofen standard and test solution (5 and 15 µg/ml) in triplicate by different analysts and on different instruments varied between 99.46 to 99.52 %.

The method of preparing the buffer solution was found to be simple and accurate and variation of the pH of the buffer between 6.2-6.6 did not affect the sensitivity of the method. The % RSD for intra- and inter-day variation was less than 6.0 %, which fall well below the acceptance criteria described by Shah et al, 1992.

Recovery studies

The method was further validated by estimation of flurbiprofen in pharmaceutical formulations by the proposed method and analysis of reference pure drug solution and the results are presented in Table 3.5. The estimated drug content with low values of standard deviation further

established the precision of the proposed method and therefore suggested the non-interference from the formulation matrix present in the studied formulations. The accuracy of the results of estimation was further tested by recovery experiments. The average recovery varied from 98.9 to 99.9 %. The reported F-value of a two-way ANOVA test without replication for samples suggested that there was no significant difference in the mean recoveries of the samples (Table 3.6).

3.2.2 Spectroflourimetric method development

3.2.2.1 Experimental

Preparation of standard curve

A stock solution was prepared by dissolving 10 mg of drug in 100 ml (final volume) of 25 % v/v acetonitrile in water to get a final concentration of 100 µg/ml. The excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) of flurbiprofen was determined by scanning a suitable dilution of the stock in 1:1 mixture of methanol and 0.1N sulphuric acid using the scanning spectroflourimeter (Figure 3.2). From the stock solution, various dilutions were made using the above solvent system to obtain solutions of 10, 25, 50, 100, 200, 250 and 300 ng/ml, and fluorescence intensity was measured for each dilution. The PMT gain mode was kept at medium for all determinations. The calibration curve values for the method are listed in Table 3.1 and the results of regression analysis and one-way ANOVA test for linearity (Duncan et al, 1983; Bolton, 1997) are presented in Table 3.2 and 3.3 respectively. The florescence characteristics, accuracy, precision and other validation parameters of the proposed methods are given in Table 3.4. The stability of flurbiprofen solutions during analysis was also investigated by analyzing samples at different time intervals on the same day and the subsequent day by storing at room temperature.

Method validation

Following procedures were employed to determine various validation parameters of the three developed methods (the results are presented in Table 3.4).

Accuracy and precision: To determine accuracy and precision of the proposed method two different (5 in each case) concentrations of flurbiprofen (25 and 250 ng/ml) standard and test

solution were analyzed as per the procedure enlisted under UV spectrophotometric method. To determine intra- and inter-day precision of the assay, replicate sets (number replicate samples = 5 at each concentration) of calibration solutions were analyzed. The percentage relative standard deviation (% RSD) for the assay results was determined.

Linearity: Five separate series of solutions of the drug, 10-300 ng/ml, were prepared from the stock solution and analyzed.

Specificity: Series of five solutions of 100 ng/ml were prepared from the stock solution meant for method validation and analyzed.

LOQ and LOD: Limits of detection and quantitation were calculated on the basis of standard error of estimate and slope of the regression equation based on replicate determinations. It was also felt necessary to investigate the lowest concentration that can be accurately quantified by the method. Therefore experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the proposed method.

Ruggedness and robustness: Ruggedness was determined for all the developed methods by varying the analyst for analyzing standard and test solution the drug (25 and 250 ng/ml) in triplicate. Robustness of the method was determined by varying the relative proportion of methanol in the solvent system (48, 50, 52 %) and its effect on the sensitivity of the method studied.

Estimation of flurbiprofen from two commercial ophthalmic formulations by the proposed method

For estimation of drug content from the two commercially available ophthalmic drops of flurbiprofen (FLUR^{TM} and OCUFLUR) from Indian market the same procedure as mentioned under UV spectrophotometric method was employed except that the initial solution was made in 25 % v/v acetonitrile. The resulting solutions in both the cases were filtered through Whatman filter paper no.1 and suitably diluted in 1:1 mixture of methanol and 0.1N sulphuric acid to get final concentration within the limits of linearity for the respective proposed methods. The drug content per ml of different brands of flurbiprofen ophthalmic drops was calculated (on an average concentration basis) from the fluorescence value and the results are tabulated in Table 3.5.

Recovery Studies

To keep an additional check on the accuracy of the developed assay methods and to study the interference of formulation additives, analytical recovery experiments were performed by adding known amount of pure drug to pre-analyzed samples of commercial dosage forms. The percent analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are also listed in Table 3.5.

3.2.2.2 Results and discussion

Method development

During the course of proposed method development drugs solubility was a major problem. In pure aqueous based solvents the drugs are difficult to be solubilized. For this reason 25 % v/v acetonitrile in water was used for preparing the primary stock solution. For the analysis of flurbiprofen various solvents (either alone or in combination, 25 to 75 % v/v, with water) include high pure water, methanol, ethanol, acetonitrile, dioxane and diethyl ether, dimethyl formamide, 0.1N H_2SO_4, 0.1N NaOH and 0.1N HCl. The above solvents were also used in combinations like methanol: 0.1N H_2SO_4 (25 to 75 %) and acetonitrile: methanol (40 to 70%). The final decision of using methanol: 0.1N H_2SO_4 (1:1) as the solvent for analysis was based on sensitivity, noise, ease of preparation, suitability for drug content estimation and stability studies, time and cost in that order. No interference was observed from various formulation additives on the fluorescence pattern of the drug in the proposed solvent systems.

Calibration curve

The λ_{ex} and λ_{em} of flurbiprofen in 1:1 mixture of methanol and 0.1N H_2SO_4 was found to be 250 nm and 314 nm respectively (the corresponding spectrum scan obtained through link search is shown in Figure 3.2a & b). The drug concentration in the selected solvent system of 1:1 mixture of methanol and 0.1N H_2SO_4 showed a linear relationship with the fluorescence intensity in the range 10-300 ng/ml. The statistical analysis of data obtained for the estimation of flurbiprofen in pure solution indicate the high level of precision for the proposed method as evidenced by the low standard deviation values (Table 3.1). The low values of coefficient of variation (Table 3.1) further established the precision of the proposed methods.

The linear regression equation was obtained as Y = 27.797.X + 46.049, where Y is the measured fluorescence intensity and X is the concentration of pure drug solution in ng/ml. Linearity of the regression equation and negligible scatter of points were demonstrated from the correlation coefficient for both the methods (Table 3.2). The precision of the fit for both the drugs was further confirmed from the standard error values of the intercept, slope and the estimate. A one-way ANOVA test (Duncan et al, 1983; Bolton, 1997) was performed based on the values observed for each pure drug concentration during the replicate measurement of the standard solutions. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significance level (Table 3.3).

Validation of the developed method

The developed method was validated according to the standard procedures (ICH Guidelines, 1996; USP, 2000b) and the results obtained are tabulated in Table 3.4. The linearity range of flurbiprofen solution was found to be 10- 300 ng/ml at λ_{ex} of 251 nm and λ_{em} of 314 nm. Since the reported slope values without intercept fell within 95 % confidence limits for the proposed method, thus linearity characteristics of the proposed method could be practically considered as 0-300ng/ml. The LOD was obtained as 0.99 ng/ml and LOQ was determined as 3.32 ng/ml for the method. In the validation tables (Table 3.4) for the developed methods, the accuracy is reported in terms of percentage relative error and precision in terms of % RSD. The low values of these parameters reflected the high degree of accuracy and precision of the proposed method for estimation of flurbiprofen. For the developed spectroflourimetric method the ruggedness (%) in the analysis of various standard and test solution by different analysts (25 and 250 ng/ml for flurbiprofen in triplicate) varied from 99.46 to 100.10 %. The % RSD for intra- and inter-day variation was less than 3.0 %, which is within the acceptable limits (Shah et al, 1992).

Recovery studies

Further validation of the developed method by estimation of flurbiprofen in pharmaceutical formulations by the proposed methods and analyses of reference pure drug solution and the results are presented in Table 3.5. The estimated drug content with low values of standard deviation further established the precision of the proposed methods and therefore suggested the non-interference from the formulation matrix present in the studied formulations. The accuracy of the results of estimation was further tested by recovery experiments. The average recovery

varied between 100.79 and 102.55 %. The reported F-value of a two-way ANOVA test without replication for samples suggested that there was no significant difference in the mean recoveries of the samples (Table 3.6).

3.2.3 Liquid chromatographic method development

3.2.3.1 Experimental

Chromatographic conditions

The chromatographic column used was a reverse phase 4.6 x 125 mm LiChroCART® Purospher® endcapped reverse phase C-18 LC column (E. Merck, Darmstadt, Germany) with 5 µm particles. The column and the LC system were kept in ambient conditions. The mobile phase was methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) delivered at a flow rate of 0.75 ml/min. The injection volume was 20 µl. Millipore sample clarification module was used for all sample clarifications before injection. The eluate was analyzed at a wavelength of 248 nm.

Preparation of standard curve

A stock solution (100 µg/ml) of pure drug was prepared by dissolving 5 mg flurbiprofen in 50 ml of 40:20:40 mixture of methanol-acetonitrile-phosphate buffer (pH 5.6). From this solution, 1ml was transferred to 10 ml volumetric flask and the volume was made to obtain a solution of 10 µg/ml. From this secondary stock solution concentration of 50, 100, 200, 400, 600, 800 and 1000 ng/ml were made in series by suitable dilution in 10ml volumetric flask for the purpose of calibration curve. Composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase methanol: acetonitrile: phosphate buffer (pH 5.6) (40:20:40) was passed through the same. The mobile phase filtered through 0.22 µm membrane filter using Millipore HPLC solvent filtration assembly, was delivered at 0.75 ml/min for column stabilization, with continuous baseline monitoring. The phosphate buffer (pH 5.6) was prepared by mixing 94.8 parts of 0.1M $NaH_2PO_4.H_2O$ and 5.2 parts of 0.1M $Na_2HPO_4.7H_2O$. The wavelength of detection was fixed at 248 nm. The prepared dilutions were injected serially in increasing order of concentration. The obtained peaks were integrated and the peak area was calculated for each. The results are listed in Table 3.1 and the results of regression analysis and one-way ANOVA test for linearity (Duncan et al, 1983; Bolton, 1997) are presented in Table 3.2

and 3.3 respectively. The validation parameters of the proposed method are given in Table 3.4. The stability of flurbiprofen solution during analysis was determined by analyzing samples at different time intervals during the course of the experiment on the same day and also on the subsequent day of the experimentation by storing at room temperature and after freeze-thaw cycle. Chromatogram parameters such as, retention time and asymmetry factor were also optimized to increase the rapidity of the method.

Method validation

Following procedures were employed to determine various validation parameters of the developed method and the results are presented in Table 3.4.

Accuracy and precision: To determine accuracy and precision of the developed method two different (5 in each case) concentrations of flurbiprofen (100 and 600 ng/ml) standard and test solution were analyzed as per the procedure enlisted under UV spectrophotometric method. To determine intra- and inter-day precision of the assay, replicate sets (number replicate samples = 5 at each concentration) of calibration solutions were analyzed. The percentage relative standard deviation (% RSD) for the assay results was determined.

Linearity: Five separate series of solutions of the drug, 50-1000 ng/ml, were prepared from the stock solution and analyzed.

Specificity: Series of five solutions of 500 ng/ml were prepared from the stock solution meant for method validation and analyzed.

LOQ and LOD: The limit of detection and quantitation were calculated on the basis of signal-to-noise ratio based on replicate determinations. It was also felt necessary to investigate the lowest concentration that can be accurately quantified by each method. Therefore experiments were performed to analyze the actual concentration that can be accurately quantified or detected by the method.

Ruggedness and robustness: Ruggedness was determined for the developed method by varying the analyst for analyzing standard and test solution of the drug (100 and 600 ng/ml) in triplicate. Also ruggedness was determined by varying the instrument by using Waters HPLC equipped with two-pump system (model 501), Rheodyne injector (7725i) fitted with a 20 µl loop, UV

detector (Lambda max model 481) and AIMIL chromatography data station with WIN-ACDS software, and different column of same make.

For the developed method the robustness was determined by studying the effect of relative percentage (up to 2 % variation level) of various components of the mobile phase on retention time and selectivity was determined.

Estimation of flurbiprofen from two commercial ophthalmic formulations by the proposed method

For estimation of drug content from the two commercially available ophthalmic drops of flurbiprofen (FLUR^{TM} and OCUFLUR) from Indian market the same procedure as mentioned under UV spectrophotometric method was employed except that the final volume was made using mixture of methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) in case of LC method. The resulting solutions in both the cases were filtered through Whatman filter paper no.1 and suitably diluted to get final concentration within the limits of linearity for the proposed method. The drug content per ml of different brands of flurbiprofen ophthalmic drops was calculated (on an average concentration basis) from the peak area values. The results are tabulated in Table 3.5.

Recovery Studies

To keep an additional check on the accuracy of the developed assay method and to study the interference of formulation additives, analytical recovery experiments were performed by adding known amount of pure drug to pre-analyzed samples of commercial dosage forms. The percent analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are also listed in Table 3.5.

3.2.3.2 Results and discussion

Method development

In case of the proposed method, mobile phase investigated were methanol: water (20 to 70%), acetonitrile: water (20 to 70%), phosphate buffer (pH 5.2 – 6.8) with methanol or acetonitrile or both. Mobile phase of methanol: acetonitrile: phosphate buffer pH 5.6 (40:20:40) and flow rate selection was based on peak parameters (height, asymmetry, tailing), baseline drift, run time, ease of preparation of the mobile phase, need for pH adjustment and cost in that order. Internal

standard was not used, as there was no extraction or separation step involved in the estimation of flurbiprofen from formulations. Though glacial acetic acid (0.5 or 1.0 %) in the mobile phase improved the peak parameters but its use was given up in favor of long-term stability of the column.

Calibration curve

A typical chromatogram for flurbiprofen using reverse phase C-18 column with mobile phase composition of methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 0.75 ml/min flow rate is shown in Figure 3.3. The wavelength of detection was fixed at 248 nm (as per results of UV analysis), so as to provide least interference from mobile phase with highest sensitivity. The statistical analysis (Duncan et al, 1983; ICH guidelines, 1996; Bolton, 1997; USP, 2000) of data obtained for the estimation of flurbiprofen in pure solution indicated high level of precision of this method. The calibration curve peak area (μV.sec) vs. concentration (ng/ml) was found to be linear. Values obtained for the calibration curve are presented along with standard deviation, coefficient of variance in Table 3.1. Statistical calculations were done at 5% level of significance. The low values of standard deviation, standard error and coefficient of variation established the precision of the proposed method.

The linear Regression equation obtained for the proposed LC method was Y = 126.52.X + 1483.0, where Y = peak area in μV.sec; X = concentration in ng/ml. The correlation coefficient value was highly significant (Table 3.2). The reported slope value without intercept on the ordinate, at 95 % confidence limits, suggested that the calibration line of flurbiprofen solution did not deviate from the origin as the above value lied within the confidence limits (Table 3.2). The retention time and asymmetry factor were found to be 4.3238 ± 0.0380 mins and 1.1153 ± 0.1914 mins respectively (Table 3.4). The drug solution was found to be stable for a period of 48 hours at room temperature in the solvent system used.

Like earlier methods, a one-way ANOVA test (Duncan et al, 1983; Bolton, 1997) was also performed for the LC method based on the values observed during the replicate measurement of the standard solutions of the pure drug. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significance level in this method as well (Table 3.3).

Validation of the developed method

The developed liquid chromatographic method was validated according to the standard procedures (ICH Guidelines, 1996; USP, 2000b) and the results obtained are tabulated in Table 3.4. The linearity range of flurbiprofen solution was obtained as 50-1000 ng/ml. Since the reported slope values without intercept fell within 95 % confidence limits, the linearity characteristics of the proposed method could be practically considered as 0-1000 ng/ml. The lowest limit of detection and limit of quantitation was obtained as 15 ng/ml and 50 ng/ml respectively. However, the lowest quantity of flurbiprofen accurately quantitated by the developed method experimentally was 25 ng/ml but was omitted form the calibration plot due to constraints of signal-to-noise ratio requirement. In the validation tables (Table 3.4) for the developed methods, the accuracy is reported in terms of percentage relative error and precision in terms of % RSD. The low values of these parameters reflected the excellent measurement accuracy and precision of the proposed methods of estimation of flurbiprofen. The ruggedness (%) in the estimation of flurbiprofen standard and test solution (100 and 600 ng/ml) in triplicate by different analysts and on different instruments varied between 99.30 to 99.82 %. The variation of the relative composition of the mobile phase (greater than or equal to 1%) effected a change in retention time of the analyte peak. However variation in the buffer pH did affect the retention and peak parameters. The % RSD for intra- and inter-day variation was less than 4 % for the method, which fall well below the acceptance criteria described by Shah et al, 1992. No internal standard was used as no extraction step was involved in estimation of flurbiprofen from the formulation. Further, the accuracy of the experimental results established no need for internal standards for the suggested methods.

Recovery studies

The proposed liquid chromatographic method could be employed successfully for the estimation of flurbiprofen in pharmaceutical formulations and analysis of reference pure drug solution with low values of standard deviation. The results (presented in Table 3.5) further established the precision of the proposed methods and therefore suggested the non-interference from the formulation matrix present in the studied formulations. The accuracy of the results of estimation was further tested by recovery experiments. The average recovery was found to vary between 99.2 and 100.1 %. The reported F-value of a two-way ANOVA test without replication for samples suggested that there was no significant difference in the mean recoveries of the samples

(Table 3.6). Due to this internal standard was not used in the analysis by LC method.

3.3 Liquid chromatographic method development for the estimation of flurbiprofen in serum and aqueous humor

3.3.1 Experimental

Materials

Flurbiprofen was obtained as a gift sample from Optho Remedies Pvt. Ltd., Allahabad, India. Chromatographic grade methanol and acetonitrile were purchased from Merck, India. Analytical grade sodium dihydrogen phosphate, disodium hydrogen phosphate and sulphuric acid were purchased from Merck, Mumbai, India. High quality pure water was prepared using Millipore purification system (Millipore, Molsheim, France, model Elix SA 67120).

Equipments

A Jasco model liquid chromatograph equipped with two-pump gradient system (PU-1580), Rheodyne injector (7725i) fitted with a 20 µl loop, UV detector (UV-1575) and BORWIN-I software was used.

Method development

Different solvent systems were used to develop rugged, quick and suitable methods for the quantitative determination of flurbiprofen in serum and aqueous humor. Efforts was made to develop a method which does not involve the use of internal standard, minimize interference from sample matrix and at the same time provide sufficient sensitivity for the method to be adopted for *in-vivo* studies. The final decision on the suitability of a solvent system for method development were based on- cost, sensitivity, solvent noise, sample preparation time and steps involved, adaptability of the method for estimation of the drug in bio-matrices. In all the methods no extraction step is utilized thus reducing the time and error involved in the estimation.

Chromatographic conditions

The chromatographic column used was a reverse phase 4.6 x 125 mm LiChroCART® Purospher® endcapped reverse phase C-18 LC column (E. Merck, Darmstadt, Germany) with 5 µm particles. The column and the LC system were kept in ambient conditions. The mobile phase was

methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) delivered at a flow rate of 1.0 ml/ min. The injection volume was 20 µl. Millipore sample clarification module was used for all sample clarifications before injection. The eluate was analyzed at a wavelength of 248 nm.

Separation of serum and aqueous humor

Blood was collected from marginal ear vein of rabbit by bleeding technique into Eppendorf tubes. After allowing sufficient time (30-45 mins) for blood to coagulate, samples were centrifuged at 4000 rpm for 15 min. Supernatant serum was then collected and pooled from different tubes. Pooled serum in small lots were transferred to Eppendorf tubes and stored at –20 °C and thawed every time before usage. Aqueous humor was collected with a half-inch 30-gauge disposable needle on a 1 ml tuberculin syringe with a transparent hub from the anterior chamber of the rabbit eye by piercing through the corneo-scleral limbus. The collected aqueous humor was mixed with equal volume of methanol and refrigerated at 4 °C for 30 mins and then centrifuged at 3000 rpm for 15 min. The supernatant collected and stored at –20°C and thawed before usage. Eppendorf tubes used in these experiments were freshly sterilized ones by moist heat sterilization at 121°C for 15 mins.

Preparation of standard curve

Blank primary aqueous humor and serum obtained by the method described above was used for spiking and constructing the standard curve. A 10 ml stock solution (100 µg/ml) was prepared by dissolving 1 mg of the drug in less than 1.2 ml of methanol and volume was made up to 10 ml using phosphate buffer pH 5.6. From this stock solution further dilutions of 10 µg/ml and 1 µg/ml were prepared using phosphate buffer pH 5.6. Spiking was done by the following method. To 50 µl of serum, in an Eppendorf tube, aliquot of the drug stock solution was added to get the final concentration in 500 µl as 50, 100, 200, 500 and 1000 ng/ml. Alternatively to 20 µl aqueous humor, in an Eppendorf tube, aliquot of the drug stock solution was added to get the final concentration in 200 µl as 50, 100, 200, 500 and 1000 ng/ml. Samples were vortexed and 300 µl of acetonitrile added to spiked serum samples and 120 µl of acetonitrile added to spiked aqueous humor samples and vortexed again. The samples were centrifuged at 11500 rpm for 12 minutes at 4 °C. Supernatant liquid was collected and as such injected into the HPLC system. Peaks obtained were integrated and the peak area was calculated. The results are listed in

Table 3.7 and the results of regression analysis and one-way ANOVA test for linearity (Duncan et al, 1983; Bolton, 1997) are presented in Table 3.8 and 3.9 respectively. The validation parameters of the proposed method are given in Table 3.10. The results of the recovery experiments are presented in Table 3.11. Stability of flurbiprofen in bio-fluid during analysis (samples analyzed at different time intervals during the course of the experiment) and after freeze-thaw cycle was also studied. The intra-day and inter day variation (% RSD) in the developed methods are shown in Table 3.12. Chromatogram parameters such as, retention time and asymmetry factor optimized to increase rapidity of the method.

Method validation

Following procedures were employed to determine various validation parameters of the developed method.

Accuracy and precision: To determine accuracy and precision of the proposed method five separate spiked standard and test samples (50 and 250 ng/ml) prepared in serum or aqueous humor blank as per the procedure enlisted in the previous sections and analyzed. To determine intra- and inter-day precision of the assay, replicate sets (number replicate samples = 5 at each concentration for each method) of calibration solutions were analyzed. The percentage relative standard deviation (% RSD) for the assay results was determined.

Linearity: Five separate series of solutions of the drug (50-1000 ng/ml) prepared by spiking in serum or aqueous humor blank and analyzed.

Specificity: Series of five spiked samples of the drug (250 ng/ml) in either serum or aqueous humor blank were prepared and analyzed.

LOD and LOQ: These parameters were calculated on the basis of signal-to-noise ratio based on replicate measurements. It was also felt necessary to investigate the lowest concentration that can be accurately quantified by each method. Therefore experiments were performed to analyse the actual concentration that can be accurately quantified or detected by the method.

Ruggedness and robustness: Ruggedness was determined by varying the analyst for analyzing standard and test solution of the drug (100 ng/ml and 750 ng/ml) in triplicate. Also ruggedness was determined by varying the instrument (Waters HPLC equipped with two-pump system (model 501), Rheodyne injector (7725i) fitted with a 20 µL loop, UV detector (Lambda max

model 481) and AIMIL chromatography data station with WIN-ACDS software), and different columns of same make. The effect of changing the pH of phosphate buffer between 5.4 –5.8 and relative % of the mobile phase (0.5 to 5%) on retention time and selectivity was determined for the method.

Recovery Studies

Along with each standard curve trial QC samples at high, medium and low concentration levels were run to calculate the recoveries from spiked samples. To keep an additional check on the accuracy of the developed assay method and to study the interference of bio-matrices analytical recovery experiments were performed by adding known amount of pure drug to pre-analyzed samples of the drug. The percent analytical recovery values calculated by comparing concentration obtained from the spiked samples with actual added concentrations are also listed in Table 3.11.

3.3.2 Results and discussion

Method development

In the development of LC method for estimation of flurbiprofen in aqueous humor and serum mobile phase investigated were methanol: water (20 to 80%), acetonitrile: water (20 to 80%), methanol: phosphate buffer (pH 5.0 – 6.2) (20 to 80%) and acetonitrile: phosphate buffer (pH 5.0 – 6.2) (20 to 80%), methanol: water (20 to 70%), acetonitrile: water (20 to 70%), methanol or acetonitrile in combination with phosphate buffer (pH 5.0 – 6.8) alone or in combinations. Mobile phase of methanol: acetonitrile: phosphate buffer pH 5.6 (40:20:40) and flow rate selection was based on peak parameters (height, asymmetry, tailing), baseline drift, run time, ease of preparation of the mobile phase, need for pH adjustment and cost (in that order). Internal standard was not used, as there was no extraction or separation step involved in the estimation of the drug.

Analysis in serum

A typical chromatogram for blank serum and flurbiprofen spiked in serum using RP C-18 column with mobile phase composition of methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 1.0 ml/min flow rate is shown in Figure 3.4a and b. The wavelength of detection was fixed at 248 nm (as per earlier reported results of our group; Sajeev et al, 2002), so as to

provide least interference from mobile phase with highest sensitivity. The statistical analysis (Duncan et al, 1983; ICH guidelines, 1996; Bolton, 1997; USP, 2000b) of data obtained for the estimation of flurbiprofen spiked in primary blank serum indicated high level of precision of this method. The calibration curve peak area (μV.sec) vs. concentration (ng/ml) was found to be linear. Values obtained for the calibration curve are presented along with standard deviation and coefficient of variance in Table 3.7. Statistical calculations were done at 5% level of significance. The low values of standard deviation and coefficient of variation established the precision of the proposed method.

The linear Regression equation obtained for the proposed LC method for estimation of flurbiprofen in serum was Y = 52.27.X + (–) 1618.70, where Y = peak area in μV.sec; X = concentration in ng/ml .The correlation coefficient value was highly significant (Table 3.8). The reported slope value without intercept on the ordinate, at 95 % confidence limits, suggested that the calibration line of flurbiprofen solution did not deviate from the origin as the above value lied within the confidence limits (Table 3.8). The retention time and asymmetry factor were found to be 3.1312 ± 0.0101 mins and 1.1310 ± 0.0091 mins respectively (Table 3.10). The drug spiked serum samples were found to be stable for approximately 8 hours under ambient conditions and the freeze-thaw cycle studies did not affect the recovery of the drug from the spiked samples.

A one-way ANOVA test (Duncan et al, 1983; Bolton, 1997) was also performed for the proposed method based on the values observed during the replicate measurement of the standard spiked samples. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significance level in this method as well (Table 3.9).

Analysis in aqueous humor

Figure 3.5a and b represents a typical chromatogram for blank aqueous humor and for flurbiprofen spiked in aqueous humor obtained using RP C-18 column with mobile phase composition of methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 1.0 ml/min flow rate. As in case of method for estimation of the drug in serum the wavelength of detection was fixed at 248 nm. The data obtained for the estimation of flurbiprofen spiked in blank aqueous humor when subjected to statistical analysis (Duncan et al, 1983; ICH guidelines, 1996; Bolton, 1997; USP, 2000b) indicated high level of precision of this method. The calibration curve peak area (μV.sec) vs. concentration (ng/ml) was found to be linear. Values obtained for the

calibration curve are presented along with standard deviation and coefficient of variance in Table 3.7. Statistical calculations were done at 5% level of significance. The low values of standard deviation and coefficient of variation established the precision of the proposed method.

The linear Regression equation obtained for the proposed LC method was Y = 61.79.X + (–) 783.24, where Y = peak area in μV.sec; X = concentration in ng/ml .The correlation coefficient value was highly significant at 5 % level (Table 3.8). The reported slope value without intercept on the ordinate, at 95 % confidence limits, suggested that the calibration line of flurbiprofen solution did not deviate from the origin as the above value lied within the confidence limits (Table 3.8). The retention time and asymmetry factor were found to be 3.1312 ± 0.0101 mins and 1.1310 ± 0.0091 mins respectively (Table 3.10). The drug spiked aqueous humor samples were found to be stable for approximately 12 hours under ambient conditions and the freeze-thaw cycle studies did not affect the recovery of the drug from the spiked samples.

Like in case of serum, a one-way ANOVA test (Duncan et al, 1983; Bolton, 1997) was also performed for the analysis of the drug in aqueous humor based on the values observed during the replicate measurement of the standard spiked samples. The calculated F-value (F_{Calc}) was found to be less than the critical F-value (F_{Crit}) at 5 % significance level in this method as well (Table 3.3).

Validation of developed method

The developed method were validated according to the standard procedures (ICH Guidelines, 1996; USP, 2000b) and the results obtained are tabulated in Table 3.10. The linearity range of spiked flurbiprofen solution in serum and aqueous humor was found to be 50-1000 ng/ml. The lowest limit of detection was obtained as 35 ng/ml and 25 ng/ml for analysis in serum and aqueous humor respectively. LOQ was determined as 50 ng/ml and 45 ng/ml for analysis in serum and aqueous humor respectively. In the validation tables (Table 3.10) for the developed methods, the accuracy is reported in terms of percentage relative error and precision in terms of % RSD. The low values of these parameters reflected the excellent measurement accuracy and precision of the proposed method of estimation of flurbiprofen (Shah et al, 1992). The ruggedness (%) in the estimation of standard and test spiked samples of flurbiprofen (50 and 250 ng/ml) in triplicate by different analysts and on different instruments gave a % RSD of 4.95 and 3.47 in serum and aqueous humor respectively. As discussed in the previous section for

estimation of flurbiprofen from pharmaceutical formulation by LC method, the method of preparing the buffer solution was found to be simple and accurate and however variation in the pH of the buffer did affect the peak parameters. The variation of the relative composition of the mobile phase ($\geq$ 1%) effected a change in retention time and shape of the analyte peak, introducing peak tailing. The % RSD for intra- and inter-day variation for both the methods at different concentration range is shown in Table 3.12 and was well below the acceptance criteria described by Shah, et al. (1992). No internal standard was used as no extraction step was involved in estimation of flurbiprofen from the bio-matrices. Also the accuracy of the experimental results established no need for internal standards for the suggested methods.

Recovery studies

Along with each standard curve, trial QC samples at high, medium and low concentration levels were run to calculate the recoveries from spiked samples, the results of which are presented in Table 3.11. The sample recoveries were in good agreement with their respective labeled claim suggesting non-interference of selected biological matrices in the estimation of flurbiprofen by the proposed method. The accuracy of the results of estimation was further tested by recovery experiments. Due to good recovery from different concentrations of spiked flurbiprofen, internal standard was not used in the analysis.

3.4 Conclusions

The proposed methods of estimation of flurbiprofen in pure form, in formulations and in biological samples were accurate, precise, rugged and reproducible. They were found to be simpler with lower LOD and LOQ compared to other reported methods. The proposed UV spectrophotometric method and spectroflourimetric method for the estimation of the drug in pure form and ophthalmic formulations showed linearity at lower range of drug concentration compared to most of the reported methods. Unlike reported methods, the proposed methods do not utilize a special extraction step for recovering the drug from the formulation excipients matrices thereby decreasing the degree of error and time in estimation. The UV method was found to be simpler and cheaper as media employed is aqueous based. The rapidity of the UV method and a lower drug concentration quantifiable at 1 μg/ml make it a method of choice for a variety of analyses, including pure drug analysis, assay of formulations, *in-vitro* dissolution and

stability studies. The proposed spectroflourimetric method has detection and quantitation limits at nanogram level making it suitable for routine analysis at par with existing chromatographic techniques.

In comparison with earlier reported and official methods for estimation of flurbiprofen in pure form and in pharmaceutical formulations, the proposed LC method gave a lower LOD and LOQ at 15 and 50 ng/ml when compared to 100 ng/ml and 1 mg/ml of earlier two proposed methods. The proposed LC method is rapid and the mobile phase flow rate is very less, only 0.75 ml/min in comparison to 1 ml/min for the official USP and BP methods.

The sample recoveries in all formulation using the above three methods were in good agreement with their respective label claims and thus suggested the validity of the methods and non-interference of formulation excipients, present in analyzed formulations, in the estimation. The proposed methods were successfully employed for estimation of flurbiprofen in developed formulations, stability samples and samples obtained from *in-vitro* dissolution and *in-vitro* permeability studies (as discussed in chapter 4 to 7). These methods can, therefore, be considered useful alternative method for routine analysis of flurbiprofen in pure form and in its formulations without interference.

The proposed method for estimation of flurbiprofen in biological samples utilizes less complicated sample preparation procedure and the samples can be analyzed accurately without using internal standards. The LOQ and LOD of the proposed methods are lower than the earlier reported works. Also, high sample recoveries (> 90 %) were obtained in all the spiked biological samples, thus suggesting non-interference of biological matrices. But one of the limitations of the developed method for estimation of the drug in biological sample, is that it involves protein precipitation using acetonitrile, thereby diluting the sample. Instead a method involving extraction of the drug from the bio-matrices could detect the drug at lower levels.

3.5 References

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Table 3.1: Calibration curve points of the proposed methods in estimation of standard solution of flurbiprofen

UV spectrophotmetric method			Spectroflourimetric method			Liquid chromatographic method		
Conc. of the solution (μg/ml)	**Mean absorbance value [a]**	**C.V. (%)**	**Conc. of the solution (ng/ml)**	**Mean fluorescence intensity [a]**	**C.V. (%)**	**Conc. of the solution (ng/ml)**	**Mean area value[a] (μV.sec)**	**C.V. (%)**
1	0.0338 ± 0.0014	4.08	10	292.42 ± 8.27	2.83	50	7133.97 ± 226.24	3.17
5	0.3318 ± 0.0146	4.40	25	733.99 ± 28.50	3.88	100	14328.30 ± 139.44	0.97
10	0.7124 ± 0.0209	2.94	50	1484.00 ± 43.19	2.91	200	28224.85 ± 379.53	1.35
15	1.0824 ± 0.0335	3.09	100	2791.00 ± 118.47	4.25	400	51703.67 ± 826.66	1.59
20	1.4798 ± 0.0648	4.38	200	5525.14 ± 276.97	5.01	600	758999.97 ± 814.96	0.94
			250	7154.80 ± 234.52	3.28	800	102839.80 ± 712.71	0.69
			300	8248.25 ± 378.60	4.59	1000	128591.00 ± 724.85	0.56

[a]: Average of ten determinations with standard deviation
CV = Coefficient of variation

Table 3.2: Results of least square regression analysis of data for the estimation of standard solution of flurbiprofen by the proposed methods

Statistical Parameters	UV spectrophotometric method	Spectroflourimetric method	Liquid chromatographic method
Regression equation [a]	Y = 0.0759.X+ (–) 0.0462	Y=27.797.X + 46.049	Y = 126.52.X + 1483.0
Correlation coefficient (r)	0.9999	0.9997	0.9998
Standard error of slope	5.1000×10^{-4}	0.2945	1.1434
Standard error of intercept on ordinate	6.2460×10^{-3}	5.0484	6.4280×10^{2}
Standard error of the estimate	7.7420×10^{-3}	8.37256×10^{1}	1.0195×10^{3}
95% confidence interval of slope	7.4284×10^{-2}, 7.7527×10^{-2}	27.0410, 28.5554	1.2358×10^{2}, 1.2946×10^{2}
95% confidence interval of intercept	-6.6080×10^{-2}, -2.6330×10^{-2}	-83.7240, 175.8200	-1.6941×10^{2}, 3.1353×10^{3}
Slope without intercept	7.4669E-02	2.77970×10^{1}	1.2712×10^{2}

[a]: Based on five calibration values.

Y= Absorbance (UV spectrophotometric method); Fluorescence intensity (Spectroflourimetric method) and Peak area (Liquid chromatographic method).

X= Concentration of the drug in µg/ml (UV spectrophotometric method) and in ng/ml (Spectroflourimetric and liquid chromatographic method).

Table 3.3: One-way ANOVA test for linearity of pure flurbiprofen solution by the proposed methods

Source of variation	Degree of freedom (DF)	Sum of squares (SS)	Mean sum of squares (MS)	F-value	
				F_{Calc}	F_{Crit}
UV spectrophotometric method					
Between Group	4	6.7750×10^{-3}	1.6940×10^{-3}	0.0053	2.8661[a]
Within Group	20	6.4223	3.2112×10^{-1}		
Total	24	6.4291			
Spectroflourimetric method					
Between Groups	4	4.1249×10^{5}	1.0312×10^{5}	0.0097	2.6896[b]
Within Group	30	3.1946×10^{8}	1.0649×10^{8}		
Total	34	3.2199×10^{8}			
Liquid chromatographic method					
Between Groups	4	8.4321×10^{5}	2.1080×10^{5}	0.9999	2.6896[b]
Within Group	30	6.3665×10^{10}	2.1222×10^{9}		
Total	34	6.3666×10^{10}			

[a]: Theoretical value of F (4, 20) based on one-way ANOVA test at p = 0.05 level of significance.
[b]: Theoretical value of F (4, 30) based on one-way ANOVA test at p = 0.05 level of significance.

Table 3.4: Validation report for the determination of flurbiprofen from its standard solutions by the proposed methods

Analytical Parameter	Results		
	UV spectrophotometric method	Spectroflourimetric method	Liquid chromatographic method
Accuracy (%)	100.00 ± 0.3116	100.13 ± 0.4533	100.09 ± 0.4892
Precision (% RSD[a])	0.49	0.45	0.31
Linearity	1-20 µg/ml	10-300 ng/ml	50-1000 ng/ml
Specificity	A 10 µg/ml solution of flurbiprofen in sodium phosphate buffer, pH 6.4 at UV detection λ of 248 nm will show an absorbance of 0.7124±0.0209 at 248 nm	A 100 ng/ml solution of flurbiprofen in methanol: 0.1N H_2SO_4 (1:1) at λ_{ex} of 250 nm and λ_{em} of 314 nm will give a fluorescence intensity of 2791.00 ±118.47	A 500 ng/ml solution of flurbiporfen in methanol:acetonitrile:phosphate buffer (pH 5.6) (2:1:2) using RP-C18 column, at a flow rate of 0.75 ml/min and at UV detection λ of 248 nm will give an area of 64743.06± 356.19
Limit of Detection[b]	0.34 µg/ml	0.99 ng/ml	15 ng/ml
Limit of Quantitation[b]	1.02 µg/ml	3.32 ng/ml	50ng/ml
Ruggedness (%RSD)	99.49 ± 0.03	99.78 ± 0.32	99.56 ± 0.26
Retention time (mins)	-	-	4.3238 ± 0.3796
Asymmetry factor	-	-	1.1153 ± 0.1914

[a]: Relative standard deviation;

[b]: Based on standard deviation of the response and the slope of the regression curve in case of UV and Spectroflourimetric method and signal-to-noise ratio in case of liquid chromatographic method.

Table 3.5: Results of the assay of flurbiprofen and its commercial formulations by the proposed methods

Method/Sample	Label claim (μg/ml)	Recovery		
		Mean [a]	Coefficient of variation (%)	Analytical recovery (%)
UV spectrophotometric method				
Pure drug solution[b]		302.01 ± 1.08	0.80	99.9 ± 0.36
FLUR®	300	305.94 ± 5.04	1.08	99.3 ± 1.68
OCUFLUR®	300	298.74 ± 1.77	0.23	98.9 ± 0.59
Spectroflourimetric method				
Pure drug solution[b]		302.40 ± 2.46	0.81	100.79 ± 0.81
FLUR®	300	307.65 ± 0.30	0.10	102.55 ± 1.10
OCUFLUR®	300	304.71 ± 0.69	0.23	101.57 ± 0.23
Liquid chromatographic method				
Pure drug solution[b]		300.69 ± 3.69	1.22	100.1± 1.23
FLUR®	300	303.09 ± 2.79	0.92	99.9 ± 0.93
OCUFLUR®	300	300.30 ± 0.51	0.17	99.2 ± 0.17

[a]: Mean in μg/ml and S.D. for five triplicate determinations.
[b]: 300 μg/ml

Table 3.6: Two-way ANOVA test (without replication) for linearity in estimation of flurbiprofen in various commercial ophthalmic drops by the proposed methods.

Source of variation	Degree of freedom (DF)	Sum of squares (SS)	Mean sum of squares (MS)	F-value	
				F_{Calc}	F_{Crit}[a]
UV spectrophotmetric method					
Within the brand	2	1.7502	0.8751	0.3802	19.0000[b]
Between the brands	1	8.6400	8.6400	3.7533	18.5128[c]
Error	2	4.6039	2.3020		
Total	5	14.9941			
Spectroflourimetric method					
Within the brand	2	1.5058	0.7529	1.4594	19.0000[b]
Between the brands	1	1.4336	1.4336	2.7789	18.5128[c]
Error	2	1.0318	0.5159		
Total	5	3.9711			
Liquid chromatographic method					
Within the brand	2	0.8264	0.4132	0.8694	19.0000[b]
Between the brands	1	1.3067	1.3067	2.7490	18.5128[c]
Error	2	0.9506	0.4753		
Total	5	3.0837			

[a]: Theoretical value of 'F' based on one-way ANOVA test at p = 0.05 level of significance.
[b]: F (2, 2); [c]: F (1, 2)

Table 3.7: Calibration curve points of the proposed methods in estimation of standard solution of flurbiprofen spiked in serum and aqueous humor

Analysis in serum			Analysis in aqueous humor		
Conc. of the solution (ng/ml)	**Mean area value[a] (μV.sec)**	**Coefficient of variation (%)**	**Conc. of the solution (ng/ml)**	**Mean area value[a] (μV.sec)**	**Coefficient of variation (%)**
50	1025.50 ± 45.56	4.44	50	2960.51 ± 88.50	2.99
100	3012.08 ± 57.48	1.91	100	5910.32 ± 98.80	1.67
200	10210.50 ± 197.68	1.94	200	10536.67 ± 343.56	3.26
500	23325.33 ± 765.98	3.28	500	29604.11 ± 592.30	2.00
1000	51023.33 ± 835.83	1.64	1000	61034.67 ± 724.46	1.18

[a]: Average of ten determinations with standard deviation

Table 3.8: Results of least square regression analysis of data for the estimation of standard solution of flurbiprofen spiked in serum and aqueous humor

Statistical Parameters	**Analysis in serum**	**Analysis in aqueous humor**
Regression equation [a]	Y=52.27.X + (–) 1618.70	Y=61.79.X + (–) 783.24
Correlation coefficient (r)	0.9989	0.9995
Standard error of slope	1.4325	1.0818
Standard error of intercept on ordinate	7.3112×10^{2}	5.5214×10^{2}
Standard error of the estimate	1.1261×10^{3}	8.5043×10^{2}
95% confidence interval of slope	4.7706×10^{1}, 5.6824×10^{1}	5.8347×10^{1}, 6.5232×10^{1}
95% confidence interval of intercept	-3.9455×10^{3}, 7.0805×10^{2}	-2.5404×10^{3}, 9.7391×10^{2}
Slope without intercept	5.15833×10^{1}	6.1459×10^{1}

[a]: Based on five calibration values.
Y= Peak area
X= Concentration of the drug in ng/ml

Table 3.8: Results of least square regression analysis of data for the estimation of standard solution of flurbiprofen spiked in serum and aqueous humor

Statistical Parameters	**Analysis in serum**	**Analysis in aqueous humor**
Regression equation [a]	Y=52.27.X + (–) 1618.70	Y=61.79.X + (–) 783.24
Correlation coefficient (r)	0.9989	0.9995
Standard error of slope	1.4325	1.0818
Standard error of intercept on ordinate	7.3112×10^{2}	5.5214×10^{2}
Standard error of the estimate	1.1261×10^{3}	8.5043×10^{2}
95% confidence interval of slope	4.7706×10^{1}, 5.6824×10^{1}	5.8347×10^{1}, 6.5232×10^{1}
95% confidence interval of intercept	-3.9455×10^{3}, 7.0805×10^{2}	-2.5404×10^{3}, 9.7391×10^{2}
Slope without intercept	5.15833×10^{1}	6.1459×10^{1}

[a]: Based on five calibration values.
Y= Peak area
X= Concentration of the drug in ng/ml

Table 3.9: One-way ANOVA test for linearity of standard solution of flurbiprofen spiked in serum and aqueous humor by the proposed method

Source of variation	Degree of freedom (DF)	Sum of squares (SS)	Mean sum of squares (MS)	F-value	
				F_{Calc}	F_{Crit} [a]
Analysis in serum					
Between Group	5	8.96199E+05	1.79239E+05	0.00042	2.6220
Within Group	24	1.01575E+10	4.23228E+08		
Total	29	1.01584E+10			
Analysis in aqueous humor					
Between Groups	5	1.17743E+06	2.35486E+05	0.00033	2.6220
Within Group	24	1.72159E+10	7.18829E+08		
Total	29	1.72531E+10			

[a]: Theoretical value of F (5, 24) based on one-way ANOVA test at p = 0.05 level of significance.

Table 3.10: Validation report for the determination of standard solution of flurbiprofen spiked in serum and aqueous humor by the proposed method

Analytical Parameters	**Results**	
	Analysis in serum	**Analysis in aqueous humor**
Accuracy (%)	100.47± 0.89	99.83 ± 0.15
Precision (% RSD[a])	1.56	1.43
Linearity	50-1000 ng/ml	50-1000 ng/ml
Specificity	A spiked concentration of 250 ng/ml solution of flurbiprofen in serum using mobile phase of methanol: acetonitrile: phosphate buffer (pH 5.6) (2:1:2) using RP-C18 column, at a flow rate of 1 ml/min and at UV detection λ of 248 nm will give an area of 11450.45 ± 356.07	A spiked concentration of 250 ng/ml solution of flurbiprofen in aqueous humor using mobile phase of methanol: acetonitrile: phosphate buffer (pH 5.6) (2:1:2) using RP-C18 column, at a flow rate of 1 ml/min and at UV detection λ of 248 nm will give an area of 14664.24 ± 265.10
Limit of Quantitation[b]	50	50
Limit of Detection[b]	35	25
Ruggedness (%)	98.96 ± 4.91	99.26 ± 3.44
Retention time (mins)	3.1312 ± 0.0101	3.1312 ± 0.0101
Asymmetry factor	1.1310 ± 0.0091	1.1310 ± 0.0091

[a]: Relative standard deviation

[b]: Based on signal-to-noise ratio in case of liquid chromatographic method.

Table 3.11: Results of the recovery experiments of pure flurbiprofen solution spiked in serum and aqueous humor

Method/Sample	Actual concentration (ng/ml)	Recovery		
		Mean [a]	Coefficient of variation (%)	Analytical recovery (%)
Analysis in serum				
QC-100	100	97.9 ± 4.80	4.90	97.9 ± 4.8
QC-500	500	514.50± 34.00	6.61	102.9 ± 6.8
QC-1000	1000	988.00± 56.00	5.67	98.8 ± 5.6
Analysis in aqueous humor				
QC-100	100	99.9 ± 2.3	2.30	99.9 ± 2.3
QC-500	500	509.50± 17.00	3.33	101.9 ± 3.4
QC-1000	1000	989.00± 15.00	1.52	98.9 ± 1.5

[a] Mean in ng/ml and S.D. for five triplicate determinations.

Table 3.12: Intra-day and inter day variation in the results of analysis of flurbiprofen by the proposed method

Conc. (ng/ml)	Variation (% RSD)			
	Analysis in serum		Analysis in aqueous humor	
	Intra-day[a]	Inter-day[b]	Intra-day[a]	Inter-day[b]
100	5.63	7.17	3.63	7.50
200	4.56	6.69	4.68	7.60
500	5.32	6.46	7.68	10.31
1000	2.03	3.52	1.89	2.61

[a]: Based on five duplicate determinations per day.
[b]: Based on five duplicate determinations for five days.

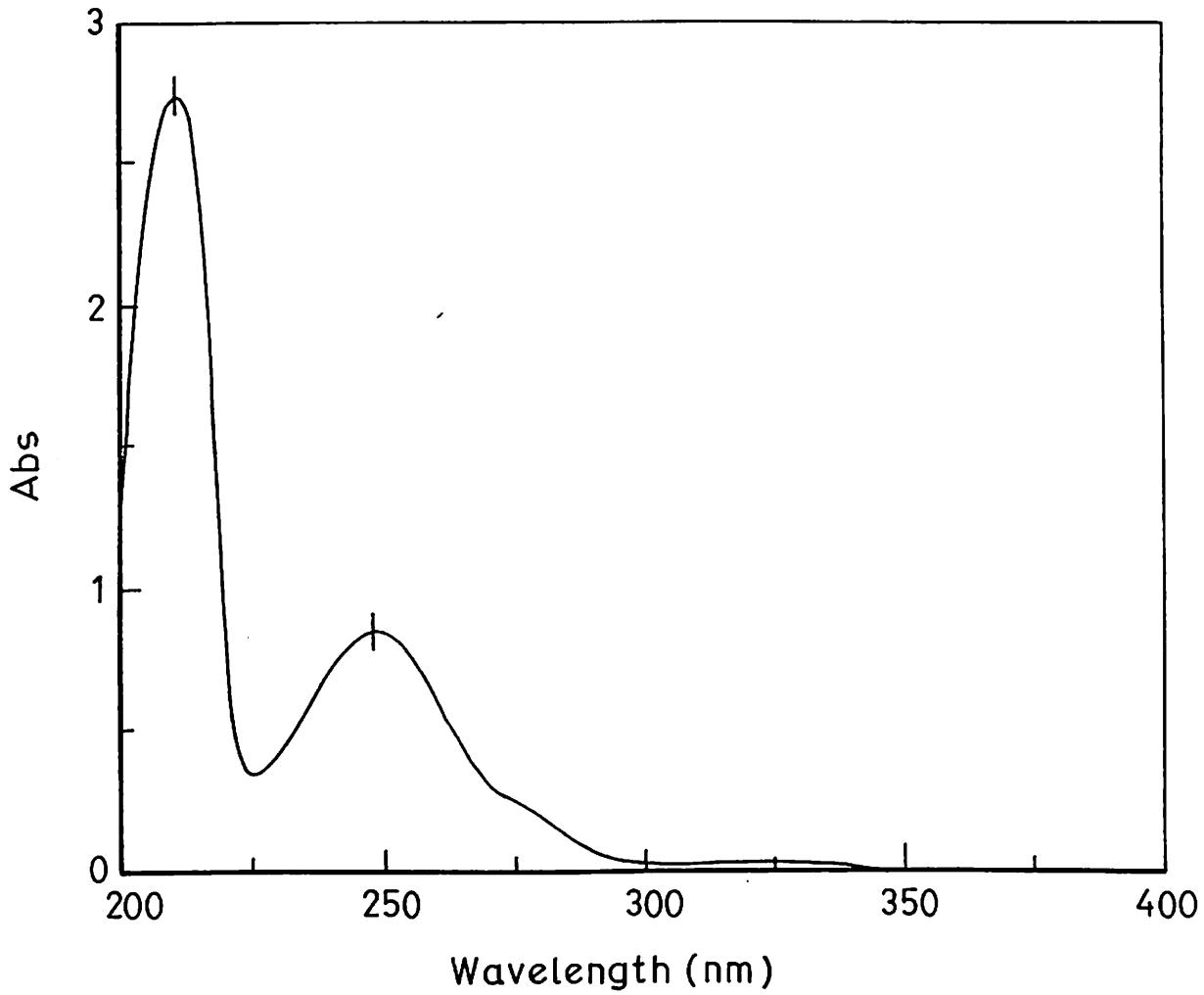

Figure 3.1: UV spectrum of flurbiprofen in sodium phosphate buffer (pH 6.4), λ_{max} -248 nm

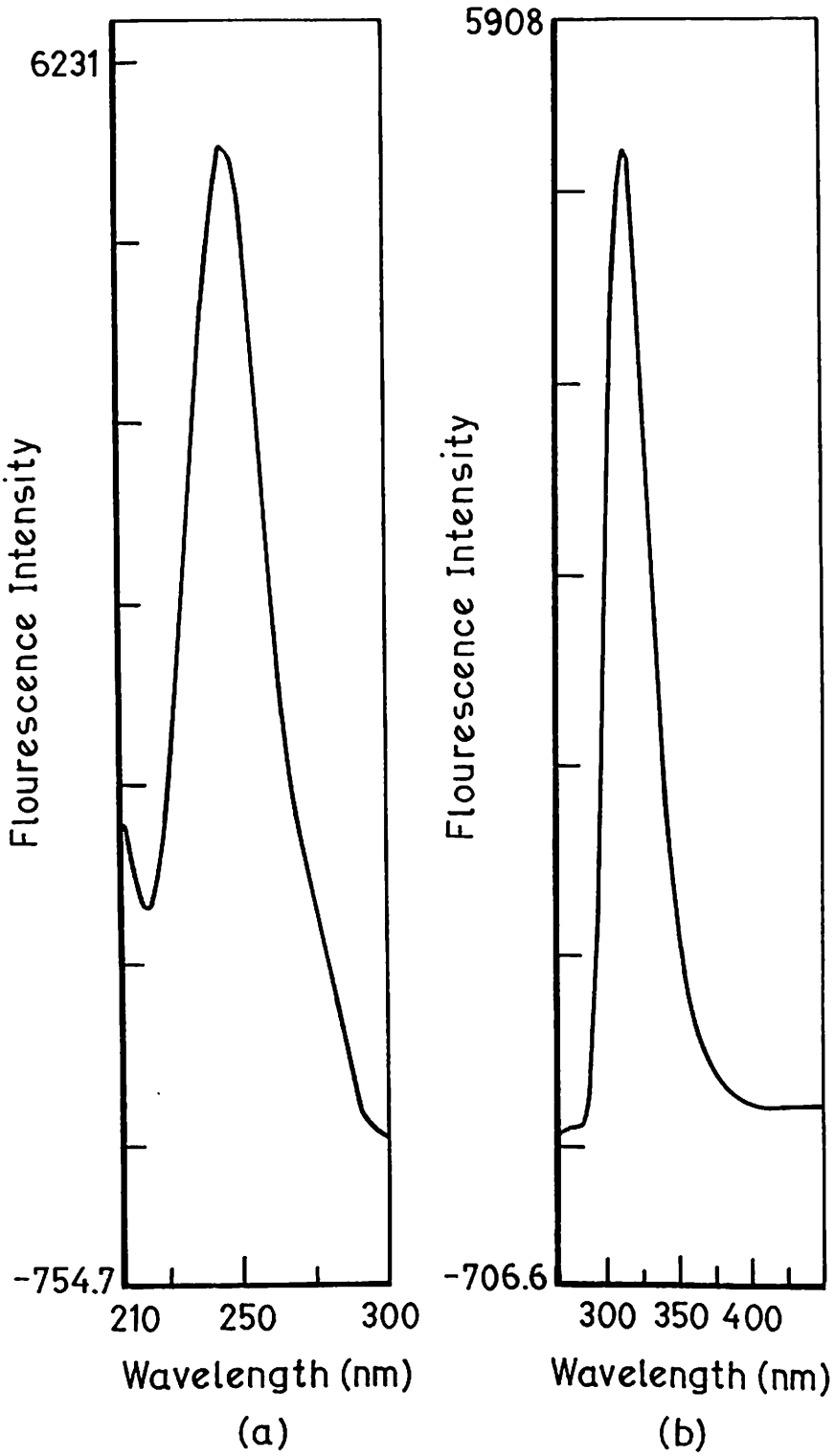

Figure 3.2: Excitation spectrum (a) and emission spectrum (b) of flurbiprofen in 1:1 mixture of methanol and 0.1N sulphuric acid. λ_{ex} - 250 nm and λ_{em} - 314.

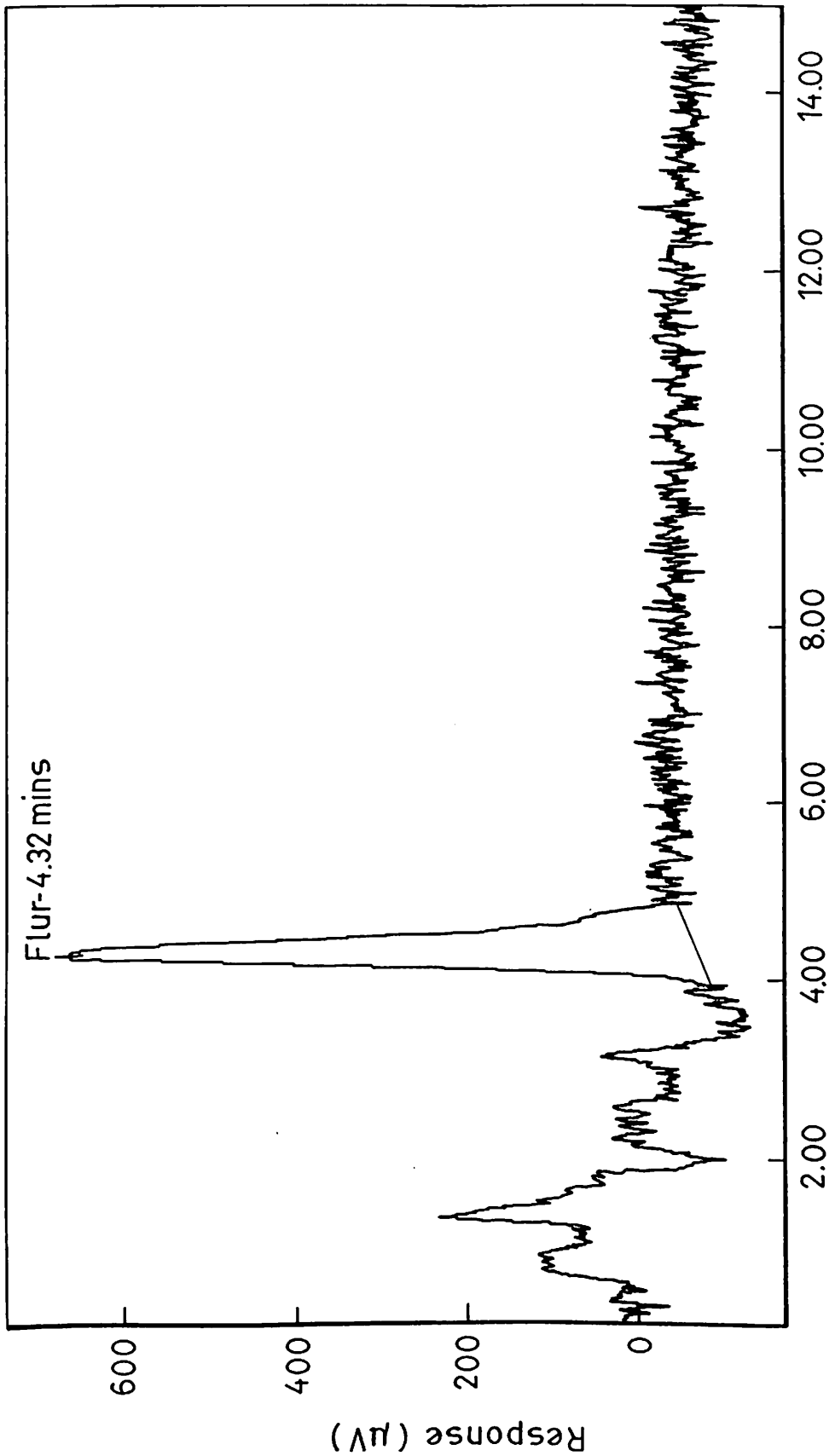

Figure 3.3: A reverse phase liquid chromatogram of pure flurbiprofen at LOQ in methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 248 nm, flow rate 0.75 ml/min on RP-C18 LiChroCART® Purospher® column.

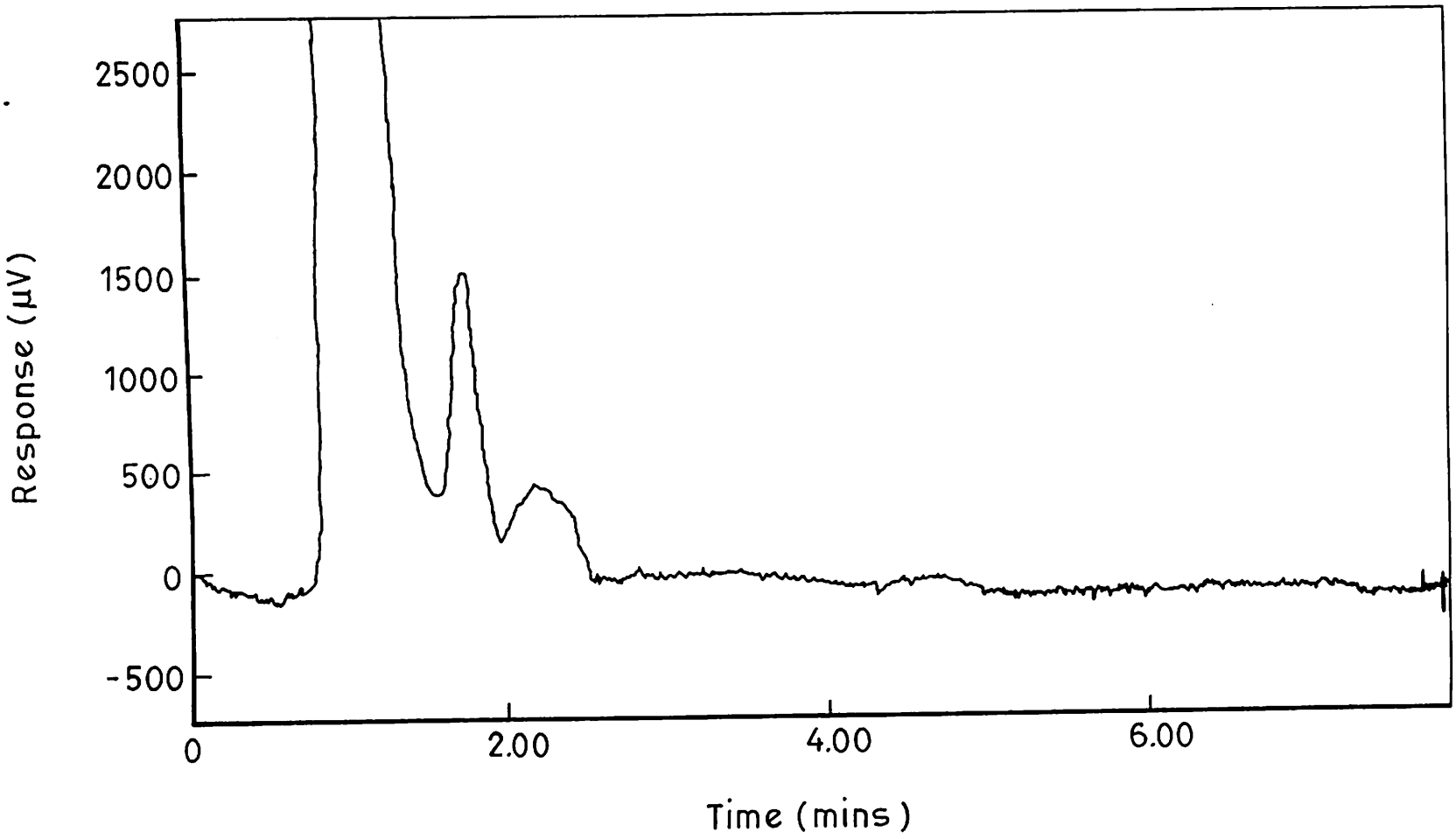

Figure 3.4a: A typical chromatogram of blank serum in methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 248 nm, flow rate 1.0 ml/min on RP-C18 LiChroCART® Purospher® column.

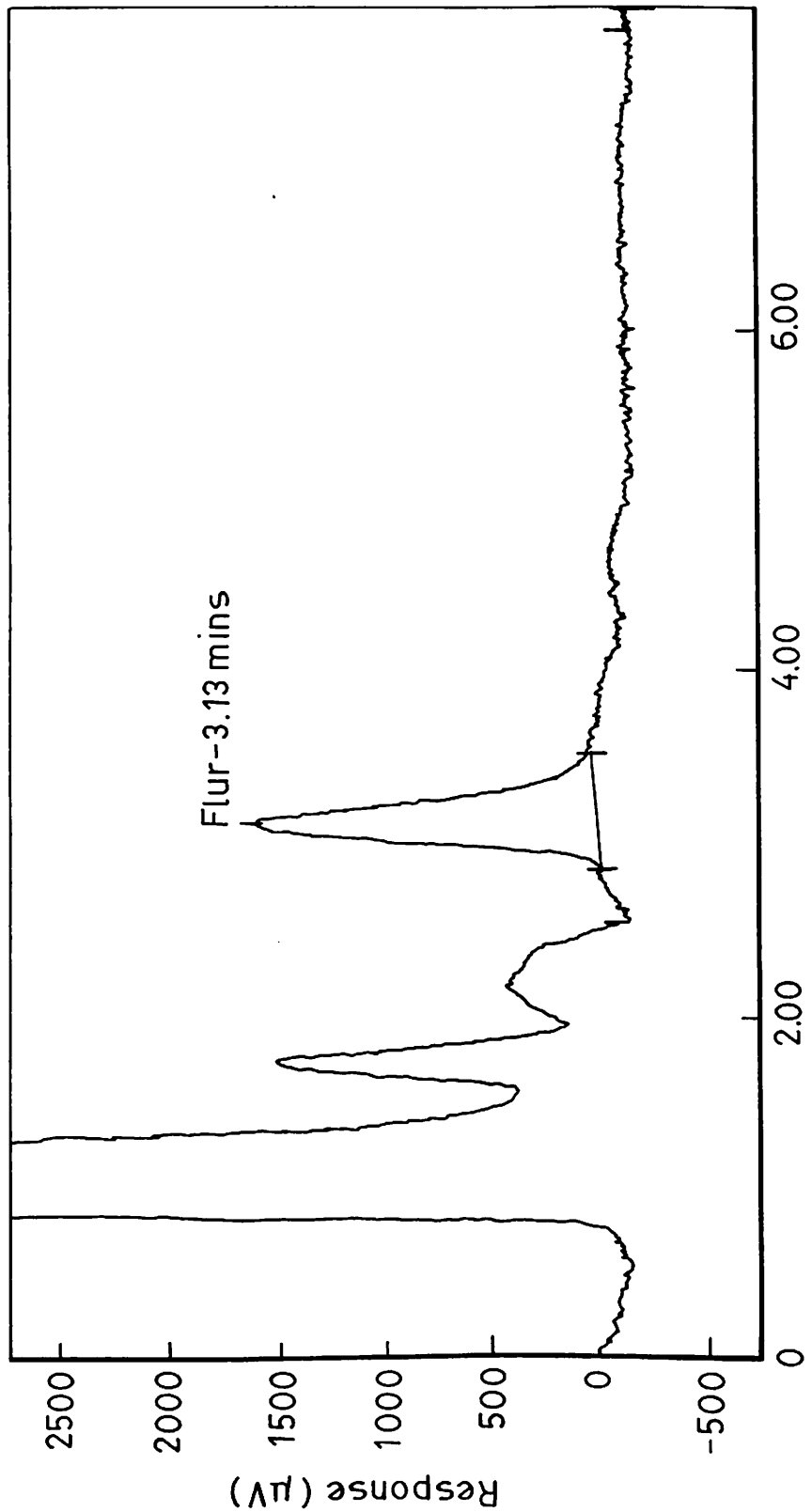

Figure 3.4b: A typical chromatogram of flurbiprofen spiked in serum blank at LOQ in methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 248 nm, flow rate 1.0 ml/min on RP-C18 LiChroCART® Purospher® column.

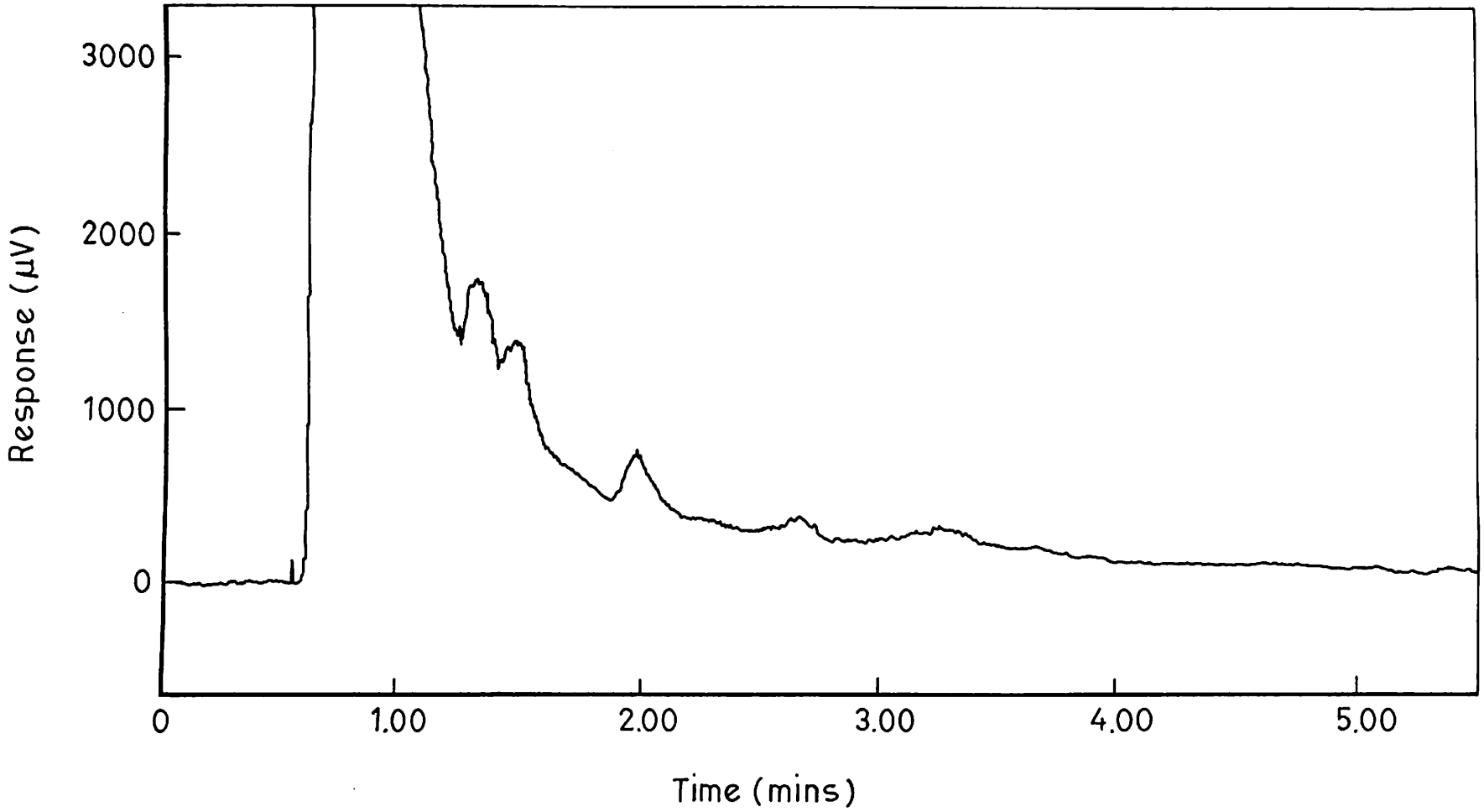

Figure 3.5a: A typical chromatogram of blank aqueous humor in methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 248 nm, flow rate 1.0 ml/min on RP-C18 LiChroCART® Purospher® column.

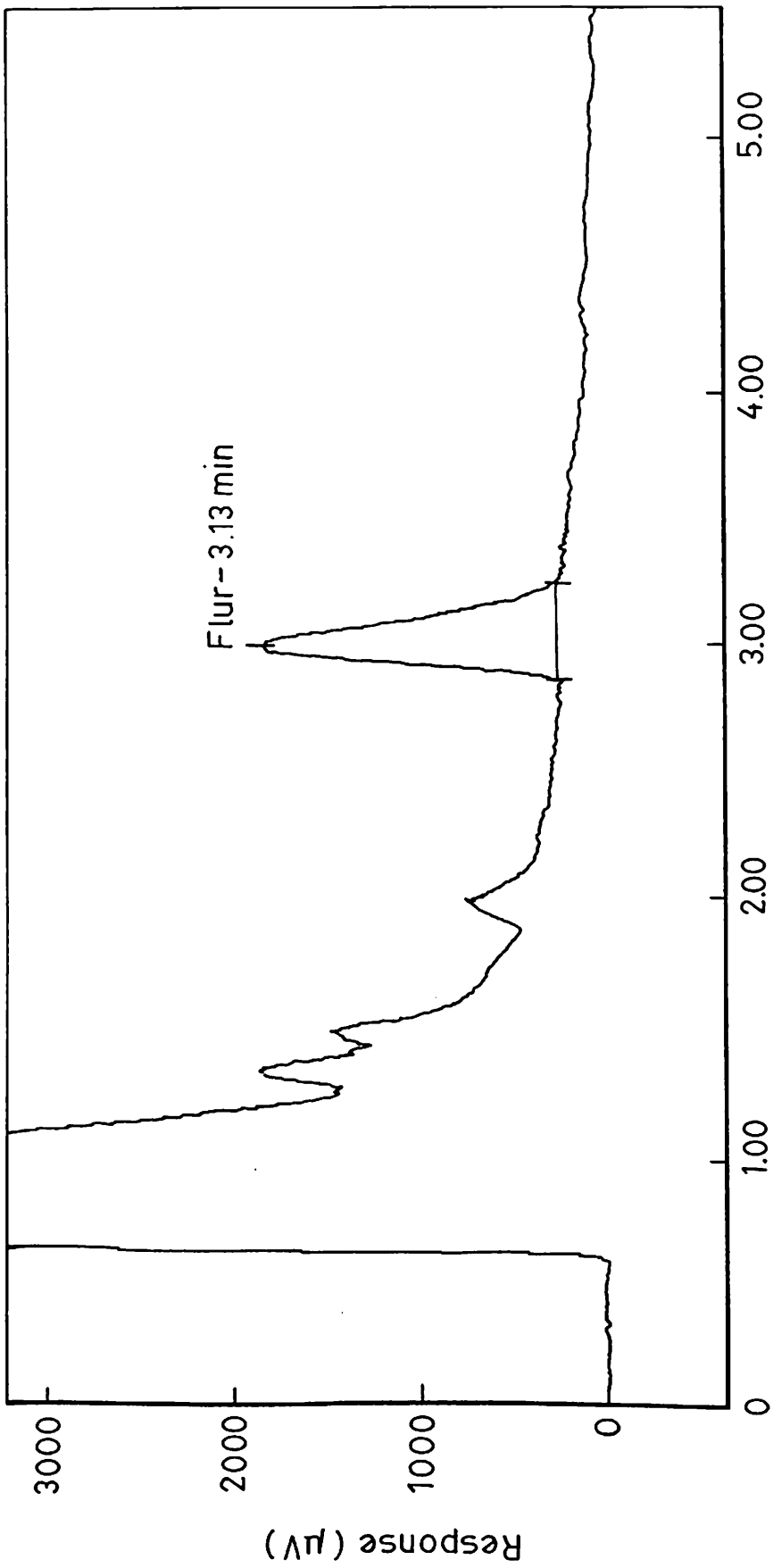

Figure 3.5b: A typical chromatogram of flurbiprofen spiked in aqueous humor blank at LOQ in methanol-acetonitrile-phosphate buffer (pH 5.6) (40:20:40) at 248 nm, flow rate 1.0 ml/min on RP-C18 LiChroCART® Purospher® column.

CHAPTER 4

PREFORMULATION STUDY

4. PREFORMULATION STUDY

4.1 Introduction

Prior to any formulation development program the formulators focus mainly hinges around evaluating those physico-chemical properties of the drug and its stability in solid form or in liquid vehicle under various conditions of temperature, humidity and other physico-chemical environment that could affect the drug performance and development of an efficacious dosage form. The variability in the bioavailability of the drug in most of the case can be traced to formulation consideration (Ravin and Radebaugh, 1990). A thorough understanding of these properties or factors may provide a rationale for formulation design whether it is a simple solution or a complex drug delivery system. The stability and preformulation studies in most of the cases confirm the absence or presence of any limitations or barriers to the development of a optimally bioavailable and stable formulation of the drug entity (Fiese and Hagen, 1987; Ravin and Radebaugh, 1990). To develop a stable dosage form for a drug entity, it is very essential to carryout stability studies in pure form, in physical mixture and in solution or suspension form under various conditions of light, humidity and temperature before any formulation development work (Connors, et al., 1986). Flurbiprofen being a well-established drug, with several ophthalmic formulations available in the Indian and international drug market, a good amount of information is available on its formulations, stability profile and degradation products (Beaulieu et al, 1991; Mathew and Gupta, 1993). Focus in the present study was on effect of different additives on stability, kinetics and predictive studies for establishing credible expiration dates for pharmaceutical products as is now accepted worldwide (Willig and Stoker, 1997). Such stability studies requirements and expiration dating are covered in the Good Manufacturing Practices guidelines (US-FDA, 1987; ICH, 1994; USP 2000).

Many factors affect the stability of a pharmaceutical product, including the stability of the active ingredient, the potential interaction between active and excipients, the manufacturing process, the decision of the dosage form, the selection of container closure-liner system and the environmental conditions encountered during shipment, storage, handling and shelf life (Vadas, 1990). There are various physico-chemical properties of a drug that can influence dosage form design, choice of additives and bioavailability of the drug from the dosage form. Some of the physico-chemical properties of the drug that have a bearing on the formulation manufacturing and the optimum ocular biovailability of the drug from the ophthalmic formulations include

solubility, log P and pK_a value. Knowledge of solubility in different media and the drug stability in those media not only aids in appropriate solvent selection for the development of ocular drops but also in the development of media for dissolution testing. Log P and pK_a are two important physico-chemical parameters extensively used by various workers in predicting the bioavailability from ocular formulations. (Mosher and Mikkelson, 1979; Kishida and Otori, 1980; Schoenwald and Ward, 1981; Huang et al, 1983; Schoenwald and Huang, 1983; Eller et al, 1985; Chein et al, 1991; Schoenwald, 1993).

Therefore, it was considered important to study the stability of the selected drug under various conditions in the presence or absence of prospective formulation additives and some of the preformulation physico-chemical evaluation of flurbiprofen relevant to ocular formulation development work envisaged.

In the present chapter results of various preformulation studies of flurbiprofen are presented. Stability of the drug was studied *per se* and in the presence of common formulation and manufacturing additives in both solid state and in aqueous solutions. The effect of buffered/ unbuffered pH and various buffering agent on the stability of flurbiprofen was also studied. The stability studies were carried out in various temperature and humidity conditions in sealed ampoules provided with inert nitrogen environment. Also investigated were the effect of different buffer type, pH of the buffered and unbuffered solvents on the solubility of flurbiprofen. Solubility of the drug in various organic solvents was also investigated. Partition coefficient and log P value of flurbiprofen in n-octanol-water system was determined and the effect of buffered and unbuffered pH of the aqueous phase on log P value studied.

4.2 Experimental

Chemicals

Flurbiprofen was obtained as a gift sample from Optho Remedies Pvt. Ltd., Allahabad, India. All other polymers, oils, excipients or chemicals used in the studies were either pharmaceutical grade or analytical grade and were used as received. High quality triple distilled water (TDW) was prepared using glass triple distillation unit.

Equipments

A UV-visible-NIR spectrophotometer (Jasco, Tokyo, Japan, model V-570) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells with Jasco spectra manager software was used for all absorbance measurements for UV analysis. Jasco Infrared spectrophotometer, model- IR Report 100, was used for obtaining the IR spectrum of the drug. For carrying out stability studies MAC model temperature and humidity control chambers were used. For studies at higher temperature of 60 °C and 90 °C MAC model thermostatic ovens were used. Studies at refrigerated conditions were performed in frost-free 200-litre Godrej refrigerator. These instruments were equipped with thermostatic temperature control unit, digital temperature recorder and relative humidity (wherever applicable) recorder. All pH measurements were performed using Elico pH meter equipped with combination glass electrode filled with potassium chloride gel and auto temperature adjustment electrode.

Analytical method

An aliquot volume of the sample, collected at different time interval, was transferred to a series of 10 ml volumetric flasks (three in each case) and final volume was made using sodium phosphate buffer (pH 6.4) prepared as described under UV spectrophotometric method of analysis in chapter 3. The resulting solutions in both the cases were filtered through Whatman filter paper no.1 and suitably diluted to get final concentration within the limits of linearity of the proposed UV method. The residual drug content of different test samples of flurbiprofen was calculated, on an average concentration basis, from the absorbance value for liquid samples and on average weight basis for solid samples.

Characterization of bulk drug

The obtained flurbiprofen in bulk were characterized by various official tests (IP, 1996; BP, 1998; USP, 2000) of identification. The IR spectrum obtained using IR spectrophotometer was compared with that of the standard.

Stability studies in solid admixture

Physical mixture of flurbiprofen (# 400 mesh) and excipients (# 100 mesh) were prepared in the ratio 1:5. The excipients that were used for the study include: starch, magnesium stearate, ethyl cellulose, polyvinyl alcohol, polyacrylamide, carboxy methyl cellulose, sodium carboxy methyl

cellulose, hydroxy propyl methyl cellulose, methyl cellulose, polyvinyl pyrolidone, polycarbophil 934, sodium alginate. Drug in combination with common ophthalmic preservatives like EDTA, phenyl mercuric nitrate, benzalknoium chloride and methyl and propyl paraben were also prepared by above method. Drug and excipients were thoroughly blended and the mix was passed through # 100 mesh to ensure uniform blending. Drug alone (used as control) or its mixture was filled and were sealed in 2 ml amber colored, neutral glass ampoules and kept at different temperature conditions. For studies at 40 °C and 75 % relative humidity (RH) the mixture were kept in open vials.

The samples thus prepared were stored at ambient as well as accelerated conditions followed by visual examination on the day of drug content estimation at different interval of time. The storage conditions used for the studies were controlled room temperature (CRT: 25 ± 3 °C and 55 ± 15 % RH), 40 °C, 60 °C, 90 °C and 40 °C/ 75 % RH and refrigerated condition (FT: 4 ± 3 °C). The samples in triplicate were withdrawn at predetermined time intervals (0, 30, 60, 90 days) and analyzed after suitable dilution for flurbiprofen content and the observed degradation rate constants at different storage conditions are listed in Table 4.1 and the predicted stability parameters are presented in Table 4.2. In an effort to establish the effect of humidity on the stability characteristics of the drug with excipients the degradation rate constants (K_{deg}) obtained at 40 °C were compared with that obtained at 40 °C/ 75 % RH and the results are presented in Table 4.3.

Stability studies in solution form

Physical incompatibility studies for the development of opalescence at CRT were carried out in various buffers (0.01M), prepared as per the composition given in Table 4.4 (Gomori, 1955), namely, citrate (pH- 6.6), phosphate (pH-5.6, 6.6, 7.0, 7.4, 7.8), citrate-phosphate (pH-7.0) and borate (pH- 7.5). Physical compatibility of the drug was also studied in various edible oils namely, cotton seed oil, olive oil, arachis oil, castor oil, sesame oil, and linseed oil. For physical compatibility studies with various buffers and edible oils the concentration of the drug used was 750 µg/ml. A solution of the drug in glass triple distilled water served as control for these studies. The above prepared samples were subjected to visual examination for the development of opalescence against a black/white background at 0, 0.5, 1, 2, 4, 8, 12, 24, 72 hours post preparation. A positive (+) score was given to formulations developing opalescence and a

negative (–) score if it was clear and free from opalescence. A score of '–/ +' was given if the solution showed mild precipitation without the generation of any opalescence. Table 4.5 represents the results of the opalescence studies.

To study the effect of various buffers on the stability of flurbiprofen, drug solutions (750 μg/ml) in different buffers (0.01M) were prepared as per the composition given in Table 4.4. To study the effect of pH of phosphate buffer on the stability of flurbiprofen, 750 μg/ml solution of the drug was prepared in pH 5.6, 6.6, 7.0, 7.4 and 7.8. Stability studies were also carried out in various edible oils namely, cottonseed, olive, arachis, castor, sesame and linseed at drug concentration of 750 μg/ml. To study the effect of pH of unbuffered solution on stability of flurbiprofen was studied at pH 1.2, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5. The pH was adjusted using varying proportion 0.1 NaOH and 0.1N HCl. A solution of the drug in glass triple distilled water served as control in all these studies.

The prepared test samples were sealed in 2 ml amber colored neutral glass ampoules and each formulation were exposed to various storage conditions. The samples in triplicate were withdrawn at predetermined time intervals (0, 7, 14, 21, 28, 60, 90 days) and analyzed after suitable dilution for flurbiprofen content. The storage conditions used for the studies were CRT (25 ± 3 °C and 55 ± 20 % RH), 40 °C, 60 °C, 90 °C and 40 °C/ 75 % RH and refrigerated condition (FT, 4 ± 3 °C). The degradation rate constants (K_{deg}) determined at different storage conditions are presented in Table 4.6 for flurbiprofen in different buffers including phosphate buffer of varying pH, in Table 4.7 in unbuffered solutions of varying pH and in Table 4.8 in different vegetable oil. The corresponding predicted stability parameters are presented in Table 4.9 for flurbiprofen in different buffers including phosphate buffer of different pH, in Table 4.10 in unbuffered solution and in Table 4.11 in different vegetable oils. The results of effect of humidity (studied as per the procedure given in the previous section) on the stability of the prepared test solution are presented in Table 4.12.

Data analysis for stability studies

Based on the results of preliminary stability studies involving flurbiprofen it was concluded that flurbiprofen in solid form and in liquid solution follow a first order degradation pattern. From the slope of the plot between log % drug content remaining and time (in days) the degradation rate constant (K_{deg}) was calculated as $K_{deg} = -2.303 \times$ Slope and used for obtaining the Arrhenius

plot (log K_{deg} vs. reciprocal of absolute temperature, in Kelvin). From the Arrhenius plot the degradation rate constant at 30 °C (K_{30}) was computed and used for the calculation of shelf life (t_{90}, considering 90% as the minimum potency at the end of shelf life). The predicted K_{30} and the corresponding t_{90} values of various test samples are presented in Table 4.2 for drug excipients stability in solid form, in Table 4.9 for stability in different buffers including phosphate buffer of different pH, in Table 4.10 for stability in different unbuffered solutions of varying pH and in different vegetable oils in Table 4.11.

Solubility studies

To study the effect of various buffers on the solubility of flurbiprofen (750 µg/ml) (Table 4.13), drug solutions in different buffers (0.01M) were prepared as per the composition given in Table 4.4 (Gomori, 1955). To study the effect of pH of phosphate buffer on the solubility of flurbiprofen, solubility of the drug was determined at 37 ± 1 °C in pH 5.6, 6.6, 7.0, 7.6 and 8.0 with ionic strength adjusted to 0.2 using sodium chloride (Table 4.14). To study the effect of pH of unbuffered solution on solubility of flurbiprofen was studied at pH 2.2, 3.2, 4.0, 5.7, 6.5, 7.0, 7.5, 8.2, 9.1, 10.4 and 12.2 (Table 4.15). The pH was adjusted using varying proportion 0.1N NaOH and 0.1N HCl and the ionic strength was adjusted to 0.2 with sodium chloride. Solubility studies were also carried out in various edible oils namely, cottonseed, olive, arachis, castor, sesame and linseed oils (Table 4.16). The organic solvents studied for the solubility of the drug include: 1,4- dioxane, acetone, acetonitrile, benzene, benzyl alcohol, chloroform, cyclo hexane, diethyl ether, dimethyl formamide, ethyl acetate, ethylalcohol, ethylene glycol, isoamyl alcohol, isobenzyl alcohol, methyl alcohol, propyl alcohol, tetrahydrofuran, toluene, triethyl amine and xylene (Table 4.17). The solubility studies in organic solvents were carried out for 48 hours at CRT (25 ± 3 °C & 55± 15 % RH), in order to minimize to loss of the solvent due to evaporation.

Log P determination

Log P (lyphophilicity) of flurbiprofen was quantified by measuring partition coefficient of the drug in n-octanol/ triple distilled water, n-octanol/phosphate buffer (pH 5.6, 6.6, 7.6) and n-octanol/ triple distilled water (pH 5.5, 6.5, 7.5 where, pH was adjusted with 0.1NaOH and/ or 0.1 N HCl) (Table 4.18). Flurbiprofen was dissolved in triple distilled water or pH adjusted triple distilled water and alternatively in phosphate buffer (pH 5.6, 6.6, 7.6) previously saturated with n-octanol to get saturated solution. To 50 ml conical flasks (in triplicate) 10 ml of flurbiprofen

solution in aqueous phase and 10 ml of n-octanol (previously saturated with the aqueous phase) was added and placed on water bath shaker maintained at 37 ± 1 °C. At the end of 24 hours contents of the flasks were transferred to the centrifuge tubes and centrifuged for 10 mins at 4000 rpm. Clear aqueous supernatant was analyzed by the UV spectrophotometeric method. Partition coefficient was determined as $[C_{aq(initial)} - C_{aq\ (final)}]/ C_{aq\ (final)}$; where, C_{aq} is the concentration of the drug in aqueous phase. And log P was calculated as logarithm to base 10 of partition coefficient.

4.3 Results and discussion

Characterization of bulk drug

Supplied flurbiprofen passed the various tests of identification and analysis as per IP, 1996; BP, 1998; USP, 2000 and the IR spectrum of the sample was found comparable with that of the standard. The excipients, chemicals or reagents used in the studies did not affect UV absorbency profile of DFS.

Stability studies in solid admixture

The first order degradation rate constant (Table 4.1) for flurbiprofen in solid state in the absence of any excipient was found to be 0.65×10^{-4} day^{-1} at CRT, 2.67×10^{-4} day^{-1} at 40 °C, 5.88×10^{-4} day^{-1} at 60 °C, 10.96×10^{-4} day^{-1} at 90 °C, 3.34×10^{-4} day^{-1} at 40°C/ 75% RH and 0.62×10^{-4} day^{-1} at FT. The observed degradation rate constants in the presence of various excipients selected for the study varied from 0.67×10^{-4} day^{-1} to 1.33×10^{-4} day^{-1}at CRT, 2.12×10^{-4} day^{-1} to 3.61×10^{-4} day^{-1} at 40 °C, 4.87×10^{-4} day^{-1} to 7.94×10^{-4} day^{-1} at 60 °C, 9.25×10^{-4} day^{-1} to 14.79×10^{-4} day^{-1} at 90 °C, 2.81×10^{-4} day^{-1} to 3.98×10^{-4} day^{-1} at 40°C/ 75% RH and 0.35×10^{-4} day^{-1} to 1.17×10^{-4} day^{-1} at FT. The corresponding values for individual drug-excipient combinations are presented in Table 4.1.

The shelf life of flurbiprofen based on observed degradation rate constant at CRT (K_{CRT}) and predicted degradation rate constant at 30 °C (K_{30}) was found to be 4.43 years and 2.70 years respectively (Table 4.2). On analysis of the predicted K_{30} values for various drug-excipient combinations, sodium alginate was found to have the most deleterious effect with 37 %

reduction in the shelf life while methylcellulose and hydroxy propyl methylcellulose did not show any deleterious effect. Among the preservatives, EDTA showed maximum deleterious effect with 40 % reduction in the shelf life of flurbiprofen and methyl and propyl paraben had no effect (Table 4.3). The excipients studied could be classified broadly into two categories namely, excipients with moderate instability and excipients with insignificant instability. Polyvinyl pyrolidone, sodium alginate, EDTA, benzalkonium chloride, and phenyl mercuric nitrate falling in the first category and all the other excipients studied coming under the second category. The above effects, which have been observed in solid state, may be due to the localized high concentration of the excipients, which may not be true for drug stability in liquid dosage form.

In an effort to establish the effect of humidity on the stability characteristics of the drug with excipients, the degradation rate constants obtained at 40°C/ 75% RH were compared with that obtained at 40 °C and the results are presented in Table 4.3. It was observed that all the excipients except benzalkonium chloride, magnesium stearate, polyvinyl alcohol, polyacrylamide and ethyl cellulose showed an increased adverse effect on the shelf life of flurbiprofen. Since most the excipients studied are polymeric in nature and have got the tendency to pick moisture, which may be the reason for increased degradation effect. The maximum protective effect of magnesium stearate at higher relative humidity is probably due to its hydrophobic nature.

No significant effect was observed on the UV absorbance profile of the drug, like shift in absorbance peak and relative absorbance, in the presence of excipients. Also there was no significant change in the physical description of the drug in the presence of the excipient during the period of study under different conditions.

Stability studies in solution form

Flurbiprofen was found to be compatible with all the buffers except citrate buffer pH 6.6 and citrate-phosphate buffer pH 7.0 (Table 4.5) in which case significant degree of opalescence was seen from 24 hours onwards when compared to control solution, which showed mild precipitation at 72 hours. However, in phosphate buffers in the pH range of 5.6 to 7.8 no incompatibility problem was observed even beyond 72 hours and up to several days.

Flurbiprofen solution (750 μg/ml) in triple distilled water was found to follow first order degradation rate constant as can be seen from the linear plot between log percentage drug content remaining and time (in days) at different temperature conditions (Figure 4.1). Similar degradation profiles were observed for the drug in citrate buffer pH 6.6 (Figure 4.2), phosphate buffer pH 7.4 (Figure 4.3), citrate-phosphate buffer pH 7.0 (Figure 4.4) and borate buffer pH 7.5 (Figure 4.5). The first order degradation rate constant of flurbiprofen in triple distilled water was found to be 3.65×10^{-4} day^{-1} at CRT, 9.50×10^{-4} day^{-1} at 40 °C, 21.00×10^{-4} day^{-1} at 60 °C, 36.98×10^{-4} day^{-1} at 90 °C, 10.93×10^{-4} day^{-1} at 40°C/ 75% RH and 1.83×10^{-4} day^{-1} at FT as given in Table 4.6. Similarly, the predicted first order degradation rate constant at 30 °C was found to be 5.10×10^{-4} day^{-1} (Table 4.9). The corresponding predicted shelf life (t_{90}) for the drug at 30 °C (using predicted K_{30}) and on the basis of observed K_{CRT} was obtained as 6.77 and 9.46 months respectively.

Arrhenius plot for degradation of flurbiprofen (750 μg/ml) in different buffers (0.01M) (Figure 4.6), in phosphate buffer of different pH (0.01M) (Figure 4.7), in different unbuffered pH solutions (Figure 4.8) and in different vegetable oils (Figure 4.9) showed a linear relationship. The predicted K_{30} along with the predicted shelf life at 30 °C from the above plots are presented in Table 4.9, 4.10 and 4.11. Also presented in these tables are the shelf life calculated on the basis of observed K_{CRT}.

The first order degradation rate constants in different buffers (citrate buffer pH 6.6, phosphate buffer pH 7.4, citrate-phosphate buffer pH 7.0 and borate buffer pH 7.5) obtained under different temperature conditions are listed in Table 4.6 and the observed shelf life at CRT and predicted shelf life at 30 °C are given in Table 4.9. Among different buffers, the drug was found to be most stable in phosphate buffer (pH 7.4) with a degradation rate constant (K_{CRT}) of 2.56×10^{-4} day^{-1} and least stable in borate buffer (pH 7.5) with K_{CRT} of 4.22×10^{-4} day^{-1}. The K_{CRT} was obtained as 2.89×10^{-4} day^{-1} and 3.55×10^{-4} day^{-1} in citrate buffer (pH 6.6) and in citrate-phosphate buffer (pH 7.0) respectively. A similar trend was observed in these buffers at accelerated stability study conditions of 40 °C, 60 °C, 90 °C and 40 °C/ 75 % RH, and refrigerated conditions (Table 4.6). The predicted shelf life at 30 °C in different buffers was found to be 8.73, 7.02, 5.11 and 4.26 months in citrate buffer (pH 6.6), phosphate buffer (pH 7.4), citrate-phosphate buffer (pH 7.0) and borate buffer (pH 7.5) respectively (Table 4.9). A comparison of corresponding shelf

life calculated for flurbiprofen at 30 °C and CRT in different buffers is also presented in Table 4.9.

Flurbiprofen showed a pH dependent stability in 0.01 M phosphate buffer of varying pH with the value of K_{deg} decreasing with increase in pH from 5.6 to 7.8 as shown in Figure 4.10. The first order degradation rate constants in different pH phosphate buffer obtained under different temperature conditions are listed in Table 4.6 and the observed shelf life at CRT and predicted shelf life at 30 °C are given in Table 4.9. The observed first order degradation rate constant at CRT was 2.83×10^{-4} day^{-1} at pH 5.6, 2.72×10^{-4} day^{-1} at pH 6.6, 2.65×10^{-4} day^{-1} at pH 7.0, 2.56×10^{-4} day^{-1} at pH 7.4 and 2.34×10^{-4} day^{-1} at pH 7.8. A similar trend was observed at these pH conditions at accelerated conditions of 40 °C, 60 °C, 90 °C and 40 °C/ 75 % RH, and refrigerated conditions (Table 4.6). The predicted shelf life (as shown in Table 4.9) at 30 °C in different pH phosphate buffer was found to be 5.98, 6.38, 6.68, 7.02 and 7.82 months at pH 5.6, 6.6, 7.0, 7.4 and 7.8 respectively. For comparison, the corresponding shelf life calculated for flurbiprofen at CRT in different pH phosphate buffer is presented in Table 4.9.

In case of stability studies in unbuffered solutions of varying pH the rate of degradation was found to be higher than the corresponding buffered pH (phosphate buffer) as is evident from the data presented in Table 4.7. A comparative profile between degradation rate constant of flurbiprofen (750 μg/ml) at different storage conditions and pH of the unbuffered solution is shown in Figure 4.11. When compared to the degradation rate constants obtained for flurbiprofen control solution in triple distilled water (pH 6.65) the drug in extreme acidic pH of 1.2 showed faster degradation with a predicted shelf life at 30 °C of 2.64 months (Table 4.10), with about 61 % reduction in the shelf life compared to the control in triple distilled water. The corresponding first order degradation rate constant obtained for flurbiprofen in pH 1.2, 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5 at different temperature conditions are presented in Table 4.7. The shelf life increased with increase in pH. A comparison of corresponding shelf life calculated for flurbiprofen at 30 °C and CRT in different pH unbuffered solutions is are presented in Table 4.10. Among unbuffered solution of pH range 4.5 to 9.5, flurbiprofen was found to be least stable at pH 4.5, around the pK_a value of 4.27 (Craig, 1990) of the drug, with a shelf life of 3.19 months and 6.13 months at 30 °C and CRT respectively (Table 4.10). And the drug was found to be most stable at pH 9.5 with a shelf life of 6.83 months and 13.48 months at 30 °C and CRT respectively (Table 4.10).

Mostly eye drops are buffered at pH 7.4, considered the physiological pH of the tear fluid, and thus the pH range investigated for drug stability was weakly acidic to weakly basic. In ophthalmic formulations there exists a limitation to the use of wide range of pH because of two reasons. Firstly eye is intolerant to a wide range of pH as extreme pH in acidic or basic range result in increased rate of blinking, lacrimation and corneal damage. Secondly some drugs are stable at specific pH that is different from the pH of optimal corneal permeability. The tears have some buffer capacity, which can neutralize the pH of the instilled solution if the solution does not have strong resistance to neutralization. Thus, buffers were formulated at low equimolar concentration of 0.01M so that marginal change in tear pH post instillation can be brought back to normal physiological pH. Flurbiprofen was found to be incompatible with citrate and citrate-phosphate buffer producing opalescence but compatible with phosphate and borate buffer. Since phosphate buffer provides an option of wide range of pH from 5.6 to 7.8, and also the degradation rate constant of the drug was minimum it was preferred for further investigations.

When the pH of the phosphate buffered solution increased from 5.6 to 7.8 the Na_2HPO_4/ NaH_2PO_4 ratio gradually increases and the shelf life of flurbiprofen gradually decreases. This may be probably due to increased concentration of HPO_4^{2-} (which is the predominant buffer species at higher pH of phosphate buffer) and thus exerting a protective effect on drug stability. At lower pH the predominant buffer species is $H_2PO_4^-$ and this being more acidic than HPO_4^{2-} may contribute to increased rate of degradation of flurbiprofen. Low shelf life at pH 1.2 can again be attributed to the role of acid catalysis by hydrochloride ions at that pH as there were very little sodium hydroxide ions used for pH adjustment at 1.2 pH. As seen in case of buffered solutions with increase in pH of the unbuffered solutions there is a marked increase in stability of the drug. Which once again reinforces the acid catalysis as the mechanism of degradation of flurbiprofen. But to attribute acid catalysis as the mechanism of degradation of flurbiprofen conclusively further studies under very controlled conditions of temperature, humidity, ionic concentration and buffer strength is required.

Flurbiprofen was found to follow first order degradation profile in the selected vegetable oils. The first order degradation rate constants in different vegetable oils obtained under different temperature conditions are listed in Table 4.8 and the observed shelf life at CRT and predicted

shelf life at 30 °C are given in Table 4.11. Among different vegetable oils studied, at CRT the drug was found to be most stable in sesame oil with a degradation rate constant of 0.75×10^{-4} day^{-1} and least stable in olive oil with a degradation rate constant of 0.98×10^{-4} day^{-1}. The degradation rate constant was obtained as 0.78×10^{-4} day^{-1} in cotton seed oil, 0.85×10^{-4} day^{-1} in arachis oil, 0.87×10^{-4} day^{-1} in castor oil and 0.91×10^{-4} day^{-1} in linseed oil (Table 4.8). A similar trend was observed in these oils at accelerated stability study conditions of 40 °C, 60 °C, 90 °C and 40 °C/ 75 % RH, and refrigerated conditions (Table 4.8). The predicted shelf life at 30 °C in these oils was found to be 23.05 months in cotton seed oil, 18.34 months in olive oil, 20.67 months in castor oil, 23.98 months in sesame oil, 21.15 months in arachis oil and 19.76 months in linseed oil (Table 4.11). A comparison of corresponding shelf life calculated for flurbiprofen at 30 °C and CRT in different oils is also presented in Table 4.11. In sesame oil, flurbiprofen was found to be most stable.

The excellent stability of the drug in sesame oil can be attributed to the presence of sesamul, a phenolic constituent, in oil (Tyler, et al, 1981). Phenols are known reducing agent (Robinson, 1970). Thus the increased stability of flurbiprofen in sesame oil can be attributed to the reducing property of the oil constituent and also thereby suggesting an oxidative degradation mechanism. However, further studies are required to confirm this.

A comparison of the degradation rate constants obtained at 40 °C with that obtained at 40°C/ 75% RH in different buffered or unbuffered solutions and in vegetable oils in order to establish the effect of humidity on the stability characteristics of drug showed an increased rate of degradation of the drug in all the test solutions (Table 4.12). An increase in 7.9 to 18.7 % in the magnitude of the first order degradation rate was observed at 40 °C/ 75 % RH when compared to the values at 40 °C.

Solubility studies

Flurbiprofen was found to have a poor solubility of 61.5 ± 3.5 µg/ml in triple distilled water (pH 6.65) at 37 ± 1 °C (Table 4.13). Buffering at weakly acidic to neutral pH was found to considerably enhance the solubility of the drug and the maximum concentration of flurbiprofen attained in different buffer types is presented in Table 4.13. Highest solubility of 3525.4 ± 23.4 µg/ml was observed in borate buffer (pH 7.5) and lowest of 1574.3 ± 28.5 µg/ml in citrate buffer

(pH 6.6). In phosphate buffer of pH 7.0 the solubility was found to be 3458.4 ± 45.0 μg/ml while in citrate buffer (pH 7.0) it had an intermediate solubility of 2362.7 ± 26.0 μg/ml. The variation in solubility of flurbiprofen in different buffer types within a narrow range of pH may be probably due to variation in the ionization profile of the drug in different buffer types. In spite of lower solubility in phosphate buffer than borate buffer it was selected to study the effect of pH on the solubility of the drug and for future formulation development work because of higher stability of flurbiprofen in phosphate buffer.

The solubility of the drug was found to increase in phosphate buffer with increase in pH from 5.6 to 8.0 (Figure 4.12 and Table 4.14). At pH 5.6 in phosphate buffer flurbiprofen was found to have a solubility of 199.1 ± 4.6 μg/ml and in at pH 8.0 it showed a solubility of 7866.7 ± 154.8 μg/ml with intermediate solubility at pH 6.6 and 7.6 (Table 4.14 and Figure 4.12). An increase in solubility with increase in pH can be attributed to the fact that flurbiprofen being a weakly acidic drug with a pK_a of 4.27 (Craig, 1990) will be ionized to a greater extend at pH above 4.27 with the degree of ionization increasing with increase in pH. This increase in ionization of the drug from weakly acidic to basic pH contributes to increase its solubility. A similar phenomenon was observed with unbuffered solutions of varying pH with the solubility of the drug increasing with the increase in pH (Figure 4.13 and Table 4.15). At pH 2.2 solubility was as low as 18.2 ± 1.4 μg/ml with the solubility increasing to 370.6 ± 4.3 μg/ml at pH 10.4. But a drastic increase in solubility to 26.41 ± 1.3 mg/ml was observed at pH 12.2 (at predominantly NaOH environment). A comparison (Table 4.14 and 4.15) of the solubility of the drug in buffered and unbuffered solution revealed a drastic difference in solubility profile. At different pH solubility was significantly higher ($p< 0.001$) in buffered solutions than in unbuffered solutions. This is probably may be due to the lesser ionization capacity of hydrochloride and sodium hydroxide ions (used for adjusting the pH) when compared to that sodium dihydrogen phosphate and disodium mono hydrogen phosphate ions (used for preparation of phosphate buffer of varying pH).

Flurbiprofen was found to have very high solubility in vegetable oils at 37 ± 1 °C. Amongst the vegetable oils studied for flurbiprofen solubility, maximum concentration of the drug was attained in sesame oil (23.86 ± 0.57 mg/ml) and minimum in castor oil (9.80 ± 0.24 mg/ml) as presented in Table 4.16. Solubility in cotton seed oil, olive oil, arachis oil and linseed oil was

obtained as 16.15 ± 0.19, 21.63 ± 0.40, 18.99 ± 0.15, 14.71 ± 0.32 mg/ml respectively. Flurbiprofen was also found to have very high solubility in organic solvents at CRT and the results are presented in Table 4.17. The solubility varying from 71.50 ± 2.20 mg/ml in case of isobenzyl alcohol to 149.12 ± 7.00 mg/ml in case of methyl alcohol. In case of benzyl alcohol, which is used for solubilizing the drug in ophthalmic formulations, flurbiprofen was found to have a solubility of 135.43 ± 2.50 mg/ml. But in the solubility study involving organic solvents saturation solubility could be attained only in case of chloroform, cyclohexane, ethyl acetate, ethylene glycol, isobenzyl alcohol, propyl alcohol, tetrahydrofuran, triethyl amine and xylene.

Log P determination

The partition coefficient of flurbiprofen in n-octanol- water system at 37 ± 1 °C was determined to be 51.62 ± 0.52 after 24 hours and the corresponding log P value was calculated as 1.7128 ± 0.0212 (Table 4.18). Replacement of n-octanol saturated triple distilled water with either n-octanol saturated unbuffered (pH adjusted) aqueous phase or n-octanol saturated phosphate buffer of varying pH showed a reduction in the partition coefficient value of the drug (Table 4.18). This can be attributed to the increased solubility of flurbiprofen in buffered and unbuffered (pH adjusted) solutions. Though the solubility of the drug is more in buffered media than unbuffered media but the partition coefficient value was found to be more in buffered media. This may be probably due to increased partitioning of the drug to the oil phase in the presence of buffering salts used in buffered media. The partition coefficient decreased with increase in pH of the aqueous phase (in case of both buffered and unbuffered aqueous phase) (Table 4.18 and Figure 4.14). The partition coefficient obtained in n-octanol-unbuffered aqueous phase at pH 5.5, 6.5 and 7.5 was 7.89 ± 0.56, 5.67 ± 0.73 and 2.81 ± 0.21. The corresponding log P value determined was 0.8963 ± 0.039, 0.7512 ± 0.0563 and 0.4479 ± 0.0325 respectively (Table 4.18 and Figure 4.15). At pH 5.6 phosphate buffer the partition coefficient was obtained as 43.64 ± 2.36 and a log P value of 1.6395 ± 0.0235. With increase in pH to 6.6 and 7.6 the partition coefficient value decreased to 28.87 ± 1.52 and 6.37 ± 0.54 respectively (Figure 4.14). The corresponding log P value was calculated as 1.4600 ± 0.0229 and 0.8031 ± 0.0369 respectively at pH 6.6 and 7.6 (Table 4.18 and Figure 4.15).

4.4 Conclusions

The shelf life of flurbiprofen in solid form based on observed K_{CRT} and predicted K_{30} was found to be 4.43 years and 2.70 years respectively whereas in triple distilled water it was obtained as 9.46 months and 6.77 months respectively. Amongst the excipients studied for drug-excipient stability studies only polyvinyl pyrolidone, sodium alginate, EDTA, benzalkonium chloride, and phenyl mercuric nitrate showed moderate instability while all other excipients showed insignificant instability. The above effects, which have been observed in solid state, may be due to the localized high concentration of the excipients, which may not be true for drug stability in liquid dosage form. Since most of the excipients studied were polymeric in nature and have got the tendency to absorb moisture, which appears to be the reason for increased degradation effect at higher humidity conditions. But hydrophobic excipients did not show such effect. There was no significant change in the physical characteristics of the drug in the presence of the excipient during the period of study under different conditions. Flurbiprofen was found to be compatible with phosphate and borate buffer whereas it was found to be incompatible with citrate and citrate-phosphate buffer. Flurbiprofen solution in triple distilled water was found to follow first order degradation rate constant and similar degradation mechanism was evident in different buffer types of varying pH, unbuffered solutions and vegetable oils.

Flurbiprofen showed a pH dependent stability in 0.01 M phosphate buffer of varying pH with the stability increasing with increase in pH from 5.6 to 7.8. In case of stability studies in unbuffered solutions of varying pH the rate of degradation was found to be higher than the corresponding buffered pH (phosphate buffer). In unbuffered solutions as well the shelf life increased with increase in pH. Acid catalysis seems to be the mechanism of degradation of flurbiprofen in buffered and unbuffered solutions. But further studies under controlled conditions are required to be done to make a final conclusion. Among different vegetable oils studied, the drug was found to be most stable in sesame oil and least stable in olive oil. Increased stability of flurbiprofen in sesame oil can be attributed to the reducing property of the phenolic constituent (sesamul) present in the oil and also thereby suggesting an oxidative degradation.

Flurbiprofen was found to have a poor solubility of 61.5 ± 3.5 μg/ml in triple distilled water. Buffering at weakly acidic to neutral pH was found to considerably enhance the solubility of the drug. Highest solubility was observed in borate buffer and lowest in citrate buffer. In phosphate

buffer the solubility was found to increase with increase in pH. The variation in solubility profile of flurbiprofen in different buffer types within a narrow range of pH may be probably due to variation in the ionization profile of the drug in different buffer types. An increase in solubility with increase in pH can be attributed to the fact that flurbiprofen being a weakly acidic drug will be ionized to a greater extend at pH above its pK_a thereby increasing the solubility with increase in pH. Amongst the vegetable oils studied for flurbiprofen solubility, maximum concentration of the drug was attained in sesame oil and minimum in castor oil. Flurbiprofen was also found to have very high solubility in organic solvents at controlled room temperature. The partition coefficient of flurbiprofen in n-octanol- water system at 37 ± 1 °C was determined to be 51.62 ± 0.52 and the corresponding log P value was determined to be 1.7128 ± 0.0212. Use of either n-octanol saturated unbuffered (pH adjusted) aqueous phase or n-octanol saturated phosphate buffer of varying pH caused a decrease in the partition coefficient value of the drug. This can be attributed to the increased solubility of flurbiprofen in unbuffered (pH adjusted) solutions and in phosphate buffer. The log P value decreased with increase in pH of the aqueous phase (in case of both buffered and unbuffered system).

4.5 References

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Table 4.1: Observed degradation rate constants obtained from flurbiprofen and excipient compatibility study at different storage conditions

Drug/ Drug +excipient	Observed degradation rate constant[a] (K_{deg}) × 10^4 (day^{-1})					
	CRT[b]	40 °C	60 °C	90 °C	40 °C/ 75 % RH	FT[c]
Flurbiprofen	0.65	2.67	5.88	10.96	3.34	0.62
Flurbiprofen + starch	0.95	2.75	6.06	11.29	3.44	0.42
Flurbiprofen + magnesium stearate	0.76	3.61	7.94	14.79	3.02	0.61
Flurbiprofen + ethyl cellulose	1.08	2.75	6.05	11.27	2.78	0.79
Flurbiprofen + polyvinyl alcohol	0.74	2.74	6.02	11.22	3.42	0.35
Flurbiprofen + polyacrylamide	0.87	2.49	5.47	10.20	3.11	0.70
Flurbiprofen + carboxy methyl cellulose	0.97	2.34	5.16	9.61	2.93	0.78
Flurbiprofen + methyl cellulose	0.67	2.28	5.02	9.35	2.85	0.54
Flurbiprofen + sodium carboxy methyl cellulose	0.97	2.34	5.16	11.54	3.52	1.17
Flurbiprofen + hydroxy propyl methyl cellulose	0.67	2.28	5.02	10.60	3.23	0.52
Flurbiprofen + polyvinyl pyrolidone	1.12	2.81	6.19	13.06	3.98	0.78
Flurbiprofen + polycarbophil 934	0.97	2.59	5.69	12.05	3.67	0.94
Flurbiprofen + sodium alginate	1.20	3.19	7.01	12.89	3.93	0.96
Flurbiprofen + EDTA	1.33	2.94	6.47	11.71	3.84	1.08
Flurbiprofen + benzalkonium chloride	1.06	3.14	6.92	11.04	3.22	1.12
Flurbiprofen +phenyl mercuric nitrate	1.17	2.55	6.35	11.07	3.45	0.92
Flurbiprofen + methyl paraben	0.73	2.12	4.87	9.48	2.89	0.66
Flurbiprofen + propyl paraben	0.69	2.18	5.04	9.25	2.81	0.64

[a] : Based on average of triplicate assay value at four time points with C.V. less than 2.3 % in all cases
[b] : CRT= 25 ± 3 °C & 55 ± 15 % RH
[c] : FT= 4 ± 3 °C

Table 4.2: Predicted degradation rate constant at 30 °C (K_{30}), correlation coefficient (r) of Arrhenius plot and shelf-life based on predicted K_{30} obtained from flurbiprofen and excipient stability study at different storage conditions

Drug/ Drug +excipient	Predicted values from Arrhenius plot at 30 °C			Shelf-life based on observed K_{CRT} (t_{90}, years)
	$K_{30} \times 10^4$ (day^{-1})	t_{90} (years)	r	
Flurbiprofen	1.06	2.70	0.9821	4.43
Flurbiprofen + starch	1.38	2.09	0.9937	3.03
Flurbiprofen + magnesium stearate	1.31	2.20	0.9770	3.79
Flurbiprofen + ethyl cellulose	1.50	1.92	0.9971	2.66
Flurbiprofen + polyvinyl alcohol	1.17	2.46	0.9855	3.89
Flurbiprofen + polyacrylamide	1.26	2.29	0.9939	3.31
Flurbiprofen + carboxy methyl cellulose	1.32	2.18	0.9983	2.97
Flurbiprofen + methyl cellulose	1.05	2.74	0.9885	4.29
Flurbiprofen + sodium carboxy methyl cellulose	1.32	2.18	0.9983	2.97
Flurbiprofen + hydroxy propyl methyl cellulose	1.05	2.74	0.9885	4.29
Flurbiprofen + polyvinyl pyrolidone	1.55	1.86	0.9975	2.57
Flurbiprofen + polycarbophil 934	1.37	2.10	0.9959	2.97
Flurbiprofen + sodium alginate	1.69	1.70	0.9960	2.40
Flurbiprofen + EDTA	1.76	1.64	0.9996	2.16
Flurbiprofen + benzalkonium chloride	1.55	1.86	0.9930	2.71
Flurbiprofen +phenyl mercuric nitrate	1.54	1.87	0.9831	2.46
Flurbiprofen + methyl paraben	1.06	2.71	0.9951	3.94
Flurbiprofen + propyl paraben	1.03	2.79	0.9931	4.17

Table 4.3: Comparison of observed degradation rate constants obtained from flurbiprofen and excipient compatibility study at 40°C and 40°C/ 75% RH

Drug/ Drug +excipient	Observed degradation rate constant[a] (K_{deg}) × 10^4 (day^{-1})		Relative rate constant[b]
	40 °C	40 °C/ 75 % RH	
Flurbiprofen	2.67	3.34	1.2509
Flurbiprofen + starch	2.75	3.44	1.2509
Flurbiprofen + magnesium stearate	3.61	3.02	0.8366
Flurbiprofen + ethyl cellulose	2.75	2.78	1.0109
Flurbiprofen + polyvinyl alcohol	2.74	3.42	1.2482
Flurbiprofen + polyacrylamide	2.49	3.11	1.2490
Flurbiprofen + carboxy methyl cellulose	2.34	2.93	1.2521
Flurbiprofen + methyl cellulose	2.28	2.85	1.2500
Flurbiprofen + sodium carboxy methyl cellulose	2.34	3.52	1.5043
Flurbiprofen + hydroxy propyl methyl cellulose	2.28	3.23	1.4167
Flurbiprofen + polyvinyl pyrolidone	2.81	3.98	1.4164
Flurbiprofen + polycarbophil 934	2.59	3.67	1.4170
Flurbiprofen + sodium alginate	3.19	3.93	1.2320
Flurbiprofen + EDTA	2.94	3.84	1.3061
Flurbiprofen + benzalkonium chloride	3.14	3.22	1.0255
Flurbiprofen +phenyl mercuric nitrate	2.55	3.45	1.3529
Flurbiprofen + methyl paraben	2.12	2.89	1.3632
Flurbiprofen + propyl paraben	2.18	2.81	1.2890

[a] : Based on average of triplicate assay value at four time points with C.V. less than 2.3 % in all cases

[b] : Based on ratio of observed degradation rate constant at 40 °C/ 75 % RH to that at 40 °C

Table 4.4: Preparation of different buffers

Buffer	Composition of stock solutions		pH	Method of preparation (for 1 litre)	
	Solution A (per litre)	Solution B (per litre)		Solution A	Solution B
Citrate buffer	21.0 g of citric acid	29.4 g of sodium citrate	6.6	40 ml	960 ml
Phosphate buffer	13.8 g of monobasic sodium monophosphate	14.2 g of dibasic sodium monophosphate	5.6	948 ml	52 ml
			6.6	685 ml	315 ml
			7.0	390 ml	610 ml
			7.4	190 ml	810 ml
			7.8	85 ml	915 ml
Citrate phosphate buffer	21.0 g of citric acid	71.5 g of disodium hydrogen phosphate	7.0	176 ml	824 ml
Borate buffer	5.0 g of sodium chloride	5.70 g of sodium tetra borate and 2.0 g of boric acid	7.5	500 ml	500 ml

[a]: Volume of stock solution A & B to be taken for preparing 1 litre buffer of the desired pH

Table 4.5: Results of physical compatibility of flurbiprofen with different buffers and vegetable oils

Vehicle	Observed opalescence		
	12 hours	24 hours	72 hours
Control	–	–	–/+
(A) Buffers			
Citrate buffer pH 6.6	–	+	+
Phosphate buffer pH 5.6	–	–	–
pH 6.6			
pH 7.0	–	–	–
pH 7.4	–	–	–
pH 7.8	–	–	–
Citrate-phosphate buffer pH 7.0	–	+	+
Borate buffer pH 7.5	–	–	–
(B) Vegetable oils			
Cotton seed oil	–	–	–
Olive oil	–	–	–
Castor oil	–	–	–/+
Sesame oil	–	–	–
Arachis oil	–	–	–
Linseed oil	–	–	–/+

'+' Opalescence observed; '–' Opalescence not observed;
'–/+' Mild precipitation without any opalescence

Table 4.6: Observed degradation rate constants obtained from flurbiprofen stability studies in different buffers types and phosphate buffers of different pH

Sample	Observed degradation rate constant[a] (K_{deg}) × 10^4 (day^{-1})					
	CRT[b]	40 °C	60 °C	90 °C	40 °C/ 75 % RH	FT[c]
Flurbiprofen control in triple distilled water	3.65	9.50	21.00	36.98	10.93	1.83
Citrate buffer pH 6.6	2.89	7.08	15.65	26.53	8.14	1.45
Phosphate buffer pH 5.6	2.83	21.84	50.58	89.64	25.92	1.47
pH 6.6	2.72	19.60	44.11	81.60	22.54	1.36
pH 7.0	2.65	18.00	39.16	75.35	20.98	1.32
pH 7.4	2.56	16.63	36.76	71.68	19.13	1.28
pH 7.8	2.34	14.44	30.62	65.52	16.61	1.17
Citrate-phosphate buffer pH 7.0	3.55	22.50	50.97	99.40	24.28	1.78
Borate buffer pH 7.5	4.22	27.42	54.59	94.16	31.53	2.11

[a]: Based on average of triplicate assay value at four time points with C.V. less than 3.0 % in all cases
[b]: CRT= 25 ± 3 °C & 55 ± 15 % RH; [c]: FT= 4 ± 3 °C

Table 4.7: Observed degradation rate constants obtained from flurbiprofen stability studies in different pH unbuffered solutions

Sample	Observed degradation rate constant[a] (K_{deg}) × 10^4 (day^{-1})					
	CRT[b]	40 °C	60 °C	90 °C	40 °C/ 75 % RH	FT[c]
Flurbiprofen control in triple distilled water (pH 6.65)	3.65	9.50	21.00	36.98	10.93	1.83
Unbuffered solution pH 1.2	6.81	44.25	97.78	190.68	50.88	3.41
pH 4.5	5.63	36.58	80.84	157.64	42.07	2.82
pH 5.5	4.89	31.77	70.22	136.92	36.54	2.45
pH 6.5	4.02	32.65	72.15	140.70	37.55	2.01
pH 7.5	3.78	22.15	54.28	105.84	25.48	1.89
pH 8.5	3.02	15.43	35.95	75.50	17.74	1.51
pH 9.5	2.56	18.02	39.82	71.68	20.72	1.28

[a]: Based on average of triplicate assay value at four time points with C.V. less than 2.8 %in all cases
[b]: CRT= 25 ± 3 °C & 55 ± 15 % RH; [c]: FT= 4 ± 3 °C

Table 4.8: Observed degradation rate constants obtained from flurbiprofen stability studies in different vegetable oils

Sample	Observed degradation rate constant[a] (K_{deg}) × 10^4 (day^{-1})					
	CRT[b]	40 °C	60 °C	90 °C	40 °C/ 75 % RH	FT[c]
Flurbiprofen control in triple distilled water (pH 6.65)	3.65	9.50	21.00	36.98	10.93	1.83
Cotton seed oil	0.78	5.07	11.20	21.84	5.83	0.39
Olive oil	0.98	6.37	14.07	27.44	7.32	0.49
Castor oil	0.87	5.65	12.49	24.36	6.50	0.44
Sesame oil	0.75	4.87	10.77	21.00	5.60	0.38
Arachis oil	0.85	5.52	12.21	23.80	6.35	0.43
Linseed oil	0.91	5.91	13.07	25.48	6.80	0.46

[a] : Based on average of triplicate assay value at four time points with C.V. less than 2.5 % in all cases
[b]: CRT= 25 ± 3 °C & 55 ± 15 % RH, [c]: FT= 4 ± 3°C

Table 4.9: Predicted degradation rate constant at 30 °C (K_{30}), correlation coefficient (r) of Arrhenius plot and shelf-life based on predicted K_{30} obtained from flurbiprofen stability studies in different buffers types and phosphate buffers of different pH

Sample	Predicted values from Arrhenius plot at 30 °C			Shelf-life based on observed K_{CRT} (t_{90}, months)
	$K_{30} \times 10^4$ (day^{-1})	t_{90} (months)	r	
Flurbiprofen control in triple distilled water	5.10	6.77	0.9967	9.46
Citrate buffer pH 6.6	3.96	8.73	0.9980	11.94
Phosphate buffer pH 5.6	5.77	5.98	0.9654	12.20
pH 6.6	5.41	6.38	0.9653	12.69
pH 7.0	5.17	6.68	0.9646	13.03
pH 7.4	4.92	7.02	0.9672	13.48
pH 7.8	4.41	7.82	0.9656	14.75
Citrate-phosphate buffer pH 7.0	6.76	5.11	0.9697	9.72
Borate buffer pH 7.5	8.10	4.26	0.9671	8.18

Table 4.10: Predicted degradation rate constant at 30 °C (K_{30}), correlation coefficient (r) of Arrhenius plot and shelf-life based on predicted K_{30} obtained from flurbiprofen stability studies in different pH unbuffered solutions

Sample		Predicted values from Arrhenius plot at 30 °C			Shelf-life based on observed K_{CRT} (t_{90}, months)
		$K_{30} \times 10^4$ (day^{-1})	t_{90} (months)	r	
Flurbiprofen control in triple distilled water (pH 6.65)		5.10	6.77	0.9967	9.46
Unbuffered solution	pH 1.2	13.08	2.64	0.9671	5.07
	pH 4.5	10.81	3.19	0.9671	6.13
	pH 5.5	9.39	3.68	0.9672	7.06
	pH 6.5	8.34	4.14	0.9605	8.59
	pH 7.5	7.01	4.93	0.9771	9.13
	pH 8.5	5.34	6.47	0.9783	11.43
	pH 9.5	5.05	6.83	0.9647	13.48

Table 4.11: Predicted degradation rate constant at 30 °C (K_{30}), correlation coefficient (r) of Arrhenius plot and shelf-life based on predicted K_{30} obtained from flurbiprofen stability studies in different vegetable oils

Sample	Predicted values from Arrhenius plot at 30 °C			Shelf-life based on observed K_{CRT} (t_{90}, months)
	$K_{30} \times 10^4$ (day^{-1})	t_{90} (months)	r	
Flurbiprofen control in triple distilled water (pH 6.65)	5.10	6.77	0.9967	9.46
Cotton seed oil	1.50	23.05	0.9671	44.26
Olive oil	1.88	18.34	0.9671	35.23
Castor oil	1.67	20.67	0.9672	39.68
Sesame oil	1.44	23.98	0.9672	46.03
Arachis oil	1.63	21.15	0.9672	40.61
Linseed oil	1.75	19.76	0.9672	37.93

Table 4.12: Comparison of observed degradation rate constants obtained from flurbiprofen stability studies in different buffer types, phosphate buffers and unbuffered solutions of varying pH and vegetable oils at 40°C and 40°C/ 75% RH

Drug/ Drug +excipient	Observed degradation rate constant[a] (K_{deg}) × 10^4 (day^{-1})		Relative rate constant[b]
	40°C	40°C/ 75% RH	
Flurbiprofen control in triple distilled water	9.50	10.93	1.1505
Citrate buffer pH 6.6	7.08	8.14	1.1497
Phosphate buffer pH 5.6	21.84	25.92	1.1868
pH 6.6	19.60	22.54	1.1500
pH 7.0	18.00	20.98	1.1655
pH 7.4	16.63	19.13	1.1503
pH 7.8	14.44	16.61	1.1503
Citrate-phosphate buffer pH 7.0	22.50	24.28	1.0791
Borate buffer pH 7.5	27.42	31.53	1.1499
Unbuffered solution pH 1.2	44.25	50.88	1.1498
pH 4.5	36.58	42.07	1.1501
pH 5.5	31.77	36.54	1.1501
pH 6.5	32.65	37.55	1.1501
pH 7.5	22.15	25.48	1.1503
pH 8.5	15.43	17.74	1.1497
pH 9.5	18.02	20.72	1.1498
Cotton seed oil	5.07	5.83	1.1499
Olive oil	6.37	7.32	1.1491
Castor oil	5.65	6.50	1.1504
Sesame oil	4.87	5.60	1.1499
Arachis oil	5.52	6.35	1.1504
Linseed oil	5.91	6.80	1.1506

[a] : Based on average of triplicate assay value at four time points with C.V. less than 3.0 % in all cases

[b] : Based on ratio of observed degradation rate constant at 40°C/ 75% RH to that at 40°C

Table 4.13: Results of solubility study of flurbiprofen in different buffer types at 37 ± 1 °C

Media	Mean[a] (µg/ml)	S.D.	C.V. (%)
Triple distilled water (pH 6.65)	61.5	3.5	5.69
Citrate buffer pH- 6.6	1574.3	28.5	1.81
Phosphate buffer pH-7.0	3458.4	45.0	1.30
Citrate phosphate buffer pH-7.0	2362.7	26.0	1.10
Borate buffer pH-7.5	3525.4	23.4	0.66

[a]: Average of triplicate determinations

Table 4.14: Results of solubility study of flurbiprofen in different pH phosphate buffer at 37 ± 1 °C

Media	Mean[a] (µg/ml)	S.D.	C.V. (%)
Triple distilled water (pH 6.65)	61.5	3.5	5.69
Phosphate buffer pH-5.6	199.1	4.6	2.31
Phosphate buffer pH-.6.6	1843.3	51.1	2.77
Phosphate buffer pH-7.0	3458.4	45.0	1.30
Phosphate buffer pH-7.6	6183.4	43.7	0.71
Phosphate buffer pH-8.0	7866.7	154.8	1.97

[a]: Average of triplicate determinations

Table 4.15: Results of solubility study of flurbiprofen in different pH unbuffered solutions at 37 ± 1 °C

Media	Mean[a] (µg/ml)	S.D.	C.V. (%)
Triple distilled water (pH 6.65)	61.5	3.5	5.69
pH 2.2	18.2	1.4	7.69
pH 3.2	53.5	3.2	5.98
pH 4.0	69.5	1.7	2.45
pH 5.7	138.8	2.0	1.44
pH 6.5	140.9	2.1	1.49
pH 7.0	144.2	3.5	2.43
pH 7.5	157.8	1.6	1.01
pH 8.2	177.7	2.5	1.41
pH 9.1	307.3	3.9	1.27
pH 10.4	370.6	4.3	1.16

[a]: Average of triplicate determinations

Solubility at 12.2 pH was found to be 26.41 ± 1.3 mg /ml

Table 4.16: Results of solubility study of flurbiprofen in different vegetable oil at 37 ± 1 °C

Media	Mean[a] (mg/ml)	S.D.	C.V. (%)
Cotton seed oil	16.15	0.19	1.18
Olive oil	21.63	0.40	1.85
Castor oil	9.80	0.24	2.45
Sesame oil	23.86	0.57	2.39
Arachis oil	18.99	0.15	0.79
Linseed oil	14.71	0.32	2.18

[a]: Average of triplicate determinations

Table 4.17: Results of solubility study of flurbiprofen in different organic solvents at CRT (25 ± 3 °C & 55 ± 15 % RH)

Solvent	Mean[a] (mg/ml)	S.D.	C.V. (%)
1,4- dioxane	111.13	8.70	7.83
Acetone	108.11	8.00	7.40
Acetonitrile	105.00	5.00	4.76
Benzene	141.96	12.00	8.45
Benzyl alcohol	135.43	2.50	1.85
Chloroform*	96.09	4.00	4.16
Cyclohexane*	81.01	2.70	3.33
Diethyl ether	122.32	6.00	4.91
Dimethyl formamide	105.02	7.90	7.52
Ethyl acetate*	86.57	3.00	3.47
Ethylalcohol	102.46	8.00	7.81
Ethylene glycol*	84.26	3.00	3.56
Isobenzyl alcohol*	71.50	2.20	3.08
Isoamyl alcohol	111.79	11.00	9.84
Methylalcohol	149.12	7.00	4.69
Propyl alcohol*	77.77	2.00	2.57
Tetrahydrofuran*	75.52	1.40	1.85
Toluene	120.12	9.00	7.49
Triethyl amine*	94.45	3.50	3.71
Xylene*	99.98	2.60	2.60

[a]: Average of triplicate determinations
*- Saturation solubility attained

Table 4.18: Results of partition coefficient and log P determination in n-octanol and water/ phosphate buffer/ unbuffered system of different pH at 37 ± 1 °C

n-octanol/ media		Partition Coefficient[a]			Log P		
		Mean[b]	S.D.	C.V. (%)	Mean[b]	S.D.	C.V. (%)
Triple distilled water		51.62	0.52	1.01	1.7128	0.0212	1.24
Phosphate buffer	pH 5.6	43.64	2.36	5.41	1.6395	0.0235	1.43
	pH 6.6	28.87	1.52	5.26	1.4600	0.0229	1.57
	pH 7.6	6.37	0.54	8.48	0.8031	0.0369	4.60
Unbuffered system	pH 5.5	7.89	0.56	7.10	0.8963	0.0309	3.45
	pH 6.5	5.67	0.73	12.9	0.7512	0.0563	7.49
	pH 7.5	2.81	0.21	7.47	0.4479	0.0325	7.26

[a] : Partition coefficient = $[C_{aq(initial)} - C_{aq\ (final)}]/ C_{aq\ (final)}$; where, C is the concentration

[b] : Average of triplicate determinations

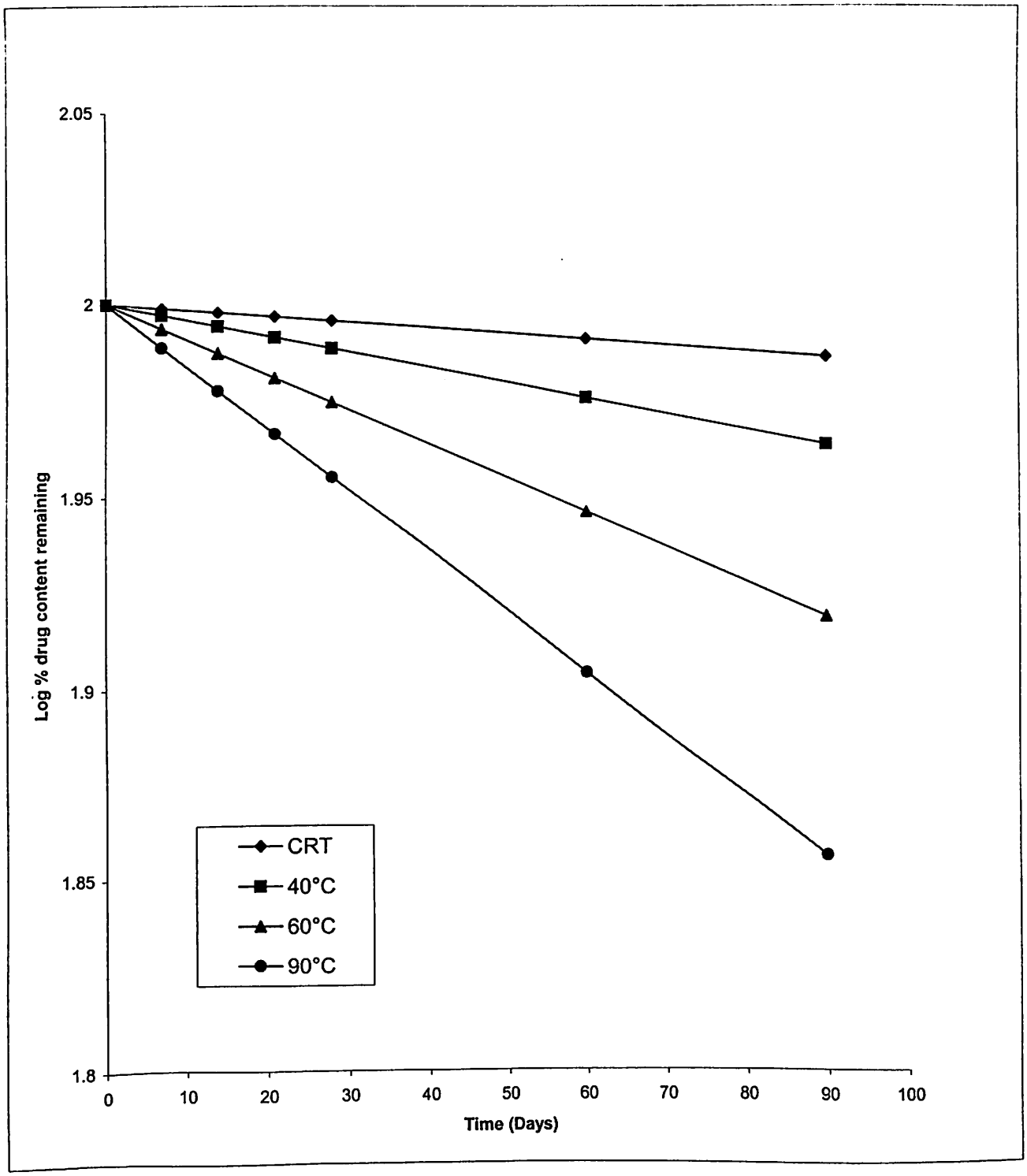

Figure 4.1: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen control solution (750 mg/ml) in triple distilled water.

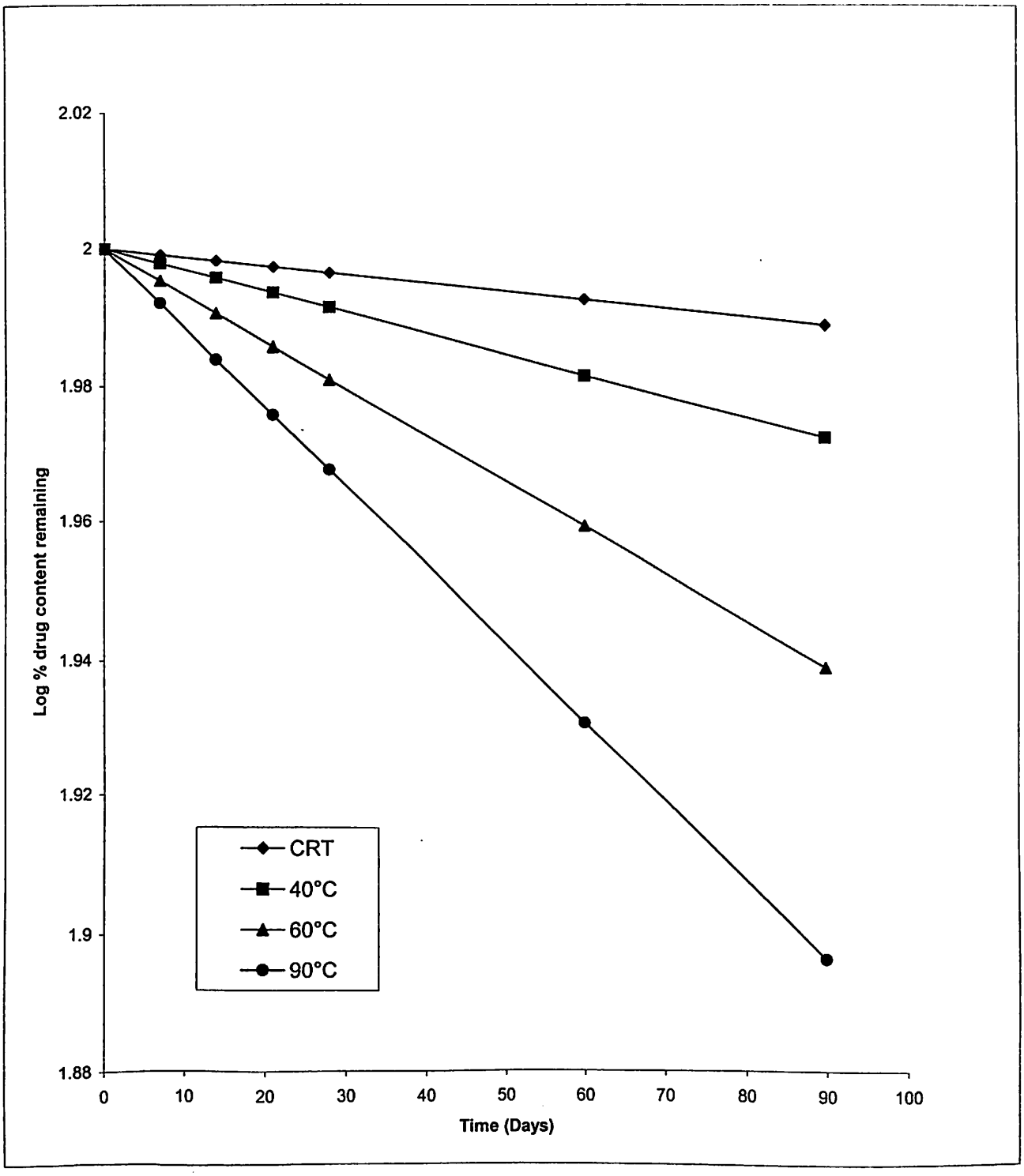

Figure 4.2: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen solution (750 mg/ml) in citrate buffer pH 6.6.

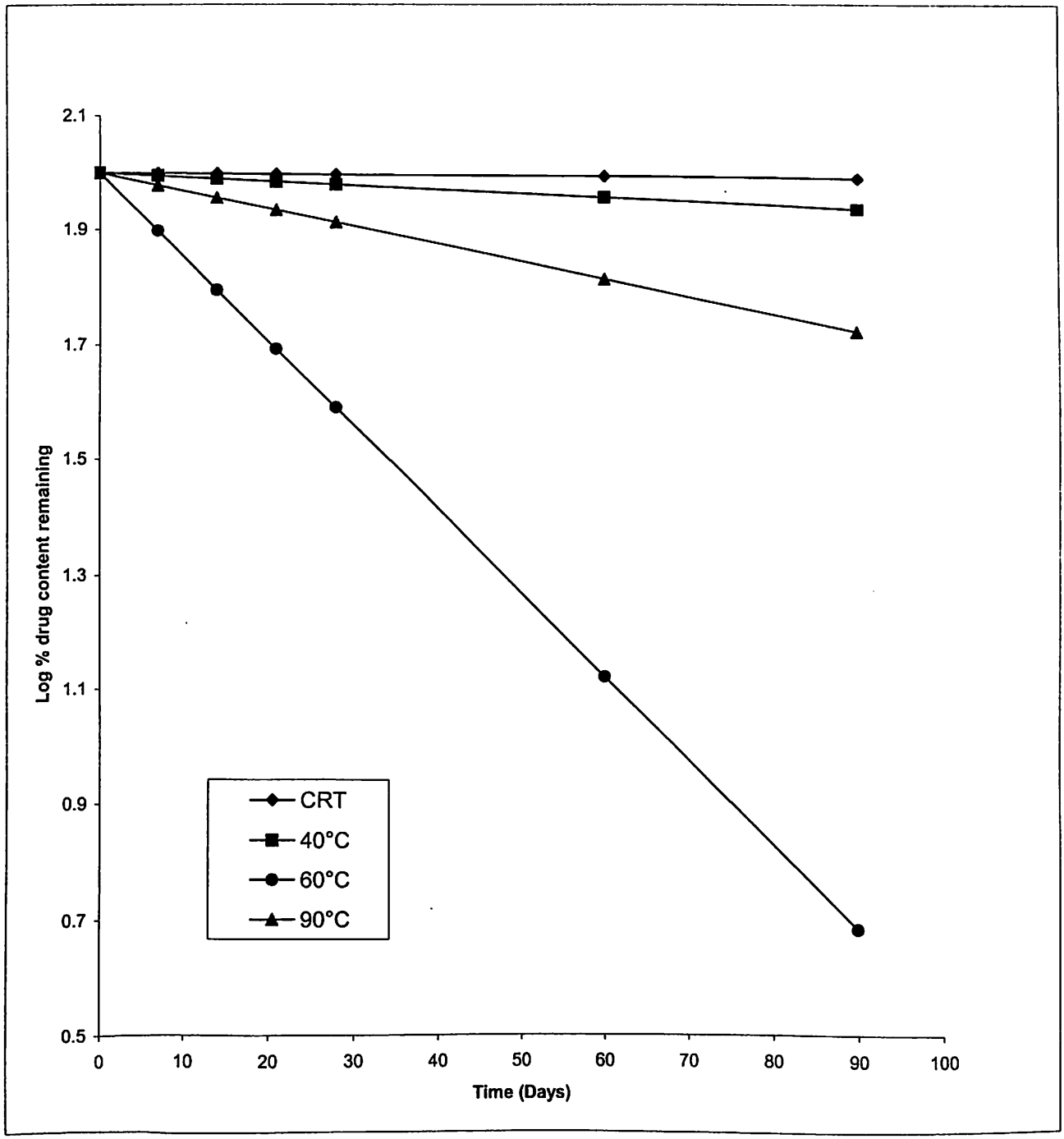

Figure 4.3: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen solution (750 mg/ml) in phosphate buffer pH 7.4.

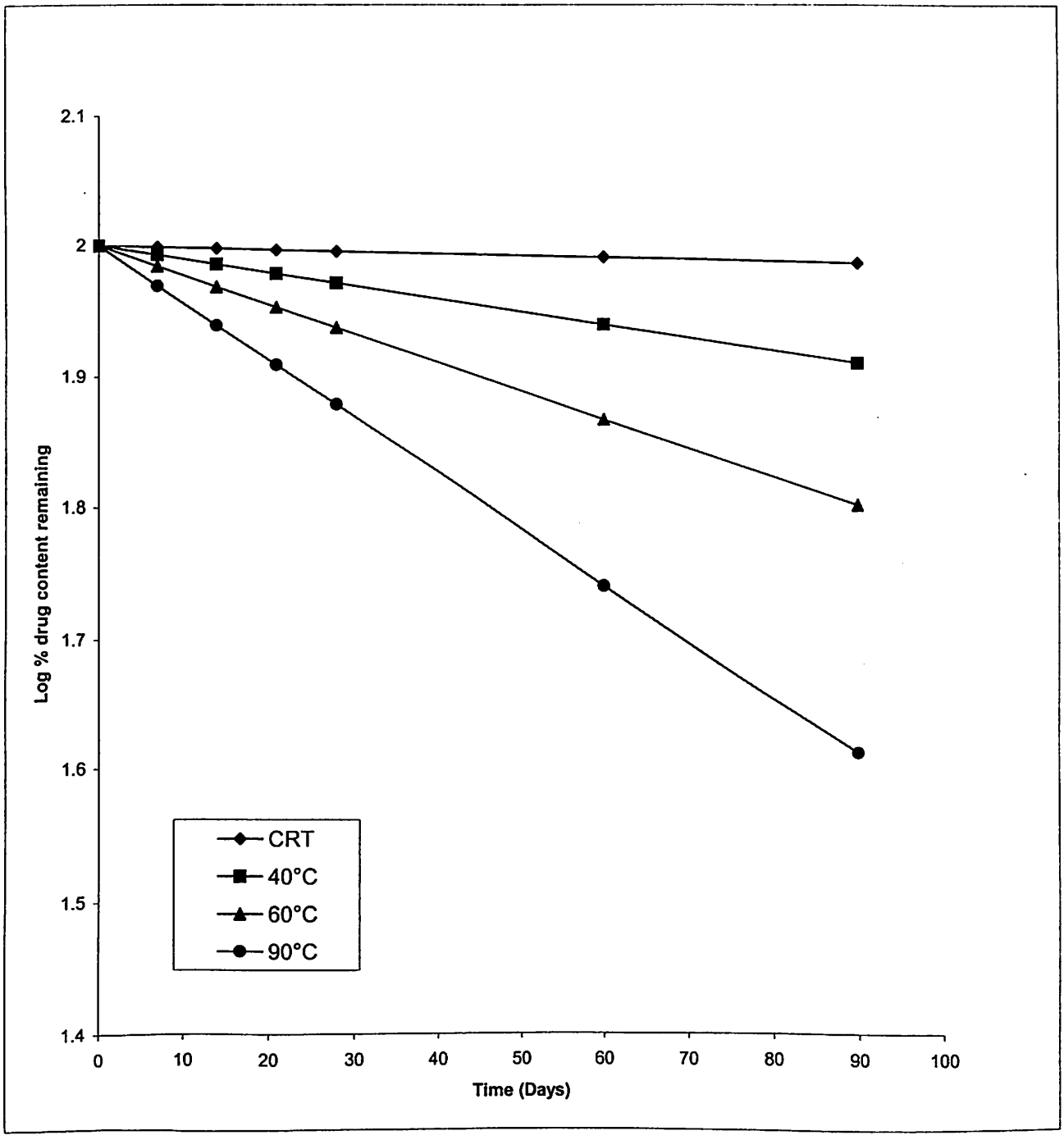

Figure 4.4: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen solution (750 mg/ml) in citrate-phosphate buffer pH 7.0.

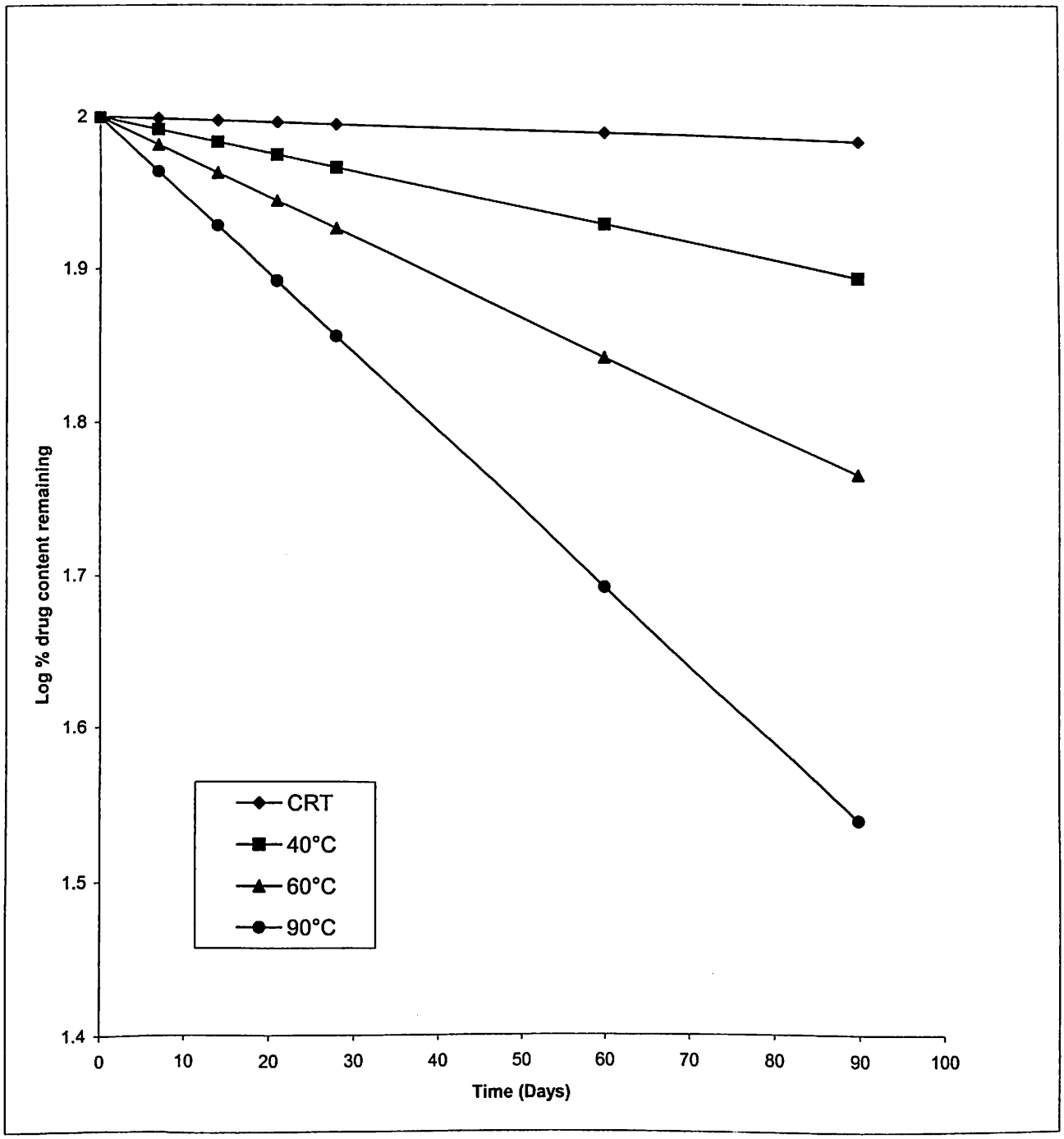

Figure 4.5: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen solution (750 mg/ml) in borate buffer pH 7.5.

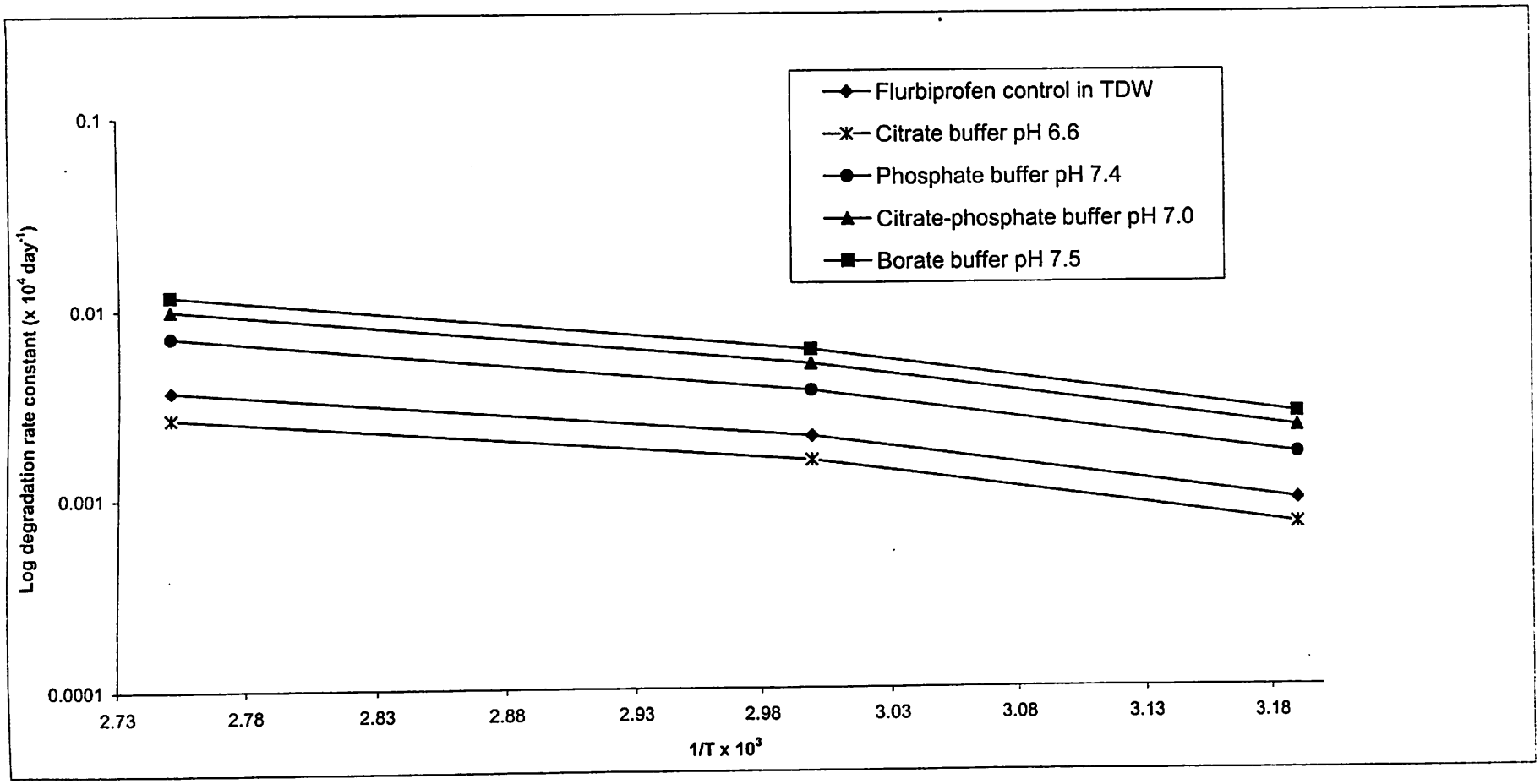

Figure 4.6: Arrhenius plot for degradation of flurbiprofen (750 mg/ml) in different buffers (0.01M), T is absolute temperature in Kelvin.

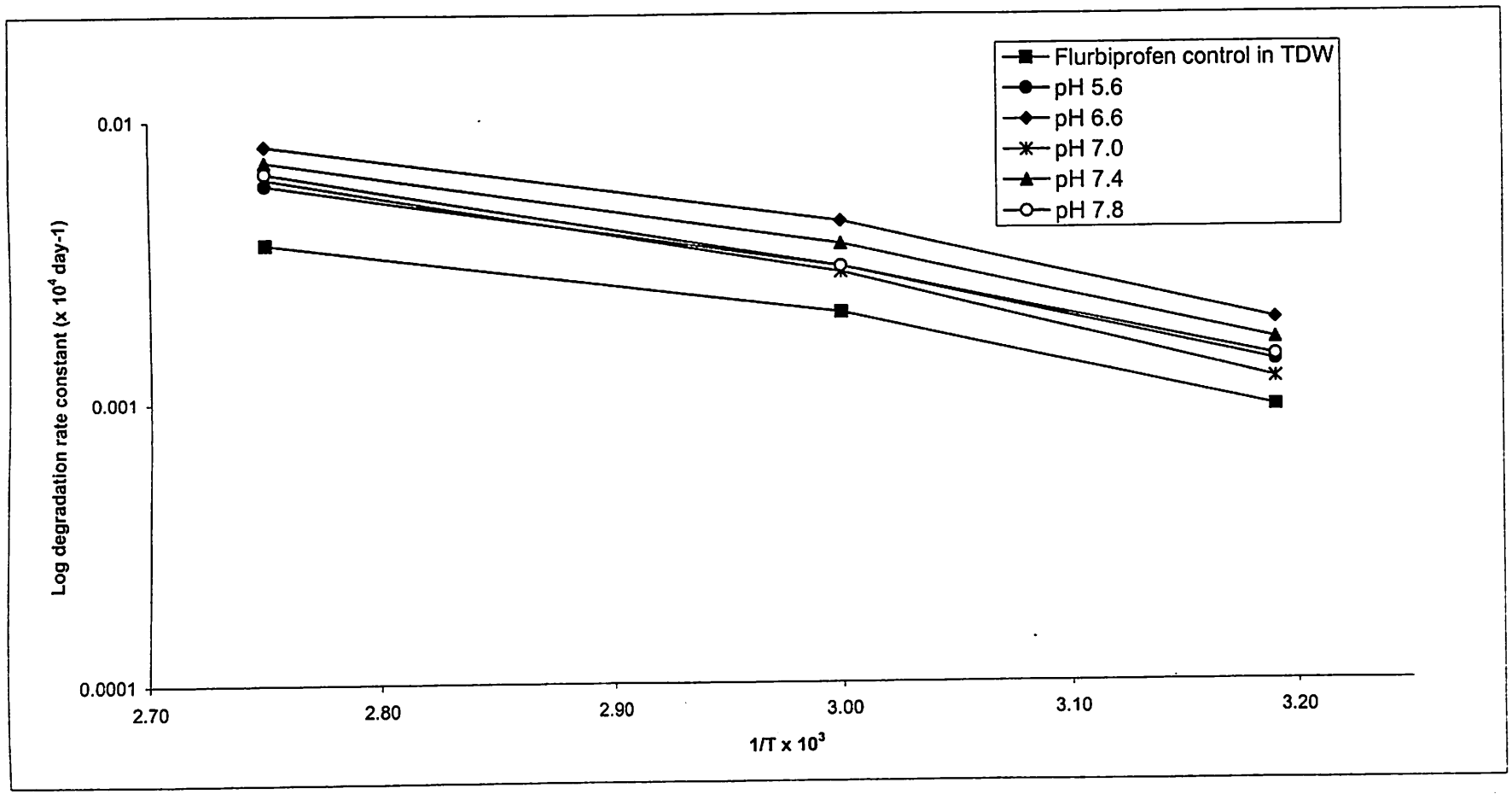

Figure 4.7: Arrhenius plot for degradation of flurbiprofen (750 mg/ml) in different pH phosphate buffer (0.01M), T is absolute temperature in Kelvin.

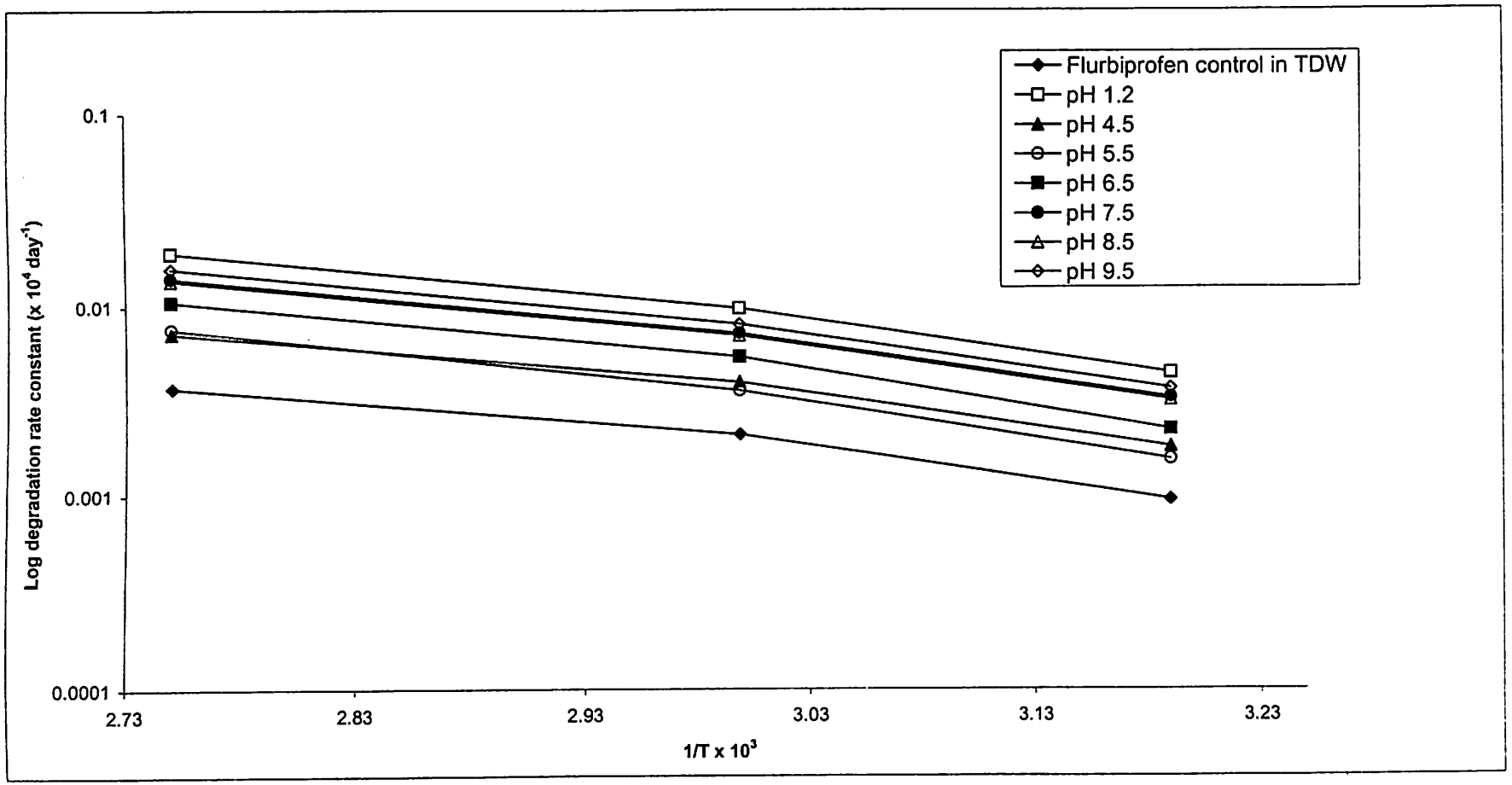

Figure 4.8: Arrhenius plot for degradation of flurbiprofen (750 mg/ml) in different pH unbuffered solutions, T is absolute temperature in Kelvin.

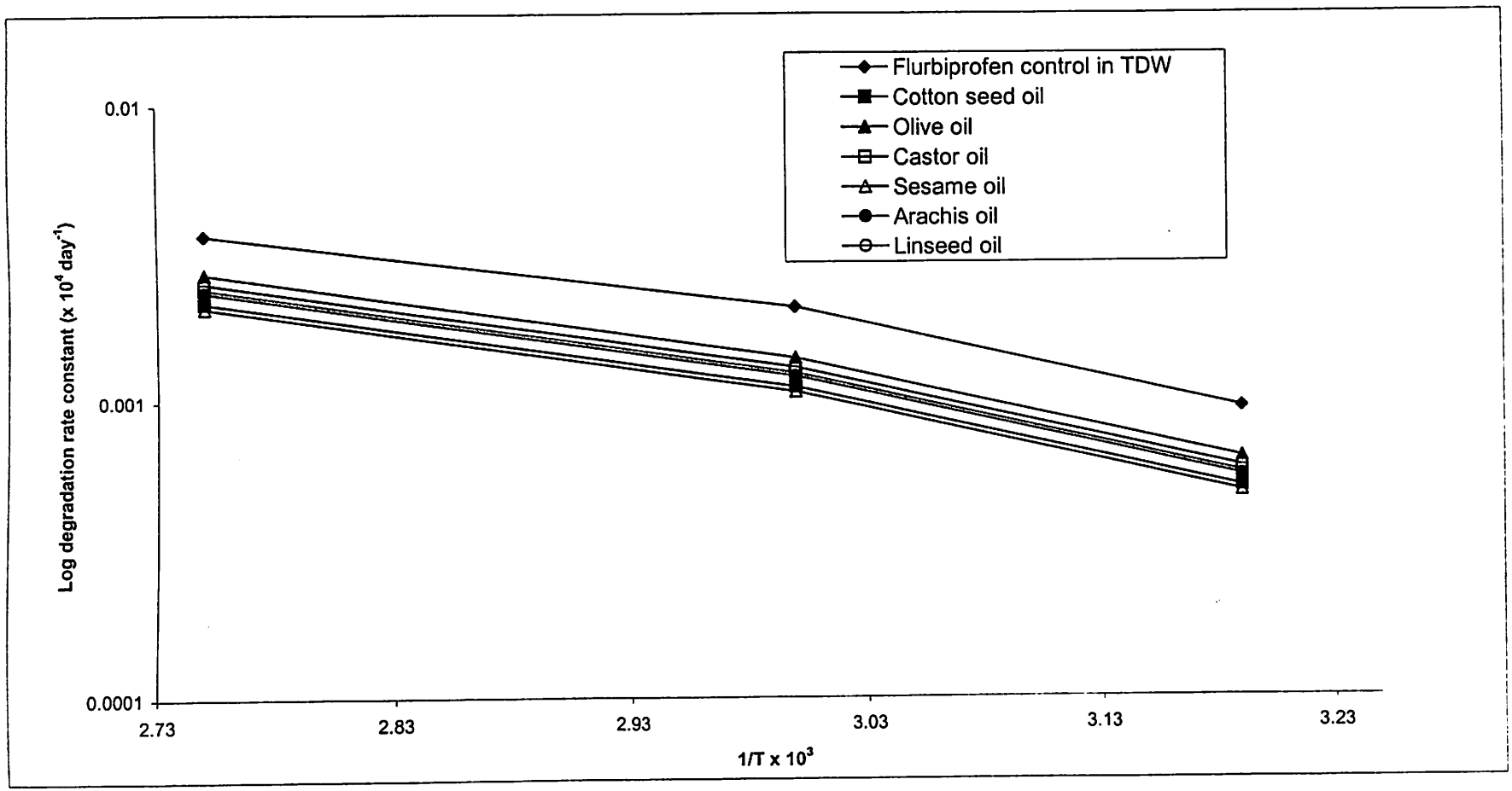

Figure 4.9: Arrhenius plot for degradation of flurbiprofen (750 mg/ml) in different vegetable oils, T is absolute temperature in Kelvin.

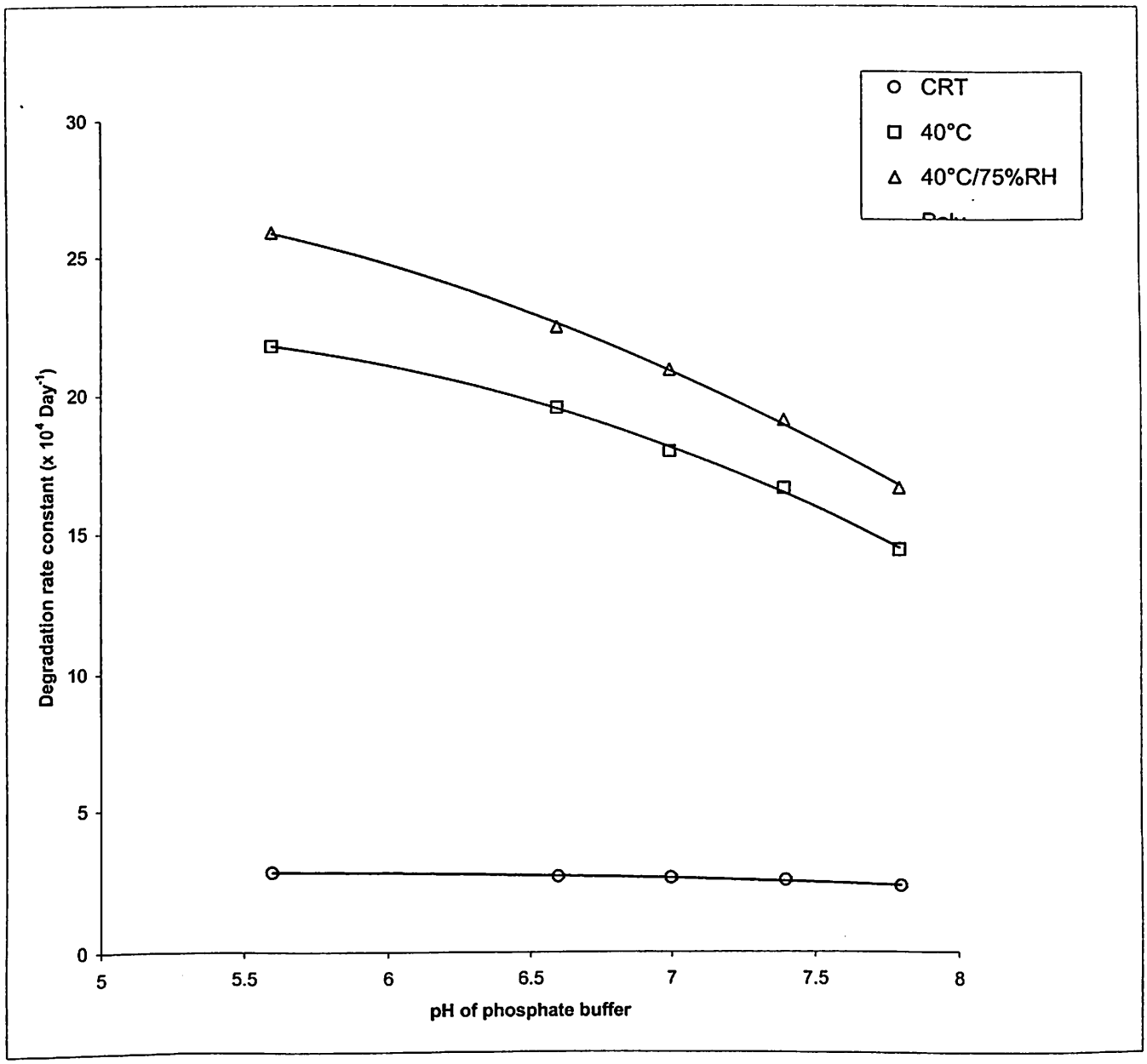

Figure 4.10: Comparative profile between degradation rate constants of flurbiprofen (750 mg/ml) at different storage conditions and pH of the phosphate buffer.

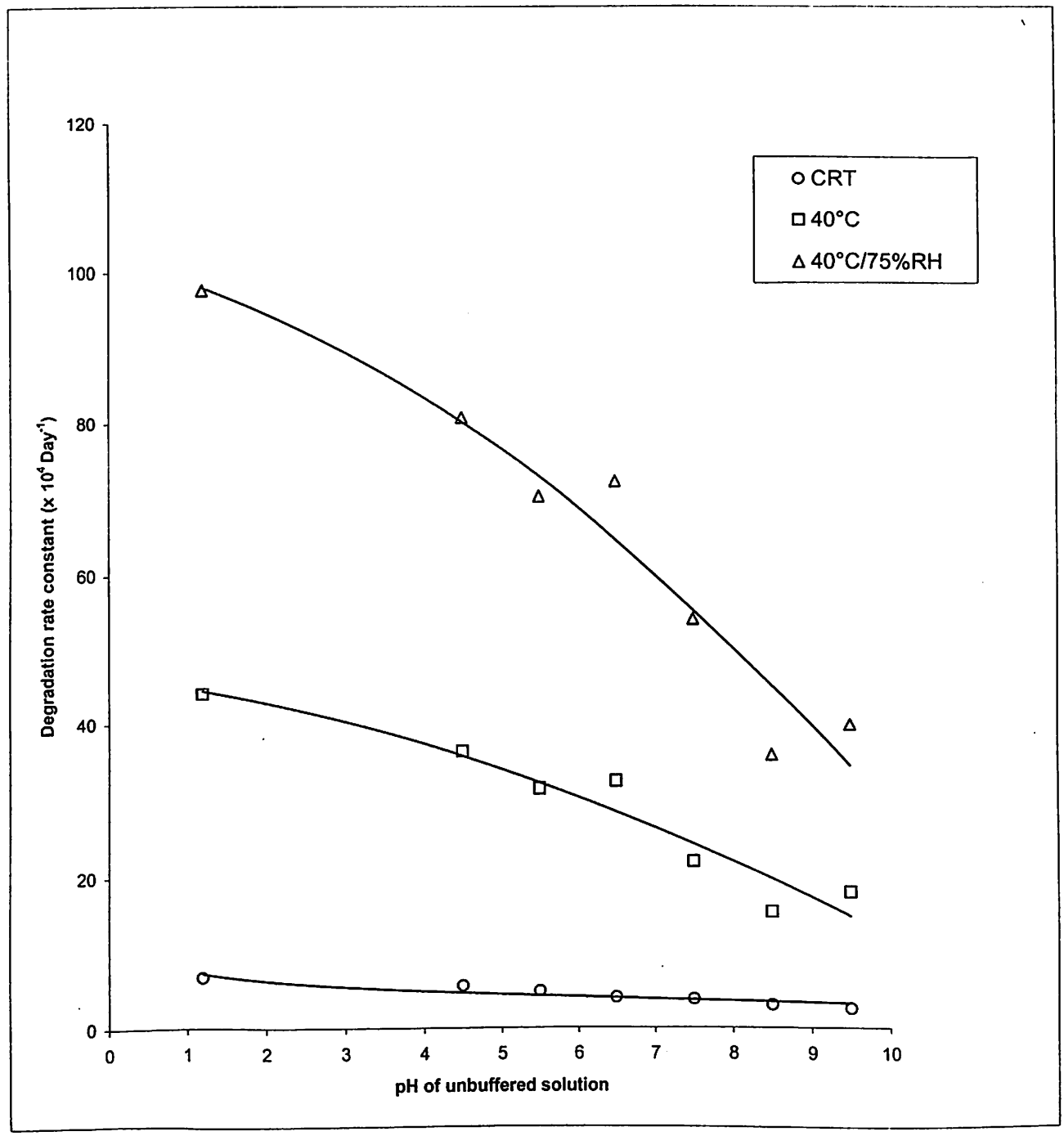

Figure 4.11: Comparative profile between degradation rate constants of flurbiprofen (750 mg/ml) at different storage conditions and pH of the unbuffered solution.

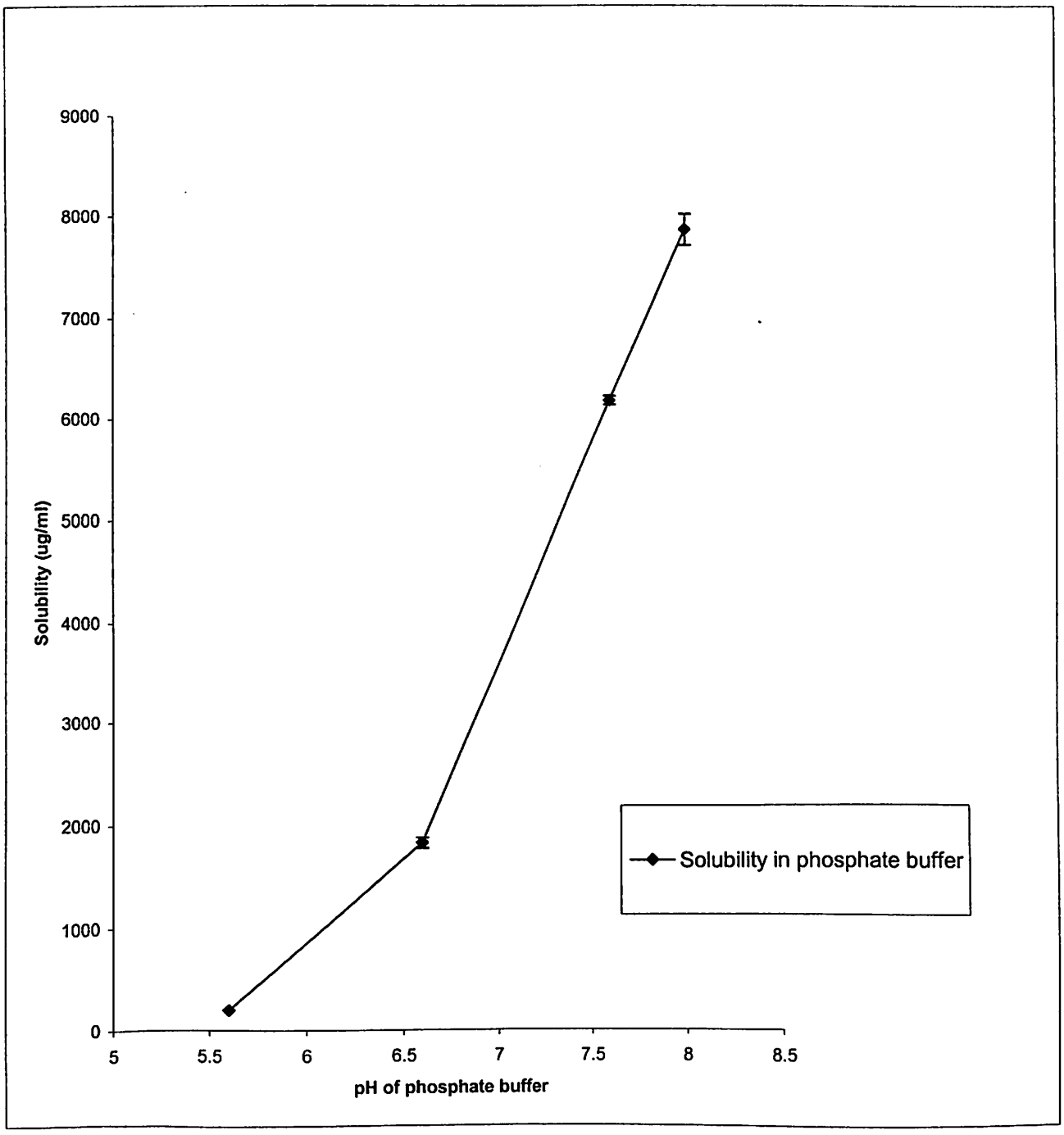

Figure 4.12: Solubility profile of flurbiprofen in phosphate buffer of varying pH.

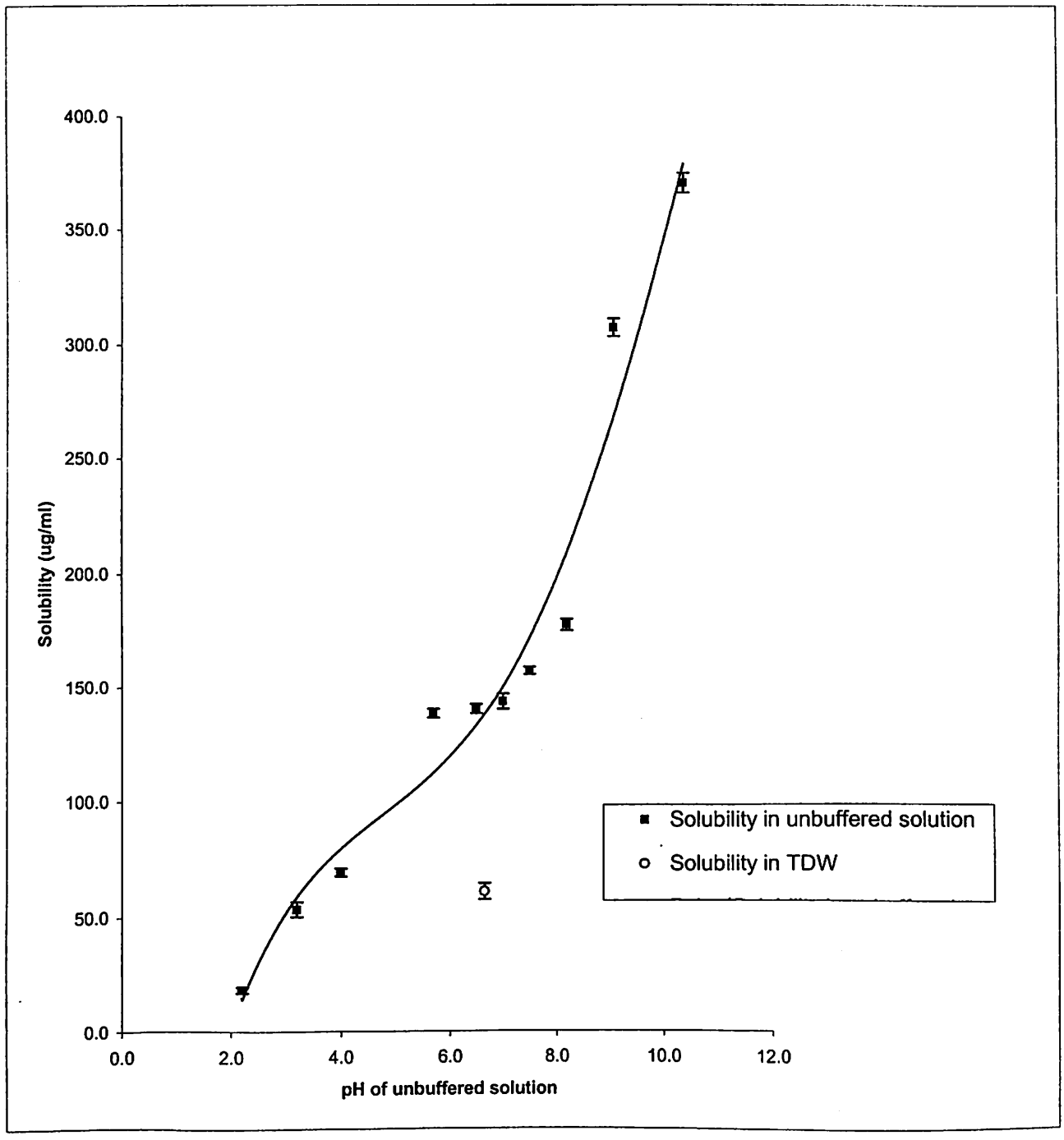

Figure 4.13: Solubility profile of flurbiprofen in unbuffered solution phosphate buffer of varying pH.

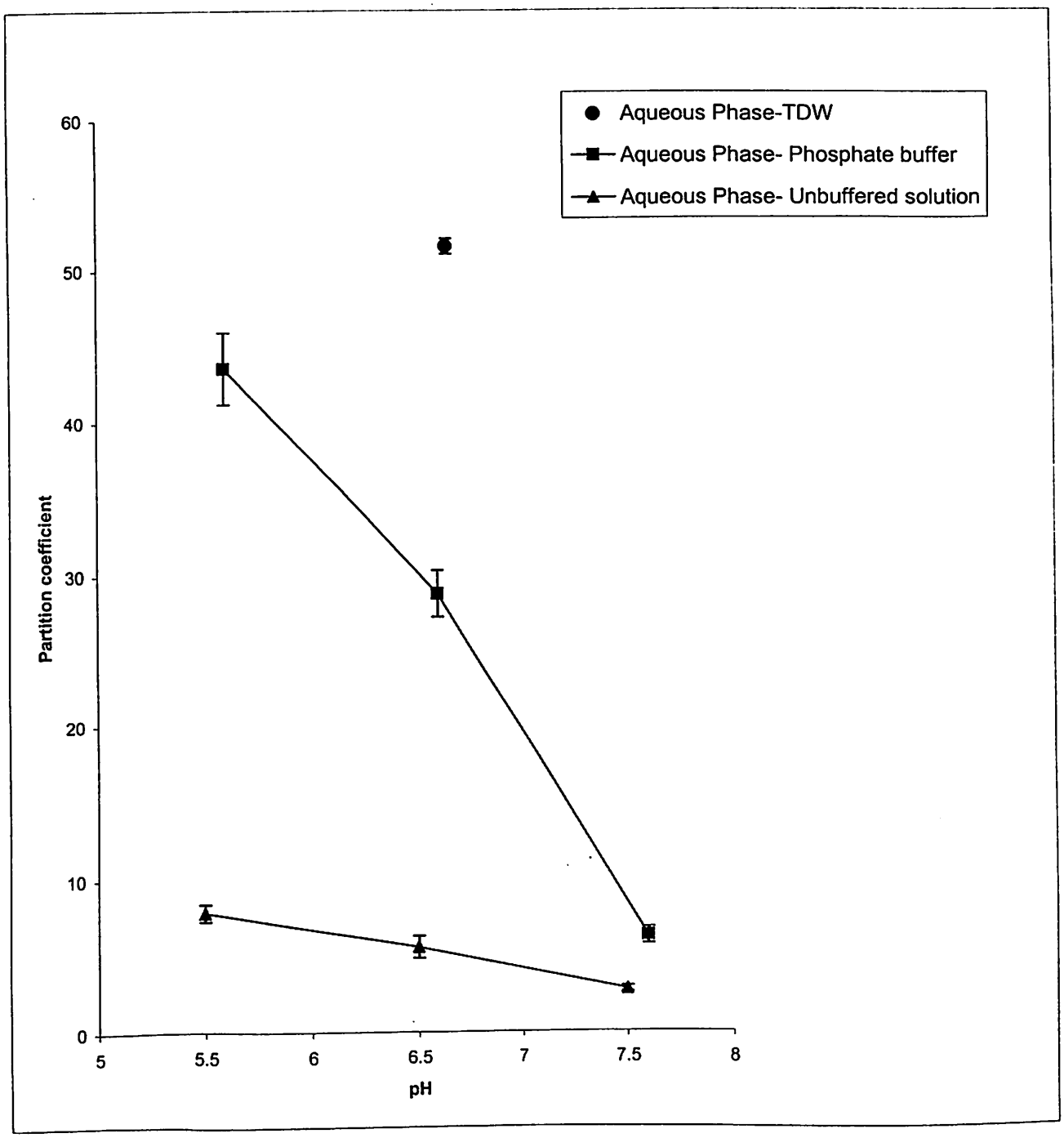

Figure 4.14: Effect of pH (buffered/ unbuffered) of the aqueous phase on the partition coefficient value of flurbiprofen.

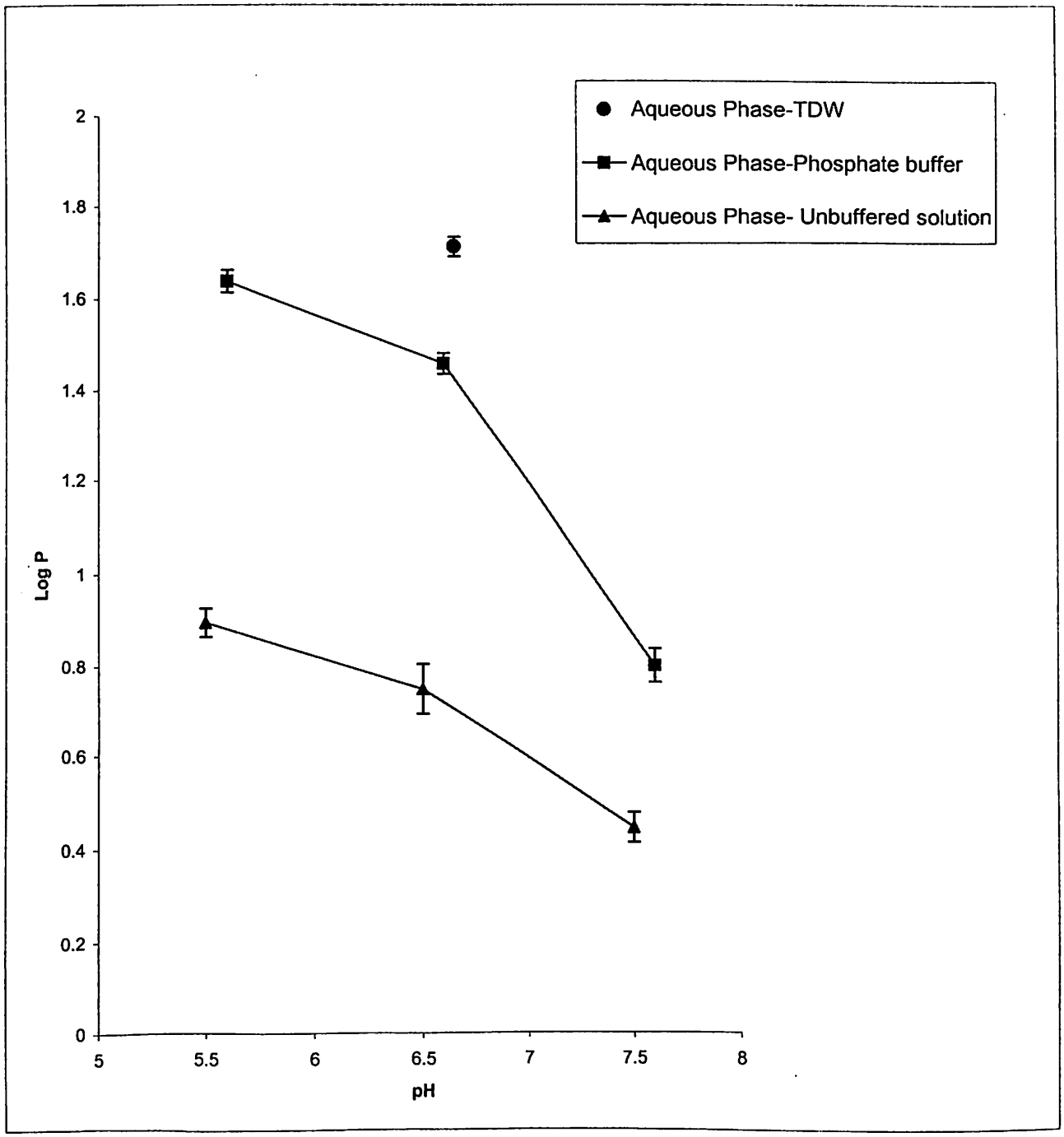

Figure 4.15: Effect of pH (buffered/ unbuffered) of the aqueous phase on the Log P value of flurbiprofen.

CHAPTER 5

POLYMERIC GEL AND OIL BASED FORMULATIONS

5. POLYMERIC GEL AND OIL BASED FORMULATIONS

5.1 Introduction

Ocular administration of drug in formulations like aqueous or oily drops in the form of solutions or suspensions fail to maintain the therapeutic drug level for the desired length of time within the ocular tissues and fluids. Less than 10 % of the topically applied dose is absorbed into the target ocular tissues as a result of constant tear flow and naso-lachrymal drainage. This drainage of tear along with the instilled solution can account for the removal of approximately 75-90 % of the instilled dose in human eye in a matter of 90 sec, and thus play a major role in naso-lachrymal absorption of a good amount of drug from instilled dose (Lee and Robinson, 1986) and decrease in ocular bioavailability of the drug from topical formulations (Chrai et al., 1973; Chrai et al., 1974). As a result relatively concentrated solution is required for instillation into the eye cavity to achieve an adequate drug level for satisfactory therapeutic effect (Lee and Robinson, 1979; Sieg and Triplett, 1980; Stratford et al., 1983; Lee and Carson, 1985; Lee and Robinson, 1986). The frequent periodic instillation of eye drops becomes a necessity to maintain a minimum level of medication for an intended period of time. Subsequently, a higher dose is potentially absorbed into the systemic circulation resulting in undesirable systemic side effects. The low absorbed fraction of the applied dose, into the ocular tissues, further undergoes rapid elimination from the intra ocular tissues and loss through the canal of Schlemn or via absorption through the ciliary body (Robinson, 1993).

Increasing the contact time of the formulation, and thus the drug, with the corneal membrane by using polymeric system provides a plausible solution for overcoming most of the above problems without compromising on patient compliance. A review of literature also revealed attempts by workers in developing mucoadhesive polymeric gel based ophthalmic formulations (Davis, et al., 1991; Saettone, et al., 1982, 1989; Marriot and Gregory, 1990, Sajeev at al, 2001a, 2002a). For most of the viscous solutions the optimum viscosity has been found to range between 12 to 15 cps (1.2 to 1.5 mPa) (Patton and Robinson, 1975). A viscosity beyond 15 cps has been shown to cause poor product-tear intermixing accompanied by distortion of the optical surface, producing visual disturbances for the patient (Eriksen, 1980).

In the present study an attempt was made to develop extended release ophthalmic formulations in the form of mucoadhesive gels for flurbiprofen using various mucoadhesive polymers like

polycarbophil (PCB), methylcellulose (MC), hydroxy propyl methyl cellulose (HPMC), sodium carboxymethylcellulose (NaCMC), polyvinyl pyrolidone (PVP), polyvinyl alcohol (PVA) and polyacrylamide (PAA) as 1 and 5 % w/v gels containing 750 µg/ml of the drug. Formulations of flurbiprofen (750 µg/ml) were also prepared using highly pure (parentral grade) vegetable oils like, cottonseed, olive, arachis, castor, sesame and linseed. Another type of formulation manufactured was using a combination of polymer and vegetable oils. These formulations consisted of 5% w/v of HPMC, PCB or MC along with the vegetable oils used in earlier studies except cottonseed oil. The formulated gels were evaluated for appearance, clarity, odour, pH, drug content, flow property, spreadability and mucoadhesiveness. Various parameters like proportion and type of the retardant mucoadhesive polymer, type of oil and polymer-oil combination were studied for their effect on release character from designed formulations by *in-vitro* dissolution studies. Effect of sterilization on stability of the drug along with polymers, vehicles and its release from the designed formulations was also investigated. Also investigated was batch reproducibility and stability on storage and the effect of storage conditions on the shelf life of selected formulations.

5.2 Experimental

Materials

Pure flurbiprofen was obtained as a gift sample from Optho Remedies Pvt. Ltd., Allahabad. Polycarbophil (Noveon AA1) was gifted by BF Goodrich, Mumbai. All the other polymers, chemicals and reagents used were pharmaceutical or analytical grade and were used as received. Parentral grade vegetable oils were used in the present study.

Equipment

A UV-visible-NIR spectrophotometer (Jasco, Tokyo, Japan, model V-570) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells with Jasco spectra manager software was used for all absorbance measurements for UV analysis. A scanning spectrofluorimeter (Jasco, Tokyo, Japan, model FP-777) with built-in compatible software, link search mode, multiple PMT gain mode, automatic wavelength accuracy of 1.5 nm, range 220-750 nm and 10 mm quartz cells was used for fluorescence intensity measurement in case of spectroflourimetric analysis. *In-vitro* dissolution studies were carried out in an in-house

fabricated double-chambered water jacketed (to maintain the desired temperature of 37 ±1°C) glass dissolution flask with an internal volume of 20 ml and fitted with magnetic stirrer. Sterilization studies were carried out in industrially fabricated moist heat sterilizer or alternatively in hot air oven.

Analytical Method

UV-visible spectrophotometric method of analysis as described in Chapter 3 (UV spectrophotometric method) (Sajeev et al., 2002) was employed for the analysis of the drug using phosphate buffer (pH 6.4) at 248 nm in the concentration range 1-20 µg/ml. The spectroflourimetric method described in Chapter 3 (Sajeev et al, 2001b) using 1:1 mixture of methanol and 0.1N H_2SO_4 at the λ_{ex} and λ_{em} of 250 nm and 314 nm respectively was employed for analysis of dissolution study samples with very low drug concentration.

Characterization of bulk drug

The obtained flurbiprofen in bulk were characterized by various official tests (IP, 1996; BP, 1998; USP, 2000) of identification and were analyzed by UV spectrophotometric method as described in the previous section. The IR spectrum obtained using IR spectrophotometer was compared with that of the standard. Effects of various formulation excipients on the UV absorbance and stability of the respective drugs were also studied and are reported in chapter 4.

Preparation of polymeric gels

Extended release polymeric gel type formulations of flurbiprofen were prepared using varying proportion (1 % and 5 % w/v of the final volume) of polymers namely, PCB, MC, HPMC, NaCMC, PVP, PVA and PAA each containing 750 µg/ml of the drug. For preparing the above formulations, finely pulverized drug was dissolved in an iso-osmotic phosphate buffer solution of pH 7.4 to obtain a concentrated stock solution of the drug and filtered. Benzyl alcohol at 0.5 % v/v in the final formulation was used to solubilise the drug and also for its preservative property. Required amount of accurately weighed polymer powder (to produce intended strength) was dispersed in sufficient quantity of phosphate buffer pH 7.4 at 80-90 °C and then rapidly cooled. The dispersion was left at 10-15 °C for 24 hours to ensure complete hydration and for effecting solution formation. To the hydrated dispersion of the polymer required amount of concentrated drug solution (for final concentration of 750 µg/ml) was added with vigorous

stirring on a magnetic stirrer fitted with warm water bath maintained at 60°C. Final volume of the homogenous blend was made up using phosphate buffer pH 7.4. The obtained gels were packaged into sealed vials, autoclaved (121 °C for 15 mins) and stored in refrigerator (4 °C) when not in use. An aqueous drop prepared by the above method but without the polymer served as control in all further studies. The composition of various formulations prepared by this method is presented in Table 5.1.

Preparation of oil based formulations

Extended release oil based formulations of flurbiprofen were prepared using different types of vegetable oils namely, cottonseed, olive, arachis, castor, sesame and linseed oils each containing 750 µg/ml of the drug. For preparing the above formulations the selected oils were sterilized at 160 °C for 1.5 hours in a hot air oven and then used for preparing the formulations aseptically. Finely pulverized drug was dissolved in the selected oils with vigorous stirring on a magnetic stirrer fitted with warm water bath maintained at 45°C on laminar flow hood. Benzyl alcohol at 0.5 % v/v in the final formulation was used to solubilise the drug and also for its preservative property and the final volume was made up with the respective oil. The obtained formulations were packaged into sealed vials and stored at room temperature (25 ± 4 °C) when not in use. An aqueous drop prepared by the above method in phosphate buffer pH 7.4 alone and without the oil served as control in all further studies. The composition of various formulations prepared by this method is presented in Table 5.2.

Preparation of formulations using polymeric gels in combination with vegetable oils

Extended release polymeric gel in combination with vegetable oil based formulations of flurbiprofen were prepared using 5% w/v concentration of HPMC, PCB or MC in combination with the vegetable oil used for formulation in the preceding section, each containing 750 µg/ml of the drug. Cotton seed oil was not used in this category of formulations as it was found to be inelegant in color and appearance for ocular purpose. For preparing the above formulations finely pulverized drug was dissolved in sufficient volume of iso-osmotic phosphate buffer solution of pH 7.4 to obtain a concentrated stock solution of the drug and filtered. Benzyl alcohol (at 0.5 % v/v in the final formulation) was used to solubilise the drug and also for its preservative property. Aliquot amount of accurately weighed polymer powder (adequate to give final concentration of 5 % w/v) was dispersed in sufficient quantity of phosphate buffer pH 7.4 at 80-

90 °C and then rapidly cooled. The dispersion left at 10-15 °C for 24 hours to ensure complete hydration. To the hydrated dispersion of the polymer aliquot amount of concentrated drug solution (for a concentration of 750 µg/ml in the final formulation) was added with vigorous stirring on a magnetic stirrer fitted with warm water bath maintained at 60°C. Final volume was made up to 50 ml using phosphate buffer pH 7.4. To this homogenous blend selected vegetable oil was added in parts with vigorous stirring on a magnetic stirrer fitted with warm water bath maintained at 45 °C to emulsify the components and the volume slowly made up to 100 ml. The obtained formulations were packaged into sealed vials, autoclaved (121°C for 15 mins) and stored in refrigerator (4 °C) when not in use. An aqueous drop prepared by the above method but without the polymer and the oil served as control in all further studies. The composition of various formulations prepared by this method is presented in Table 5.3.

Characterization of designed formulations

Developed formulations were subjected to following physical characterization studies and the results are presented in Table 5.4, 5.5 and 5.6 for polymeric gel based, oil based and polymeric gel in combination with oil based formulation respectively.

(a) Appearance, clarity and odour: All the formulations prepared were observed against a black/white background for appearance and clarity. Different research scholars working in the laboratory tested freshly prepared formulations for any odour. The response of a maximum out of five respondents was taken as the final odour.

(b) Pourability and spreadability: Pourability was evaluated checking the ease at which the formulation can be poured from the container after proper shaking of the container. Spreadability (expressed in cm^2/min) was determined by calculating the area of spread in unit time obtained by placing a constant volume of 2 ml formulation between two very thin 12 cm × 12 cm glass sheets with an applied external force of 1.5 g in 2 mins.

(c) pH measurement: pH measurements of all the freshly prepared formulations were measured using Elico pH meter under room temperature using combined glass electrode.

(d) Mucoadhesiveness: It was determined on an in-house fabricated modified pan balance apparatus. The right hand side pan of the balance was replaced with a hanging circular wooden block kept in contact with another stationary circular wooden block of identical dimensions. The

pan was balanced in such a way that under no weight condition the right hand side hanging wooden block just touches the stationary block. Freshly excised guinea pig ileum were mounted on both the wooden blocks and properly secured with nylon thread so that the mucosal surface of both the tissues are in contact with each other over an area of 5 cm^2. Care was taken to prevent any damage to the mucin layer on the mucosal surface of the ileum. Between the two mounted tissues surface 1 ml of the test formulation was placed and the two blocks held tightly together for 5 mins so as to provide sufficient time for the preparation to interact with the mucin layer of the tissue. Now, on the left hand side pan weights were increased carefully so as to avoid any mechanical jerk. Mucoadhesiveness (expressed as g/cm^2) of the formulation was determined as the weight (g) at which the two closely held tissues secured on the wooden block with a contact surface area of 5 cm^2 just separate out.

(e) Drug content: For determining drug content (µg/ ml) in all the prepared formulations, 1 ml of the formulation was taken in duplicate from three different batches and was extracted using sufficient volume (< 8 ml) pH 6.4 phosphate buffer for 5 min on a platform shaker. The volume of the content was made up to 10 ml and then transferred to centrifuge tube and centrifuged for 5 mins at 4000 rpm. The supernatant clear aqueous phase separated and the solid sediments collected for further processing. The solid residues obtained after centrifugation of the previous step were extracted consecutively two more times using pH 6.4 phosphate buffer as described above and the supernatant of all the three extractions pooled and volume made up to 50 ml. This solution was suitably diluted to obtain samples in the linearity range of the UV spectrophotometric method and analyzed. From the absorbance and concentration data the drug content was calculated on average volume basis and expressed in µg/ml.

(f) Sterility: All the manufactured formulations after autoclaving were tested for compliance with Pharmacopeial requirement for sterility. The formulations were tested for the absence of bacteria, fungi, yeast and mould as per the testing procedure enlisted in USP-2000.

***In-vitro* dissolution studies**

In-vitro dissolution studies were carried out in an in-house fabricated double-chambered water jacketed (to maintain the desired temperature of 37 ± 1°C) glass dissolution flask with an internal volume of 20 ml and fitted with magnetic stirrer. The assembly was mounted on a Remi model magnetic stirrer with thermostatic temperature control. The dissolution media used was 20 ml

phosphate buffer pH 7.4 at 37 ± 1°C. Stirring speed was set at very low level to give very mild shearing action corresponding to the situation in eyes. A 5 ml sample was collected and replaced with fresh media at different time intervals and analyzed. Volume of sample withdrawn was kept high so as to simulate sink condition inside the dissolution flask. The results of the study are presented in Table 5.7 to 5.12. Plot between cumulative percentage flurbiprofen released vs. time from various designed formulations are shown in Figure 5.1 (polymeric gel formulations using PAA and HPMC), Figure 5.2 (polymeric gel formulations using PCB and NaCMC), Figure 5.3 (polymeric gel formulations using MC, PVP and PVA), Figure 5.4 (vegetable oil based formulations), Figure 5.5 (oil based formulations containing PCB), Figure 5.6 (oil based formulations containing MC) and Figure 5.7 (oil based formulations containing HPMC).

Batch reproducibility

To study batch variation in the manufacturing of the designed formulations three batches of each formulation were manufactured and evaluated for its physical characteristic as described earlier and the results are presented in Table 5.4, 5.5 and 5.6 for polymeric gel based, oil based and polymeric gel in combination with oil based formulations respectively. *In-vitro* release data pertaining to reproducibility studies from duplicate study per three batches of each formulations are presented in Table 5.7 (polymeric gel formulations using PAA, HPMC, PCB and NaCMC), Table 5.8 (polymeric gel formulations using MC, PVP and PVA), Table 5.9 (vegetable oil based formulations), Table 5.10 (oil based formulations containing PCB), Table 5.11 (oil based formulations containing MC) and Table 5.12 (oil based formulations containing HPMC).

Effect of sterilization method on the stability and release kinetics

To study the effect of method of sterilization on the stability of the drug and release characters, the drug content and dissolution profile before and after sterilization for the formulations were studied. Comparative release profiles of flurbiprofen release from selected formulations (PCB-5, MC-5, MC-SO and HPMC-OO), selected on the basis of drug release profile from earlier studies, before and after sterilization, are presented in Figure 5.8. The determined drug content before and after sterilization process for above selected formulations along with the results of one-way ANOVA is enlisted in Table 5.19.

Stability studies

Stability studies of the formulation were carried out at different temperature and RH conditions [controlled room temperature (CRT)- 25 ± 3 °C and 55 ± 15 % RH; 40° C and 45° C, 75 % RH] as per the procedure enlisted in chapter 4. The prepared formulation samples were sealed in 2 ml amber colored, neutral glass ampoules and exposed to above storage conditions. The samples in triplicate were withdrawn at predetermined time intervals (0, 1, 2, 3, 4, 5, 6 months) and analyzed after suitable dilution for flurbiprofen content.

From the absorbance values of the samples, after adjusting for dilution factor, the residual amount of flurbiprofen was calculated on average concentration basis. From the slope of the plot between log % residual flurbiprofen and time (as shown in Figure 5.9 to 5.21 for various developed formulations) the degradation rate constant (K_{deg}) was calculated as $K_{deg} = -2.303 \times$ Slope. From the Arrhenius plot (Figure 5.22 to 5.24) the degradation rate constant at 25 °C was predicted for various formulations and compared with the observed K_{CRT}. Observed K_{deg} at CRT (K_{CRT}) and predicted K_{25} were used to determine the shelf life or t_{90} (time for drug content to fall to 90%) of the product as presented in Table 5.20, 5.21 and 5.22 for polymeric gel based, oil based and polymeric gel in combination with oil based formulations respectively. A comparative analysis of the predicted and observed shelf life for various developed formulations are presented in Figure 5.25 to 5.27.

5.3 Results and discussion

Characterization of bulk drug

The obtained flurbiprofen passed the tests of identification and analysis as per official tests (IP, 1996; BP, 1998; USP, 2000). The IR spectrum obtained using IR spectrophotometer was comparable with that of the standard. The formulation additives (in the concentration used) did not affect the stability and analysis.

Characterization of designed formulations

Physical properties of the designed polymeric gel based ophthalmic formulations of flurbiprofen are presented in Table 5.4. The 1 % w/v and 5% w/v gels of flurbiprofen prepared using different mucoadhesive polymers were devoid of any odour except in case of formulation prepared with

MC and PAA (at both 1% and 5% w/v level), which possessed faint odour. In terms of appearance and clarity when these formulations were compared to the colorless and transparent nature of flurbiprofen aqueous drop formulations prepared using PCB, HPMC, PVP, PVA at both 1 % w/v and 5 % w/v concentration and MC, PAA and NaCMC at 1 % w/v were found to be colorless and translucent. On the other hand gels of NaCMC, PAA and MC at 5 % w/v concentration showed colorless to white/turbid appearance and clarity. The degree of hydration of the polymers with aqueous phase was found to affect the appearance and clarity of the gels. The clarity decreased or turbidity increased with decrease in the time for which the polymers were soaked with the aqueous phase at 10-15 °C. This further confirmed reports of earlier workers (Chrai and Robinson, 1974; Patton and Robinson, 1975) about the need for polymer hydration for producing elegant ophthalmic formulations. The formulations were evaluated for the pourability characteristics on the basis of the ease with which the formulation can be poured from the container after proper shaking. All the formulations at 1 % and 5 % w/v polymeric concentration were found to possess very good pourability (flow) characteristics with 1 % w/v gels more easily pourable than 5 % w/v gels in all the cases. Though 10 % w/v polymeric gels using same polymers were prepared but were not selected for further study since they possessed very poor flow characteristics, which would hamper dose dispensation into the lower cul-de-sac.

The mean spreadability value did not vary much between the polymer type though it increased when the respective polymer proportion was increased from 1 to 5 % w/v. The mean spreadability of 1% gels varied between 8.0 ± 0.5 cm^2/min (PCB-1) to 9.0 ± 0.8 cm^2/min (MC-1) and for 5 % w/v gels varied from 3.1 ± 0.5 cm^2/ min (NaCMC-5) to 3.9 ± 0.7 cm^2/min (in case of PVA-5). Low standard deviations of the determined spreadability values for three different batches (Table 5.4) indicate the absence of batch-to-batch variation in formulation consistency. All the gels were found to be weakly basic with a pH ranging from 6.8 to 7.8. Mucoadhesive property of the designed gels of flurbiprofen determined on guinea pig ileum was found to be directly proportional to the concentration of the polymer in the gel (Table 5.4) but a 5 times increase in the concentration of the polymer in the gel (from 1 % w/v to 5 % w/v) resulted in approximately two fold increase in the mucoahesiveness of the gel. The drug content uniformity (Table 5.4) of the developed polymeric gel type formulations were found to be satisfactory and varied ± 4.0 % of the theoretical claim indicating the reliability and reproducibility of the manufacturing process. Sterility studies (as per USP-2000) of the designed sterile formulations

demonstrated absence of contamination from bacteria, fungi, yeast and mould thus validating the efficiency of the sterilization process employed.

Table 5.5 represents the physical properties of the designed ophthalmic formulations of flurbiprofen in vegetable oils. All the oil based formulations possessed a characteristic odour peculiar to each oil type used. Formulations prepared using olive oil and arachis oil were found to be transparent in nature with light greenish yellow and pale light yellow tint respectively. When sesame oil was used the formulation possessed a translucent clarity with characteristic yellow tint. Castor oil and linseed oil formulations were viscous and turbid. Only cottonseed oil based preparation was found to be dark brown-black and opaque because of this reason cotton seed oil was not used for formulating polymeric gels in combination with vegetable oil type formulations as it was considered to possess inelegant appearance for ophthalmic purpose. Flow property in all case was found to be satisfactory as all formulations were easily pourable after shaking the container. The pH varied from 7.6 to 7.8 and mucoadhesiveness was less than 1 g/cm^2 in all cases. The drug content uniformity (Table 5.5) of the developed oil based formulations varied ± 4.0 % of the theoretical amount indicating the reliability and reproducibility of the manufacturing process. As in case of polymeric gels absence of contamination from bacteria, fungi, yeast and mould was demonstrated by the sterility studies (as per USP-2000) of the designed formulations.

Physical properties of the designed polymeric gel in combination with vegetable oil based ophthalmic formulations of flurbiprofen are presented in Table 5.6. All formulations possessed the characteristic odour peculiar to each oil type. Appearance and clarity of polymer in combination with vegetable oil based formulations (Table 5.6) when compared with pure vegetable oil based formulations (Table 5.5) and polymeric gel (5 % w/v) (Table 5.4) reveal that formulations prepared using either HPMC, PCB or MC in five vegetable oils namely, olive oil, arachis oil, castor oil, sesame oil and linseed oil possessed properties predominantly of the oil type used in the formula. As earlier discussed (in case of polymeric gel type formulations) the degree of hydration of the polymers with aqueous phase was found to affect the appearance and clarity of the formulations. Pourability of the formulations, which was evaluated on the basis of the ease with which the formulation can be poured from the container after proper shaking, was found to be satisfactory for all the formulations. The mean spreadability of the PCB based formulations varied between 13.9 ± 0.3 cm^2/ min (PCB-CO) to 18.4 ± 0.6 cm^2/ min (PCB-OO),

for MC based formulations from 14.0 ± 0.9 cm^2/ min (MC-SO) to 17.2 ± 0.1 cm^2/ min (MC-LSO) and for HPMC based formulations from 14.8 ± 0.6 cm^2/ min (HPMC-OO) to 19.2 ± 0.4 cm^2/ min (HPMC-CO). All the formulations were found to be weakly basic with a pH ranging from 6.8 to 7.8. No significant difference (at $p < 0.05$) was observed in the mucoadhesive property between various designed formulations in this category (Table 5.6). This category of formulations (Table 5.6) showed a wider variation of drug content uniformity with a maximum ± 5.0 % variation. Sterility studies (as per USP-2000) result indicated the absence of contamination from bacteria, fungi, yeast and mould in the designed formulations after autoclaving at 121 °C for 15 mins.

***In-vitro* dissolution studies**

Dissolution study data indicate that compared to aqueous drops the duration of drug release was increased in case of all the designed formulations. Mean cumulative percentage of flurbiprofen released along with standard deviation from polymeric gel formulations (of three batches with duplicate determination per batch) in pH 7.4 phosphate buffer prepared using PAA, HPMC, PCB and NaCMC is presented in Table 5.7 and for formulations prepared using MC, PVP and PVA is presented in Table 5.8. The cumulative percentage released in the first 5 mins was used to compare the initial release characteristics. The duration of release was extended with the increase in the percentage of polymer in the gel from 1 % w/v to 5 % w/v in case of all the polymers. In case of gels prepared from PAA (1% w/v) the initial release in first 5 mins was found to be 29.1 ± 2.3 % (PAA-1) however complete release was obtained within 60 mins. Increasing the percentage of PAA to 5 % w/v (PAA-5) the initial release (5 mins) was decreased to 4.5 ± 3.3 % and the duration of release extended up to 300 mins with 97.8 ± 2.5 % release (Figure 5.1). In case of gels prepared with HPMC, initial release in 5 mins with 1 % w/v polymer (HPMC-1) was found to be high (63.5 ± 2.0 %) and complete release observed within 45 mins. Increasing the percentage of HPMC to 5 % w/v though decreased the initial release to 12.6 ± 1.2 % but the duration of release was extended beyond 90 mins (96.2 ± 4.1 % release) (Figure 5.1).

In case of PCB gels (PCB-1 and PCB-5), increasing the proportion of the polymer from 1 to 5 % w/v did not seem to have a drastic effect. The initial release (in 5 mins) from formulations containing 1 and 5 % w/v PCB gel was found to be high at 34.7 ± 3.5 % and 30.4 ± 2.5 % respectively with duration of release extended up to 180 and 240 mins respectively (Figure 5.2).

As observed in case of HPMC-1 the formulation prepared with 1 % w/v NaCMC (Na CMC-1) showed high initial release (within 5 mins) of 67.3 ± 1.9 % but the duration of release was extended up to 90 mins. Increasing the percentage of NaCMC to 5 % w/v (NaCMC-5) decreased the initial release only to 28.6 ± 2.9 % and the duration of release was extended only up to 180 mins (Figure 5.2). At 1 % w/v level, MC formulation (MC-1) showed the maximum extension of release up to 240 mins inspite of the high initial release of 53.1 ± 1.7 % in 5 mins. Upon increasing the percentage of MC to 5 % w/v (MC-5) the initial release was decreased to 25.3 ± 1.5 % and the duration of release extended up to 420 mins with 98.5 ± 3.9 % release (Figure 5.3). With PVP and PVA gels at 1 % w/v concentration (PVP-1 and PVA-1) high initial release of 78.7 ± 1.7 % and 75.9 ± 2.5 % respectively was observed with complete release within 60 mins. But increasing the proportion of the polymer to 5 % w/v (PVP-5 and PVA-5) the duration of release was extended up to 240 mins with 56.2 ± 2.3 % and 58.5 ± 2.1 % initial release respectively (Figure 5.3).

Of all the polymers studied for the development of polymeric gel type formulations of flurbiprofen, MC seems to be the best with maximum retardation of the duration of release up to 7 hours (in case of MC-5). All the polymers showed little control on the initial release with PVP and PVA showing high initial release at both 1 % w/v and 5 % w/v. In case of PAA, HPMC, NaCMC and MC, increasing the proportion of the polymer to 5 % w/v considerably controlled the high initial release observed at 1 % w/v level. On the contrary in case of PCB increasing the percentage of polymer from 1 to 5 % w/v did not have any significant effect.

The dissolution data from different vegetable oil based formulations (Table 5.9 and Figure 5.4) exhibited mostly similar release pattern with release extended up to 300 mins in case of formulations prepared using cotton seed oil (CSO), olive oil (OO), arachis oil (AO), castor oil (CO) and linseed oil (LSO) whereas in case of sesame oil formulation (SO) the release was extended only up to 240 mins. When compared to polymeric gel formulations (discussed earlier) the initial release from oil based formulations was not very high with 9.6 ± 2.5 % release in case of OO (being the lowest) and 19.7 ± 3.6 % in case of CSO (being the highest). Initial release in case of AO, CO, LSO and SO was found to be 14.3 ± 1.9 %, 10.7 ± 3.3 %, 14.9 ± 1.4 % and 18.5 ± 2.4 % respectively. The reason for controlled release of flurbiprofen from oily vehicle

could be higher solubility of the drug in the selected oils (Chapter 4) thereby decreasing the partitioning of the drug into the phosphate buffer (pH 7.4).

Based on the dissolution study results of polymeric gel and oil based formulations of flurbiprofen and there physical properties it was decided to study the combined effect of oil and polymer on the release profile of flurbiprofen in phosphate buffer (pH 7.4). PCB, MC and HPMC were selected for formulation as 5 % w/v polymeric gel in combination with vegetable oil (50 % w/v) based ophthalmic preparations of flurbiprofen using olive oil, arachis oil, castor oil, linseed oil and sesame oil.

For PCB based vegetable oil formulations duration of release was extended up to 480 mins (8 hours) in case of PCB-AO, PCB-CO and PCB-SO with slow initial release of 6.3 ± 2.1 %, 10.0 ± 1.9 % and 10.6 ± 2.9 % respectively (Table 5.10 and Figure 5.5). In case of PCB-OO and PCB-LSO the initial release was found to be 7.9 ± 2.3 % and 9.2 ± 2.3 % respectively. The duration of release was extended beyond 480 mins in case of PCB-OO and PCB-LSO with 81.6 ± 3.0 % and 92.6 ± 3.0 % release in 480 mins respectively. MC based vegetable oil formulations of flurbiprofen showed a comparatively higher initial release in first 5 mins as compared to PCB based formulations except in case of MC-SO with only 8.0 ± 2.3 % release in that period (Table 5.11 and 5.6). Other formulations of MC (MC-OO, MC-LSO, MC-AO, MC-CO) showed an initial release of 15.7 ± 2.1 to 23.1 ± 2.5 %. The duration was extended beyond 480 mins in all case except MC-LSO (complete release was observed within 360 mins). The lowest cumulative percentage of flurbiprofen released from these formulations was 53.9 ± 3.9 % in case of MC-SO followed by 64.3 ± 4.2 % in case of MC-CO, 73.3 ± 3.6 % in case of MC-OO and 82.1 ± 1.9 % in case of MC-AO at the end of 480 mins. Formulations prepared using combination of HPMC with vegetable oils showed a slow initial release (Table 5.12 and Figure 5.7) with 4.9 ± 2.3 to 9.0 ± 2.0 % release in first 5 mins. The duration of release was extended beyond 480 mins in case of all the formulations in this category. The fastest release profile was exhibited by HPMC-AO and HPMC-CO with 90.7 ± 3.8 % and 90.1 ± 4.2 % release respectively in 480 mins. Slowest release profile was exhibited by HPMC-OO with only 49.8 ± 3.0 % release in 480 mins. HPMC-SO and HPMC-LSO exhibited an intermediate release rate with 60.1 ± 3.9 % and 78.2 ± 3.4 % release in 480 mins respectively.

Characterization of the release kinetics

In order to describe the kinetics of the drug release from controlled or extended release formulations various mathematical equations are used. Since the controlled release system designed in this study did not fall in the category of insoluble matrix with fixed geometry delivery systems it was considered pertinent to investigate only whether the release rate followed zero or first order release kinetics. Criteria for selecting the most appropriate model was based on goodness of fit of the regression line with correlation coefficient approaching 1.0 and smallest mean sum of squared residuals (Parab, et al, 1986). Using the slope and intercept value of the regression plot, release rate constant and time for release of fixed percentage of the drug from the formulation for comparison purpose were calculated. Upon subjecting the drug release data to the above models in order to establish the drug release mechanism it was found that the drug release from designed polymeric gel type formulations followed first order release kinetics as evident by good 'r' value and very small mean sum of squared residuals (MSSR) value (Table 5.13 and 5.14). Calculated release rate constant (K, in min^{-1}) and time for 50 % ($t_{50\%}$), 70 % ($t_{70\%}$) and 90 % ($t_{90\%}$) of drug release from polymeric gel type formulations are also enlisted in Table 5.13 and 5.14. Amongst 1 % w/v gels the release rate constant for drug release from MC-1 was found to be the slowest with a K value of 5.390×10^{-3} min^{-1}, $t_{50\%}$ of 18 mins, $t_{70\%}$ of 71 mins and $t_{90\%}$ of 274 mins and the fastest release rate was exhibited by PVA-1 with a K value of 5.954×10^{-2} min^{-1}, $t_{50\%}$ of 3 mins, $t_{70\%}$ of less than 5 mins and $t_{90\%}$ of 19 mins. In case of 5 % w/v gels, MC-5 was found to possess the slowest release rate with a K value of 4.800×10^{-3} min^{-1}, $t_{50\%}$ of 104 mins, $t_{70\%}$ of 211 mins and $t_{90\%}$ of 440 mins. The fastest release rate was exhibited by PVP-5 with a K value of 3.228×10^{-2} min^{-1}, $t_{50\%}$ of 4 mins, $t_{70\%}$ of 13 mins and $t_{90\%}$ of 47 mins

Similarly, the drug release from vegetable oil based formulations was also found to follow first order drug release kinetics as proved by high 'r' value and very small mean sum of squared residuals value (Table 5.15). Calculated K value and $t_{50\%}$, $t_{70\%}$ and $t_{90\%}$ of drug release from the formulations are also enlisted in Table 5.15. Among various oil based formulations LSO (linseed oil) exhibited the slowest release kinetics with a K value of 9.530×10^{-3} min^{-1}, $t_{50\%}$ of 61 mins, $t_{70\%}$ of 114 mins and $t_{90\%}$ of 230 mins. The fastest release rate was exhibited by SO (sesame oil) with a K value of 2.302×10^{-2} min^{-1}, $t_{50\%}$ of 42 mins, $t_{70\%}$ of 64 mins and $t_{90\%}$ of 112 mins.

In case of polymeric gel in oil based formulations containing PCB with different vegetable oil, the release mechanism was found to follow first order release kinetics (based on 'r' value and MSSR value) (Table 5.16). Calculated K value and $t_{50\%}$, $t_{70\%}$ and $t_{90\%}$ of drug release from PCB based oil formulations are presented in Table 5.16. The fastest release rate was exhibited by PCB-AO with a K value of 8.280×10^{-3} min^{-1}, $t_{50\%}$ of 66 mins, $t_{70\%}$ of 128 mins and $t_{90\%}$ of 261 mins. In this category of formulations PCB-OO was found to have slowest release rate with a K value of 3.310×10^{-3} min^{-1}, $t_{50\%}$ of 165 mins, $t_{70\%}$ of 319 mins and $t_{90\%}$ of 651 mins. Among MC based oil formulations (Table 5.17) the slowest release rate was shown by MC-SO with a K value of 8.200×10^{-4} min^{-1}, $t_{50\%}$ of 413 mins (6.9 hours), $t_{70\%}$ of 1040 mins (17.3 hours) and $t_{90\%}$ of 2387 mins (39.8 hours) and the fastest in case of MC-LSO with a K value of 1.071×10^{-2} min^{-1}, $t_{50\%}$ of 31 mins, $t_{70\%}$ of 79 mins and $t_{90\%}$ of 182 mins. The calculated K value and $t_{50\%}$, $t_{70\%}$ and $t_{90\%}$ of drug release from MC based oil formulations are presented in Table 5.17.

In case of 5 % w/v HPMC gel type formulation in oil, HPMC-OO was found to possess the slowest release rate with a 'K' value of 1.010×10^{-3} min^{-1}, $t_{50\%}$ of 437 mins (7.3 hours), $t_{70\%}$ of 944 mins (15.7 hours) and $t_{90\%}$ of 2034 mins (33.9 hours) (Table 5.18). The fastest release rate was exhibited by HPMC-AO with a 'K' value of 4.380×10^{-3} min^{-1}, $t_{50\%}$ of 91 mins, $t_{70\%}$ of 208 mins and $t_{90\%}$ of 459 mins (Table 5.18).

Batch reproducibility

Batch to batch variability and reproducibility of the manufacturing process was studied based on evaluation of the physical properties and release characteristics of drug from three batches of each of the designed formulations. The evaluated physical characteristic of the formulations presented in Table 5.4, 5.5 and 5.6 for polymeric gel based, oil based and polymeric gel in combination with oil based formulation respectively showed low value of standard deviation of triplicate sample analysis per batch for three batches of each formulation. Low standard deviations of the determined spreadability, mucoadhesiveness, drug content, pH and pourability values for three different batches of each formulation indicate absence of significant batch-to-batch variation. No significant difference was observed in the release profile of different batches of the developed flurbiprofen formulations as seen from the low value of standard deviation of the cumulative release data at different time points obtained from replicate dissolution studies of

samples from different batches (as discussed in previous sections) indicating that the manufacturing process employed was reliable and reproducible.

Effect of sterilization method on the stability and release kinetics

No effect was observed on the release kinetics of the developed formulations after sterilization. A representative comparative dissolution profile of flurbiprofen release from selected formulations (PCB-5, MC-5, MC-OO, HPMC-SO) before and after sterilization, as shown in Figure 5.8, did not reveal any change in the release profile. Analysis of drug content before and after sterilization process for above selected formulations also did not show any difference as evident from the low calculated F value (based on replicate measurement per batch per formulation) of one-way ANOVA of drug data before and after sterilization (Table 5.19). Further, no detrimental effect of moist heat on the polymer character was also observed. The results of drug content determination and dissolution studies reveal that the method of sterilization does not have adverse effect on the stability and release kinetics of the drug from designed formulations.

Stability studies

Flurbiprofen in polymeric gels (1% and 5% w/v) was found to follow first order degradation rate as can be seen from the linear plot between log percentage drug content remaining and time profile (Figure 5.9 to 5.15) and also from different vegetable oil based formulations (Figure 5.16 to 5.18) and from polymer based oil formulations (Figure 5.19 to 5.21). From the Arrhenius plot (log K_{deg} vs. reciprocal of absolute temperature) (Figure 5.22 for polymeric gel type formulations, Figure 5.23 for vegetable oil based formulations and Figure 5.24 for polymeric gel in combination with different vegetable oil based formulations the degradation rate constant at 25°C (K_{25}) was predicted and compared with the observed K_{CRT}. Observed K at CRT and predicted K_{25} was used to determine the shelf life or t_{90} (time for drug content to fall to 90%) of the product as presented in Table 5.20 for polymeric gel based, 5.21 for oil based and 5.22 polymeric gel in combination with oil based formulations respectively.

Among various polymeric gel type formulations the predicted K_{25} as well as K_{CRT} decreased with increase in percentage of polymer in the formulation except in case of PVP where the observed and predicted degradation rate constant increased with increase percentage of polymer from 1 % w/v to 5 % w/v. Out of 1 % w/v gels NaCMC-1 was found to have the lowest predicted

and observed shelf life of 8.79 and 9.24 months respectively and PVP-1 was found to have the highest predicted and observed shelf life of 15.65 months and 16.30 months respectively (Table 5.20). Among 5 % w/v gels, PVA-5 was found to have the highest predicted and observed shelf life of 17.27 months and 17.98 months respectively and lowest in case of PVP-5 with predicted and observed shelf life of 10.50 months and 10.97 months respectively. A comparative analysis of the predicted and observed shelf life of polymeric gel type formulations of flurbiprofen did not reveal any significant difference (at $p< 0.01$) (Figure 5.25).

The highest shelf life in case of oil based formulations of flurbiprofen was observed in case of SO with the observed (at CRT) and predicted (at 25 °C) shelf life as 25.08 months and 23.99 months respectively. Among oil based formulations, lowest observed and predicted shelf life was observed as 14.52 months and 13.89 months respectively in case of CSO (Table 5.21). An analysis of the predicted and observed shelf life did not show any difference at $p < 0.01$ in case of oil based formulations (Figure 5.26).

The maximum predicted shelf life (t_{90}) for the drug at 25 °C and at CRT for polymer gel in combination with oil based ophthalmic formulations of flurbiprofen was obtained with linseed oil (PCB-LSO, MC-LSO and HPMC-LSO) (Table 5.22). The lowest observed and predicted shelf life in case of PCB with oil based formulation was of PCB-CO (22.66 and 21.68 months respectively). Whereas in case of MC with oil based formulations lowest shelf life was observed in case of MC-OO (19.98 and 19.34 months respectively) and for HPMC with oil based formulation in case of HPMC-AO (19.63 and 18.77 months respectively). No significant difference was observed in the predicted and observed shelf life of polymeric gel in oil type of formulations of the drug at $p< 0.05$ (Figure 5.27).

5.4 Conclusions

The designed and developed polymeric gel based, vegetable oil based and polymeric gel in combination with vegetable oil based formulations of flurbiprofen were found to possess good physical properties in terms of physical appearance, odour, mucoadhesiveness, spreadability, pourability, pH and drug content uniformity. The extent of polymer hydration in the aqueous phase at the time of manufacturing was found to affect the clarity and appearance of the final product and this influenced its elegance and patient acceptability. Low standard deviations of the

determined physical property indicate the absence of batch-to-batch variation in formulation consistency and quality. It also reinstates the reliability and reproducibility of the manufacturing process employed. All the formulations were found to be weakly basic with a pH ranging from 6.8 to 7.8 thus rendering them compatible with tear pH. Mucoahesiveness of the gel was found to increase with increase in the proportion of the polymer in the formulation. Autoclaving at 121 °C for 15 mins in case of aqueous based formulations demonstrated valid efficiency in ensuring the sterility of the formulations. Also the sterilization method did not affect the stability and the release kinetics of the drug.

The duration of release was extended with the increase in the percentage of polymer in the gel (from 1 % w/v to 5 % w/v) in case of all the polymers. Of all the polymers studied for the development of polymeric gel type formulations of flurbiprofen MC seems to be the best with maximum extension of the duration of release up to 7 hours (MC-5). The dissolution data from different vegetable oil based formulations exhibited mostly similar release pattern with release extended up to 300 mins except for SO (240 mins). The probable reason for controlled release of flurbiprofen from oily vehicle could be higher solubility of the drug in the selected oils (Chapter 4) thereby decreasing the partitioning of the drug into the phosphate buffer (pH 7.4). For PCB based vegetable oil formulations the duration of release was extended beyond 480 mins (8 hours) in case of PCB-OO and PCB-LSO, with only 81.6 % and 92.6 % release in 480 mins respectively. In case of MC based vegetable oil formulations comparatively higher initial release in first 5 mins was observed and the duration was extended beyond 480 mins in all case except MC-LSO (lasted for 360 mins). Only 53.9 % and 64.3 % drug was released from MC-SO and MC-CO respectively by 480 mins. The duration of release was extended beyond 480 mins in case of all the formulations of HPMC with vegetable oils with only 49.8 % and 60.1 % drug release from HPMC-OO and HPMC-SO respectively at the end of 480 mins. The prolonged duration of release of flurbiprofen from this category of formulations is probably due to the dispersion of the drug loaded oil globules in polymeric gels.

The release profile was found to follow first order release kinetics in case of all the designed formulations as demonstrated by highly significant goodness of fit of the regression line (correlation coefficient approaching 1.0) and smallest mean sum of squared residuals. However the duration of release was extended maximum with satisfactory initial release when dual retardant system of polymer and oil was used. Flurbiprofen in the designed formulation matrix

was found to be fairly stable with predicted shelf life at 25 °C varying from 8.79 to 15.65 months and from 10.50 to 17.27 months in case of 1 % w/v and 5 % w/v polymeric gels respectively. The predicted shelf life varied between 13.89 and 23.99 months in case of oil based formulations and between 18.77 and 31.88 months in case of various polymeric gel in oil type formulations. No significant difference was observed in the predicted and observed shelf life of polymeric gel in oil type of formulations of the drug at $p< 0.05$.

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Table 5.1: Composition of various polymeric gel type ophthalmic preparations of flurbiprofen

Formulation code	Components[a]			
	Flurbiprofen (mg)	Polymer[b]	Benzyl alcohol (ml)	Phosphate buffer[c] (pH 7.4)
Aqueous drop	75.0	Absent	0.5	q.s.
PCB-1	75.0	PCB-1.0 g	0.5	q.s.
PCB-5	75.0	PCB-5.0 g	0.5	q.s.
MC-1	75.0	MC-1.0 g	0.5	q.s.
MC-5	75.0	MC-5.0 g	0.5	q.s.
HPMC-1	75.0	HPMC-1.0 g	0.5	q.s.
HPMC-5	75.0	HPMC-5.0 g	0.5	q.s.
NaCMC-1	75.0	NaCMC-1.0 g	0.5	q.s.
NaCMC-5	75.0	NaCMC-5.0 g	0.5	q.s.
PVP-1	75.0	PVP-1.0 g	0.5	q.s.
PVP-5	75.0	PVP-5.0 g	0.5	q.s.
PVA-1	75.0	PVA-1.0 g	0.5	q.s.
PVA-5	75.0	PVA-5.0 g	0.5	q.s.
PAA-1	75.0	PAA-1.0 g	0.5	q.s.
PAA-5	75.0	PAA-5.0 g	0.5	q.s.

[a]: In final volume of 100 ml

[b]: PCB- polycarbophil 934 NF, MC- methylcellulose, HPMC- hydroxy propyl methyl cellulose, NaCMC- sodium carboxymethyl cellulose, PVP- polyvinyl pyrolidone, PVA- polyvinyl alcohol, PAA- polyacrylamide

q.s.- Quantity sufficient to make the final volume of 100 ml

Table 5.2: Composition of various oil based ophthalmic preparations of flurbiprofen

Formulation code	Components[a]		
	Flurbiprofen (mg)	**Benzyl alcohol (ml)**	**Vegetable oil[b] (q.s to 100 ml)**
Aqueous drop[c]	75.0	0.5	Absent
CSO	75.0	0.5	Cotton seed oil
OO	75.0	0.5	Olive oil
AO	75.0	0.5	Arachis oil
CO	75.0	0.5	Castor oil
SO	75.0	0.5	Sesame oil
LSO	75.0	0.5	Linseed oil

[a]: In final volume of 100 ml
[b]: CSO- cottonseed oil, OO- olive oil, AO- arachis oil, CO- castor oil, SO- sesame oil, LSO- linseed oil
[c]: Incase of aqueous drops volume was made up using phosphate buffer pH 7.4
q.s.- Quantity sufficient to make the final volume of 100 ml

Table 5.3: Composition of various polymeric gel in vegetable oil based ophthalmic preparations of flurbiprofen

Formulation code	Components[a]			
	Flurbiprofen (mg)	**Polymer[b] (5 g)**	**Benzyl alcohol (ml)**	**Vegetable oil[c] (50 ml)**
Aqueous drop[d]	75.0	Absent	0.5	Absent
HPMC-OO	75.0	HPMC	0.5	Olive oil
HPMC-AO	75.0	HPMC	0.5	Arachis oil
HPMC-CO	75.0	HPMC	0.5	Castor oil
HPMC-SO	75.0	HPMC	0.5	Sesame oil
HPMC-LSO	75.0	HPMC	0.5	Linseed oil
PCB-OO	75.0	PCB	0.5	Olive oil
PCB-AO	75.0	PCB	0.5	Arachis oil
PCB-CO	75.0	PCB	0.5	Castor oil
PCB-SO	75.0	PCB	0.5	Sesame oil
PCB-LSO	75.0	PCB	0.5	Linseed oil
MC-OO	75.0	MC	0.5	Olive oil
MC-AO	75.0	MC	0.5	Arachis oil
MC-CO	75.0	MC	0.5	Castor oil
MC-SO	75.0	MC	0.5	Sesame oil
MC-LSO	75.0	MC	0.5	Linseed oil

[a]: In final volume of 100 ml
[b]: HPMC- hydroxy propyl methyl cellulose, PCB- polycarbophil 934 NF, MC- methyl cellulose
[c]: OO- olive oil, AO- arachis oil, CO- castor oil, SO- sesame oil, LSO- linseed oil
[d]: In case of aqueous drops volume was made up using phosphate buffer pH 7.4

Table 5.4: Physical properties of designed polymeric gel type ophthalmic preparation of flurbiprofen

Formulation code	Physical properties[a]				
	Appearance/ Clarity	Odour	Mucoadhesiveness[b] (g/cm^2)	Spreadability[b] (cm^2/min)	Drug content[b] (% w/v)
Aqueous drop	Colorless/ Transparent	Absent	-	-	100.5 ± 0.2
PCB-1	Colorless/ Translucent	Absent	2.5 ± 0.8	8.0 ± 0.5	101.7 ± 2.7
PCB-5	Colorless/ Translucent	Absent	6.5 ± 1.2	3.5 ± 0.4	99.8 ± 1.7
MC-1	Colorless/ Translucent	Faint	3.3 ± 0.3	9.0 ± 0.8	101.7 ± 2.3
MC-5	Colorless to white/ Turbid	Faint	5.6 ± 0.9	3.2 ± 0.1	100.8 ± 1.0
HPMC-1	Colorless/ Translucent	Absent	2.9 ± 0.6	8.4 ± 0.2	102.7 ± 2.0
HPMC-5	Colorless/ Translucent	Absent	6.3 ± 1.7	3.7 ± 0.7	99.6 ± 1.3
NaCMC-1	Colorless/ Translucent	Absent	3.1 ± 0.7	8.2 ± 0.8	99.8 ± 2.3
NaCMC-5	Colorless to white/ Turbid	Absent	6.6 ± 0.4	3.1 ± 0.5	100.6 ± 3.0
PVP-1	Colorless/ Translucent	Absent	2.1 ± 0.6	8.9 ± 0.9	101.2 ± 1.0
PVP-5	Colorless/ Translucent	Absent	6.1 ± 1.0	3.6 ± 0.4	99.5 ± 2.0
PVA-1	Colorless/ Translucent	Absent	2.9 ± 0.6	8.5 ± 0.3	99.3 ± 0.3
PVA-5	Colorless/ Translucent	Absent	5.8 ± 1.5	3.9 ± 0.7	98.7 ± 0.7
PAA-1	Colorless/ Translucent	Faint	2.7 ± 0.3	8.3 ± 0.2	102.4 ± 0.6
PAA-5	Colorless to white/ Turbid	Faint	6.1 ± 0.2	3.8 ± 0.4	102.1 ± 0.7

[a]: Flow property in all case was found to be easily pourable after shaking the container
pH in all case was weakly basic (pH 6.8-7.8)
Absence of contamination from bacteria, fungi, yeast and mould

[b]: Average ± S.D. of three batches with duplicate determination per batch

Table 5.5: Physical properties of designed oil based ophthalmic preparations of flurbiprofen

Formulation code	Physical properties[a]		
	Appearance/ Clarity	Spreadability[b] (cm^2/min)	Drug content[b] (% w/v)
Aqueous drop	Colorless/ Transparent	-	100.5 ± 0.2
CSO	Dark brown-black/ Opaque	28.2 ± 1.5	101.3 ± 2.0
OO	Light greenish yellow/ Transparent	23.8 ± 1.4	100.1 ± 2.7
AO	Pale light yellow/ Transparent	29.8 ± 1.8	101.7 ± 2.3
CO	Yellow/ Viscous/ Turbid	23.6 ± 1.1	99.0 ± 0.7
SO	Yellow/ Translucent	28.6 ± 1.2	100.5 ± 1.7
LSO	Colorless to white/ Viscous/ Turbid	23.3 ± 1.7	101.8 ± 2.0

[a]:Flow property in all case was found to be easily pourable after shaking the container
pH in all case was weakly basic (pH 7.6 -7.8)
Absence of contamination from bacteria, fungi, yeast and mould
All oil based formulations have characteristic odour peculiar to each oil type
Mucoadhesiveness in all the case was less than 1 g/cm^2

[b]: Average ± S.D. of three batches with duplicate determination per batch

Table 5.6: Physical properties of designed polymeric gel in combination with vegetable oil based ophthalmic preparations of flurbiprofen

Formulation code	Physical properties[a]			
	Appearance/ Clarity	Mucoadhesiveness[b] (g/cm^2)	Spreadability[b] (cm^2/min)	Drug content[b] (% w/v)
Aqueous drop	Colorless/ Transparent	-	-	100.5 ± 0.2
PCB-OO	Light greenish yellow/ Translucent	4.7± 0.3	18.4 ± 0.6	97.3 ± 2.0
PCB-AO	Pale light yellowish-white/ Translucent	4.1 ± 0.8	16.5 ± 0.2	101.2 ± 2.7
PCB-CO	Yellow/ Viscous/ Turbid	3.6 ± 0.6	13.9 ± 0.3	98.3 ± 2.0
PCB-SO	Yellowish-white/ Translucent	3.9 ± 0.7	18.0 ± 0.7	99.9 ± 1.3
PCB-LSO	White/ Viscous/ Turbid	3.8 ± 0.4	17.9 ± 0.6	101.5 ± 2.0
MC-OO	Light greenish yellow/ Translucent	5.1 ± 0.3	16.8 ± 0.4	102.1 ± 2.7
MC-AO	Pale light yellowish-white/ Translucent	3.5 ± 0.7	16.0 ± 0.1	98.3 ± 3.3
MC-CO	Yellow/ Viscous/ Turbid	3.6 ± 0.4	15.5 ± 0.3	102.7 ± 1.7
MC-SO	Yellowish-white/ Translucent	3.4 ± 0.6	14.0 ± 0.9	100.4 ± 3.0
MC-LSO	White/ Viscous/ Turbid	4.9 ± 0.4	17.2 ± 0.1	101.0 ± 2.7
HPMC-OO	Light greenish yellow/ Translucent	4.3 ± 1.2	14.8 ± 0.6	100.2 ± 3.0
HPMC-AO	Pale light yellowish white/ Translucent	2.9 ± 1.3	19.2 ± 0.4	100.8 ± 2.0
HPMC-CO	Yellowish-white/ Viscous/ Turbid	4.5 ± 0.4	18.4 ± 0.9	99.0 ± 0.7
HPMC-SO	Yellowish-white/ Translucent	3.6 ± 0.3	16.4 ± 0.6	99.5 ± 2.3
HPMC-LSO	White/ Viscous/ Turbid	3.2 ± 0.4	15.7 ± 0.3	98.2 ± 2.6

[a]: Flow property in all case was found to be easily pourable after shaking the container
pH in all case was weakly acidic (pH 6.8-7.8)
Absence of contamination from bacteria, fungi, yeast and mould
All formulations have characteristic odor peculiar to each oil type used
[b]: Average ± S.D. of three batches with duplicate determination per batch

Table 5.7: Cumulative percentage release of flurbiprofen from polymeric gel formulations prepared using PAA, HPMC, PCB and NaCMC in pH 7.4 phosphate buffer

Time (mins)	Cumulative percentage released[a]							
	PAA-1	PAA-5	HPMC-1	HPMC-5	'PCB-1	PCB-5	NaCMC-1	NaCMC-5
5	29.1 ± 2.3	4.5 ± 3.3	63.5 ± 2.0	12.6 ± 1.2	34.7 ± 3.5	30.4 ± 2.5	67.3 ± 1.9	28.6 ± 2.9
10	38.0 ± 5.6	6.1 ± 4.2	67.6 ± 3.9	20.7 ± 5.6	43.2 ± 4.9	32.6 ± 4.1	70.0 ± 2.3	40.5 ± 5.3
20	60.2 ± 2.3	7.6 ± 5.1	75.8 ± 3.4	30.1 ± 6.1	44.0 ± 5.6	36.4 ± 5.0	75.4 ± 2.6	49.5 ± 4.3
30	71.9 ± 5.0	9.9 ± 4.6	82.7 ± 2.4	41.2 ± 4.5	46.5 ± 2.6	40.1 ± 3.0	81.9 ± 3.5	52.6 ± 2.3
45	97.5 ± 4.7	16.3 ± 5.3	101.7 ± 1.9	55.3 ± 4.7	59.2 ± 5.4	41.1 ± 7.0	85.5 ± 3.8	59.4 ± 5.1
60	101.2 ± 3.2	31.2 ± 1.8		72.6 ± 3.9	62.8 ± 5.1	47.8 ± 2.1	86.7 ± 2.2	65.5 ± 4.1
90		54.2 ± 4.2		96.2 ± 4.1	68.8 ± 3.6	62.3 ± 3.4	99.5 ± 3.3	74.6 ± 6.3
120		74.8 ± 3.9			79.1 ± 2.5	72.5 ± 3.5		88.5 ± 2.8
180		84.8 ± 5.3			98.7 ± 6.2	85.0 ± 3.8		98.2 ± 1.5
240		88.2 ± 3.2				98.3 ± 3.7		
300		97.8 ± 2.5						

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 5.9: Cumulative percentage release of flurbiprofen from oil based formulations prepared using various vegetable oils in pH 7.4 phosphate buffer

Time (mins)	Cumulative percentage released[a]					
	CSO	OO	AO	CO	SO	LSO
5	19.7 ± 3.6	9.6 ± 2.5	14.3 ± 1.9	10.7 ± 3.3	18.5 ± 2.4	14.9 ± 1.4
10	28.2 ± 3.9	19.6 ± 2.9	21.4 ± 2.3	23.6 ± 4.5	23.2 ± 5.6	22.6 ± 1.9
20	37.4 ± 4.5	30.2 ± 3.5	25.9 ± 2.2	24.8 ± 4.1	26.6 ± 4.8	29.3 ± 1.4
30	48.1 ± 4.8	34.1 ± 3.1	31.7 ± 1.5	28.0 ± 5.2	37.6 ± 4.2	36.1 ± 2.1
45	54.8 ± 2.8	45.3 ± 2.4	41.6 ± 2.8	38.6 ± 4.8	40.9 ± 4.3	41.6 ± 2.3
60	66.6 ± 4.7	55.7 ± 3.8	47.4 ± 2.4	49.9 ± 3.9	46.2 ± 3.8	50.2 ± 2.5
90	73.6 ± 5.1	71.7 ± 3.7	72.7 ± 2.5	61.7 ± 3.7	74.6 ± 3.7	57.2 ± 2.8
120	85.1 ± 5.0	78.5 ± 2.4	78.6 ± 1.9	73.6 ± 3.5	90.9 ±4.1	69.1 ± 3.1
180	87.5 ± 3.9	91.7 ± 4.0	85.7 ± 2.0	85.5 ± 4.1	98.7 ± 3.0	83.7 ± 2.9
240	94.3 ± 4.5	96.7 ± 4.1	94.3 ± 2.1	97.9 ± 4.5	103.1 ± 4.9	91.7 ± 2.4
300	101.1 ± 4.4	99.6 ± 2.3	102.9 ± 2.6	103.1 ± 4.9		100.8 ± 1.7

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 5.10: Cumulative percentage release of flurbiprofen from oil based formulations containing PCB in pH 7.4 phosphate buffer

Time (mins)	Cumulative percentage released[a]					
	PCB-OO	PCB-LSO	PCB-AO	PCB-CO	PCB-SO	PCB-5
5	7.9 ± 2.3	9.2 ± 2.3	6.3 ± 2.1	10.0 ± 1.9	10.6 ± 2.9	30.4 ± 2.5
10	8.8 ± 2.9	15.9 ± 2.9	18.3 ± 1.3	16.0 ± 2.1	16.1 ± 3.1	32.6 ± 4.1
20	15.2 ± 3.1	23.3 ± 3.1	21.2 ± 2.0	21.8 ± 2.5	22.9 ± 3.7	36.4 ± 5.0
30	21.6 ± 3.5	32.6 ± 3.5	30.0 ± 1.4	26.9 ± 2.7	29.3 ± 3.8	40.1 ± 3.0
45	26.8 ± 3.4	39.8 ± 3.4	45.2 ± 1.3	35.7 ± 2.9	38.7 ± 4.2	41.1 ± 7.0
60	32.4 ± 3.2	50.9 ± 3.2	54.9 ± 2.6	45.1 ±3.1	47.9 ± 3.2	47.8 ± 2.1
120	45.7 ± 2.7	67.6 ± 2.7	74.3 ± 3.5	56.9 ± 2.8	66.7 ± 3.1	62.3 ± 3.4
180	58.8 ± 2.5	74.5 ± 2.5	79.8 ± 3.6	64.9 ± 3.3	82.6 ± 4.2	72.5 ± 3.5
240	63.2 ± 3.7	82.9 ± 3.7	85.7 ± 3.4	76.3 ± 3.5	88.2 ± 4.0	85.0 ± 3.8
300	68.3 ± 3.4	85.8 ± 3.4	92.0 ± 2.8	84.2 ± 2.7	91.6 ± 3.9	98.3 ± 3.7
360	73.1 ± 3.1	89.5 ± 3.1	95.8 ± 2.9	95.5 ± 2.9	88.9 ± 2.9	
420	77.3 ± 2.8	91.1 ± 2.8	97.5 ± 2.1	96.5 ± 2.1	93.3 ± 3.4	
480	81.6 ± 3.0	92.6 ± 3.0	99.8 ± 1.9	97.5 ± 1.9	96.7 ± 3.5	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 5.11: Cumulative percentage release of flurbiprofen from oil based formulations containing MC in pH 7.4 phosphate buffer

	Cumulative percentage released[a]					
Time (mins)	**MC-OO**	**MC-LSO**	**MC-AO**	**MC-CO**	**MC-SO**	**MC-5**
5	17.3 ± 2.1	23.1 ± 2.5	15.7 ± 2.1	17.5 ±2.3	8.0 ± 2.3	25.3 ± 1.5
10	30.8 ± 1.9	31.2 ± 2.6	25.1 ± 1.3	20.0 ± 2.9	15.9 ± 3.6	26.0 ± 1.9
20	34.8 ± 2.6	39.4 ± 3.2	37.8 ± 2.0	25.3 ± 3.5	31.4 ± 3.8	27.6 ± 2.3
30	42.9 ± 2.5	52.4 ± 3.1	42.3 ± 1.4	31.8 ± 3.7	29.5 ± 4.1	31.7 ± 2.4
45	44.0 ± 2.4	56.5 ± 3.5	48.9 ± 1.3	32.6 ± 4.2	31.0 ± 4.0	35.6 ± 6.4
60	49.7 ± 3.1	67.6 ± 3.8	53.9 ± 2.6	38.6 ± 4.8	35.5 ± 3.9	39.4 ± 3.5
120	53.8 ± 3.0	86.0 ± 2.9	60.3 ± 3.5	41.9 ± 5.2	37.5 ± 3.8	45.8 ± 3.7
180	57.2 ± 2.9	89.5 ± 3.8	65.4 ± 3.6	44.2 ± 5.1	40.2 ± 3.6	57.8 ± 4.3
240	60.5 ± 2.8	93.7 ± 1.6	71.1 ± 3.4	50.9 ± 4.8	42.2 ± 3.5	69.1 ± 4.4
300	63.9 ± 2.6	97.9 ± 3.5	74.1 ± 2.8	54.0 ± 4.7	44.7 ± 4.5	78.7 ± 4.1
360	67.2 ± 2.7	101.0 ± 1.5	78.0 ± 2.9	57.4 ± 4.5	47.2 ± 4.2	88.8 ± 4.0
420	70.2 ± 3.5		81.2 ± 2.1.	60.9 ± 4.3	51.5 ± 4.0	98.5 ± 3.9
480	73.3 ± 3.6		82.1 ± 1.9	64.3 ± 4.2	53.9 ± 3.9	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 5.12: Cumulative percentage release of flurbiprofen from oil based formulations containing HPMC in pH 7.4 phosphate buffer

Time (mins)	Cumulative percentage released[a]					
	HPMC-OO	HPMC-LSO	HPMC-AO	HPMC-CO	HPMC-SO	HPMC-5
5	7.4 ± 1.9	5.8 ± 3.6	7.7 ± 2.8	9.0 ± 2.0	4.9 ± 2.3	12.6 ± 1.2
10	8.4 ± 2.3	9.7 ± 3.8	16.4 ± 2.7	10.1 ± 2.9	10.6 ± 3.6	20.7 ± 5.6
20	10.9 ± 2.5	14.3 ± 3.9	22.6 ± 3.2	13.4 ± 3.5	17.8 ± 3.8	30.1 ± 6.1
30	17.1 ± 3.1	19.9 ± 4.2	35.6 ± 3.1	17.8 ± 3.7	26.0 ± 4.1	41.2 ± 4.5
45	22.2 ± 2.8	27.8 ± 4.5	41.3 ± 3.0	27.3 ± 4.2	30.4 ± 4.0	55.3 ± 4.7
60	25.6 ± 2.4	32.4 ± 3.8	51.3 ± 2.8	33.5 ± 4.8	37.4 ± 3.9	72.6 ± 3.9
120	36.5 ± 2.1	39.6 ± 3.7	58.2 ± 2.9	50.4 ± 5.2	46.5 ± 3.8	101.6 ± 4.1
180	41.0 ± 3.5	45.3 ± 3.5	65.7 ± 3.8	58.6 ± 5.1	46.8 ± 3.6	
240	41.9 ± 3.7	49.5 ± 4.1	76.2 ± 3.9	66.7 ± 4.8	48.8 ± 3.5	
300	44.0 ± 2.8	55.8 ± 4.0	80.7 ± 4.1	70.5 ± 4.7	52.1 ± 4.5	
360	45.3 ± 2.9	62.7 ± 3.9	83.9 ± 4.0	79.8 ± 4.5	54.4 ± 4.2	
420	47.5 ± 3.1	70.2 ± 3.5	87.5 ± 3.7	83.5 ± 4.3	58.5 ± 4.0	
480	49.8 ± 3.0	78.2 ± 3.4	90.7 ± 3.8	90.1 ± 4.2	60.1 ± 3.9	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 5.13: First order release rate parameters of designed polymeric gel formulations of flurbiprofen containing PAA, HPMC, PCB and NaCMC.

Parameters	PAA-1	PAA-5	HPMC-1	HPMC-5	PCB-1	PCB-5	NaCMC-1	NaCMC-5
r^a	0.9976	0.9820	0.9992	0.9838	0.9868	0.9902	0.9680	0.9752
$MSSR^b$	0.0003	0.0052	0.0001	0.0014	0.0008	0.0012	0.0019	0.0035
K^c ($mins^{-1}$)	0.03958	0.01029	0.03138	0.02091	0.00914	0.00899	0.01683	0.01373
$t_{50\%}{}^d$ (mins)	15	80	4	35	30	53	4	27
$t_{70\%}{}^e$ (mins)	28	130	13	60	85	110	6	64
$t_{90\%}{}^f$ (mins)	56	237	48	112	206	232	72	144

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: First order release rate constant
[d]: Time for 50% of the drug release
[e]: Time for 70% of the drug release.
[f]: Time for 90% of the drug release.

Table 5.14: First order release rate parameters of designed polymeric gel formulations of flurbiprofen MC, PVP and PVA.

Parameters	MC-1	MC-5	PVP-1	PVP-5	PVA-1	PVA-5
r^a	0.9375	0.9779	0.9689	0.9972	0.9764	0.9873
$MSSR^b$	0.0029	0.0032	0.0068	0.0006	0.0038	0.0008
K^c ($mins^{-1}$)	0.00539	0.00480	0.04056	0.03228	0.05954	0.00639
$t_{50\%}{}^d$ (mins)	18	104	3	4	3	4
$t_{70\%}{}^e$ (mins)	71	211	2	13	< 5	32
$t_{90\%}{}^f$ (mins)	274	440	29	47	19	204

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: First order release rate constant
[d]: Time for 50% of the drug release
[e]: Time for 70% of the drug release
[f]: Time for 90% of the drug release

Table 5.15: First order release rate parameters of designed oil based formulations of flurbiprofen

Parameters	CSO	OO	AO	CO	SO	LSO
r^a	0.9974	0.9983	0.9927	0.9976	0.9684	0.9964
MSSR[b]	0.0005	0.0009	0.0025	0.0004	0.0257	0.0009
K^c ($mins^{-1}$)	0.01660	0.01379	0.01135	0.01018	0.02302	0.00953
$t_{50\%}{}^d$ (mins)	32	49	54	60	42	61
$t_{70\%}{}^e$ (mins)	63	86	99	110	64	114
$t_{90\%}{}^f$ (mins)	129	166	196	218	112	230

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: First order release rate constant
[d]: Time for 50% of the drug release
[e]: Time for 70% of the drug release
[f]: Time for 90% of the drug release

Table 5.16: First order release rate parameters of designed polymeric gel in oil based formulations of flurbiprofen containing PCB

Parameters	PCB-OO	PCB-LSO	PCB-AO	PCB-CO	PCB-SO	PCB-5
r^a	0.9930	0.9862	0.9953	0.9921	0.9968	0.9909
MSSR[b]	0.0009	0.0047	0.0029	0.0037	0.0014	0.0010
K^c ($mins^{-1}$)	0.00331	0.00534	0.00828	0.00627	0.00797	0.00614
$t_{50\%}{}^d$ (mins)	165	79	66	97	70	64
$t_{70\%}{}^e$ (mins)	319	175	128	178	134	147
$t_{90\%}{}^f$ (mins)	651	381	261	353	272	326

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[b]: First order release rate constant
[d]: Time for 50% of the drug release
[e]: Time for 70% of the drug release
[f]: Time for 90% of the drug release

Table 5.17: First order release rate parameters of designed polymeric gel in oil based formulations of flurbiprofen containing MC

Parameters	MC-OO	MC-LSO	MC-AO	MC-CO	MC-SO	MC-5
r^a	0.9782	0.9856	0.9786	0.9782	0.9892	0.9942
$MSSR^b$	0.0008	0.0051	0.0020	0.0007	0.0001	0.0002
K^c ($mins^{-1}$)	0.00180	0.01071	0.00281	0.00159	0.00082	0.00367
$t_{50\%}{}^d$ (mins)	118	31	72	252	413	115
$t_{70\%}{}^e$ (mins)	403	79	254	573	1040	254
$t_{90\%}{}^f$ (mins)	1015	182	645	1263	2387	553

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: First order release rate constant
[d]: Time for 50% of the drug release
[e]: Time for 70% of the drug release
[f]: Time for 90% of the drug release

Table 5.18: First order release rate parameters of designed polymeric gel in oil based formulations of flurbiprofen containing HPMC

Parameters	HPMC-OO	HPMC-LSO	HPMC-AO	HPMC-CO	HPMC-SO	HPMC-5
r^a	0.9377	0.9845	0.9928	0.9924	0.9592	0.9857
$MSSR^b$	0.0008	0.0012	0.0016	0.0016	0.0007	0.0012
K^c ($mins^{-1}$)	0.00101	0.00258	0.00438	0.00426	0.00123	0.02012
$t_{50\%}{}^d$ (mins)	437	214	91	140	269	35
$t_{70\%}{}^e$ (mins)	944	412	208	260	686	60
$t_{90\%}{}^f$ (mins)	2034	837	459	517	1583	115

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: First order release rate constant
[d]: Time for 50% of the drug release
[e]: Time for 70% of the drug release
[f]: Time for 90% of the drug release

Table 5.19: Effect of sterilization method on the drug content in designed polymeric gel type ophthalmic preparation of flurbiprofen

Formulation code	Drug content[a] (μg/ ml)		Results of one-way ANOVA of drug content results[b]	
	Before sterilization	After sterilization	F_{calc}	F_{crit}
Aqueous drop	301.5 ± 0.5	298.9 ± 4.0	1.248	7.709
PCB-5	299.3 ± 5.0	298.6 ± 2.0	0.051	7.709
MC-5	292.4 ± 3.0	289.7 ± 5.0	0.643	7.709
HPMC-SO	288.5 ± 7.0	294.0 ± 4.0	1.396	7.709
MC-OO	316.4 ± 8.2	310.2 ± 5.0	1.250	7.709

[a]: Average ± S.D. of triplicate determinations
[b]: Based on degree of freedom (1, 4) at 5% level of significance

Table 5.20: Observed and predicted degradation rate constants along with the observed and predicted shelf life in months for flurbiprofen from designed polymeric gel type ophthalmic preparation of flurbiprofen

Formulation code	Observed degradation rate constant[a] (K_{deg}) × 10^3 ($months^{-1}$)			Shelf-life[c] based on observed K_{CRT} (months)	Predicted values from the Arrhenius plot	
	CRT[b]	40°C/ 75% RH	45°C/ 75% RH		K_{25} × 10^3 ($months^{-1}$)	Predicted Shelf-life[c] (months)
Aqueous drop	9.16	30.12	39.63	11.46	9.44	11.12
PCB-1	9.63	35.15	40.93	10.90	10.07	10.43
PCB-5	6.58	22.36	27.97	15.96	6.82	15.40
MC-1	8.75	30.66	37.19	12.00	9.10	11.54
MC-5	7.02	20.13	29.84	14.96	7.13	14.73
HPMC-1	10.56	39.63	44.88	9.94	11.08	9.48
HPMC-5	6.89	23.45	29.28	15.24	7.14	14.70
NaCMC-1	11.36	43.65	48.28	9.24	11.95	8.79
NaCMC-5	9.53	33.20	40.50	11.02	9.91	10.60
PVP-1	6.44	22.89	27.37	16.30	6.71	15.65
PVP-5	9.57	34.93	40.67	10.97	10.00	10.50
PVA-1	7.01	21.78	29.79	14.98	7.19	14.61
PVA-5	5.84	20.63	24.82	17.98	6.08	17.27
PAA-1	10.73	39.52	45.60	9.79	11.23	9.35
PAA-5	6.94	22.36	29.50	15.13	7.15	14.69

[a] : Based on average of triplicate assay value at six time points with C.V. less than 3.8 % in all cases

[b] : CRT= 25 ± 3 °C & 55 ± 15 % RH

[c] : Shelf life in months

Table 5.21: Observed and predicted degradation rate constants along with the observed and predicted shelf life in months for flurbiprofen from designed oil based ophthalmic preparation of flurbiprofen

Formulation code	Observed degradation rate constant[a] (K_{deg}) × 10^3 ($months^{-1}$)			Shelf-life[c] based on observed K_{CRT} (months)	Predicted values from the Arrhenius plot	
	CRT[b]	40°C/ 75% RH	45°C/ 75% RH		K_{25} × 10^3 ($months^{-1}$)	Predicted shelf-life[c] (months)
Aqueous drop	9.16	30.12	39.63	11.46	9.44	11.12
CSO	7.23	26.39	30.73	14.52	7.56	13.89
OO	5.53	18.24	23.49	19.00	5.71	18.40
AO	5.86	21.39	24.91	17.92	6.13	17.14
CO	4.99	18.20	21.19	21.06	5.21	20.14
SO	4.19	15.28	17.79	25.08	4.38	23.99
LSO	5.21	12.69	22.14	20.15	5.19	20.24

[a] : Based on average of triplicate assay value at six time points with C.V. less than 2.8% in all cases
[b]: CRT= 25 ± 3 °C & 55 ± 15 % RH
[c]: Shelf life in months

Table 5.22: Observed and predicted degradation rate constants along with the observed and predicted shelf life in months for flurbiprofen from designed polymeric gel in vegetable oil based ophthalmic preparation of flurbiprofen

Formulation code	Observed degradation rate constant[a] (K_{deg}) × 10^3 ($months^{-1}$)			Shelf-life[c] based on observed K_{CRT} (months)	Predicted values from the Arrhenius plot	
	CRT[b]	40°C/ 75% RH	45°C/ 75% RH		K_{25} × 10^3 ($months^{-1}$)	Predicted Shelf-life[c] (months)
Aqueous drop	9.16	30.12	39.63	11.46	9.44	11.12
PCB-OO	4.37	15.94	18.56	24.05	4.56	23.00
PCB-AO	4.51	16.47	19.17	23.28	4.72	22.26
PCB-CO	4.63	16.91	19.69	22.66	4.84	21.68
PCB-SO	3.41	12.43	14.47	30.83	3.56	29.49
PCB-LSO	3.28	11.98	13.96	31.98	3.43	30.59
MC-OO	5.26	17.34	22.34	19.98	5.43	19.34
MC-AO	4.76	17.36	20.21	22.08	4.97	21.12
MC-CO	3.87	14.13	16.46	27.12	4.05	25.94
MC-SO	3.54	12.92	15.04	29.67	3.70	28.38
MC-LSO	3.40	12.41	14.45	30.88	3.55	29.54
HPMC-OO	4.86	17.74	20.66	21.60	5.08	20.66
HPMC-AO	5.35	19.53	22.74	19.63	5.59	18.77
HPMC-CO	4.36	15.92	18.53	24.08	4.56	23.03
HPMC-SO	3.32	10.94	14.09	31.66	3.43	30.65
HPMC-LSO	3.15	11.50	13.39	33.33	3.29	31.88

[a] : Based on average of triplicate assay value at six time points with C.V. less than 3.8 % in all cases
[b] : CRT= 25 ± 3 °C & 55 ± 15 % RH
[c] : Shelf life in months

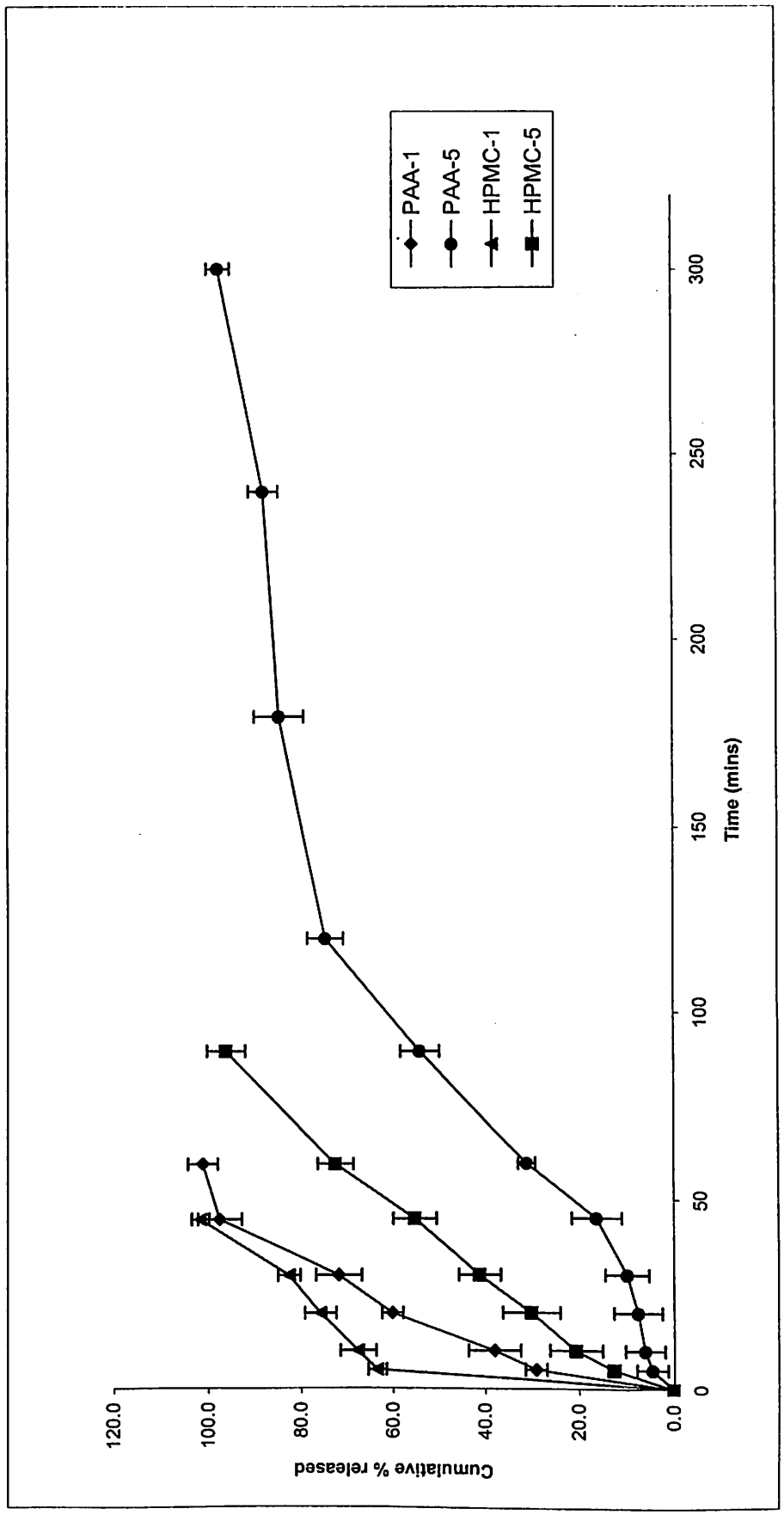

Figure 5.1: Release profile of flurbiprofen from polymeric gel formulations prepared using PAA and HPMC in pH 7.4 phosphate buffer.

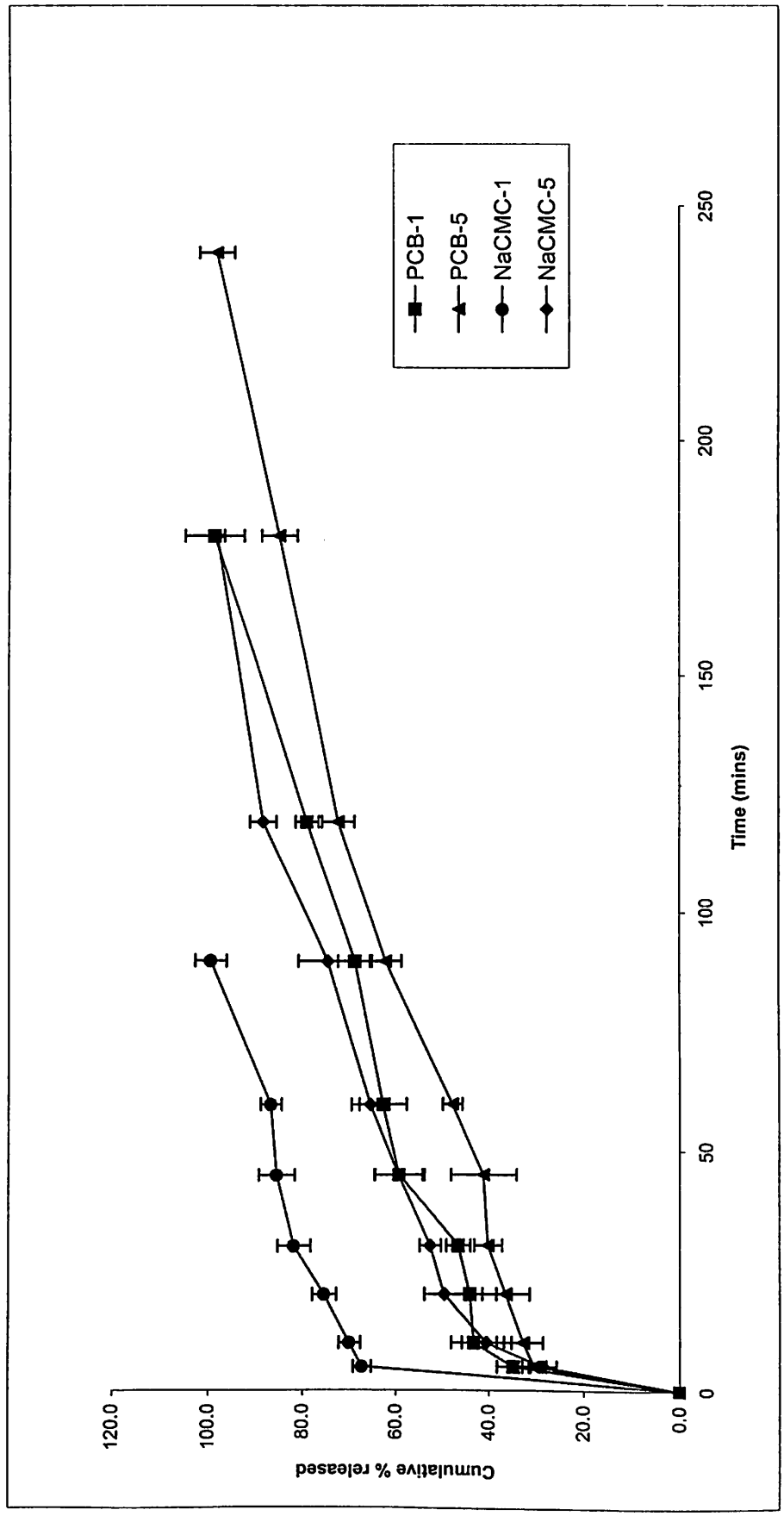

Figure 5.2: Release profile of flurbiprofen from polymeric gel formulations prepared using PCB and NaCMC in pH 7.4 phosphate buffer.

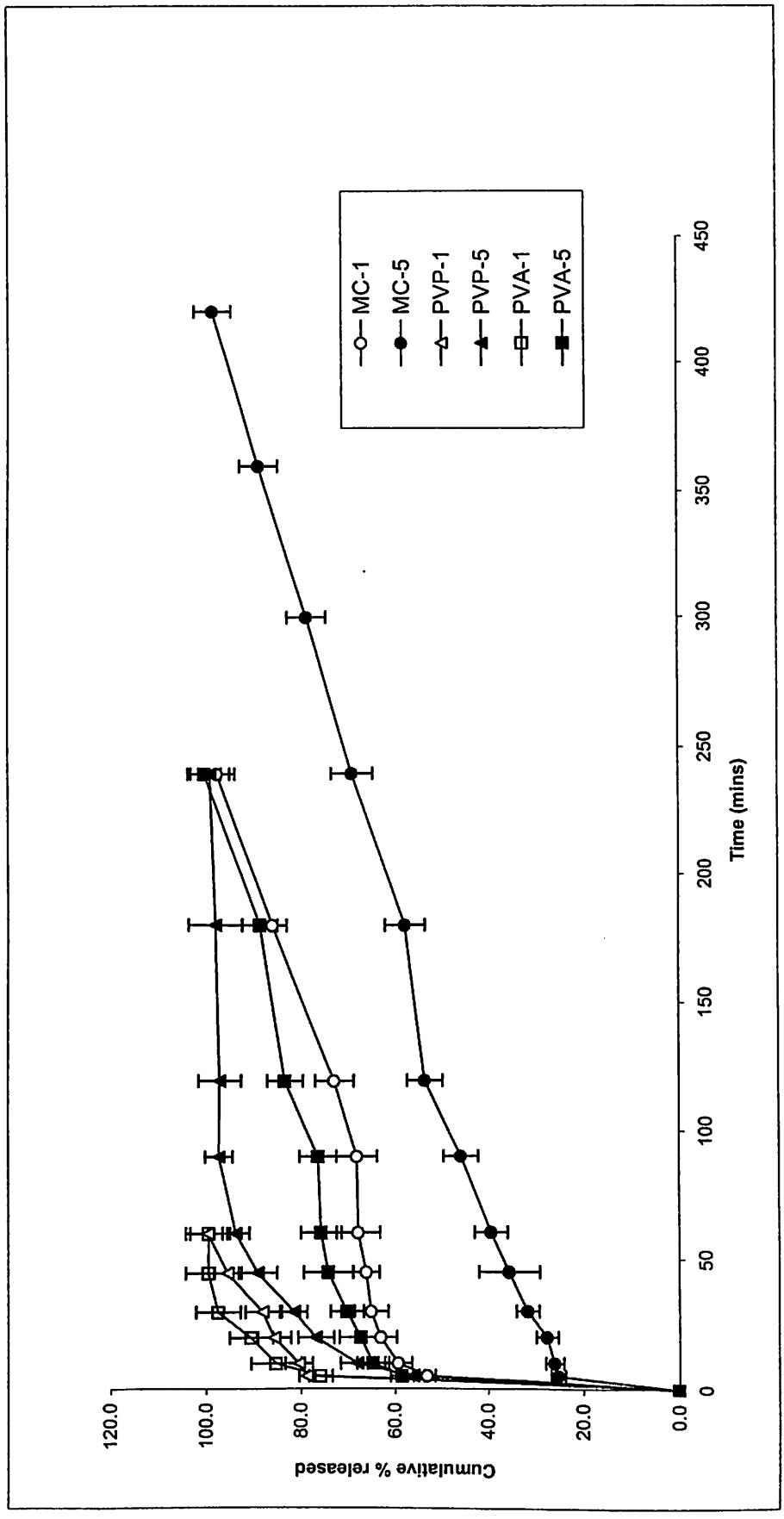

Figure 5.3: Release profile of flurbiprofen from polymeric gel formulations prepared using MC, PVP and PVA in pH 7.4 phosphate buffer.

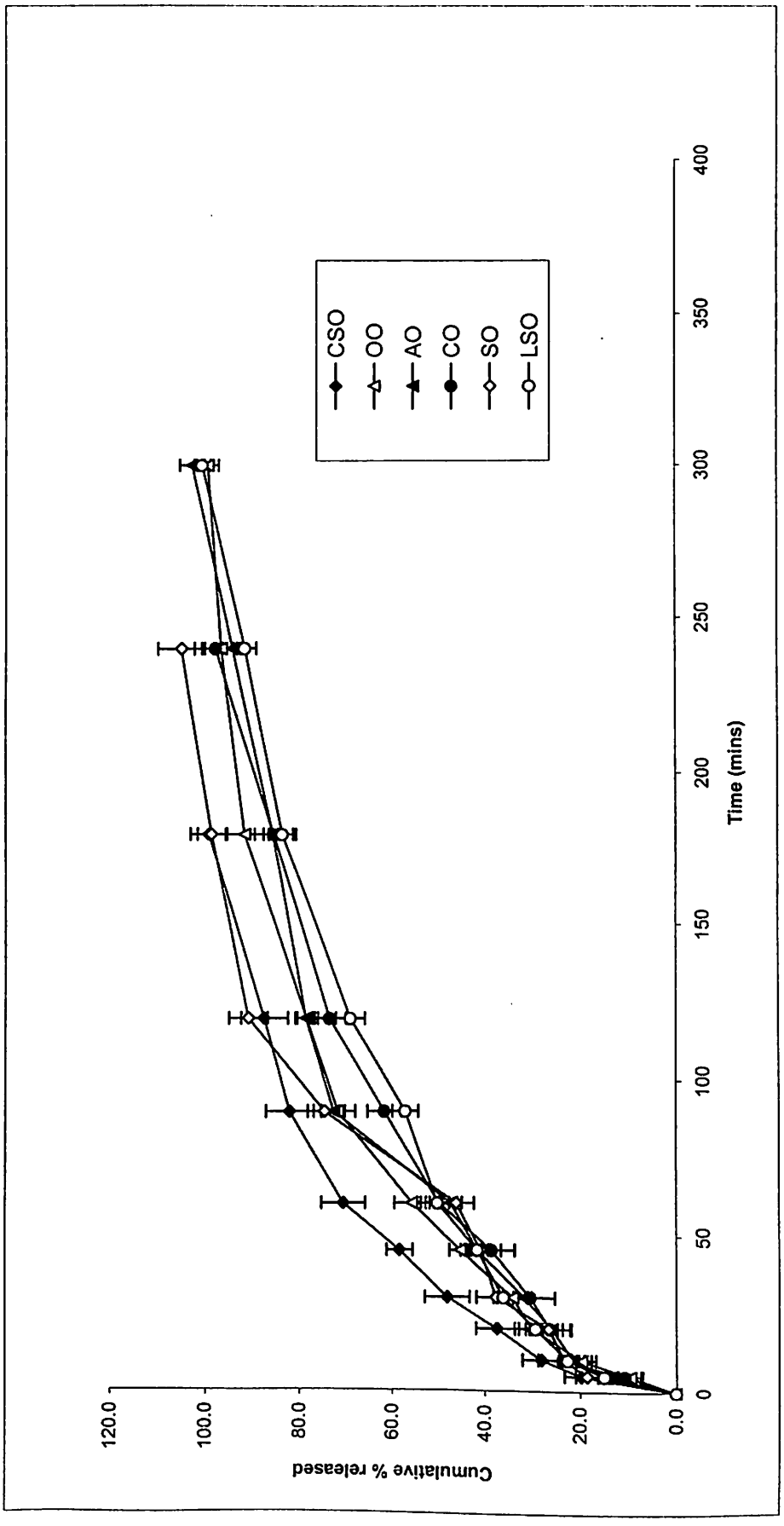

Figure 5.4: Release profile of flurbiprofen from oil based formulations prepared using various vegetable oils in pH 7.4 phosphate buffer.

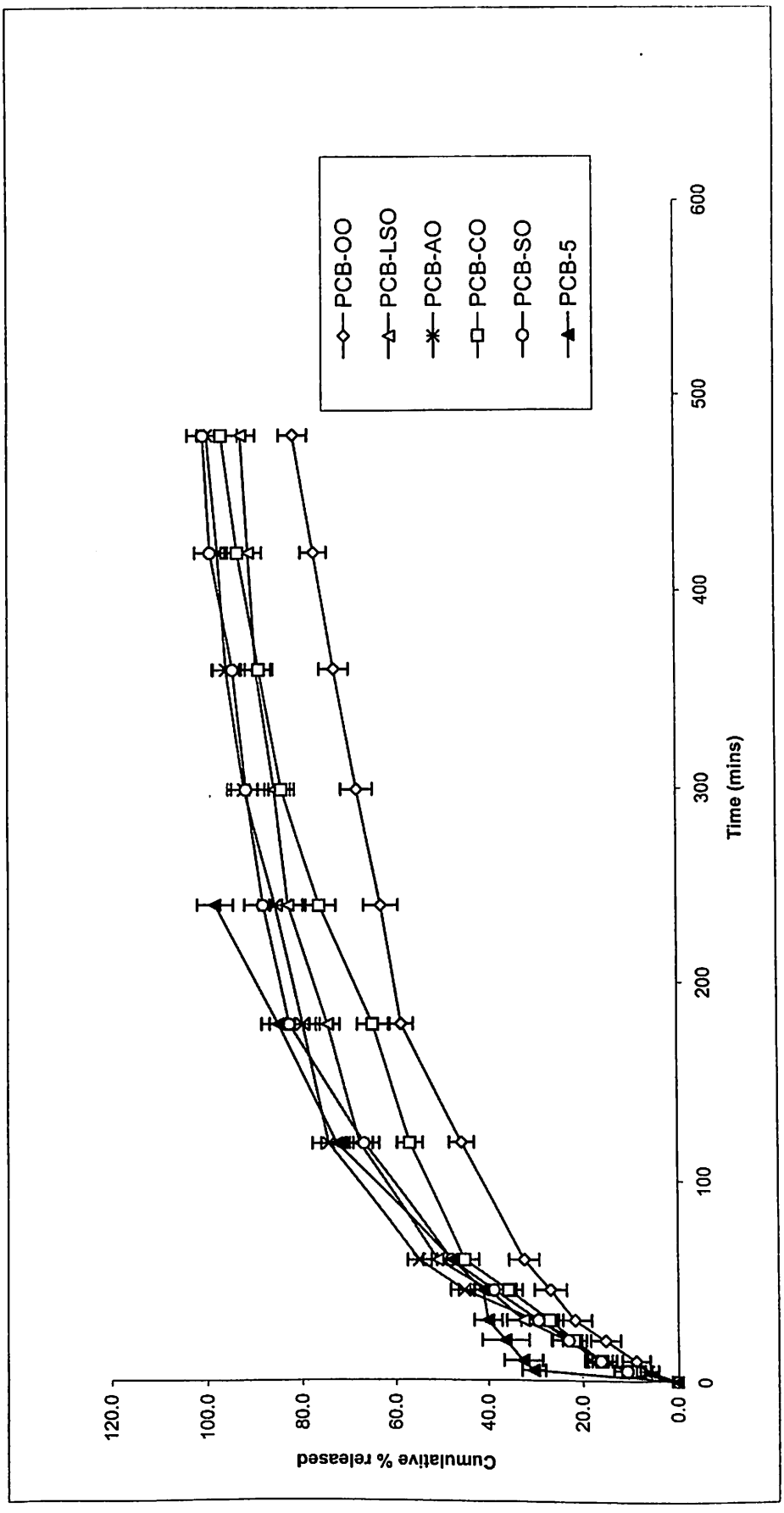

Figure 5.5: Release profile of flurbiprofen from oil based formulations containing PCB in pH 7.4 phosphate buffer.

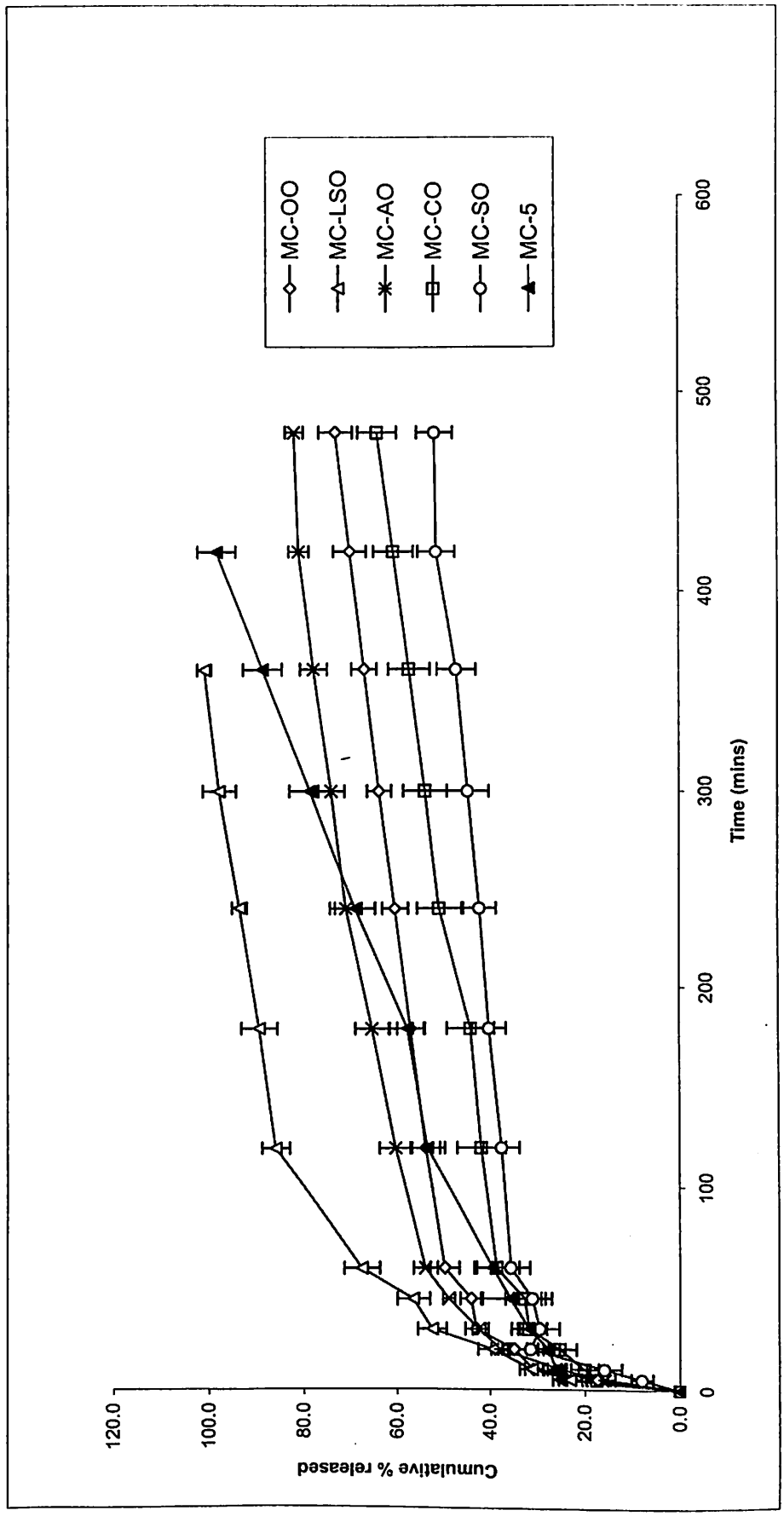

Figure 5.6: Release profile of flurbiprofen from oil based formulations containing MC in pH 7.4 phosphate buffer.

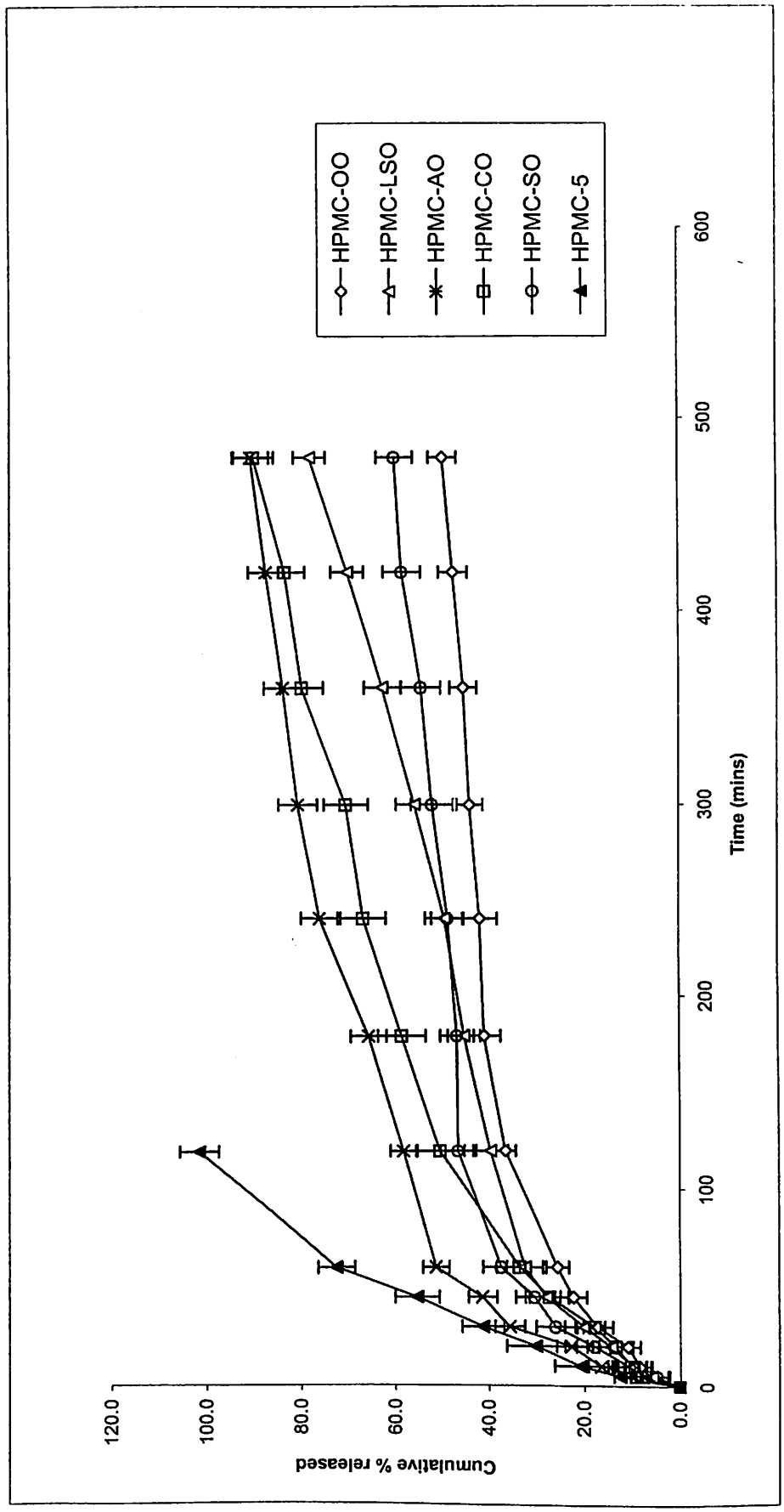

Figure 5.7: Release profile of flurbiprofen from oil based formulations containing HPMC in pH 7.4 phosphate buffer.

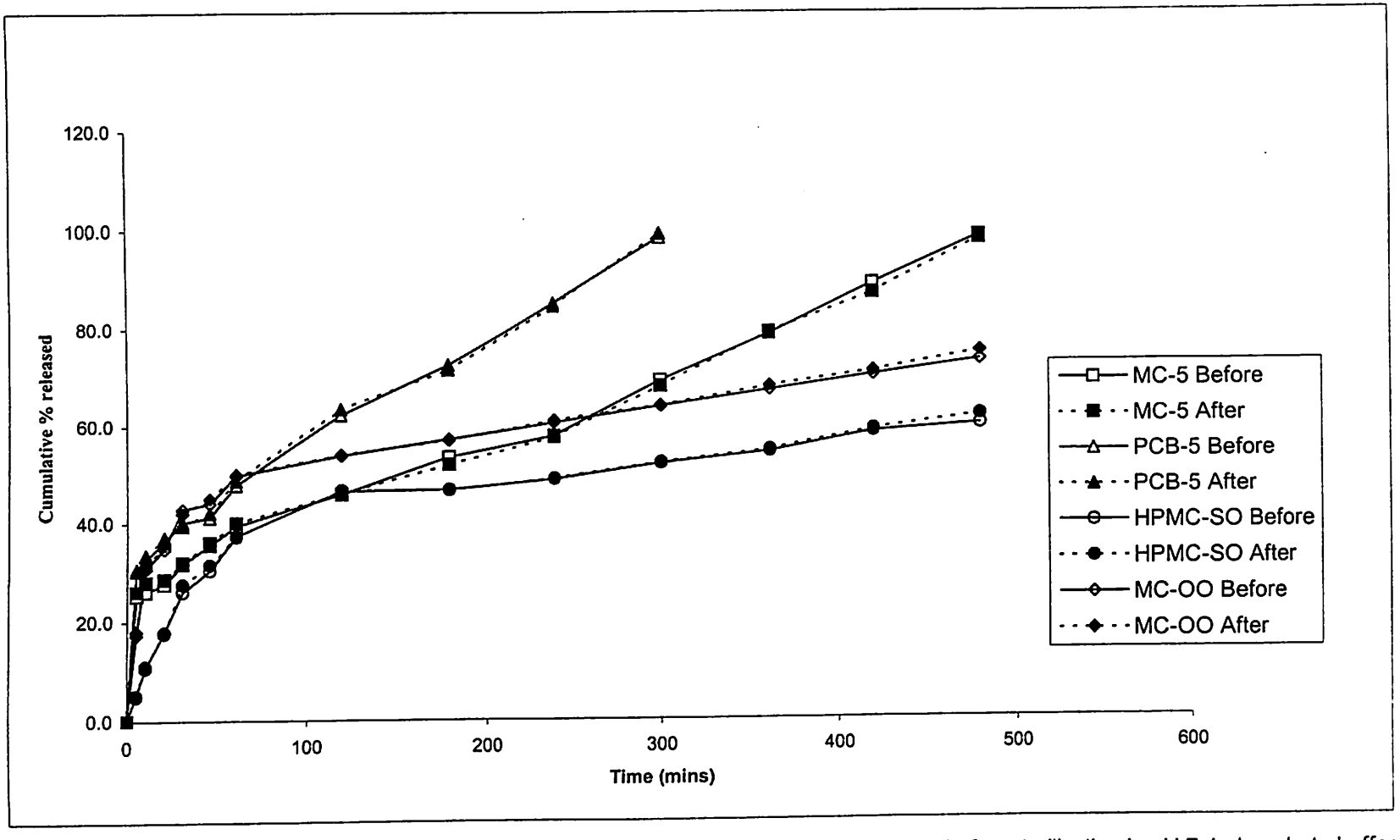

Figure 5.8: Comparative release profile of flurbiprofen from selected formulations before and after sterilization in pH 7.4 phosphate buffer.

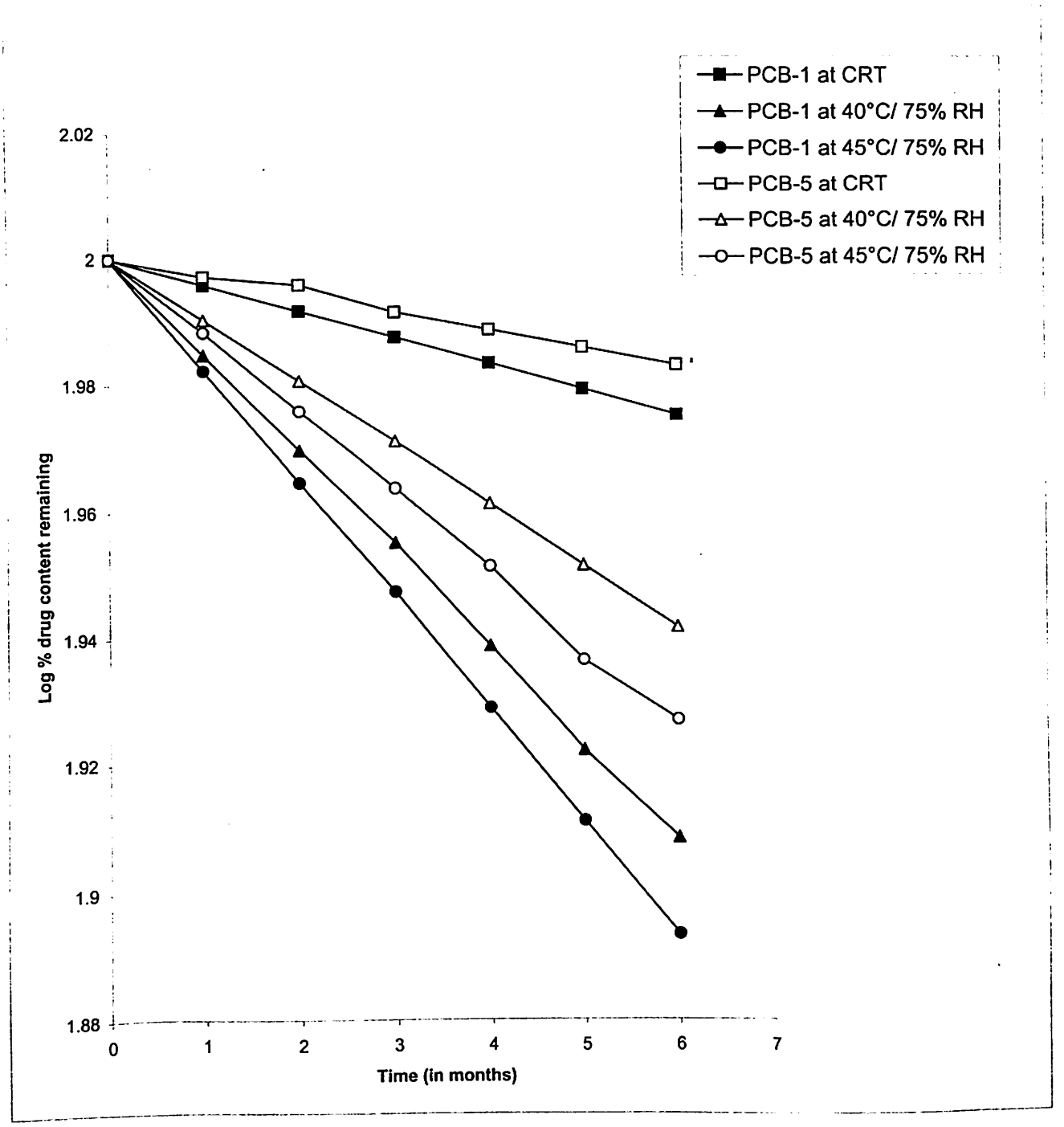

Figure 5.9: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric gel formulations prepared using 1% and 5% w/v PCB.

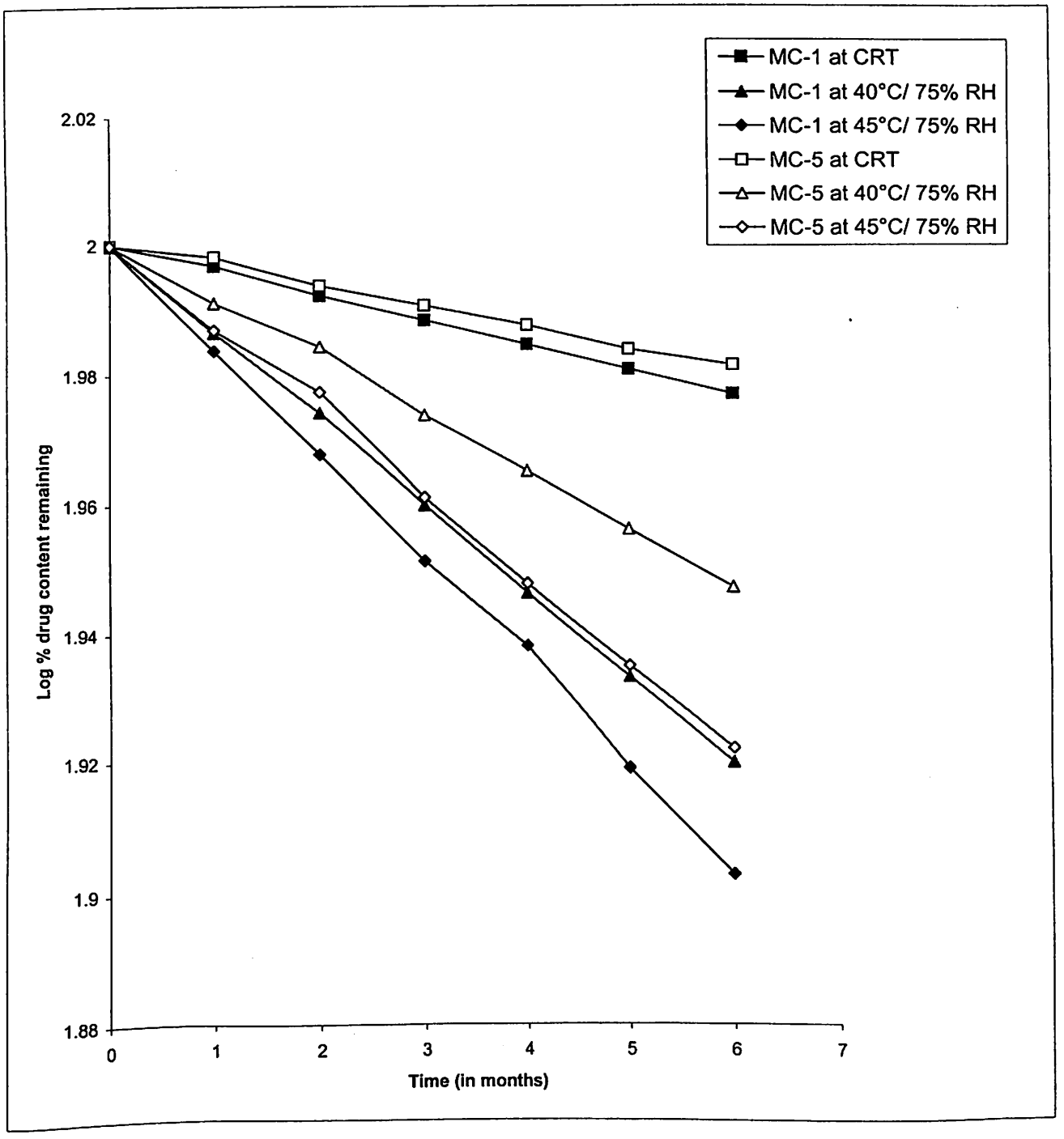

Figure 5.10: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric gel formulations prepared using 1% and 5% w/v MC.

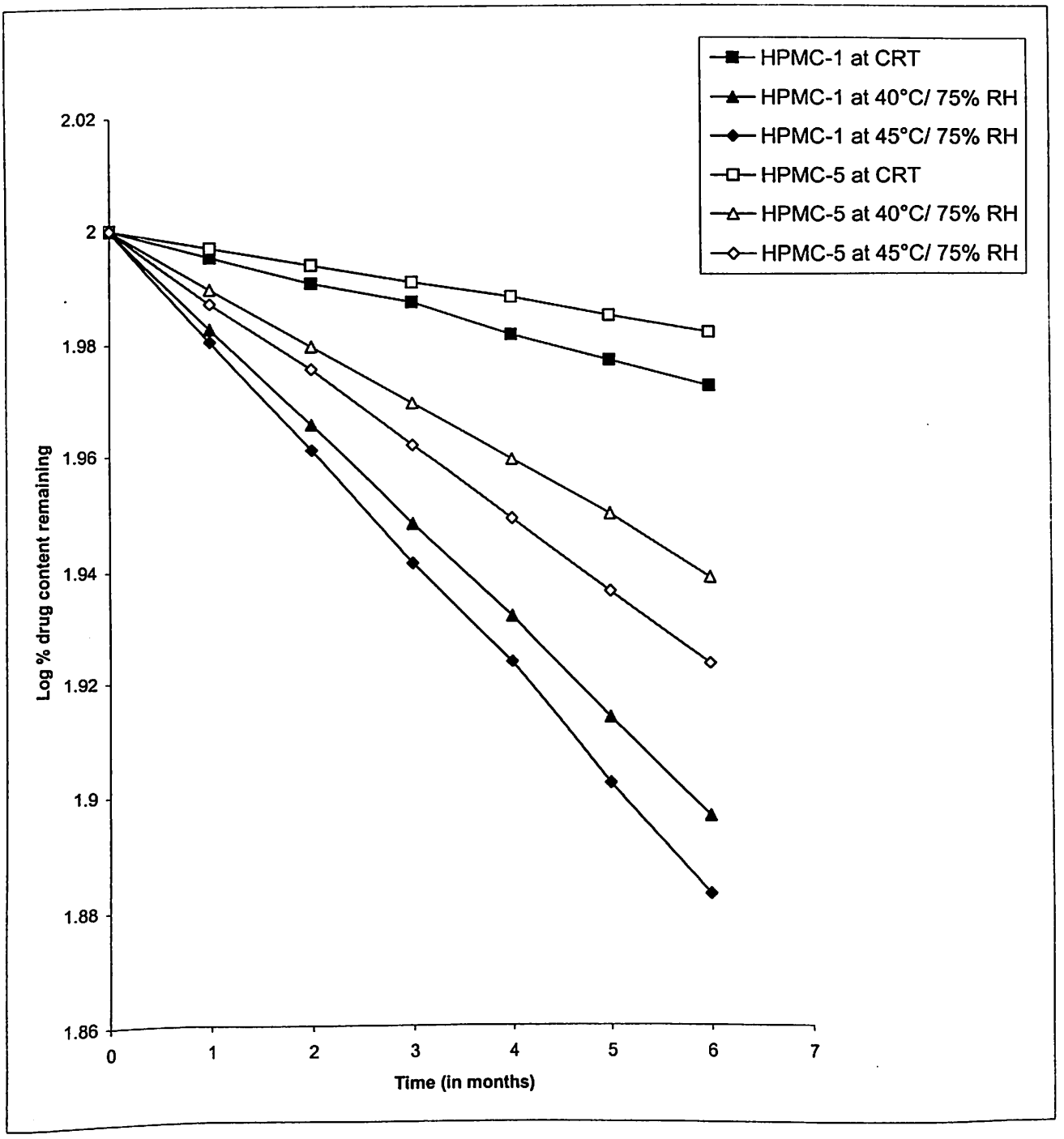

Figure 5.11: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric gel formulations prepared using 1% and 5% w/v HPMC.

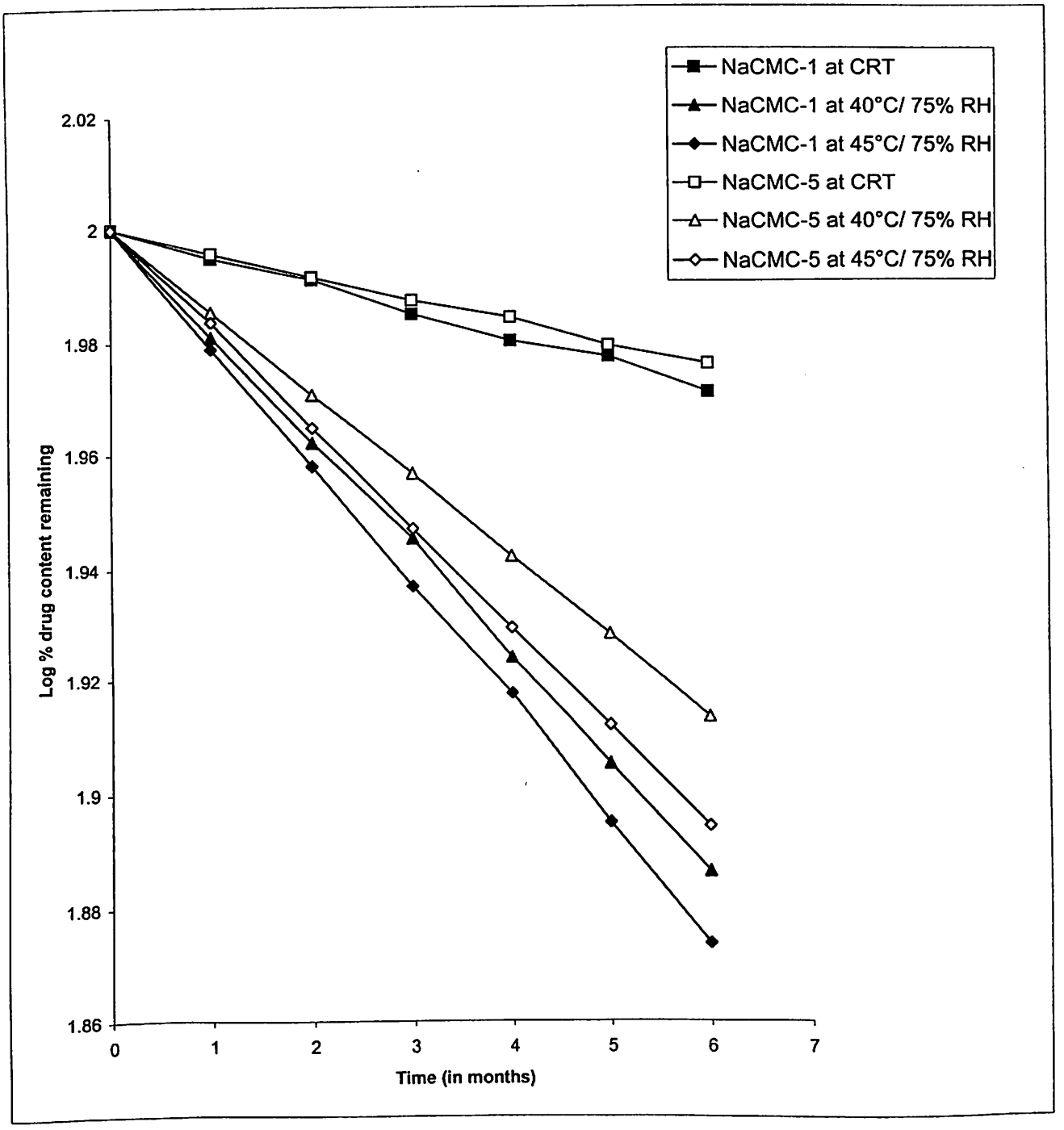

Figure 5.12: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric gel formulations prepared using 1% and 5% w/v NaCMC.

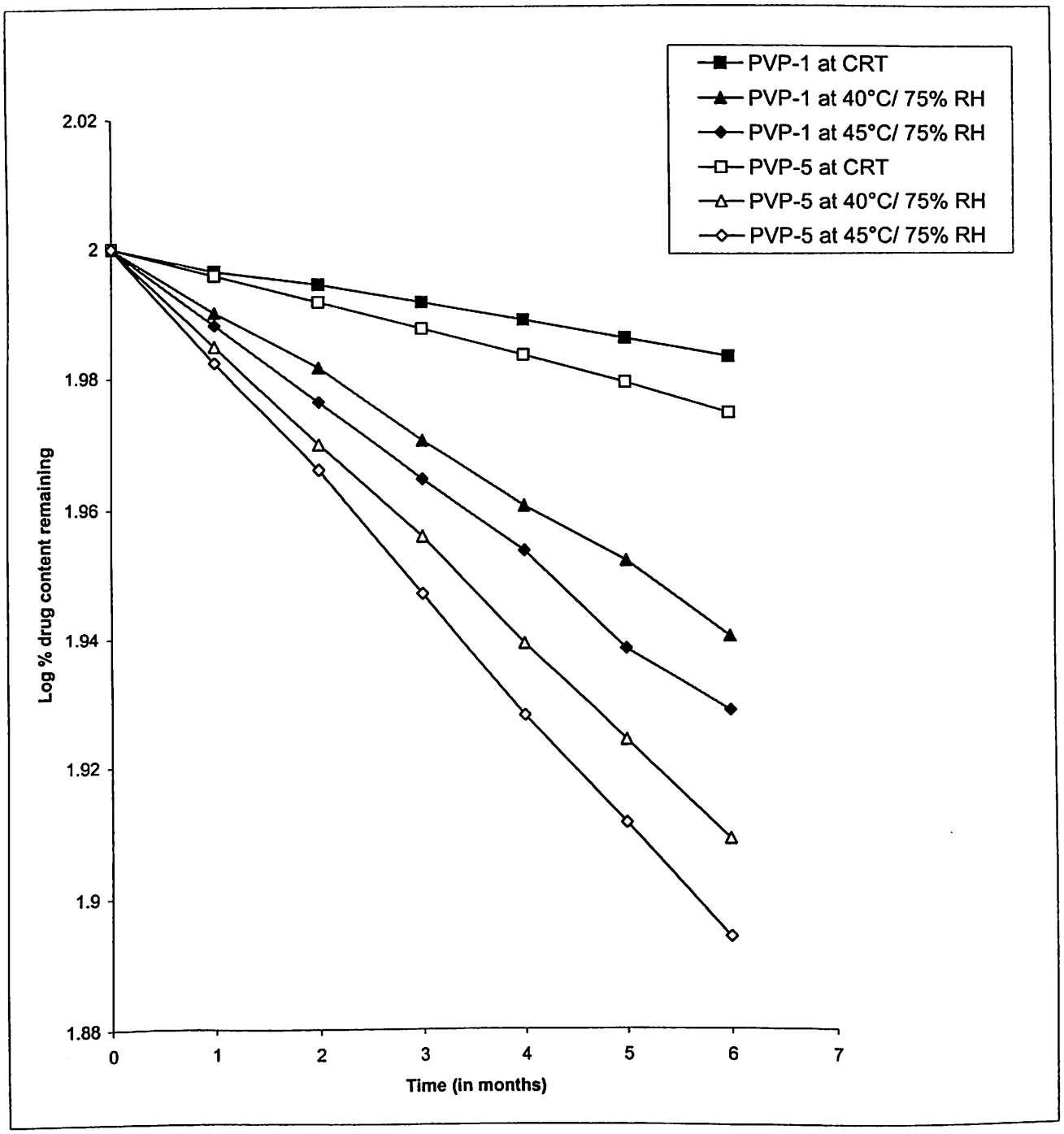

Figure 5.13: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric gel formulations prepared using 1% and 5% w/v PVP.

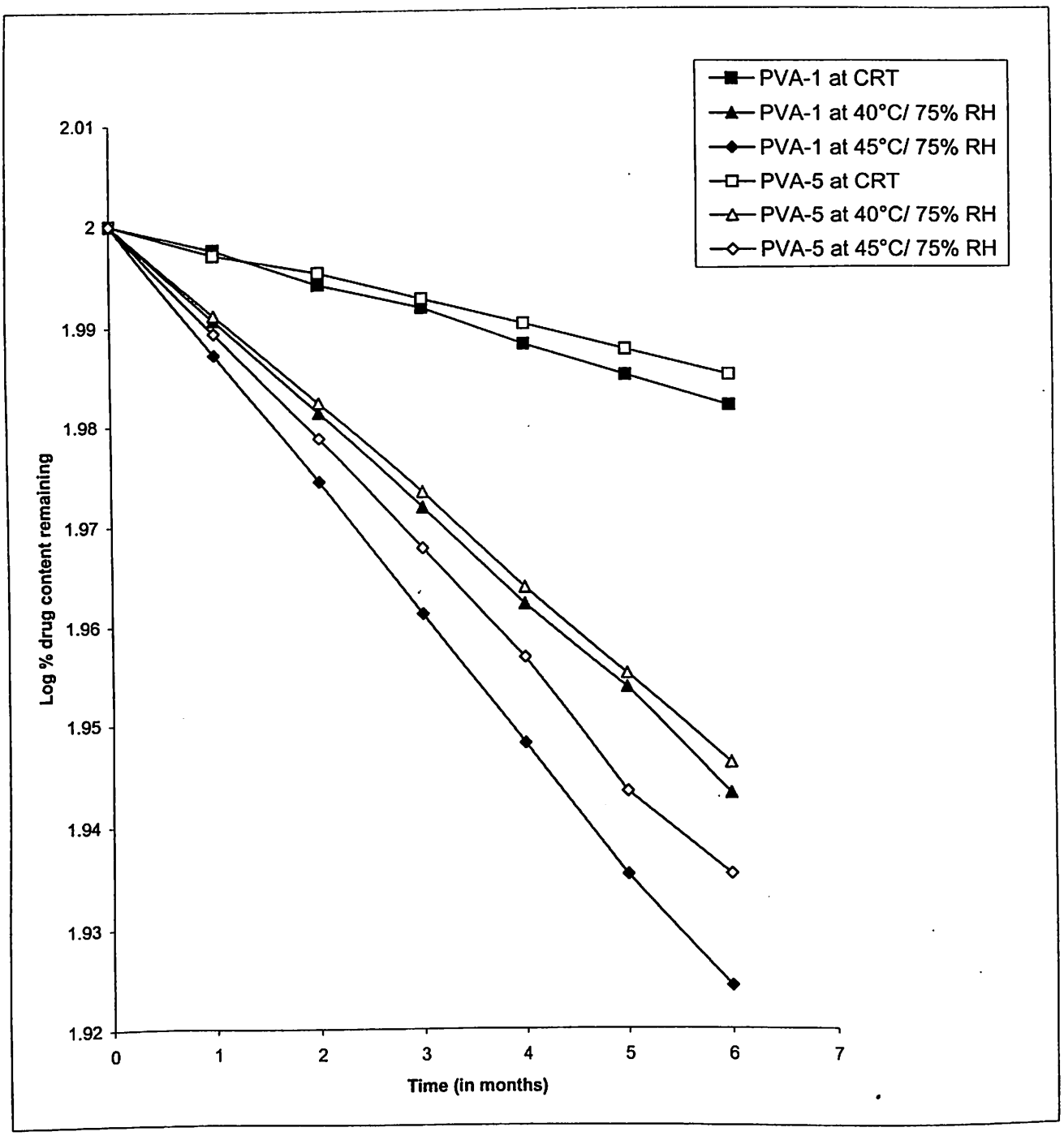

Figure 5.14: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric gel formulations prepared using 1% and 5% w/v PVA.

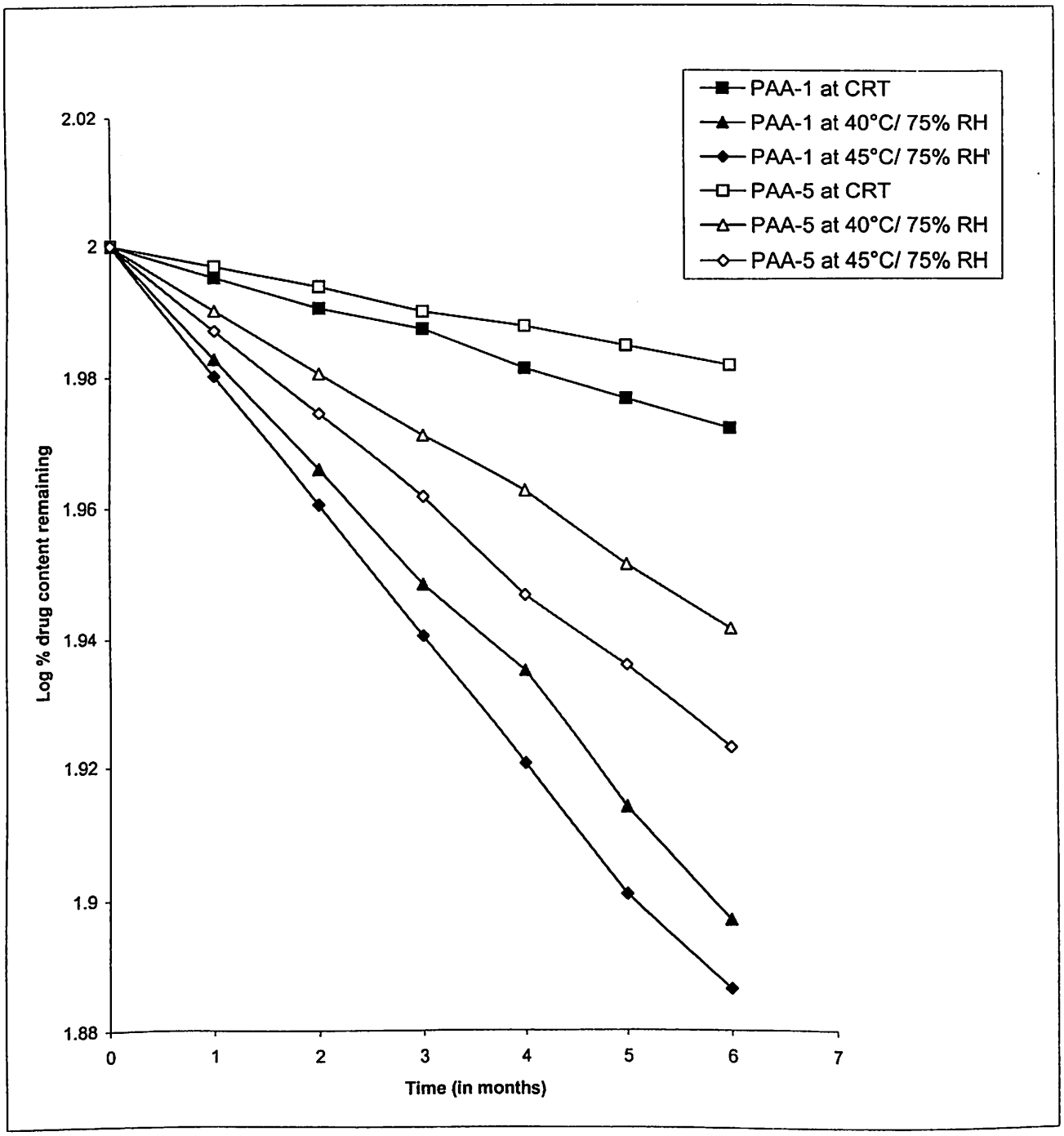

Figure 5.15: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric gel formulations prepared using 1% and 5% w/v PAA.

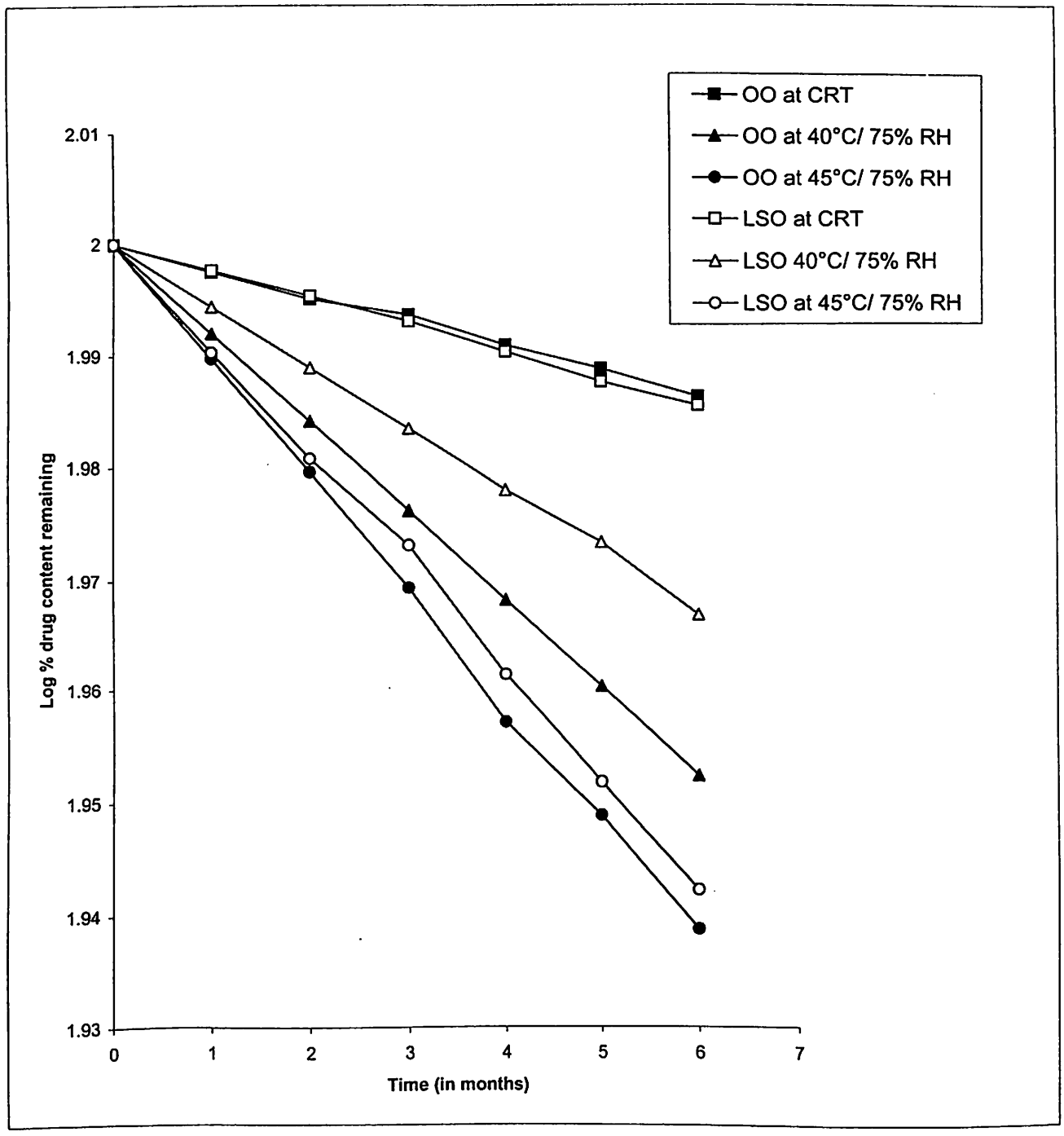

Figure 5.16: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from oil-based formulations prepared using olive oil (OO) and linseed oil (LSO).

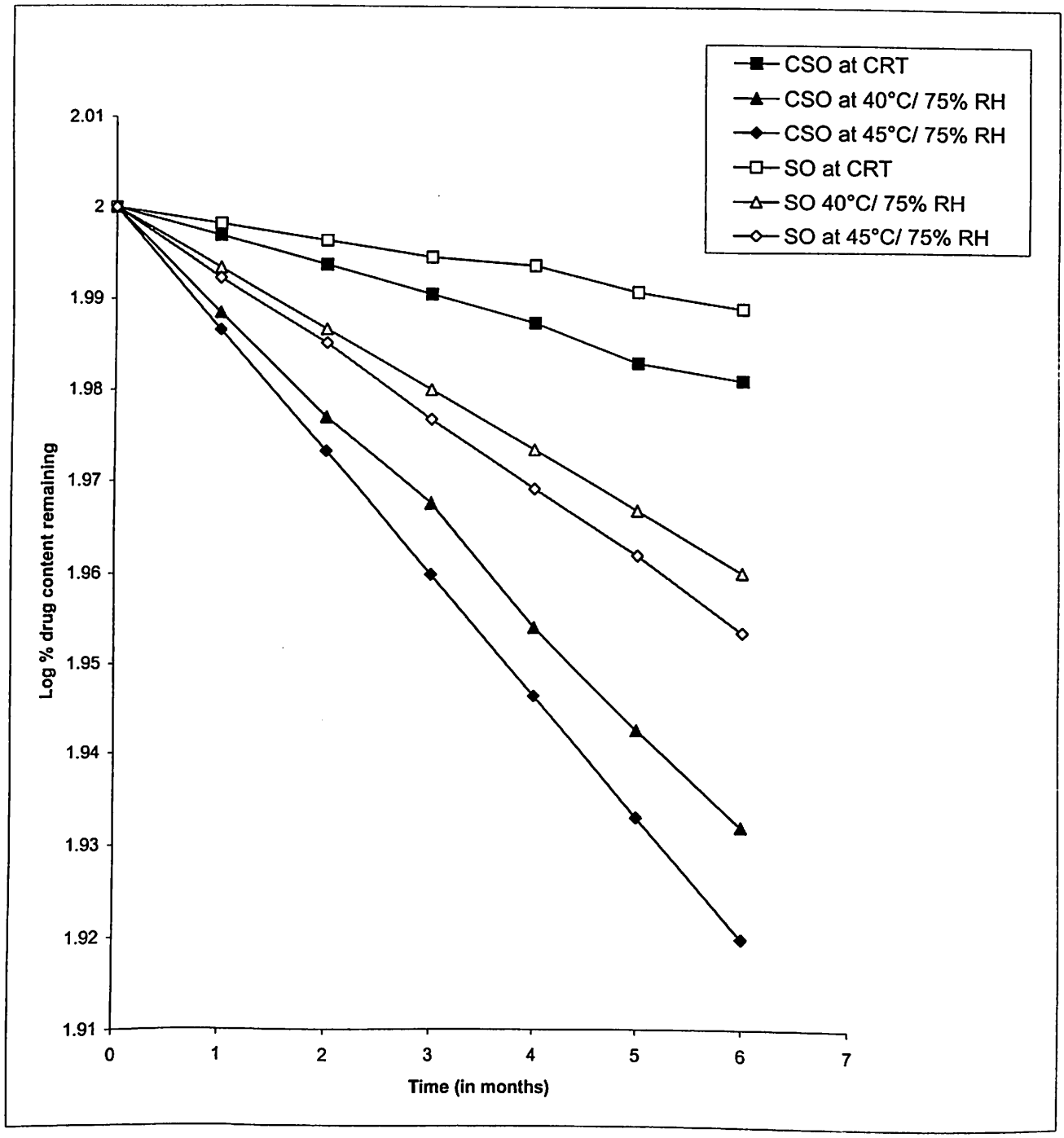

Figure 5.17: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from oil-based formulations prepared using cotton seed oil (CSO) and sesame oil (SO).

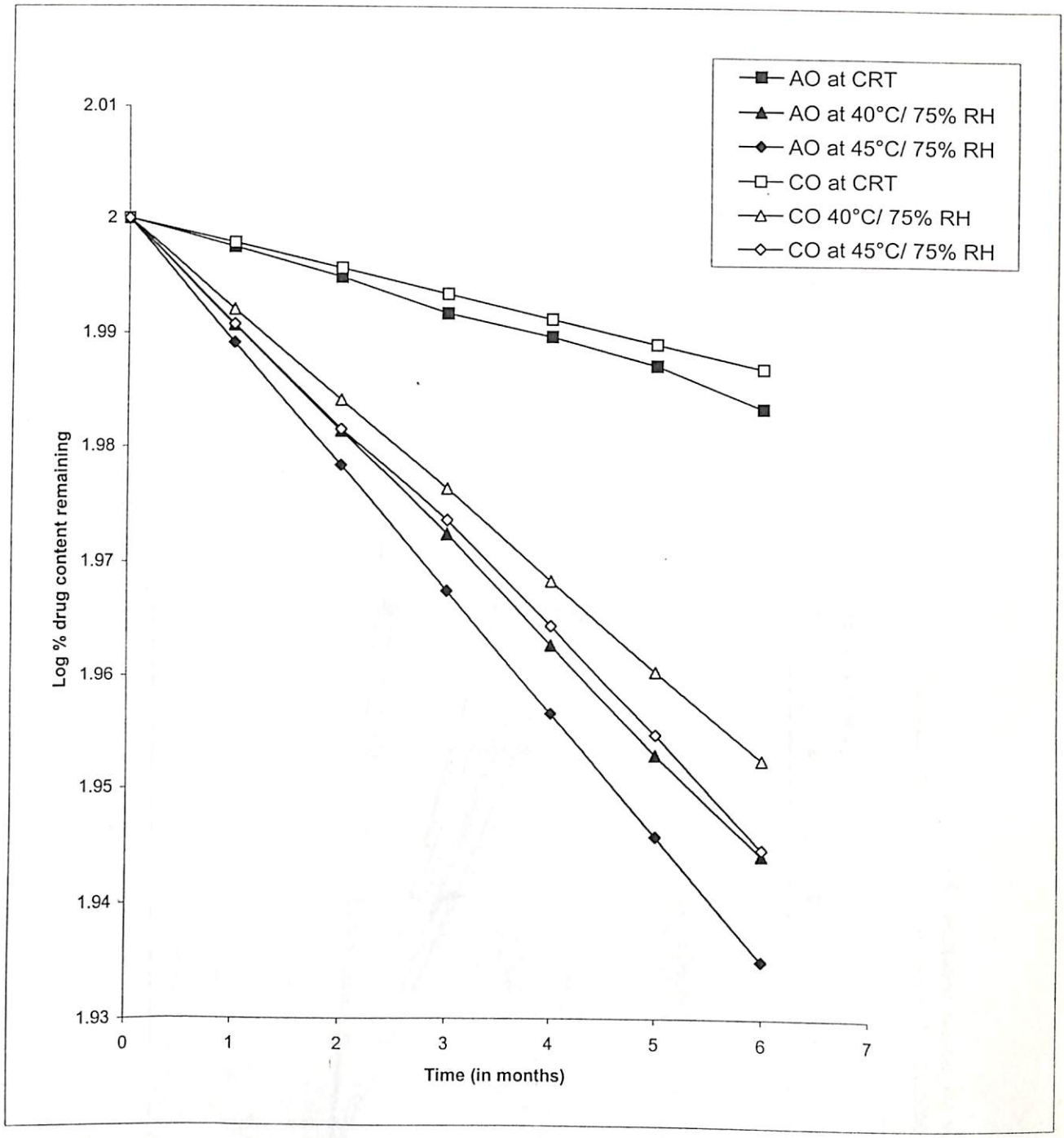

Figure 5.18: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from oil-based formulations prepared using arachis oil (AO) and castor oil (CO).

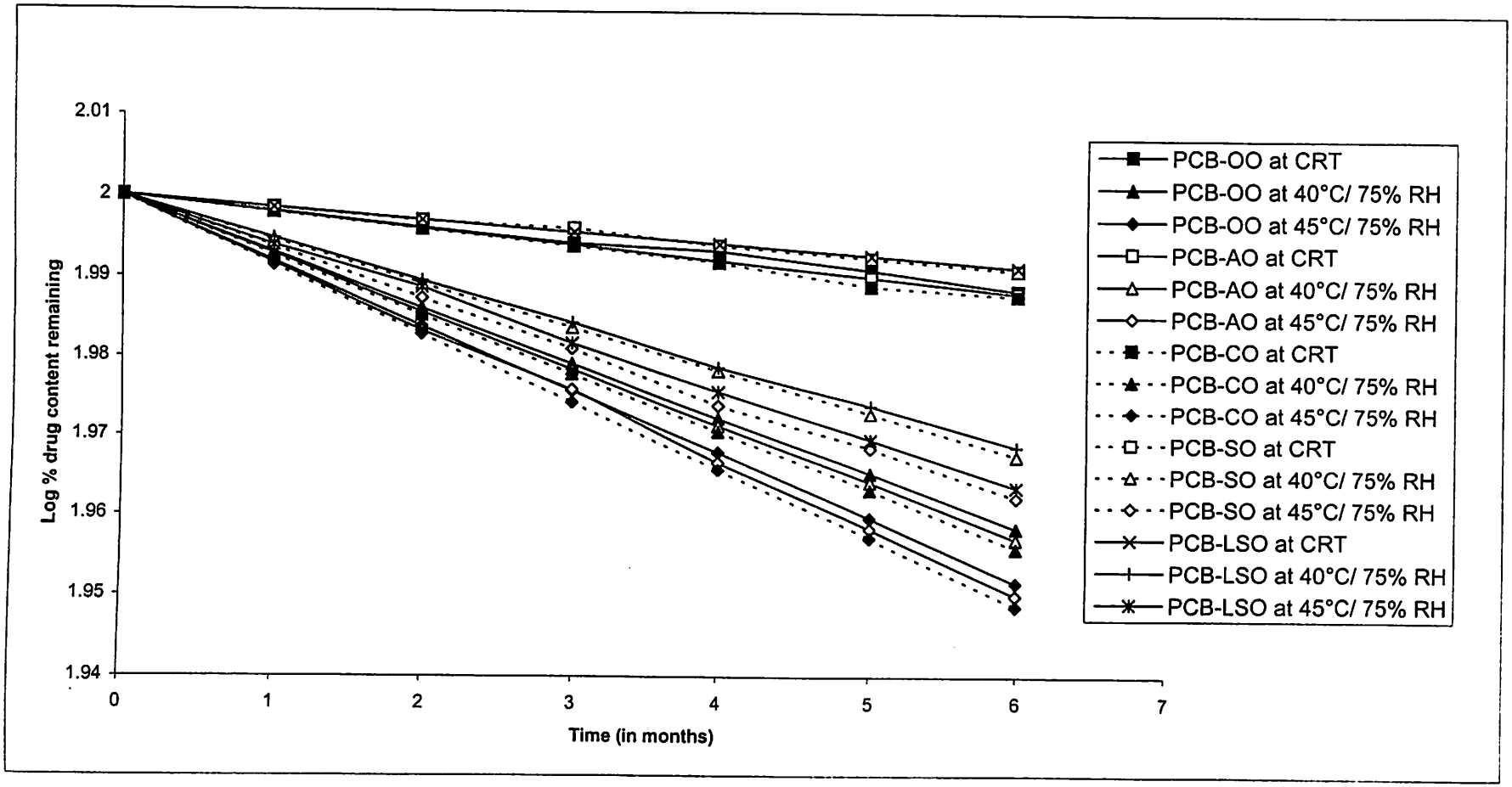

Figure 5.19: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from PCB based formulations in different vegetable oils.

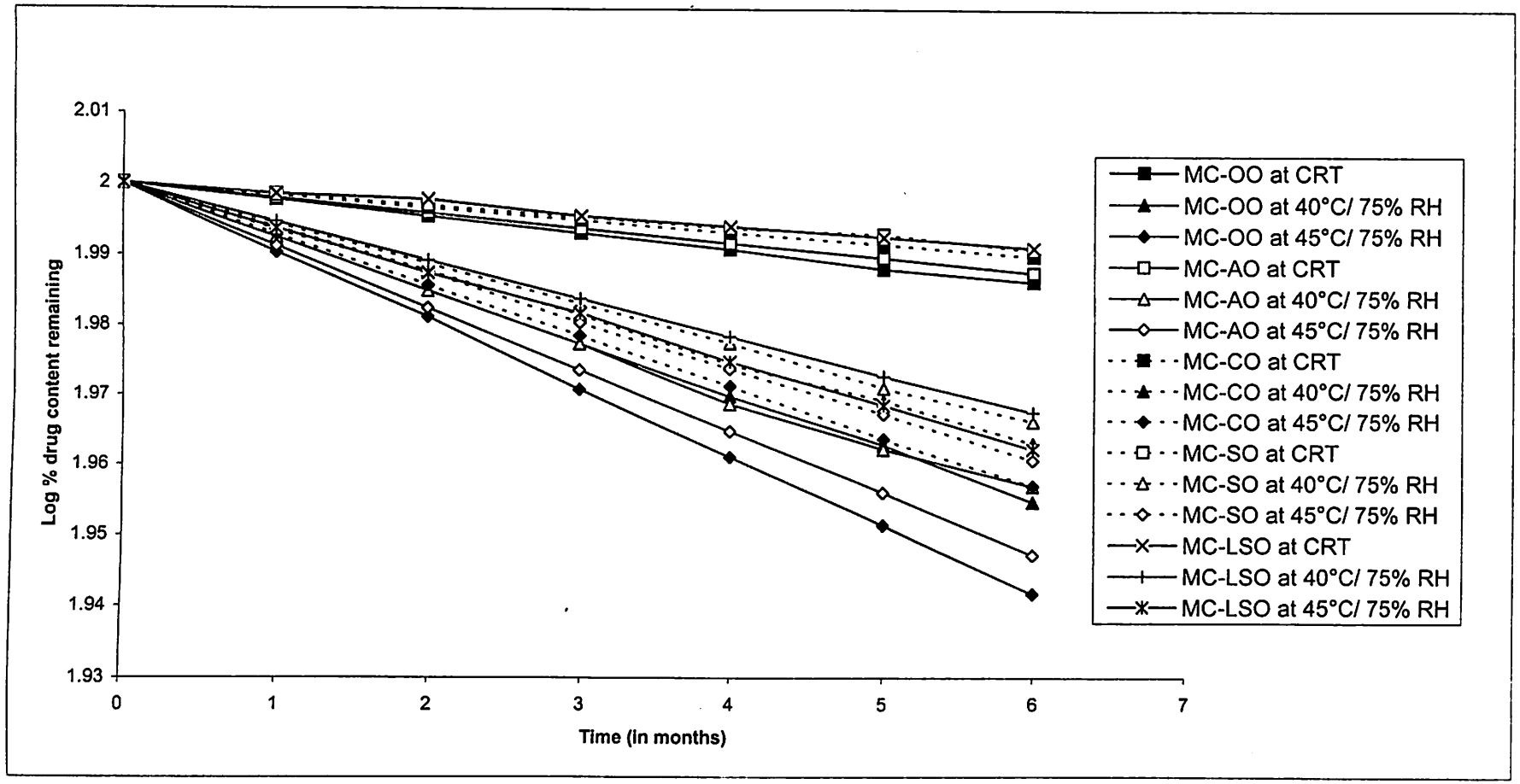

Figure 5.20: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from MC based formulations in different vegetable oils.

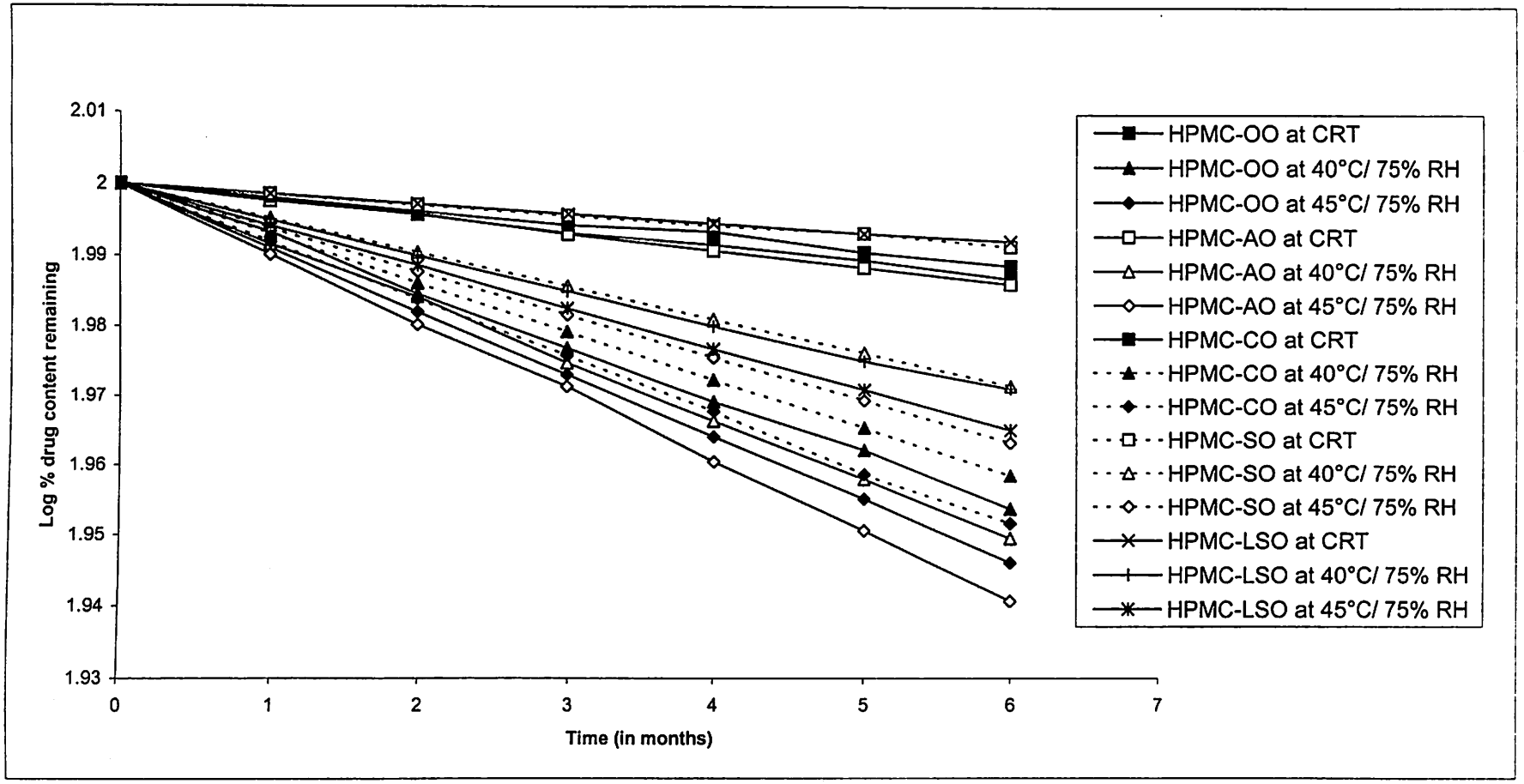

Figure 5.21: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from HPMC based formulations in different vegetable oils.

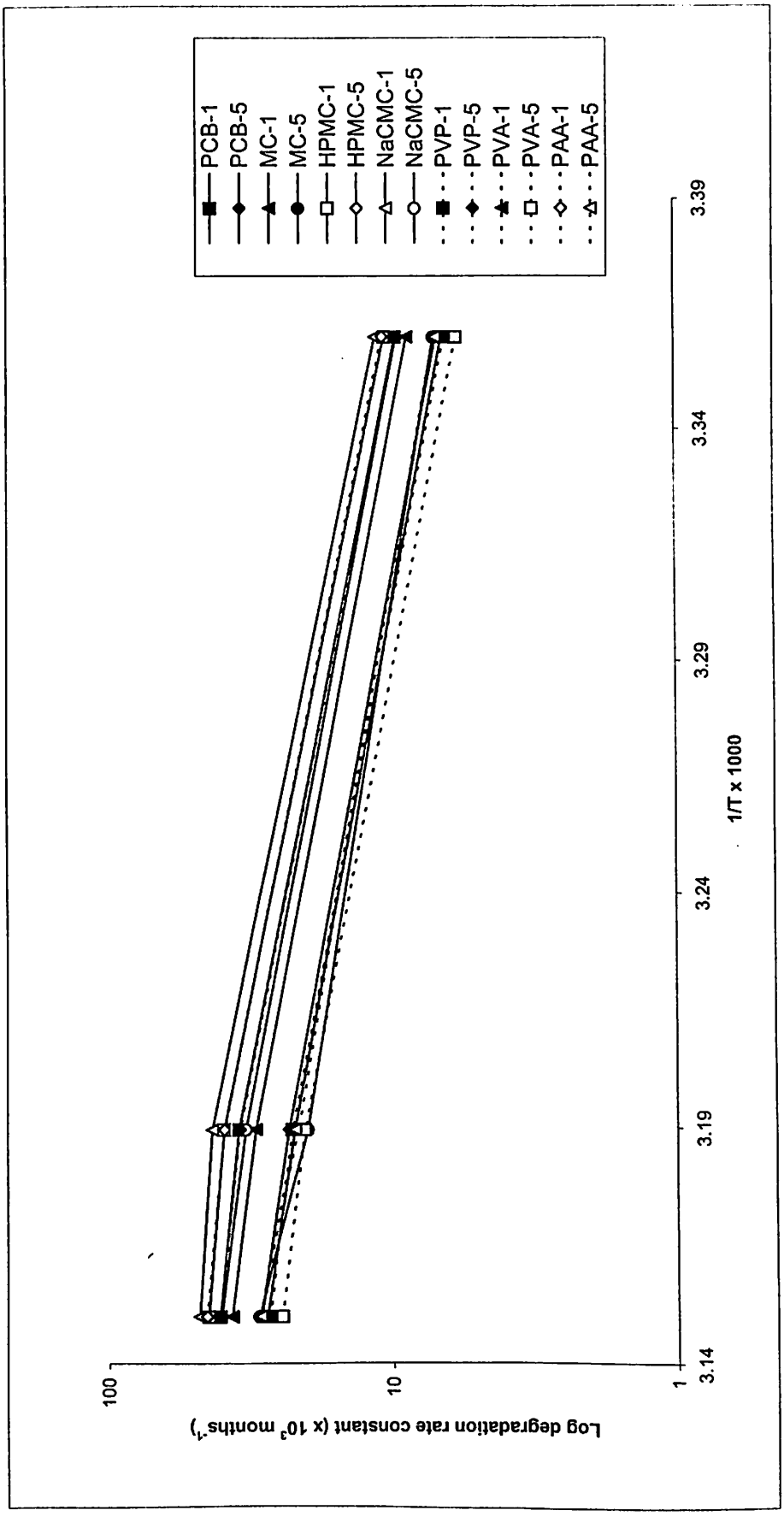

Figure 5.22: Arrhenius plot for degradation of flurbiprofen from polymeric gel type formulations.

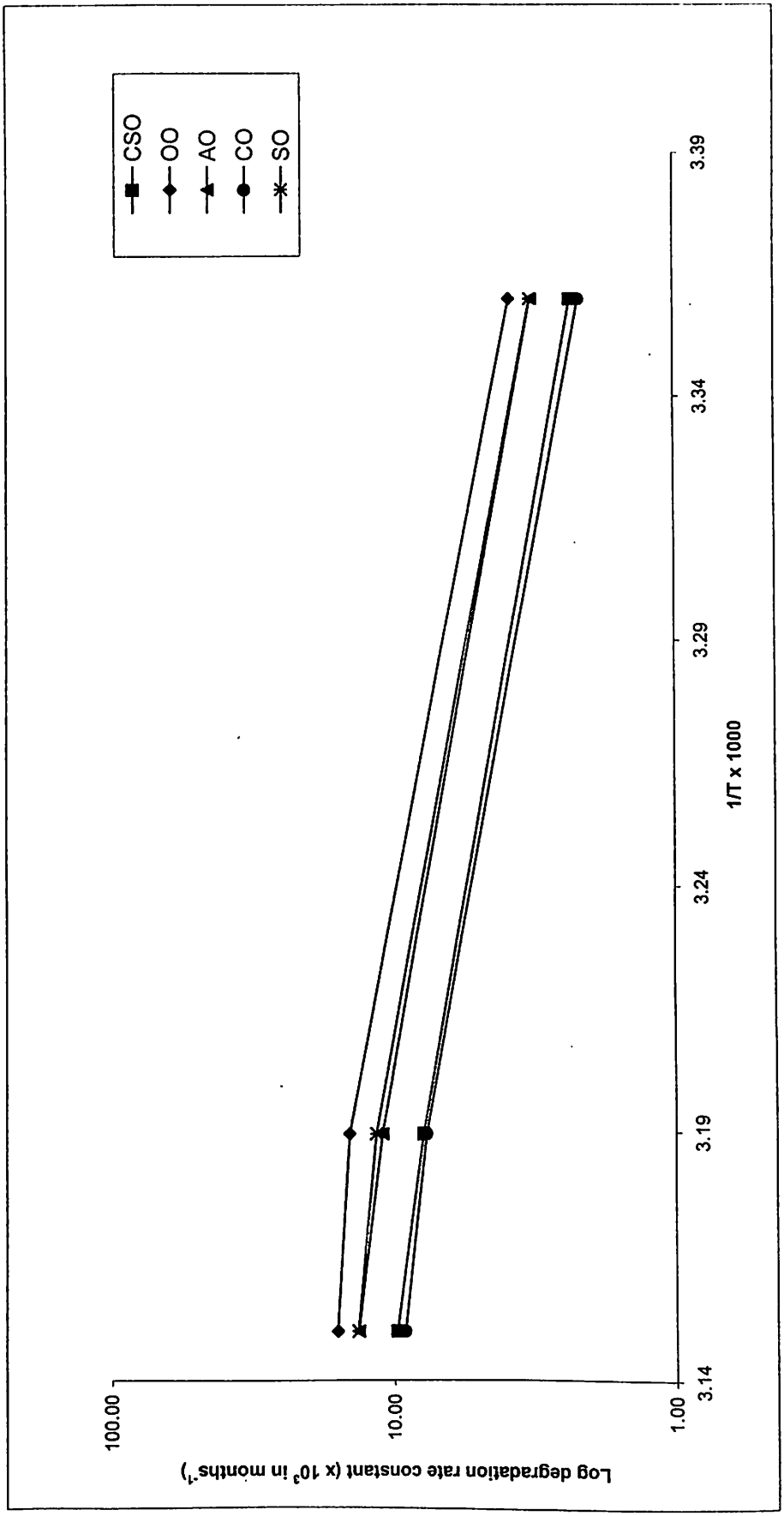

Figure 5.23: Arrhenius plot for degradation of flurbiprofen from vegetable oil based formulations.

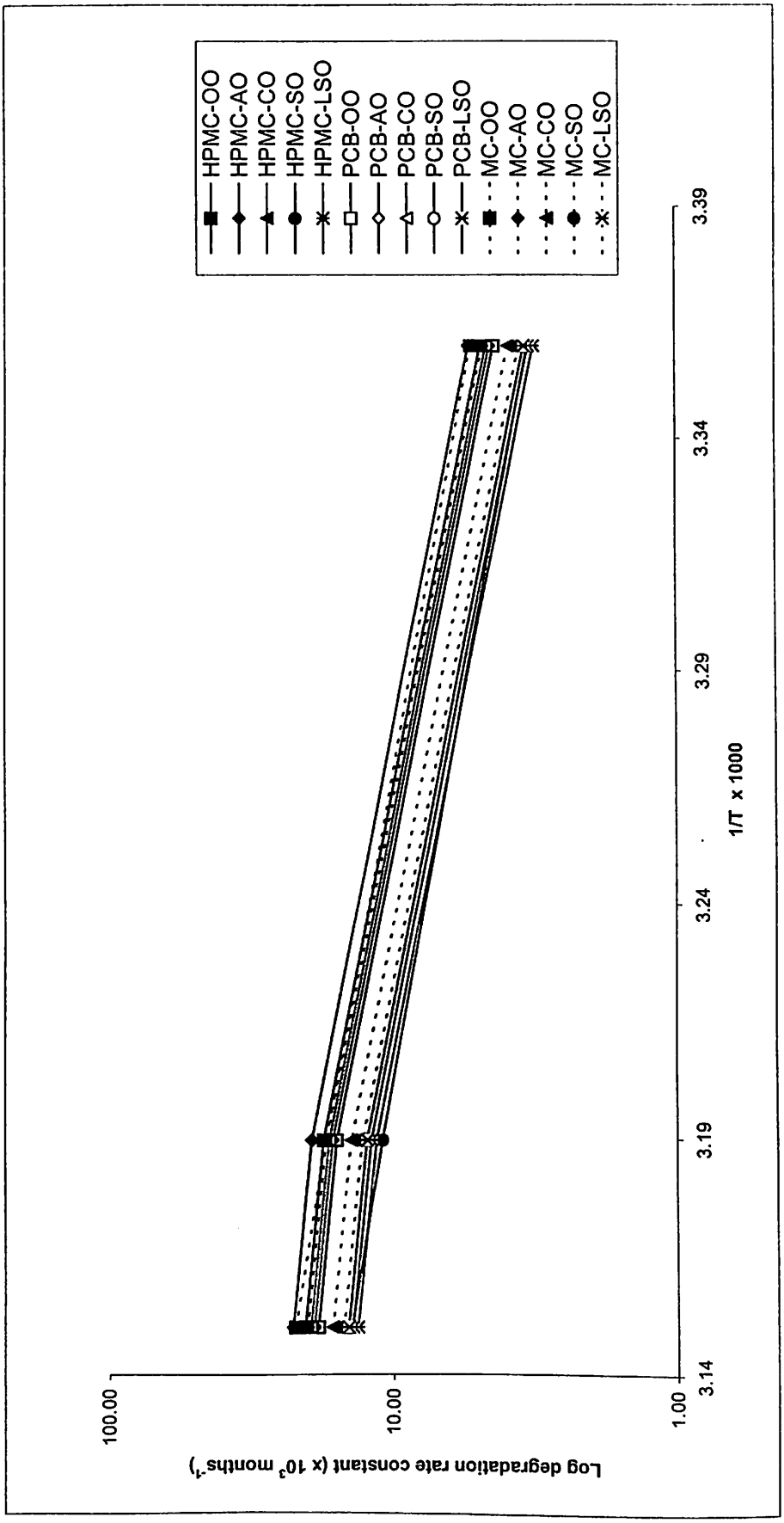

Figure 5.24: Arrhenius plot for degradation of flurbiprofen from polymeric gel based formulations in different vegetable oils.

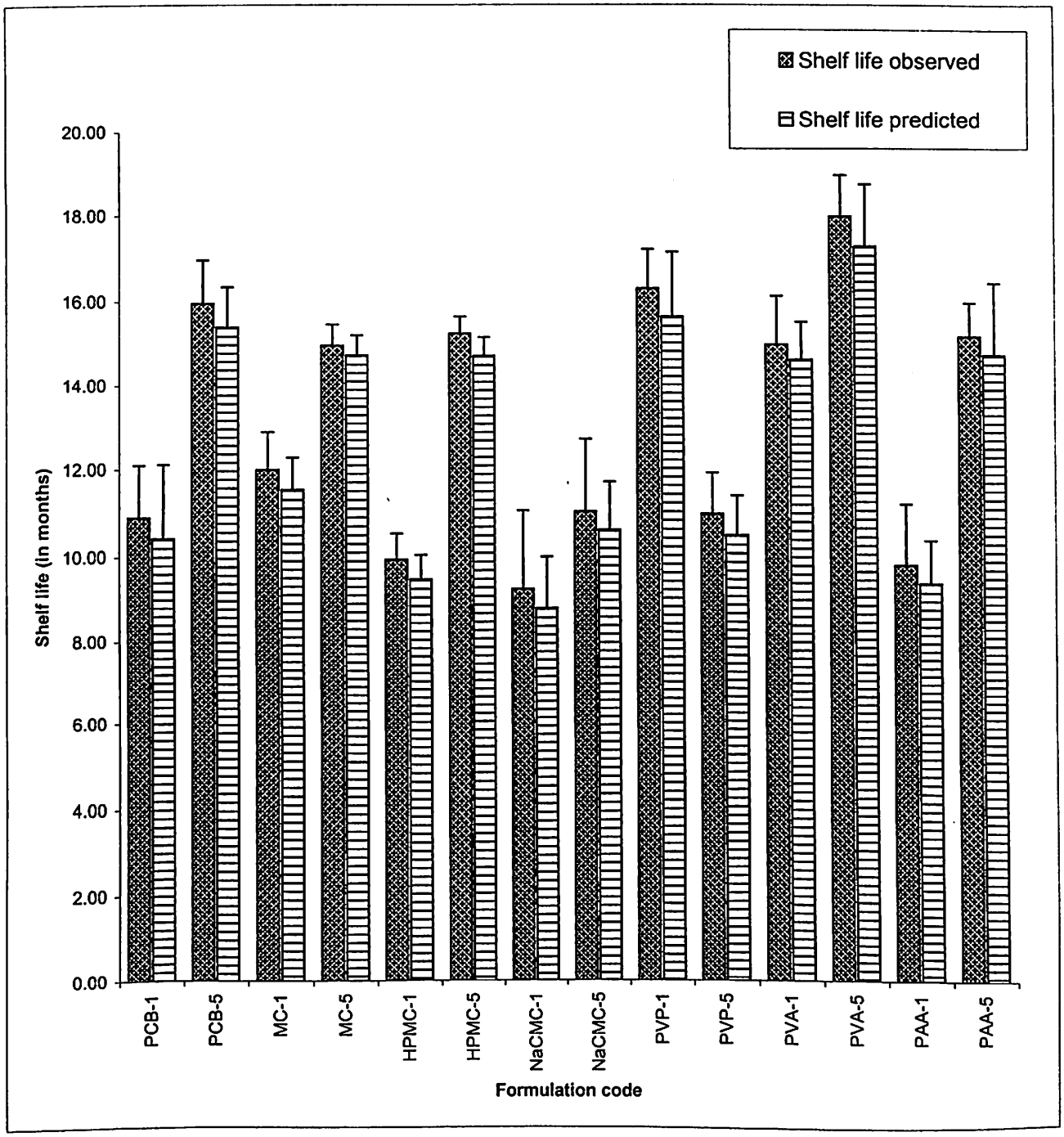

Figure 5.25: Comparative analysis of the predicted and observed shelf life for polymeric gel type flurbiprofen formulations.

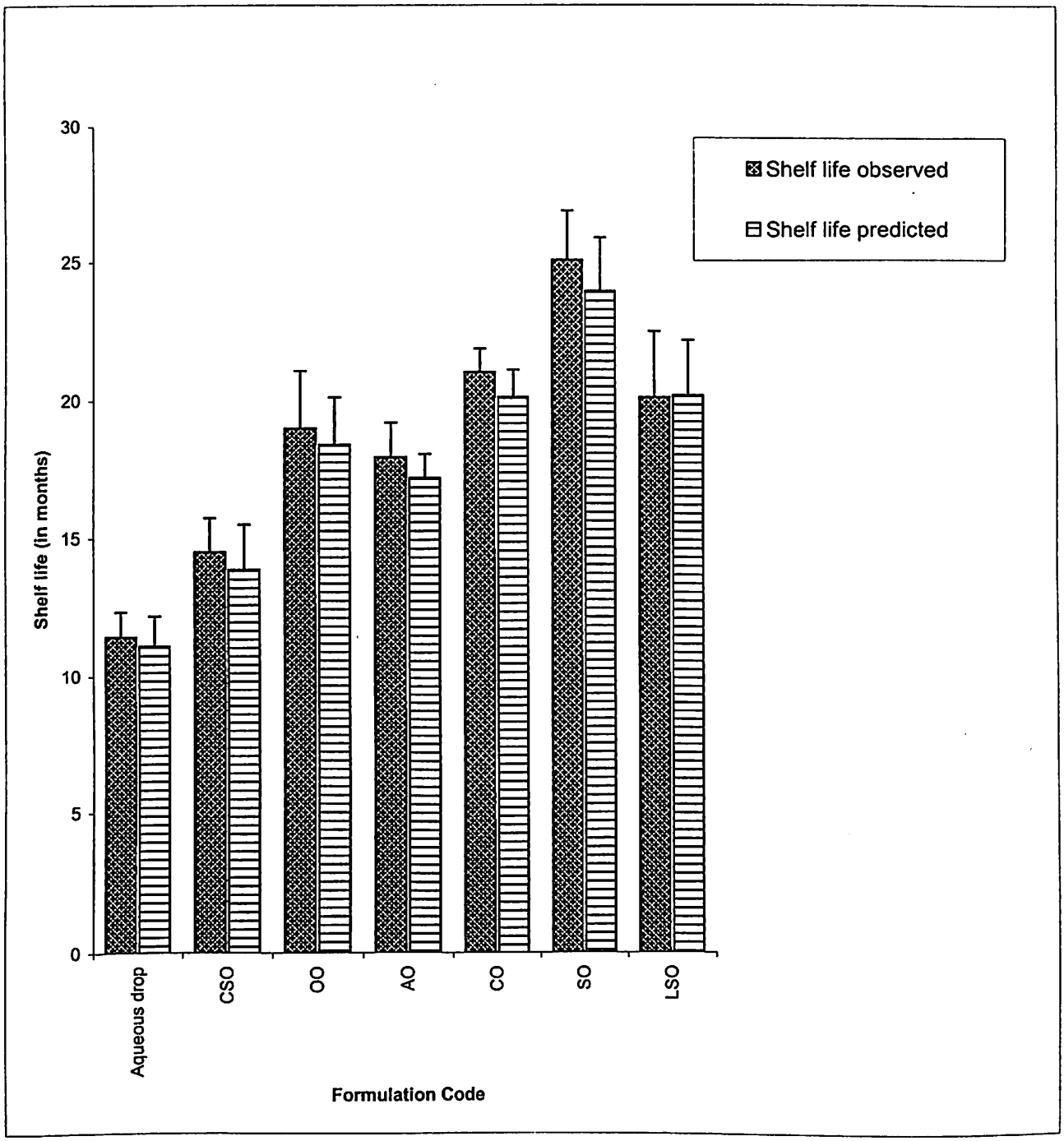

Figure 5.26: Comparative analysis of the predicted and observed shelf life for vegetable oil based flurbiprofen formulations.

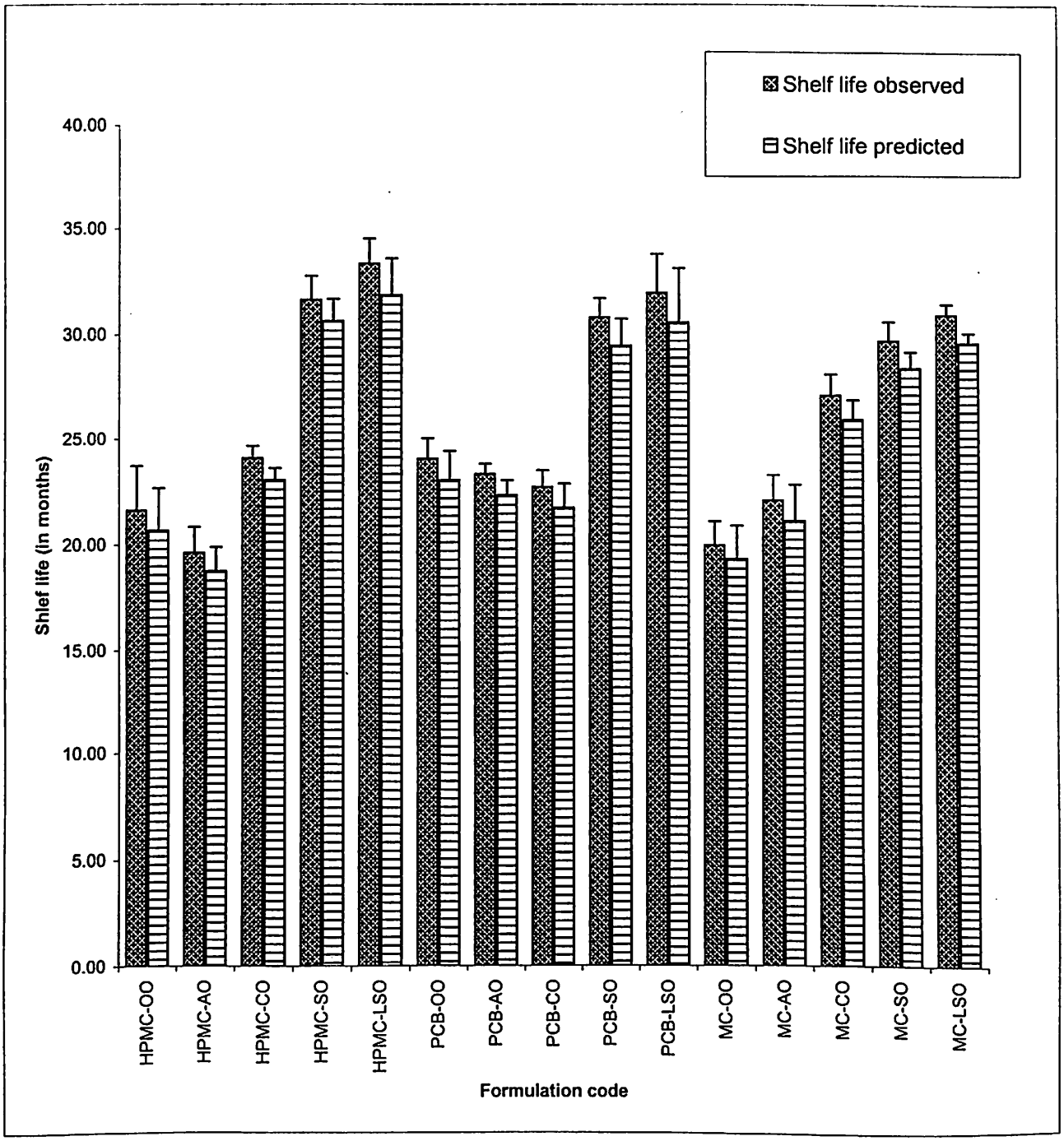

Figure 5.27: Comparative analysis of the predicted and observed shelf life for polymeric gel in combination with oil based formulations of flurbiprofen

CHAPTER 6

MATRIX EMBEDDED POLYMERIC DISC BASED FORMULATIONS

6. MATRIX EMBEDDED POLYMERIC DISC BASED FORMULATIONS

6.1 Introduction

To achieve required level of medication continuously, with better drug bioavailability and without compromising on patient compliance, several workers have attempted drug impregnated polymeric discs. The non-compliance issues, the poor intraocular drug bioavailability and the potential systemic side effects associated with pilocarpine eye drops were significantly improved with development of pilocarpine releasing ocusert system (Shell and Baker 1974). Several bio-erodible drug inserts have been developed and evaluated for ocular use: for the drug pilocarpine CMC wafers (Haddad, 1974; Haddad and Loucas, 1975), PVA disc (Grass et al., 1984) and PVA rod (Bondi and Harwood, 1988). Collagen waffers of gentamycin sulfate (Bloomfield et al., 1978; Slatter et al., 1982; Punch et al., 1985), erodible ocular inserts containing hydrophobic polycarboxylic acid (Heller and Baker, 1974) and various NSAID containing matrix embedded polymeric discs (Sajeev et al, 2001a; Saha et al, 2002) have also been studied. Despite the efforts only few devices have been successfully marketed till date because of inherent problems of patient comfort in the ocular spaces (a detailed review has been presented in chapter 1).

In this chapter, studies involving development and evaluation of controlled release ocular disc type formulations of flurbiprofen prepared by the matrix-embedded technique alone or in combination with membrane barrier technique have been presented. Drug impregnated small polymeric discs of flurbiprofen were prepared using varying proportion (10 to 50 % w/v of the drug content) of polymers using ethyl cellulose (EC), hydroxy propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC), sodium carboxy methyl cellulose (NaCMC), polyvinyl alcohol (PVA), polyacrylamide (PAA) and sodium alginate (NaAL). Proportion and type of the retardant polymer were varied to formulate discs and to study their effect on drug release kinetics from designed formulations. Effect of hydrophobic polymeric coat on the release kinetics from matrix-embedded polymeric discs was also studied to demonstrate dual control. Fabricated discs were evaluated for physico-chemical properties, drug content and *in-vitro* drug release. Effect of sterilization on stability of the drug and its release from the designed formulations was also investigated. Also investigated was batch reproducibility and stability on storage and the effect of storage conditions on the shelf life of selected formulations.

6.2 Experimental

Materials

Pure flurbiprofen was obtained as a gift sample from Optho Remedies Pvt. Ltd., Allahabad. All other the polymers, chemicals and reagents used were pharmaceutical or analytical grade and were used as received.

Equipment

A UV-visible-NIR spectrophotometer (Jasco, Tokyo, Japan, model V-570) with automatic wavelength accuracy of 0.1 nm, a 10 mm matched quartz cells with Jasco spectra manager software was used for all absorbance measurements for UV analysis. A scanning spectrofluorimeter (Jasco, Tokyo, Japan, model FP-777) with built-in compatible software, link search mode, multiple PMT gain mode, automatic wavelength accuracy of 1.5 nm, range 220-750 nm and 10 mm quartz cells was used for fluorescence intensity measurement in case of spectroflourimetric analysis. A single station four-punch disc compression machine (Cadmach, Ahmedabad, India) was used for manufacturing polymeric discs. Afcoset, Type ER182A, electronic balance was used for all weighing. Hardness was determined using Monsanto (standard type) hardness tester and friability on a Campbell Electronic friabilator.

In-vitro dissolution studies were carried out in in-house fabricated double-chambered water jacketed (to maintain the desired temperature of 37 ± 1°C) glass dissolution flask with an internal volume of 20 ml and fitted with magnetic stirrer. All sterilization procedures were carried out in the sterilization unit of Chethana Pharmaceuticals Ltd., Perianthalmanna, Kerala using radioisotope cobalt-60 at dose of 2.0 to 2.5 megarads.

Analytical method

UV-visible spectrophotometric method of analysis (Sajeev et al., 2002) as described in Chapter 3 was employed for the analysis of the drug using phosphate buffer (pH 6.4) at 248 nm in the concentration range 1-20 μg/ml. The spectroflourimetric method (Sajeev et al, 2001b) described in Chapter 3 using 1:1 mixture of methanol and 0.1N H_2SO_4 at the λ_{ex} and λ_{em} of 250 nm and 314 nm respectively was employed for analysis of dissolution study samples with very low drug concentration.

Characterization of bulk drug

The obtained flurbiprofen in bulk were characterized by various official tests (IP, 1996; BP, 1998; USP, 2000) of identification and were analyzed by UV spectrophotometric method as described in the previous section. The IR spectrum obtained using IR spectrophotometer was compared with that of the standard. Effects of various formulation excipients on the UV absorbance and stability of the respective drugs were also studied as reported in Chapter 4.

Preparation of matrix-embedded polymeric discs

Controlled release matrix-embedded polymeric discs of flurbiprofen were prepared by the wet granulation technique using varying proportions (10 %, 20 %, 30 %, 40 % and 50 % w/w of the drug taken) of polymers namely EC, HPMC, CMC, NaCMC, PVA, PAA and NaAL. For each formulation, finely pulverized drug (# 400) and the polymer (# 100) in required quantity (as per the composition given in Table 6.1 to 6.4) were dry blended and then granulated with ethyl alcohol. The granules were passed through # 20 mesh and dried in a tray drier at 55°C. The dry granules were passed through # 40 sieve and compressed on a single station four-punch disc compression machine to get discs 4.0 mm in diameter and 0.5 to 1.5 mm in thickness. Three batches of discss were prepared for each formulation. The obtained discs were packaged into sealed airtight cellophane packets and sterilized by gamma irradiation. After sterilization formulations were stored in controlled room temperature conditions (25 ± 3 °C and 55 ± 15 % RH) when not in use.

Preparation of matrix-embedded polymer coated discs

Polymer coated matrix-embedded discs were prepared by coating 40 % w/w matrix embedded discs of different polymers with one coat of 10 % w/v or alternatively with 20 % w/v EC solution in ethyl alcohol by dip coating. Compositions of formulations prepared by this method are presented in Table 6.5 and 6.6. The prepared formulations were packaged into sealed airtight cellophane packets and sterilized by gamma irradiation. After sterilization formulations were stored in controlled room temperature conditions (25 ± 3 °C and 55 ± 15 % RH) when not in use.

Characterization of designed formulations

For all manufactured formulations disc diameter and thickness were determined. Developed formulations were subjected to following physical characterization studies and the results are

presented in Table 6.1 to 6.4 for various matrix-embedded polymeric discs and in Table 6.5 and 6.6 for matrix-embedded polymer coated discs.

(a) Physical characteristics: The weight variation was evaluated taking 20 discs using an electronic balance (Afcoset, Type ER182A). Hardness was determined for 10 discs using a Monsanto (standard type) disc hardness tester. Friability was determined by testing 10 discs in Campbell Electronic friabilator for 5 min at 25 rpm.

(b) Drug content: To determine the drug content of the manufactured discs of each batch, 20 discs were taken, weighed, and finely ground. An aliquot amount of this powder equivalent to 10 mg of flurbiprofen was accurately weighed, suitably dissolved, diluted in pH 6.4 phosphate buffer to obtain samples in the linearity range of the UV spectrophotometric method and analyzed. From the absorbance data the drug content was calculated on average weight basis and expressed as mg/ disc (Table 6.1 to 6.6).

(c) Sterility: All the manufactured formulations after sterilization were tested for compliance with Pharmacopeial requirement for sterility. The formulations packed in sterilized packets were opened and representative discs ground into powder under aseptic conditions using laminar hood. Required amount of the powdered samples were tested for the absence of bacteria, fungi, yeast and mould by direct transfer method as per the testing procedure enlisted in USP-2000.

***In-vitro* dissolution studies**

In-vitro dissolution studies were carried out in in-house fabricated double-chambered water jacketed (to maintain the desired temperature of 37 ± 1°C) glass dissolution flask with an internal volume of 20 ml and fitted with magnetic stirrer. The assembly was mounted on a Remi model magnetic stirrer with thermostatic temperature control. The dissolution media used was 20 ml phosphate buffer pH 7.4 at 37 ± 1°C. Stirring speed was set at very low level to give very mild shearing action corresponding to the situation in eyes. A 5 ml sample was collected and replaced with fresh media at different time intervals and analyzed. Volume of sample withdrawn was kept high so as to simulate sink condition inside the dissolution flask. Plot between cumulative percentage flurbiprofen release from various designed matrix embedded formulations are shown in Figure 6.1 to 6.7 and that of polymer coated matrix embedded formulations in Figure 6.8 to 6.14. Corresponding drug release data are presented in Table 6.7 to 6.13 for matrix embedded formulations and from 6.14 to 6.20 for polymer coated matrix embedded formulations.

Batch reproducibility

To study batch variation in the manufacturing of the designed formulations three batches of each formulation was manufactured and duplicate samples from each batch was evaluated for its physical characteristic as per the procedure described earlier and the results are presented in Table 6.1 to 6.4 for matrix-embedded polymeric discs and in Table 6.5 and 6.6 for matrix-embedded polymer coated discs. *In-vitro* release data pertaining to reproducibility studies from duplicate study per three batches of each formulations type are presented in Table 6.7 to 6.20.

Effect of sterilization on the stability and release kinetics

To study the effect of method of sterilization on the stability of the drug and release kinetics, the drug content and dissolution profile before and after sterilization for all the formulations were studied. A representative comparative profiles of flurbiprofen released from selected formulations (NaCMC-40, PVA-40, NaAL-40, CMC-40, HPMC-40, PAA-40, EC-40, NaCMC-40/EC-20, PVA-40/EC-20, NaAL-40/EC-20, CMC-40/EC-20, HPMC-40/EC-20, PAA-40/EC-20, EC-40/EC-10, EC-40/EC-20), selected on the basis of drug release profile from earlier studies, are presented in Figure 6.15 and 6.16. The drug content determined before and after sterilization process for above selected formulations along with the results of one-way ANOVA is enlisted in Table 6.28.

Stability studies

Stability studies of the formulation were carried out at different temperature and RH conditions [controlled room temperature (CRT, 25 ± 3 °C and 55 ± 15 % RH); 40 °C, 75 % RH; and 45 ° C, 75 % RH] as per the procedure enlisted in chapter 4. The prepared formulations were packaged into sealed airtight cellophane packets and exposed to above storage conditions. The samples in triplicate were withdrawn at predetermined time intervals (0, 1, 2, 3, 4, 5, 6 months) and analyzed after suitable dilution for flurbiprofen content (as per the procedure enlisted in earlier section). From the absorbance values of the samples, after adjusting for dilution factor, the residual amount of flurbiprofen was calculated on average weight basis. From the slope of the plot between log % residual flurbiprofen content and time (as shown in Figure 6.17 to 6.23 for matrix-embedded polymeric discs and in Figure 6.27 to 6.29 for matrix-embedded polymer coated discs) the degradation rate constant (K_{deg}) was calculated as $K_{deg} = -2.303 \times$ Slope. From the Arrhenius plot (Figure 6.24 to 6.26 for matrix-embedded polymeric discs and in Figure 6.30

for matrix-embedded polymer coated discs) the degradation rate constant at 25 °C (K_{25}) was predicted for various formulations and compared with the observed K_{CRT}. Observed K_{CRT} and predicted K_{25} was used to determine the shelf life or t_{90} (time for drug content to fall to 90%) of the product as presented in Table 6.29 and 6.30 for matrix-embedded polymeric discs and for matrix-embedded polymer coated discs type formulations respectively. A comparative analysis of the predicted and observed shelf life for various developed formulations are presented in Figure 6.31 to 6.32.

6.3 Results and discussion

Characterization of bulk drug

The obtained flurbiprofen passed the tests of identification and analysis as per official tests (IP, 1996; BP, 1998; USP, 2000). The IR spectrum obtained using IR spectrophotometer was comparable with that of the standard. The formulation additives (in the concentration used) did not affect the stability and analysis of the drug.

Characterization of designed formulations

The designed matrix embedded polymeric discs of flurbiprofen were found to possess very good physical properties and the results are presented in Table 6.1 (NaCMC and PVA), Table 6.2 (NaAL and CMC), Table 6.3 (HPMC and PAA) and Table 6.4 (EC). Weight variation in case of all the matrix embedded discs was found to be within ± 3.9 % of the average weight, which falls within the acceptance criteria (Banker and Anderson, 1991). Hardness of the manufactured matrix embedded discs increased with the increase in the proportion of the polymer in the disc. Because of the low thickness of the order of 0.5 mm to 0.75 mm in case of discs with 10 % w/w and 20 % w/w polymers, it was very difficult to manufacture such polymeric discs with hardness above 3.0 Kg/ cm^2. Polymeric discs with 30 % w/w to 50 % w/w polymeric proportion had a hardness value ranging between 3.0 to 4.0 Kg/ cm^2. Friability in case of all the matrix embedded formulations was found to be less than 0.5 % indicating wet granulation to be an acceptable method for manufacturing good quality matrix embedded polymeric discs for controlled release of flurbiprofen. The drug content of all the developed formulations was found to vary between 95 % and 105 % of the theoretical claim further indicating the reliability and reproducibility of the manufacturing process. Sterility studies (as per USP-2000) of the designed formulations after

gamma irradiation demonstrated absence of contamination from bacteria, fungi, yeast and mould thus validating the efficiency of the sterilization process employed.

Matrix embedded polymer coated formulations of flurbiprofen using different polymeric matrix base (40 % w/w) coated with EC solution were also found to possess excellent physical properties and the results are presented in Table 6.5 and 6.6. Weight variation in case of these formulations was also found to be within the acceptance criteria (Banker and Anderson, 1991) with a value within ± 3.9 % of the average weight. Hardness of the manufactured matrix embedded discs were found to be almost similar to that seen in case of 40 % w/w matrix embedded formulations of the corresponding polymers. Coating 40 % w/w matrix discs with EC solution drastically reduced the friability of the formulations to less than 0.2 % and in some cases less than 0.1 %. The drug content of all the developed formulations in this category was found to vary between 95 % and 105 % of the theoretical claim. Sterility studies (as per USP-2000) of the designed formulations after gamma irradiation indicated absence of any contamination from bacteria, fungi, yeast and mould thus demonstrating the efficiency of the sterilizing agent.

***In-vitro* dissolution studies**

In-vitro dissolution study data indicate that compared to aqueous drop (as discussed in Chapter 5) the duration of drug release was substantially increased in case of matrix embedded polymeric discs and was found to be directly proportional to the percentage of the selected polymers used in the formulations. Mean cumulative percentage of flurbiprofen released of three batches with duplicate determination per batch from designed polymeric discs in pH 7.4 phosphate buffer prepared are presented in Table 6.7 for NaCMC discs, in Table 6.8 for PVA discs, in Table 6.9 for NaAL discs, in Table 6.10 for CMC discs, in Table 6.11 for HPMC discs, in Table 6.12 for PAA discs and in Table 6.13 for EC discs with varying proportion of the polymers. The corresponding cumulative percentage released vs. time profiles are presented in Figure 6.1 to 6.7 respectively. The duration of release (in hours) was determined on the basis of time taken for more than 97 % to complete drug release. The cumulative percentage released in the first 15 mins was used to compare the initial release characteristics. The duration of release was extended with the increase in the percentage of polymer in the discs (from 10 % w/w to 50 % w/v) in case of all the polymers though in general, the effect of increasing the polymer concentration from 40 % w/w to 50 % w/w had less pronounced effect on sustaining the release during the later part except in case of PVA, CMC and HPMC.

In case of polymeric discs prepared from 10 % w/w NaCMC (NaCMC-10) the initial release in first 15 mins was found to be 56.6 ± 2.6 % and complete release was obtained in 2 hours. Increasing the percentage of NaCMC to 20 % w/w (NaCMC-20) decreased the initial release only to 48.2 ± 2.5 % with complete release within 4 hours. But increasing the proportion of NaCMC beyond 20 % (i.e. to 30 % w/w and higher), as in case of NaCMC-30, NaCMC-40 and NaCMC-50, the initial release was reduced considerably to 16.9 ± 1.3 %, 12.3 ± 3.2 % and 8.5 ± 1.5 % respectively (Table 6.7 and Figure 6.1). The duration of release was extended up to 8 hours in case of NaCMC-30 with 97.7 ± 3.4 % release, up to 10 hour in case of both NaCMC-40 and NaCMC-50 (Figure 6.1). Though the initial release was better controlled in case of NaCMC-50 (50 % w/w polymer) compared to NaCMC-40 (40 % w/w polymer) but the duration of release was found to be the same.

PVA matrix embedded discs (Table 6.8 Figure 6.2) showed much controlled initial release when compared to NaCMC discs with only 23.7 ± 1.9 % release in case of PVA-10 (10 % w/w polymer) and 4.0 ± 1.5 % in case of PVA-50 (50 % w/w polymer). Duration of release was not drastically increased when the polymer proportion was increased from 10 % w/w to 30 % w/w with the release extending up to 2 hours, 3 hours and 4 hours in case of PVA-10, PVA-20 and PVA-30 respectively. But increasing the proportion to 40 % w/w (PVA-40) or to 50 % w/w (PVA-50) increased the duration of release considerably to 6 hours and 10 hours respectively (Figure 6.2).

In case of discs prepared with NaAL, initial release in first 15 mins was found to be 25.8 ± 3.6 %, 20.1 ± 1.1 %, 16.4 ± 2.3 %, 10.5 ± 3.5 % and 6.5 ± 4.9 % for NaAL-10, NaAL-20, NaAL-30, NaAL-40 and NaAL-50 respectively (Table 6.9 and Figure 6.3). The corresponding duration of release was found to be 2 hours, 3 hours, 4 hours, 8 hours and 10 hours respectively, though very little difference was observed in post 90 % release in case of 40 % w/w (NaAl-40) and 50 % w/w (NaAL-50) discs. Increasing the percentage of CMC from 10 % w/w (CMC-10) to 50 % w/w (CMC-50) though decreased the initial release from 37.0 ± 2.4 % to 5.3 ± 3.5 % but the duration of release could be extended only from 3 hours to 8 hours (Table 6.10 and Figure 6.4). A 10 % increase in the proportion of CMC in the matrix increased the duration of release in the corresponding formulation by another 1 hour.

In case of HPMC based matrix embedded polymeric discs, initial release was found be the lowest when compared to NaCMC, PVA, NaAL and CMC based formulations with 22.3 ± 2.1 % and 3.5 ± 1.2 % release in case of HPMC-10 and HPMC-50 respectively (Table 6.11 and Figure 6.5). The longest duration of release was obtained in case of HPMC-40 and HPMC-50 with release extending up to 10 hours and 12 hours respectively and the least in case of HPMC-10 with complete release in 4 hours. Of all the polymers studied, PAA discs were found to be least efficient in controlling the release at lower proportion of the polymer (10 % w/w and 20 % w/w) with complete release in 1.5 hours and 2 hours respectively, though the corresponding initial release of 24.9 ± 1.4 % and 20.2 ± 2.3 % was considerably lower compared to NaCMC and CMC discs at the same polymer proportion (Table 6.12 and Figure 6.6). Initial release in case of PAA discs with higher polymer proportion (30 % w/w to 50 % w/w) was found to vary from 17.6 ± 1.8 % (PAA-30) to 8.9 ± 1.5 % (PAA-50) and the corresponding duration of release extended from 4 hours to 8 hours.

EC based matrix embedded discs were found to control the release of flurbiprofen better than all other formulations developed in this category (Table 6.13 and Figure 6.7). This could be attributed to the fact that of all the polymers investigated, EC is the most hydrophobic. Initial release was found to be very low in case of EC discs with only 5.3 ± 2.3 % release for 10 % w/w polymer (EC-10) and 2.4 ± 1.5 % release for 50 % w/w polymer (EC-50). The duration of release was extended up to 16 hours, 20 hours and 24 hours in case of EC-10, EC-20 and EC-30 respectively. Though the initial release was not affected much by increase in the proportion of EC, a considerable increase in the duration of release was observed with increase in polymer proportion to 40 % w/w (EC-40) and 50 % w/w (EC-50) with release extending up to 48 hours (2 days) and beyond 54 hours (beyond 2 days) (with only 87.6 ± 3.3 % release) respectively.

The developed matrix embedded formulations were found to be hard compact discs and were designed as non-disintegrating matrices that were left as a polymeric cage/ flap like structures at the end of dissolution studies after complete release of the drug. This would be particularly useful for removable of non-degradable matrix structures from the lower cul-de-sac and also would cause less irritation in the precorneal space in the eye. Since the increase in the duration of release upon increasing the proportion of the polymer from 40 % w/w to 50 % w/w was found not to be significant in case of all the polymers, except in NaCMC and also 40 % w/w polymeric

matrix discs gave 5.5 % to 12.3 % initial release (except in case of EC-40 which gave 2.9 %) it was decided to use 40 % w/w matrix embedded discs for fabricating matrix embedded polymer coated formulations to study the effect of dual control on the release pattern of flurbiprofen.

Mean cumulative percentage of flurbiprofen released along with standard deviation of three batches with duplicate determination per batch from designed matrix embedded polymer coated discs in pH 7.4 phosphate buffer prepared are presented in Table 6.14 for NaCMC formulations, in Table 6.15 for PVA formulations, in Table 6.16 for NaAL formulations, in Table 6.17 for CMC formulations, in Table 6.18 for HPMC formulations, in Table 6.19 for PAA formulations and in Table 6.20 for EC formulations with 10 % w/v and 20 % w/v EC coat on 40 % w/w matrix discs. The corresponding cumulative percentage released vs. time profiles are presented in Figure 6.8 to 6.14 respectively. For easy comparison, the cumulative percentage of drug released from respective 40 % w/w polymeric discs (without EC coat) is also presented in corresponding Tables and Figures. As mentioned earlier, the duration of release (in hours) was considered to be time taken for more than 97 % to complete drug release and the cumulative percentage released in the first 15 mins was used to compare the initial release. *In-vitro* dissolution study data indicate that compared to matrix embedding alone an additional control of hydrophobic polymeric coat of EC drastically extends the duration of drug release. Here again, the duration of release was found to further extended with increased polymeric coat thickness (increase in the percentage of polymer in the coating solution).

In case of NaCMC polymeric discs prepared with 40 % w/w NaCMC-40 and coated with different concentration of EC solution (10 % w/v and 20 % w/v) separately, the initial release in first 15 mins was found to be almost unaffected for 10 % w/v EC coated discs (NaCMC-40/EC-10) but reduced to 7.1 ± 2.2 % in case of 20 % w/v EC coat (NaCMC-40/EC-20). The duration of release was extended from 12 hours (in case of NaCMC-40) to 24 hours (in case of NaCMC-40/EC-10) and 60 hours (2.5 days) (in case of NaCMC-40/EC-20) (Table 6.14 and Figure 6.8). Again in case of PVA polymeric discs (Table 6.15 and Figure 6.9), on coating the 40 % w/w disc (PVA-40) with EC solution, the initial release in first 15 mins was found to be reduced to 2.4 ± 1.8 % in case of PVA-40/EC-20 (coating with 20 % w/v EC solution). The duration of release was extended from 6 hours (in case of PVA-40) to 12 hours (in case of PVA-40/EC-10) and 49 hours (more than 2 days) (in case of PVA-40/EC-20).

Coating with 10 % w/v and 20 % w/v EC solution on NaAL-40 discs reduced the initial release to 4.9 ± 1.5 % (NaAL-40/EC-10) and to only 0.7 ± 0.3 % (NaAL-40/EC-20) with duration of release extended up to 18 hours and 96 hours (4 days) respectively (Table 6.16 and Figure 6.10). Similar results were obtained with CMC discs with reduction in the initial release to 4.4 ± 2.3 % (CMC-40/EC-10) and to 1.9 ± 1.3 % (CMC-40/EC-20) with duration of release extended up to 32 hours and 120 hours (5 days) respectively (Table 6.17 and Figure 6.11). In case of HPMC based matrix embedded polymer coated discs the initial release with HPMC-40 was found to be 5.5 ± 2.8 % and coating with EC decreased the release only to 3.4 ± 1.3 % and 2.3 ± 1.1 % respectively in case of HPMC-40/EC-10 and HPMC-40/EC-20 (Table 6.18 and Figure 6.12). The duration of release in this case was extended only up to 32 hours (HPMC-40/EC-10) and to 49 hours (more than 2 days) (HPMC-40/EC-20) (Table 6.18 and Figure 6.12).

In case of PAA polymeric discs prepared from 10 % w/v and 20 % w/v coat on 40 % w/w matrix discs (PAA-40), the initial release in first 15 mins was found to be reduced to 3.5 ± 1.9 % in case of 10 % w/v EC coated discs (PAA-40/EC-10) and only to 3.0 ± 2.3 % in case of PAA-40/EC-20 (20 % w/v EC coat). But the duration of release was extended from 6 hours in case of PAA-40 to 40 hours in case of PAA-40/EC-10 and 120 hours (5 days) in case of PAA-40/EC-20 (Table 6.19 and Figure 6.13). EC coated matrix discs prepared from EC matrix base showed only 0.5 ± 0.2 % release (in case of EC-40/EC-10) and no detectable release up to 30 mins in case of EC-40/EC-20 (Table 6.20 and Figure 6.14). The duration of release in case of EC-40/EC-10 was extended beyond 168 hours (7 days) with only 67.4 ± 0.2 % release in that period. In case of EC-40/EC-20, the duration of release was also extended beyond 168 hours (7 days) with just 34.5 ± 0.8 % drug release. The predicted duration of release could be more than 9 days and 26 days for EC-40/EC-10 and EC-40/EC-20 respectively (Table 6.27). It has been observed in case of all the polymeric discs that coating with 20 % EC solution the release rate decreased and duration of release extended drastically. The corresponding release rate constant and other calculated release parameters are presented in next section.

Characterization of the release kinetics

In order to describe the kinetics of the drug release from controlled or extended release formulations various mathematical equations are used by different workers. Since the controlled release system designed in this study fall in the category of insoluble matrix with fixed geometry

delivery systems it was considered pertinent to investigate the release kinetics using power equation (Ritger and Peppas, 1987). This was done by fitting the dissolution data after 1 hour in the below given equation.

$M_t/M_\infty = K.t^n$

Where, M_t/M_∞ is the fraction of drug released at any time 't'; K is the release rate constant incorporating the structural and geometric characteristics of the discs; n is the diffusional exponent, indicative of the release mechanism. (The value of n for a cylinder is <0.45 for Fickian release, 0.45 to <0.89 for non-Fickian release, 0.89 for case II release, and >0.89 for super II release). The regression analysis between log (M_t/M_∞) and log (t) was done to check the correlation coefficient value (approaching 1.0) of the regression line and smallest mean sum of squared residuals (Parab, et al, 1986). Using the slope and intercept value of the regression plot various parameters like, diffusional exponent, release rate constant and time for release of fixed percentage of the drug from the formulation for comparison purpose were calculated. Calculated release rate constant (K, in $hour^{-n}$) and time for 30 % ($t_{30\%}$), 50 % ($t_{50\%}$), 70 % ($t_{70\%}$) and 90 % ($t_{90\%}$) of drug release from matrix embedded polymeric discs type formulations are enlisted in Table 6.21 to 6.24 and in Table 6.25 to 6.27 for matrix embedded polymer coated discs.

Upon subjecting the drug release data to the above model in order to establish the drug release mechanism it was found that the drug release from designed polymeric discs composed of NaCMC was found to follow Fickian release at lower polymer concentration of 10 % and 20% (in case of NaCMC-10 and NaCMC-20) wherein value of 'n' was obtained as 0.2853 and 0.2662 respectively with good 'r' value and small mean sum of squared residuals (MSSR) value (Table 6.21). But in case of NaCMC-30, NaCMC-40 and NaCMC-50, the release kinetics was found to follow non-Fickian release kinetics as the 'n' value was more than 0.45 but less than 0.89 (Table 6.21). Amongst NaCMC matrix embedded formulations, the release rate constant for drug release from NaCMC-10 was found to be the fastest with a K value of 85.72 $hour^{-0.2853}$ with $t_{30\%}$ of 0.03 hour, $t_{50\%}$ of 0.15 hour, $t_{70\%}$ of 0.49 hour and $t_{90\%}$ of 1.19 hours and the slowest release rate was exhibited by NaCMC-50 with a K value of 24.43 $hour^{-0.6474}$ with $t_{30\%}$ of 1.37 hour, $t_{50\%}$ of 3.02 hour, $t_{70\%}$ of 5.09 hour and $t_{90\%}$ of 7.50 hours (Table 6.21).

In case of NaCMC disc (NaCMC-40) coated with 10 % w/v or 20 % w/v solution of EC the release was found to be Fickian with 'n' value obtained as 0.3139 (NaCMC-40/EC-10) and

0.4352 (NaCMC-40/EC-20) (Table 6.25). The release rate constant for drug release from NaCMC-40/EC-10 was found to be 38.23 $hour^{-0.3139}$ with $t_{30\%}$ of 0.46 hour, $t_{50\%}$ of 2.35 hours, $t_{70\%}$ of 6.87 hours and $t_{90\%}$ of 15.29 hours. And in case of NaCMC-40/EC-20, 'K' was obtained as 19.78 $hour^{-0.4352}$ with $t_{30\%}$ of 2.60 hour, $t_{50\%}$ of 8.42 hour, $t_{70\%}$ of 18.25 hour and $t_{90\%}$ of 32.50 hours (Table 6.25).

In case of PVA matrix embedded discs, the value of 'n' varied from 0.7133 to 0.8287 at different level of polymer concentration indicating that the release of flurbiprofen from PVA matrix base followed non-Fickian release kinetics (Table 6.21). For PVA matrix embedded formulations, the release rate constant in PVA-50 was found to be the slowest with a K value of 24.78 $hour^{-0.8287}$ with $t_{30\%}$ of 1.26 hours, $t_{50\%}$ of 2.33 hours, $t_{70\%}$ of 3.50 hours and $t_{90\%}$ of 4.74 hours and the fastest in case of PVA-10 with a K value of 69.15 $hour^{-0.7282}$ with $t_{30\%}$ of 0.32 hour, $t_{50\%}$ of 0.64 hour, $t_{70\%}$ of 1.02 hour and $t_{90\%}$ of 1.44 hours. In case of PVA disc (PVA-40) coated with 10 % w/v or 20 % w/v solution of EC, the release was found to be Fickian in case of PVA-40/EC-10 with a 'n' value of 0.3758 and non-Fickian in case of PVA-40/EC-20 with a 'n' value of 0.6270 (Table 6.25). The release rate constant for drug release from PVA-40/EC-10 was found to be 41.55 $hour^{-0.3758}$ with $t_{30\%}$ of 0.42 hour, $t_{50\%}$ of 1.64 hours, $t_{70\%}$ of 4.01 hours and $t_{90\%}$ of 7.82 hours. And in case of PVA-40/EC-20 'K' was obtained as 10.63 $hour^{-0.6270}$ with $t_{30\%}$ of 5.23 hours, $t_{50\%}$ of 11.81 hours, $t_{70\%}$ of 20.20 hours and $t_{90\%}$ of 30.17 hours.

NaAL discs followed non-Fickian release kinetics as seen from the value of 'n' of the power equation which varied from 0.5963 to 0.8002 for various formulations of NaAL (Table 6.22). NaAL-10 exhibited the fastest release with a 'K' value of 67.70 $hour^{-0.6802}$, $t_{30\%}$ of 0.30 hour, $t_{50\%}$ of 0.64 hour, $t_{70\%}$ of 1.05 hour and $t_{90\%}$ of 1.52 hours and the slowest in case of NaAL-50 with a K value of 21.86 $hour^{-0.8002}$, $t_{30\%}$ of 1.49 hours, $t_{50\%}$ of 2.81 hours, $t_{70\%}$ of 4.28 hours and $t_{90\%}$ of 5.86 hours. The release rate constant for drug release from NaAL-40/EC-10 was found to be 30.50 $hour^{-0.4477}$ with $t_{30\%}$ of 0.96 hour, $t_{50\%}$ of 3.02 hours, $t_{70\%}$ of 6.40 hours and $t_{90\%}$ of 11.21 hours (Table 6.25). And in case of NaAL-40/EC-20 it was obtained as 4.02 $hour^{-0.7190}$, $t_{30\%}$ of 16.37 hours, $t_{50\%}$ of 33.31 hours, $t_{70\%}$ of 53.19 hours and $t_{90\%}$ of 75.45 hours (Table 6.25). Both the Ec coated formulations of NaAL-40 were found to possess non-Fickian release kinetics.

For CMC based formulations the release kinetics was found to follow Fickian release kinetics for CMC-10 with 'n' value of 0.4399 and non-Fickian release kinetics for CMC-20 to CMC-50 (Table 6.22). Calculated release rate constant (K, in hour^{-n}) and time for 30 % ($t_{30\%}$), 50 % ($t_{50\%}$), 70 % ($t_{70\%}$) and 90 % ($t_{90\%}$) of drug release from CMC matrix embedded polymeric discs type formulations are enlisted in Table 6.22 and for CMC matrix embedded polymer coated discs in Table 6.26. Amongst CMC matrix embedded formulations, the release rate constant for drug release from CMC-10 was found to be the fastest with a K value of 68.60 hour$^{-0.4399}$ with $t_{30\%}$ of 0.15 hour, $t_{50\%}$ of 0.49 hour, $t_{70\%}$ of 1.05 hour and $t_{90\%}$ of 1.85 hours and the slowest release rate was exhibited by CMC-50 with a K value of 25.46 hour$^{-0.8367}$ with $t_{30\%}$ of 1.22 hour, $t_{50\%}$ of 2.24 hours, $t_{70\%}$ of 3.35 hours and $t_{90\%}$ of 4.52 hours (Table 6.22). In case of CMC disc (CMC-40) coated with 10 % w/v or 20 % w/v solution of EC the release was found to be non-Fickian with value of 'n' obtained as 0.5894 (CMC-40/EC-10) and 0.6210 (CMC-40/EC-20) (Table 6.26). The release rate constant for drug release from CMC-40/EC-10 was found to be 14.51 hour$^{-0.5894}$ with $t_{30\%}$ of 3.43 hours, $t_{50\%}$ of 8.16 hours, $t_{70\%}$ of 14.45 hours and $t_{90\%}$ of 22.13 hours. And in case of CMC-40/EC-20 it was obtained as 7.16 hour$^{-0.6210}$ with $t_{30\%}$ of 10.04 hours, $t_{50\%}$ of 22.87 hours, $t_{70\%}$ of 39.31 hours and $t_{90\%}$ of 58.92 hours.

All HPMC matrix embedded formulations were found to follow non-Fickian release kinetics (Table 6.23) except HPMC-50, which showed an 'n' value of 0.9045 thus approaching first order release kinetics. The release rate constant for drug release from HPMC-10 was found to be the fastest with a K value of 50.24 hour$^{-0.6141}$ with $t_{30\%}$ of 0.43 hour, $t_{50\%}$ of 0.99 hour, $t_{70\%}$ of 1.72 hours and $t_{90\%}$ of 2.58 hours and the slowest release rate was exhibited by HPMC-50 with a K value of 14.24 hour$^{-0.9045}$ with $t_{30\%}$ of 2.28 hours, $t_{50\%}$ of 4.01 hours, $t_{70\%}$ of 5.82 hours and $t_{90\%}$ of 7.68 hours (Table 6.23). In case of HPMC disc (HPMC-40) coated with 10 % w/v or 20 % w/v solution of EC the release was found to be non-Fickian with value of 'n' obtained as 0.6472 (HPMC-40/EC-10) and 0.7583 (HPMC-40/EC-20) (Table 6.26). The release rate constant for drug release from HPMC-40/EC-10 was found to be 12.97 hour$^{-0.6472}$ with $t_{30\%}$ of 3.66 hours, $t_{50\%}$ of 8.05 hours, $t_{70\%}$ of 13.54 hours and $t_{90\%}$ of 19.96 hours. And in case of HPMC-40/EC-20 it was obtained as 5.75 hour$^{-0.7583}$ with $t_{30\%}$ of 8.83 hours, $t_{50\%}$ of 17.31 hours, $t_{70\%}$ of 26.98 hours and $t_{90\%}$ of 37.58 hours.

PAA discs followed non-Fickian release kinetics as seen from the value of 'n' of the power equation which varied from 0.6299 to 0.7877 for various formulations of PAA (Table 6.23). PAA-10 exhibited the fastest release with a 'K' value of 81.25 $hour^{-0.7877}$, $t_{30\%}$ of 0.28 hour, $t_{50\%}$ of 0.54 hour, $t_{70\%}$ of 0.83 hour and $t_{90\%}$ of 1.14 hours and the slowest in case of PAA-50 with a K value of 31.23 $hour^{-0.6802}$, $t_{30\%}$ of 0.94 hour, $t_{50\%}$ of 2.00 hours, $t_{70\%}$ of 3.28 hours and $t_{90\%}$ of 4.74 hours. In case of PAA matrix coated discs the release rate constant for drug release from PAA-40/EC-10 was found to be 12.17 $hour^{-0.0.5919}$ with $t_{30\%}$ of 4.59 hours, $t_{50\%}$ of 10.88 hours, $t_{70\%}$ of 19.20 hours and $t_{90\%}$ of 29.36 hours (Table 6.27). And in case of PAA-40/EC-20, 'K' was obtained as 5.51 $hour^{-0.6212}$, $t_{30\%}$ of 15.28 hours, $t_{50\%}$ of 34.78 hours, $t_{70\%}$ of 59.78 hours and $t_{90\%}$ of 89.58 hours (Table 6.27).

As discussed in the previous section the EC matrix discs produced slowest release profile with the release pattern following non-Fickian release kinetics as inferred from the calculated 'n' values for the formulations (Table 6.24). EC-10 exhibited the fastest release rate in this category of formulations with a K value of 17.62 $hour^{-0.6954}$ with $t_{30\%}$ of 2.15 hours, $t_{50\%}$ of 4.48 hours, $t_{70\%}$ of 7.27 hours and $t_{90\%}$ of 10.43 hours and the slowest release rate was exhibited by EC-50 with a K value of 8.58 $hour^{-0.6560}$ with $t_{30\%}$ of 6.74 hours, $t_{50\%}$ of 14.69 hours, $t_{70\%}$ of 24.53 hours and $t_{90\%}$ of 35.98 hours (Table 6.24). In case of EC disc (EC-40) coated with 10 % w/v or 20 % w/v solution of EC, the release was found to be non-Fickian with value of 'n' obtained as 0.7657 (EC-40/EC-10) and 0.6803 (EC-40/EC-20) (Table 6.27). The release rate constant for drug release from EC-40/EC-10 was found to be only 1.49 $hour^{-0.7657}$ with $t_{30\%}$ of 50.56 hours (> 2 days), $t_{50\%}$ of 98.53 hours (> 4 days), $t_{70\%}$ of 152.91 hours (> 6 days) and a predicted $t_{90\%}$ of 212.32 hours (approx. 9 days). And in case of EC-40/EC-20, 'K' was obtained as 1.12 $hour^{-0.6803}$ with $t_{30\%}$ of 125.29 hours (> 5 days), predicted $t_{50\%}$ of 265.48 hours (> 11 days), predicted $t_{70\%}$ of 435.35 hours (> 18 days) and predicted $t_{90\%}$ of 629.90 hours (> 26 days) which comes close to one month.

Batch reproducibility

Batch to batch variability and reproducibility of the manufacturing process was studied based on evaluation of the physical properties and release characteristics of drug from three batches of each of the designed formulations. The evaluated physical characteristic of the formulations presented in Table 6.1 to 6.4 for matrix embedded polymeric discs and in Table 6.5 and 6.6 for

matrix embedded polymer coated discs showed low value of standard deviation of duplicate sample analysis per batch for three batches of each formulation. Low standard deviations of the drug content, weight variation, hardness and friability for three different batches of each formulation indicate absence of significant batch-to-batch variation. No significant difference was observed in the release profile of different batches of the developed flurbiprofen formulations as seen from the low value of standard deviation of the cumulative release data at different time points obtained from replicate dissolution studies of samples (Table 6.7 to 6.20) from different batches (as discussed in previous sections) indicating that the wet granulation is an acceptable method for manufacturing good quality matrix embedded discs of flurbiprofen for ocular delivery.

Effect of sterilization method on the stability and release kinetics

Gamma irradiation method was not found to have an adverse effect on the drug stability and release kinetics from the developed formulations. Analysis of drug content before and after sterilization process for above selected formulations also did not show any difference as evident from the low calculated F value (based on replicate measurement per batch per formulation) of one-way ANOVA of drug content data (Table 6.28). A representative comparative dissolution profile of flurbiprofen release from selected formulations discussed in this chapter before and after sterilization, as shown in Figure 6.15 (matrix embedded discs) and Figure 6.16 (polymer coated matrix embedded discs), did not reveal any change in the release profile. The results of drug content determination and dissolution studies reveal that the gamma irradiation method could be used for sterilizing such formulations with out affecting the drug stability and release kinetics.

Stability studies

Flurbiprofen in matrix embedded polymeric discs was found to follow first order degradation as can be seen from the linear plot between log percentage drug content remaining and time profile for the lowest (10 % w/w) and highest (50 % w/w) of each polymer type (Figure 6.17 to 6.23) and also from various polymer coated matrix discs based formulations (Figure 6.27 to 6.29, plot for discs coated with 20 % w/v EC solutions are only shown). From the Arrhenius plot (log K_{deg} vs. reciprocal of absolute temperature) (Figure 6.24 to 6.26 for matrix embedded formulations and Figure 6.30 for polymer coated matrix embedded formulations), the degradation rate

constant at 25°C (K_{25}) was extrapolated and compared with the observed K_{CRT}. Observed K_{CRT} and predicted K_{25} were used to determine the shelf life or t_{90} (time for drug content to fall to 90%) of the product as presented in Table 6.29 (matrix embedded discs) and Table 6.30 (polymer coated matrix discs).

Among various matrix embedded polymeric discs type formulations, the predicted degradation rate constant (K_{25}) as well as the observed degradation rate constant (K_{CRT}) decreased with increase in percentage of polymer in the formulation from 10 % w/w to 50 % w/w (Table 6.29). The observed and predicted first order degradation rate constant was found to be higher ($> 3 \times 10^{-3}$ months^{-1}) in case of NaAL, CMC and PAA at all the polymer concentration when compared to NaCMC, PVA, HPMC and EC. The predicted or observed shelf life varied between 37.0 to 49.5 months in case NaCMC, PVA, HPMC and EC discs. Whereas in case of NaAL, CMC and PAA discs it varied between 27 to 34 months. A comparative analysis of the predicted and observed shelf life of matrix embedded polymeric discs type formulations of flurbiprofen did not reveal any significant difference (at $p< 0.01$) (Figure 6.31) indicating validity of the accelerated stability study.

A substantial decrease in the degradation rate constant (both observed at CRT and predicted at 25 °C) was observed in case of most of the polymer coated matrix discs (Table 6.30). Maximum shelf life among this type of formulation was obtained for EC matrix discs coated with EC solution (approximately 50-52 months) whereas lowest shelf life was observed in case PVA, CMC and PAA based formulations (38 to 43 months) (Table 6.30). A comparative analysis of the predicted and observed shelf life of EC coated matrix embedded discs of various polymers of flurbiprofen did not reveal any significant difference (at $p< 0.01$) (Figure 6.32).

6.4 Conclusions

The designed and developed matrix embedded discs with or with out EC coat were found to possess good physical properties like uniform drug content, very low weight variation, friability and hardness. Low value of standard deviation of triplicate sample analysis per batch for three batches of each formulation suggest absence of significant batch-to-batch variation. No significant difference was observed in the release profile of different batches of the developed flurbiprofen formulations thus suggesting wet granulation technique using ethyl alcohol to be an

acceptable method for manufacturing good quality matrix embedded discs of flurbiprofen for ocular delivery. The duration of release was extended with the increase in the percentage of polymer in the matrix embedded discs. EC matrix discs prolonged the duration of flurbiprofen release to the maximum and PAA and CMC were found to have the least control. In case of matrix embedded formulations at 40 % w/w of the polymer, satisfactory initial release in the first 15 mins was seen (except in case of EC-40) thus ruling out the need for separate initial dose. Maximum duration of release was obtained in case of EC matrix discs (beyond 54 hours for EC-50). Duration of release was extended to several days by providing a hydrophobic polymeric coat (EC coat) to 40 % w/w polymeric discs to several days. In this case also EC matrix coated discs extended the release up to 10 days (EC-40/EC-10) and beyond 26 days (EC-40/EC-20). Though the duration of release was extended drastically in case of formulations with dual control, a very low initial release was observed in case of these formulations thereby calling for incorporation of loading dose. Combination of polymers may also be studied in future.

The release profile was found to follow non-Fickian release kinetics in case of most of the designed formulations as demonstrated by highly significant correlation coefficient approaching to 1.0, smallest mean sum of square of the residuals and 'n' value ranging from 0.45 to 0.89. Only in case of NaCMC-10, NaCMC-20, CMC-10, NaCMC-40/EC-10, NaCMC-40/EC-20, PVA-40/EC-10 and NaAL-40/EC-10, Fickian release pattern was observed.

Flurbiprofen in the designed formulations was found to be fairly stable with predicted shelf life at 25 °C varying from 27 to 48 months for matrix embedded discs and from 38 to 52 months in case of polymer coated matrix embedded formulations. No significant difference was observed in the predicted and observed shelf life of the developed formulations (at $p< 0.05$). The products were found to be stable to sterilization and normal storage conditions.

6.5 References

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Table 6.1: Composition and physical properties of various matrix embedded polymeric discs of flurbiprofen prepared using NaCMC and PVA.

Formulations	**NaCMC-10**	**NaCMC-20**	**NaCMC-30**	**NaCMC-40**	**NaCMC-50**	**PVA-10**	**PVA-20**	**PVA-30**	**PVA-40**	**PVA-50**
Components										
Flurbiprofen (mg/ disc)	15	15	15	15	15	15	15	15	15	15
Polymer[a]										
NaCMC	10%	20%	30%	40%	50%	-	-	-	-	-
PVA	-	-	-	-	-	10%	20%	30%	40%	50%
Ethyl alcohol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Physical properties										
Drug content (mg/ disc)[b]	15.1± 0.6	14.8± 0.6	14.9± 0.7	15.0± 0.3	15.0± 0.4	14.7± 0.5	14.6± 0.1	15.1± 0.7	14.6± 0.4	14.3± 0.2
Weight variation (%)[c]	± 2.9	± 2.7	± 3.1	± 3.8	± 3.9	± 3.1	± 2.3	± 2.8	± 2.6	± 3.5
Hardness (kg/ cm^2)[d]	2.2 ± 0.2	3.1 ± 0.4	3.0 ± 0.5	3.5 ± 0.1	3.6 ± 0.7	2.3 ± 0.3	2.4 ± 0.3	2.6 ± 0.3	3.1 ± 0.2	3.4 ± 0.5
Friability (%)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

NaCMC- Sodium carboxy methyl cellulose, PVA- Polyvinyl alcohol
q.s.- Quantity sufficient
[a]: % w/w of the drug content
[b]: Mean of duplicate determination with S.D. per three batch
[c]: ± max. % variation from the mean value
[d]: Mean of 10 discs with S.D.

Table 6.2: Composition and physical properties of various matrix embedded polymeric discs of flurbiprofen prepared using NaAL and CMC.

Formulations	**NaAL-10**	**NaAL-20**	**NaAL-30**	**NaAL-40**	**NaAL-50**	**CMC-10**	**CMC-20**	**CMC-30**	**CMC-40**	**CMC-50**
Components										
Flurbiprofen (mg/ disc)	15	15	15	15	15	15	15	15	15	15
Polymer[a]										
NaAL	10%	20%	30%	40%	50%	-	-	-	-	-
CMC	-	-	-	-	-	10%	20%	30%	40%	50%
Ethyl alcohol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Physical properties										
Drug content (mg/ disc)[b]	15.3± 0.7	14.5± 0.3	14.7± 0.7	14.8± 0.3	15.0± 0.4	14.9± 0.6	14.8± 0.4	14.9± 0.3	15.2± 0.7	15.8± 0.7
Weight variation (%)[c]	± 2.6	± 3.0	± 3.1	± 3.3	± 3.4	± 3.7	± 3.4	± 3.3	± 3.2	± 3.5
Hardness (kg/ cm^2)[d]	2.1 ± 0.5	2.4 ± 0.1	2.8 ± 0.6	3.2 ± 0.4	3.7 ± 0.6	2.5 ± 0.5	2.8 ± 0.8	2.9 ± 0.9	3.5 ± 0.3	3.6 ± 0.4
Friability (%)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

NaAL- Sodium alginate, CMC- Carboxy methyl cellulose
q.s.- Quantity sufficient
[a]: % w/w of the drug content
[b]: Mean of duplicate determination with S.D. per three batch
[c]: ± max. % variation from the mean value
[d]: Mean of 10 discs with S.D.

Table 6.3: Composition and physical properties of various matrix embedded polymeric discs of flurbiprofen prepared using HPMC and PAA.

Formulations	**HPMC-10**	**HPMC-20**	**HPMC-30**	**HPMC-40**	**HPMC-50**	**PAA-10**	**PAA-20**	**PAA-30**	**PAA-40**	**PAA-50**
Components										
Flurbiprofen (mg/ disc)	15	15	15	15	15	15	15	15	15	15
Polymer[a]										
HPMC	10%	20%	30%	40%	50%	-	-	-	-	-
PAA	-	-	-	-	-	10%	20%	30%	40%	50%
Ethyl alcohol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Physical properties										
Drug content (mg/ disc)[b]	14.8± 0.6	14.9± 0.7	15.0± 0.3	15.1± 0.5	15.0± 0.4	14.7± 0.5	14.6± 0.1	15.1± 0.7	14.6± 0.4	14.9± 0.2
Weight variation (%)[c]	± 2.7	± 3.1	± 3.4	± 3.8	± 3.9	± 3.1	± 2.3	± 2.8	± 2.6	± 3.5
Hardness (kg/ cm^2)[d]	2.6 ± 0.3	2.8 ± 0.4	3.2 ± 0.6	3.4 ± 0.7	3.5 ± 0.1	2.6 ± 0.2	2.5 ± 0.9	3.1 ± 0.1	3.7 ± 0.3	3.9 ± 0.6
Friability (%)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

HPMC- Hydroxy propyl methyl cellulose, PAA- Polyacrylamide
q.s.- Quantity sufficient
[a]: % w/w of the drug content
[b]: Mean of duplicate determination with S.D. per three batch
[c]: ± max. % variation from the mean value
[d]: Mean of 10 discs with S.D.

Table 6.4: Composition and physical properties of various matrix embedded polymeric discs of flurbiprofen prepared using EC.

Formulations	**EC-10**	**EC-20**	**EC-30**	**EC-40**	**EC-50**
Components					
Flurbiprofen (mg/ disc)	15	15	15	15	15
Polymer[a]					
EC	10%	20%	30%	40%	50%
Ethyl alcohol	q.s.	q.s.	q.s.	q.s.	q.s.
Physical properties					
Drug content (mg/ disc)[b]	14.7± 0.5	14.6± 0.1	15.1± 0.1	14.7± 0.4	15.3± 0.2
Weight variation (%)[c]	± 3.5	± 2.9	± 3.8	± 3.6	± 3.2
Hardness (kg/ cm^2)[d]	2.4 ± 0.3	2.9 ± 0.1	3.0 ± 0.6	3.5 ± 0.5	3.5 ± 0.1
Friability (%)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

EC- Ethyl cellulose
q.s.- Quantity sufficient
[a]: % w/w of the drug content
[b]: Mean of duplicate determination with S.D. per three batch
[c]: ± max. % variation from the mean value
[d]: Mean of 10 discs with S.D.

Table 6.5: Composition and physical properties of various matrix embedded polymer coated discs of flurbiprofen.

Formulations	**NaCMC-40/ EC-10**	**NaCMC-40/ EC-20**	**PVA-40/ EC-10**	**PVA-40/ EC-20**	**NaAL-40/ EC-10**	**NaAL-40/ EC-20**	**CMC-40/ EC-10**	**CMC-40/ EC-20**
Components								
Flurbiprofen (mg/ disc)	15	15	15	15	15	15	15	15
Polymer[a]	40%	40%	40%	40%	40%	40%	40%	40%
Ethyl alcohol	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Concentration of EC coating solution (% w/v)	10	20	10	20	10	20	10	20
Physical properties								
Drug content (mg/ disc)[b]	15.1± 0.2	15.4± 0.3	15.2± 0.4	15.3± 0.6	15.2± 0.7	15.3± 1.0	15.4± 0.4	15.0± 0.4
Weight variation (%)[c]	± 3.7	± 3.5	± 3.9	± 2.8	± 2.9	± 3.1	± 3.3	± 3.8
Hardness (kg/ cm^2)[d]	3.5 ± 0.6	3.6 ± 0.7	3.1 ± 0.2	3.5 ± 0.9	3.1 ± 0.6	3.3 ± 0.8	3.9 ± 1.0	3.5 ± 0.6
Friability (%)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.1	< 0.1	< 0.1

NaCMC- Sodium carboxy methyl cellulose, PVA- Polyvinyl alcohol, NaAL- Sodium alginate, CMC- Carboxy methyl cellulose
q.s. – Quantity sufficient
[a]: % w/w of the drug content
[b]: Mean of duplicate determination with S.D. per three batch
[c]: ± max. % variation from the mean value
[d]: Mean of 10 discs with S.D.

Table 6.6: Composition and physical properties of various matrix embedded polymer coated discs of flurbiprofen.

Formulations	**HPMC-40/ EC-10**	**HPMC-40/ EC-20**	**PAA-40/ EC-10**	**PAA-40/ EC-20**	**EC-40/ EC-10**	**EC-40/ EC-20**
Components						
Flurbiprofen (mg/ disc)	15	15	15	15	15	15
Polymer[a]	40%	40%	40%	40%	40%	40%
Concentration of EC coating solution (% w/v)	10	20	10	20	10	20
Physical properties						
Drug content (mg/ disc)[b]	15.3± 0.4	15.1± 0.5	14.9 ± 0.5	15.4± 0.7	15.3± 0.6	15.0± 0.5
Weight variation (%)[c]	± 3.1	± 3.2	± 3.5	± 3.1	± 3.5	± 3.4
Hardness (kg/ cm^2)[d]	3.4 ± 0.7	3.3 ± 0.2	3.2 ± 0.1	3.8 ± 0.5	3.4 ± 0.4	3.0 ± 0.3
Friability (%)	< 0.1	< 0.1	< 0.1	< 0.2	< 0.1	< 0.1

HPMC- Hydroxy propyl methyl cellulose, PAA- Polyacrylamide, EC-Ethyl cellulose
q.s. – Quantity sufficient
[a]: % w/w of the drug content
[b]: Mean of duplicate determination with S.D. per three batch
[c]: ± max. % variation from the mean value
[d]: Mean of 10 discs with S.D.

Table 6.7: Cumulative percentage release of flurbiprofen from polymeric matrix embedded discs prepared using NaCMC in pH 7.4 phosphate buffer

Time (hrs)	Cumulative percentage released[a]				
	NaCMC-10	NaCMC-20	NaCMC-30	NaCMC-40	NaCMC-50
0.25	56.6 ± 2.6	48.2 ± 2.5	16.9 ± 1.3	12.3 ± 3.2	8.5 ± 1.5
0.5	69.4 ± 4.3	62.2 ± 2.4	24.0 ± 1.8	21.2 ± 3.1	16.5 ± 1.8
0.75	81.0 ± 2.1	70.7 ± 2.2	33.5 ± 1.9	24.4 ± 3.0	21.3 ± 1.9
1	89.5 ± 5.6	76.8 ± 2.1	43.1 ± 2.3	29.1 ± 2.9	26.5 ± 2.1
1.5	97.7 ± 4.5	85.2 ± 2.0	58.8 ± 2.5	38.1 ± 2.7	33.2 ± 2.5
2	99.3 ± 2.1	91.3 ± 0.9	68.8 ± 2.7	45.8 ± 2.5	38.7 ± 2.4
3		97.2 ± 1.8	80.9 ± 2.8	56.9 ±2.6	49.1 ± 2.0
4		101.4 ± 1.7	89.1 ± 3.1	66.6 ± 2.3	60.1 ± 3.1
5			91.2 ± 3.3	75.9 ± 3.0	70.4 ± 3.2
6			93.3 ± 3.5	82.6 ± 3.4	77.8 ± 1.9
8			97.7 ± 3.4	96.7 ± 3.8	92.9 ± 2.7
10				100.7 ± 3.9	100.1 ± 2.3

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.8: Cumulative percentage release of flurbiprofen from polymeric matrix embedded discs prepared using PVA in pH 7.4 phosphate buffer

Time (hrs)	Cumulative percentage released[a]				
	PVA-10	**PVA-20**	**PVA-30**	**PVA-40**	**PVA-50**
0.25	23.7 ± 1.9	19.9 ± 1.1	14.8 ± 2.5	9.7 ± 2.9	4.0 ± 1.5
0.5	39.9 ± 2.3	29.7 ± 2.9	23.3 ± 2.8	18.2 ± 3.6	12.4 ± 1.8
0.75	62.9 ± 2.6	56.4 ± 3.5	43.1 ± 2.9	27.6 ± 3.5	20.8 ± 1.9
1	76.5 ± 2.9	69.9 ± 4.1	58.2 ± 2.3	39.1 ± 4.1	35.2 ± 2.1
1.5	95.4 ± 3.1	90.8 ± 4.0	78.9 ± 3.8	57.9 ± 4.5	50.4 ± 2.5
2	100.0 ± 3.5	97.1 ± 4.5	89.5 ± 3.9	72.1 ± 3.7	69.1 ± 2.4
3		100.0 ± 3.8	96.0 ± 4.5	93.2 ± 3.3	86.6 ± 2.0
4			100.1 ± 5.3	98.2 ± 3.1	93.8 ± 3.1
5				99.0 ± 3.9	94.2 ± 3.2
6				99.8 ± 3.4	96.3 ± 1.9
8					98.7 ± 2.7
10					101.2 ± 2.3

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.9: Cumulative percentage release of flurbiprofen from polymeric disc formulations prepared using NaAL in pH 7.4 phosphate buffer

Time (hrs)	Cumulative percentage released[a]				
	NaAL-10	NaAL-20	NaAL-30	NaAL-40	NaAL-50
0.25	25.8 ± 3.6	20.1 ± 1.1	16.4 ± 2.3	10.5 ± 3.5	6.5 ± 4.9
0.5	42.9 ± 3.9	37.0 ± 2.9	32.6 ± 3.5	26.7 ± 1.2	10.5 ± 2.9
0.75	53.4 ± 3.5	47.1 ± 3.5	42.9 ± 3.7	37.6 ± 1.8	17.6 ± 3.5
1	72.9 ± 3.1	63.5 ± 4.1	48.4 ± 6.8	44.7 ± 3.6	22.8 ± 3.6
1.5	94.1 ± 4.5	83.2 ± 4.0	66.4 ± 4.1	56.6 ± 4.2	33.8 ± 3.4
2	100.2 ± 4.6	91.9 ± 4.5	87.2 ± 4 .6	71.8 ± 1.5	48.4 ± 3.1
3		101.3 ±3.8	95.4 ± 3.9	82.0 ± 1.9	61.9 ± 3.5
4			100.9 ± 3.4	88.9 ± 2.4	78.0 ± 3.8
5				92.2 ± 2.9	89.3 ± 3.7
6				95.5 ± 2.1	92.5 ± 4.2
8				99.5 ± 3.1	98.5 ± 3.8
10					101.2 ± 3.1

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.10: Cumulative percentage release of flurbiprofen from polymeric matrix embedded discs prepared using CMC in pH 7.4 phosphate buffer

Time (hrs)	Cumulative percentage released[a]				
	CMC-10	CMC-20	CMC-30	CMC-40	CMC-50
0.25	37.0 ± 2.4	24.0 ± 2.5	15.1 ± 2.9	7.6 ± 2.3	5.3 ± 3.5
0.5	46.3 ± 2.6	34.7 ± 2.9	25.0 ± 2.3	20.3 ± 2.6	12.7 ± 3.6
0.75	59.5 ± 2.8	42.1 ± 2.8	34.2 ± 2.1	33.3 ± 3.0	21.9 ± 3.1
1	78.0 ± 2.9	47.4 ± 2.7	40.5 ± 2.5	36.6 ± 3.1	33.1 ± 3.9
1.5	88.6 ± 3.1	61.1 ± 2.6	57.3 ± 2.6	54.4 ± 2.8	45.2 ± 3.4
2	93.8 ± 3.3	75.8 ± 2.3	70.1 ± 3.0	66.6 ± 2.9	62.9 ± 3.6
3	100.4 ± 3.6	88.7 ± 2.1	87.8 ± 1.9	83.5 ± 2.4	75.8 ± 3.2
4		99.0 ± 1.9	94.5 ± 1.8	89.7 ± 2.5	85.3 ± 3.5
5			99.9 ± 2.0	95.6 ± 2.8	93.8 ± 3.7
6				99.9 ± 2.4	95.0 ± 3.8
8					98.7 ± 3.1

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.11: Cumulative percentage release of flurbiprofen from polymeric matrix embedded discs prepared using HPMC in pH 7.4 phosphate buffer

Time (hrs)	Cumulative percentage released[a]				
	HPMC-10	HPMC-20	HPMC-30	HPMC-40	HPMC-50
0.25	22.3 ± 2.1	13.4 ± 2.9	7.7 ± 4.1	5.5 ± 2.8	3.5 ± 1.2
0.5	32.3 ± 1.9	19.3 ± 2.3	13.8 ± 3.9	10.0 ± 3.9	7.5 ± 4.1
0.75	39.1 ± 2.3	26.7 ± 2.1	18.0 ± 3.6	12.7 ± 3.2	10.9 ± 3.9
1	49.7 ± 2.1	38.6 ± 2.5	24.3 ± 3.8	18.2 ± 3.1	15.3 ± 3.5
1.5	66.6 ± 2.5	55.0 ± 2.6	34.3 ± 3.7	25.4 ± 2.7	20.8 ± 3.4
2	81.5 ± 2.0	71.2 ± 3.0	49.7 ± 4.0	37.3 ± 2.5	31.5 ± 3.4
3	95.8 ± 2.8	90.0 ± 1.9	63.9 ± 3.8	46.6 ± 1.9	41.6 ± 3.8
4	100.3 ± 2.9	96.3 ± 1.8	73.9 ± 4.2	59.3 ± 2.6	52.3 ± 3.6
5		100.0 ± 2.3	89.4 ± 4.3	69.6 ± 2.9	63.1 ± 3.4
6			93.9 ± 4.0	79.4 ± 3.5	73.8 ± 3.9
8			100.0 ±4.2	92.6 ± 3.6	84.5 ± 4.1
10				100.1 ± 2.6	96.8 ± 4.2
12					100.1 ± 3.6

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.12: Cumulative percentage release of flurbiprofen from polymeric matrix embedded discs prepared using PAA in pH 7.4 phosphate buffer

Time (hrs)	Cumulative percentage released[a]				
	PAA-10	**PAA-20**	**PAA-30**	**PAA-40**	**PAA-50**
0.25	24.9 ± 1.4	20.2 ± 2.3	17.6 ± 1.8	10.9 ± 3.2	8.9 ± 1.5
0.5	51.6 ± 1.0	46.9 ± 3.6	32.5 ± 1.9	22.8 ± 3.9	17.6 ± 1.2
0.75	68.2 ± 1.9	61.3 ± 3.5	50.9 ± 2.1	36.4 ± 4.0	26.8 ± 2.3
1	86.5 ± 2.3	76.3 ± 3.4	67.3 ± 2.3	51.3 ± 4.1	38.9 ± 1.8
1.5	99.6 ± 2.5	87.5 ± 3.8	80.8 ± 2.1	66.1 ± 3.8	53.6 ± 3.3
2		100.0 ± 3.9	90.1 ± 2.0	74.5 ± 3.3	63.0 ± 1.5
3			95.8 ± 2.8	81.4 ± 3.5	72.3 ± 3.1
4			99.8 ± 3.1	90.2 ± 3.6	81.7 ± 3.5
5				95.6 ± 3.1	88.9 ± 1.8
6				99.9 ± 2.5	93.4 ± 2.2
8					96.9 ± 2.8

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.13: Cumulative percentage release of flurbiprofen from polymeric matrix embedded discs prepared using EC in pH 7.4 phosphate buffer

Time (hrs)	Cumulative percentage released[a]				
	EC-10	EC-20	EC-30	EC-40	EC-50
0.25	5.3 ± 2.3	4.6 ± 2.3	3.0 ± 1.2	2.9 ± 2.0	2.4 ± 1.5
0.5	9.1 ± 2.8	7.6 ± 2.6	5.3 ± 1.6	4.6 ± 3.6	3.9 ± 3.6
0.75	14.4 ± 2.9	16.6 ± 2.8	8.5 ± 1.9	7.6 ± 3.1	6.4 ± 2.5
1	20.6 ± 3.1	19.0 ± 3.1	11.3 ± 2.1	10.1 ± 1.3	8.5 ± 3.6
1.5	28.1 ± 3.8	24.8 ± 3.0	15.4 ± 2.2	14.5 ± 2.8	12.2 ± 3.4
2	33.7 ± 3.4	29.3 ± 2.7	21.9 ± 2.3	19.9 ± 2.9	16.7 ± 3.1
3	44.1 ± 3.5	36.0 ± 2.4	26.6 ± 2.5	24.3 ± 4.1	20.4 ± 2.5
4	51.8 ± 3.0	43.2 ± 3.5	32.6 ± 2.4	30.6 ± 3.1	25.7 ± 2.1
5	56.9 ± 2.9	49.8± 3.8	39.3 ± 3.8	38.0 ± 2.9	32.0 ± 3.8
6	64.0 ± 3.4	56.9 ± 3 .9	48.7 ± 1.4	44.9 ± 3.1	37.7 ± 2.5
8	71.3 ± 2.7	67.0 ± 1 .2	53.5 ± 1.8	50.8 ± 3.5	42.7 ± 2.9
10	78.2 ± 2.4	75.7 ± 2.5	60.1 ± 1.4	54.3 ± 3.6	45.6 ± 2.7
12	89.4 ± 2.1	80.5 ± 2.8	66.9 ± 1.1	59.6 ± 3.1	50.1 ± 3.2
16	100.3 ± 2.2	96.2 ± 3.4	82.8 ± 3.6	66.7 ± 1.5	56.0 ± 3.1
20		100.0 ± 3.1	91.5 ± 3.9	71.2 ± 1.7	59.8 ± 3.0
24			99.6 ± 4.2	77.9 ± 2.4	65.4 ± 3.6
36				92.5 ± 2.9	77.7 ± 2.4
42				97.3 ± 3.8	81.7 ± 2.8
48				99.5 ± 3.4	83.6 ± 3.5
54					87.6 ± 3.3

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.14: Cumulative percentage release of flurbiprofen from 40% w/v NaCMC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

Time (hrs)	Cumulative percentage released[a]		
	NaCMC-40/EC-10	NaCMC-40/EC-20	NaCMC-40
0.25	12.2 ± 4.1	7.1 ± 2.2	12.3 ± 3.2
0.5	29.6± 4.3	13.8 ± 3.1	21.2 ± 3.1
0.75	32.8 ± 5.5	15.1 ± 2.3	24.4 ± 3.0
1	38.7 ± 5.3	18.7 ± 2.5	29.1 ± 2.9
1.5	42.3 ± 5.4	22.7 ± 2.6	38.1 ± 2.7
2	45.8 ± 4.8	25.9 ± 2.9	45.8 ± 2.5
3	53.2 ± 3.4	30.5 ± 3.1	56.9 ±2.6
4	58.9 ± 3.8	35.9 ± 3.0	66.6 ± 2.3
5	62.8 ± 3.9	41.8 ± 2.9	75.9 ± 3.0
6	71.9 ± 3.4	47.6 ± 2.8	82.6 ± 3.4
8	78.2 ± 3.1	54.8 ± 2.5	96.7 ± 3.8
12	85.9 ± 3.5	62.7 ± 2.1	100.7 ± 3.9
18	91.6 ± 3.6	72.4 ± 2.0	
24	98.6 ± 3.7	77.4 ± 1.9	
32		83.6 ± 2.5	
40		88.5 ± 2.1	
48		94.1 ± 3.4	
60		100.0 ± 2.2	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.15: Cumulative percentage release of flurbiprofen from 40% w/v PVA polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

Time (hrs)	Cumulative percentage released[a]		
	PVA-40/EC-10	PVA-40/EC-20	PVA-40
0.25	10.7 ± 2.1	2.4 ± 2.8	9.7 ± 2.9
0.5	27.5 ± 3.2	4.4 ± 2.5	18.2 ± 3.6
0.75	33.7 ± 3.1	8.5 ± 2.3	27.6 ± 3.5
1	37.8 ± 2.5	10.5 ± 2.2	39.1 ± 4.1
1.5	50.2 ± 2.6	13.3 ± 2.9	57.9 ± 4.5
2	54.3 ± 2.8	16.6 ± 3.5	72.1 ± 3.7
3	67.8 ± 2.4	21.2 ± 3.6	93.2 ± 3.3
4	71.9 ± 2.1	24.2 ± 3.1	98.2 ± 3.1
5	76.9 ± 2.9	26.9 ± 2.5	99.0 ± 3.9
6	81.8 ± 3.1	31.8 ± 2.8	99.8 ± 3.4
8	89.9 ± 3.0	46.1 ± 3.5	
12	99.6 ± 3.1	55.0 ± 3.4	
18		71.1 ± 2.4	
24		80.2 ± 2.8	
32		87.7 ± 3.2	
40		95.6 ± 3.0	
48		100.9 ±2.6	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.16: Cumulative percentage release of flurbiprofen from 40% w/v NaAL polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

Time (hrs)	Cumulative percentage released[a]		
	NaAL-40/EC-10	NaAL-40/EC-20	NaAL-40
0.25	4.9 ± 1.5	0.7 ± 0.3	10.5 ± 3.5
0.5	15.6 ± 1.9	2.5 ± 1.1	26.7 ± 1.2
0.75	23.5 ± 2.1	3.3 ± 0.9	37.6 ± 1.8
1	29.2 ± 2.3	4.8 ± 1.2	44.7 ± 3.6
1.5	35.6 ± 1.4	5.7 ± 1.3	56.6 ± 4.2
2	41.8 ± 1.8	6.8 ± 1.1	71.8 ± 1.5
3	49.3 ± 2.0	8.1 ± 1.2	82.0 ± 1.9
4	56.9 ± 2.1	9.8 ± 0.8	88.9 ± 2.4
5	65.4 ± 1.9	11.2 ± 1.9	92.2 ± 2.9
6	74.5 ± 2.5	12.9 ± 2.1	95.5 ± 2.1
8	81.9 ± 2.6	19.4 ± 2.3	99.5 ± 3.1
12	92.5 ± 1.2	23.4 ± 2.6	
18	99.4 ± 1.3	31.6 ± 2.8	
24		40.6 ± 2.7	
32		48.1 ± 3.1	
40		55.6 ± 3.0	
48		71.3 ± 2.9	
60		82.0 ± 2.7	
72		92.0 ± 2.8	
96		102.2 ± 2.5	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.17: Cumulative percentage release of flurbiprofen from 40% w/v CMC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

Time (hrs)	Cumulative percentage released[a]		
	CMC-40/EC-10	CMC-40/EC-20	CMC-40
0.25	4.4 ± 2.3	1.9± 1.3	7.6 ± 2.3
0.5	8.9 ± 2.6	4.5 ± 1.2	20.3 ± 2.6
0.75	11.5 ± 3.1	6.7 ± 2.1	33.3 ± 3.0
1	15.3 ± 1.5	7.3 ± 1.8	36.6 ± 3.1
1.5	19.6 ± 1.6	8.9 ± 2.5	54.4 ± 2.8
2	21.3 ± 3.7	9.6 ± 2.5	66.6 ± 2.9
3	24.6 ± 3.4	11.9 ± 1.4	83.5 ± 2.4
4	29.8 ± 2.5	14.3 ± 1.8	89.7 ± 2.5
5	34.8 ± 3.4	17.8 ± 1.9	95.6 ± 2.8
6	40.1 ± 3.0	21.4 ± 2.3	99.9 ± 2.4
8	56.9 ± 2.9	29.7 ± 2.5	
12	73.7 ± 3.1	42.7 ± 2.8	
18	84.4 ± 3.9	56.2 ± 1.7	
24	92.6 ± 3.4	67.9 ± 1.8	
32	101.1 ± 3.6	74.3 ± 2.8	
40		80.6 ± 2.3	
48		83.8 ± 1.4	
60		88.3 ± 2.1	
72		93.5 ± 3.1	
96		97.2 ± 1.6	
120		100.6 ± 1.5	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.18: Cumulative percentage release of flurbiprofen from 40% w/v HPMC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

Time (hrs)	Cumulative percentage released[a]		
	HPMC-40/EC-10	HPMC-40/EC-20	HPMC-40
0.25	3.4 ± 1.3	2.3 ± 2.1	5.5 ± 2.8
0.5	5.6 ± 1.2	3.1 ± 2.3	10.0 ± 3.9
0.75	10.7 ± 1.4	5.8 ± 2.0	12.7 ± 3.2
1	12.4 ± 1.6	7.4 ± 3.1	18.2 ± 3.1
1.5	15.6 ± 2.1	8.1 ± 2.4	25.4 ± 2.7
2	18.2 ± 2.5	9.8 ± 2.3	37.3 ± 2.5
3	25.2 ± 2.6	10.9 ± 2.1	46.6 ± 1.9
4	34.7 ± 1.2	13.6 ± 2.0	59.3 ± 2.6
5	40.1 ± 2.9	16.1 ± 1.9	69.6 ± 2.9
6	47.9 ± 2.5	21.8 ± 1.8	79.4 ± 3.5
8	54.7 ± 2.2	30.5 ± 2.1	92.6 ± 3.6
12	71.0 ± 1.8	39.0 ± 2.2	100.1 ± 2.6
18	86.0 ± 1.7	54.8 ± 1.4	
24	95.3 ±1.6	71.6 ± 1.8	
32	101.1 ± 1.9	87.6 ± 2.6	
40		95.4 ± 2.4	
48		100.6 ± 2.5	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.19: Cumulative percentage release of flurbiprofen from 40% w/v PAA polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

Time (hrs)	Cumulative percentage released[a]		
	PAA-40/EC-10	PAA-40/EC-20	PAA-40
0.25	3.5± 1.9	3.0 ± 2.3	10.9 ± 3.2
0.5	8.4 ± 2.3	4.2 ± 2.6	22.8 ± 3.9
0.75	11.7 ± 2.5	5.8 ± 2.9	36.4 ± 4.0
1	13.1 ± 2.4	7.5 ± 3.1	51.3 ± 4.1
1.5	16.3 ± 2.8	8.1 ± 2.5	66.1 ± 3.8
2	19.2 ± 2.9	8.7 ± 2.6	74.5 ± 3.3
3	22.1 ± 3.1	9.5 ± 3.5	81.4 ± 3.5
4	23.7 ± 2.4	10.2 ± 3.1	90.2 ± 3.6
5	27.4 ± 2.6	12.7 ± 3.0	95.6 ± 3.1
6	32.1 ± 2.8	16.3 ± 3.6	99.9 ± 2.5
8	45.2 ± 2.1	19.5 ± 2.5	
12	60.4 ± 1.9	22.3 ± 2.7	
18	73.6 ± 2.6	30.9 ± 2.9	
24	84.8 ± 2.5	40.0 ± 3.1	
32	93.5 ± 2.1	49.6 ± 3.4	
40	100.1 ± 1.7	58.9 ± 3.5	
48		67.0 ± 2.8	
60		79.2 ± 2.7	
72		85.6 ± 3.1	
96		95.8 ± 3.0	
120		100.4 ± 2.8	

[a]: Mean and S.D. of three batches with duplicate determination per batch

Table 6.20: Cumulative percentage release of flurbiprofen from 40% w/v EC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

Time (hrs)	Cumulative percentage released[a]		
	EC-40/EC-10	EC-40/EC-20	EC-40
0.25	0.5± 0.2	0.0 ± 0.0	2.9 ± 2.5
0.5	1.1 ± 0.1	0.0 ±0.0	4.6 ± 3.6
0.75	1.4 ± 0.1	0.9 ± 0.3	7.6 ± 3.1
1	1.5 ± 0.3	1.2 ± 0.5	10.1 ± 1.3
1.5	2.0 ± 0.4	1.6 ± 0.6	14.5 ± 2.8
2	2.4 ± 0.4	2.0 ± 0.2	19.9 ± 2.9
3	3.0 ± 0.4	2.4 ± 0.3	24.3 ± 4.1
4	4.1 ± 0.6	2.6 ± 0.4	30.6 ± 3.1
5	5.3 ± 0.7	2.9 ± 0.1	38.0 ± 2.9
6	6.6 ± 0.2	3.4 ± 0.2	44.9 ± 3.1
8	8.1 ± 0.3	4.2 ± 0.6	50.8 ± 3.5
12	9.9 ± 0.5	5.3 ± 0.4	59.6 ± 3.1
18	14.1 ± 0.6	8.4 ± 0.5	69.5 ± 1.5*
24	17.1 ± 0.3	11.1 ± 0.2	77.9 ± 2.4
32	21.8 ± 0.5	12.9 ± 0.3	87.6 ± 2.9*
40	25.4 ± 0.3	14.9 ± 0.1	95.4 ± 3.8*
48	32.1 ± 0.4	17.3 ± 0.3	99.5 ± 3.4
60	37.2 ± 0.6	18.6 ± 0.4	
72	43.1 ± 0.4	20.6 ± 0.2	
96	47.6 ± 0.2	25.3 ± 0.3	
120	54.2 ± 0.6	28.4 ± 0.4	
144	60.8 ± 0.3	31.4 ± 0.7	
168	67.4 ± 0.2	34.5 ± 0.8	

[a]: Mean and S.D. of three batches with duplicate determination per batch

*: Extrapolated from the profile on Figure 6.7

Table 6.21: Release rate parameters based on power equation for designed formulations of flurbiprofen polymeric discs prepared using NaCMC and PVA

Parameters	NaCMC-10	NaCMC-20	NaCMC-30	NaCMC-40	NaCMC-50	PVAC-10	PVA-20	PVA-30	PVA-40	PVA-50
r[a]	0.9876	0.9874	0.9758	0.9975	0.9963	0.9844	0.9603	0.9625	0.9745	0.9351
MSSR[b]	0.0003	0.0003	0.0037	0.0005	0.0009	0.0023	0.0070	0.0082	0.0072	0.0278
n[c]	0.2853	0.2662	0.5341	0.5729	0.6474	0.7282	0.7133	0.7352	0.7612	0.8287
K[d] ($hour^{-n}$)	85.72	74.04	39.80	29.48	24.43	69.15	58.41	47.04	34.12	24.78
$t_{30\%}$[e] (hours)	0.03	0.03	0.59	1.03	1.37	0.32	0.39	0.54	0.84	1.26
$t_{50\%}$[f] (hours)	0.15	0.23	1.53	2.51	3.02	0.64	0.80	1.09	1.65	2.33
$t_{70\%}$[g] (hours)	0.49	0.81	2.88	4.52	5.09	1.02	1.29	1.72	2.57	3.50
$t_{90\%}$[h] (hours)	1.19	2.08	4.61	7.02	7.50	1.44	1.83	2.42	3.58	4.74

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: Diffusional exponent indicative of the release mechanism
[d]: Release rate constant
[e]: Time for 30% of the drug release
[f]: Time for 50% of the drug release
[g]: Time for 70% of the drug release
[h]: Time for 90% of the drug release

Table 6.22: Release rate parameters based on power equation for designed formulations of flurbiprofen polymeric discs prepared using NaAL and CMC

Parameters	NaAL-10	NaAL-20	NaAL-30	NaAL-40	NaAL-50	CMC-10	CMC-20	CMC-30	CMC-40	CMC-50
r^a	0.9936	0.9842	0.9834	0.9514	0.9851	0.9763	0.9979	0.9924	0.9835	0.9653
$MSSR^b$	0.0008	0.0024	0.0028	0.0096	0.0055	0.0016	0.0002	0.0014	0.0056	0.0132
n^c	0.6802	0.6721	0.6586	0.5963	0.8002	0.4399	0.5238	0.6513	0.8432	0.8367
K^d ($hour^{-n}$)	67.70	56.91	47.91	37.80	21.86	68.60	49.46	40.25	27.51	25.46
$t_{30\%}{}^e$ (hours)	0.30	0.39	0.49	0.68	1.49	0.15	0.38	0.64	1.11	1.22
$t_{50\%}{}^f$ (hours)	0.64	0.82	1.07	1.60	2.81	0.49	1.02	1.40	2.03	2.24
$t_{70\%}{}^g$ (hours)	1.05	1.36	1.78	2.81	4.28	1.05	1.94	2.34	3.03	3.35
$t_{90\%}{}^h$ (hours)	1.52	1.98	2.60	4.28	5.86	1.85	3.14	3.44	4.08	4.52

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: Diffusional exponent indicative of the release mechanism
[d]: Release rate constant
[e]: Time for 30% of the drug release
[f]: Time for 50% of the drug release
[g]: Time for 70% of the drug release
[h]: Time for 90% of the drug release

Table 6.23: Release rate parameters based on power equation for designed formulations of flurbiprofen polymeric discs prepared using HPMC and PAA

Parameters	HPMC-10	HPMC-20	HPMC-30	HPMC-40	HPMC-50	PAA-10	PAA-20	PAA-30	PAA-40	PAA-50
r[a]	0.9961	0.9913	0.9966	0.9973	0.9958	0.9846	0.9705	0.9543	0.9546	0.9677
MSSR[b]	0.0005	0.0020	0.0011	0.0010	0.0020	0.0023	0.0047	0.0074	0.0099	0.0081
n[c]	0.6141	0.7781	0.8145	0.8401	0.9045	0.7877	0.7484	0.6299	0.6593	0.6802
K[d] ($hour^{-n}$)	50.24	37.00	24.34	17.92	14.24	81.25	68.15	53.12	38.75	31.23
$t_{30\%}$[e] (hours)	0.43	0.76	1.29	1.85	2.28	0.28	0.33	0.40	0.68	0.94
$t_{50\%}$[f] (hours)	0.99	1.47	2.42	3.39	4.01	0.54	0.66	0.91	1.47	2.00
$t_{70\%}$[g] (hours)	1.72	2.27	3.66	5.06	5.82	0.83	1.04	1.55	2.45	3.28
$t_{90\%}$[h] (hours)	2.58	3.13	4.98	6.83	7.68	1.14	1.45	2.31	3.59	4.74

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: Diffusional exponent indicative of the release mechanism
[d]: Release rate constant
[e]: Time for 30% of the drug release
[f]: Time for 50% of the drug release
[g]: Time for 70% of the drug release
[h]: Time for 90% of the drug release

Table 6.24: Release rate parameters based on power equation for designed formulations of flurbiprofen polymeric discs prepared using EC

Parameters	EC-10	EC-20	EC-30	EC-40	EC-50
r^a	0.9865	0.9842	0.9935	0.9817	0.9804
$MSSR^b$	0.0044	0.0054	0.0029	0.0082	0.0089
n^c	0.6954	0.6810	0.7628	0.6713	0.6560
K^d ($hour^{-n}$)	17.62	15.94	10.55	10.11	8.58
$t_{30\%}{}^e$ (hours)	2.15	2.53	3.93	5.05	6.74
$t_{50\%}{}^f$ (hours)	4.48	5.36	7.69	10.82	14.69
$t_{70\%}{}^g$ (hours)	7.27	8.79	11.95	17.86	24.53
$t_{90\%}{}^h$ (hours)	10.43	12.71	16.61	25.96	35.98

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: Diffusional exponent indicative of the release mechanism
[d]: Release rate constant
[e]: Time for 30% of the drug release
[f]: Time for 50% of the drug release
[g]: Time for 70% of the drug release
[h]: Time for 90% of the drug release

Table 6.25: Release rate parameters based on power equation for designed formulations of flurbiprofen containing 40% w/v NaCMC, PVA and NaAL polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol

Parameters	NaCMC-40/EC-10	NaCMC-40/EC-20	PVA-40/EC-10	PVA-40/EC-20	NaAL-40/EC-10	NaAL-40/EC-20
r^a	0.992341	0.991414	0.986286	0.994735	0.990666	0.996767
MSSR[b]	0.00033	0.000957	0.00056	0.001212	0.000678	0.00141
n^c	0.3139	0.4352	0.3758	0.6270	0.4477	0.7190
K^d ($hour^{-n}$)	38.23	19.78	41.55	10.63	30.50	4.02
$t_{30\%}{}^e$ (hours)	0.46	2.60	0.42	5.23	0.96	16.37
$t_{50\%}{}^f$ (hours)	2.35	8.42	1.64	11.81	3.02	33.31
$t_{70\%}{}^g$ (hours)	6.87	18.25	4.01	20.20	6.40	53.19
$t_{90\%}{}^h$ (hours)	15.29	32.50	7.82	30.17	11.21	75.45

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: Diffusional exponent indicative of the release mechanism
[d]: Release rate constant
[e]: Time for 30% of the drug release
[f]: Time for 50% of the drug release
[g]: Time for 70% of the drug release
[h]: Time for 90% of the drug release

Table 6.26: Release rate parameters based on power equation for designed formulations of flurbiprofen containing 40% w/v CMC and HPMC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol

Parameters	CMC-40/EC-10	CMC-40/EC-20	HPMC-40/EC-10	HPMC-40/EC-20
r^a	0.9898	0.9825	0.9899	0.9909
MSSR[b]	0.0018	0.0063	0.0022	0.0034
n^c	0.5894	0.6210	0.6472	0.7583
K^d ($hour^{-n}$)	14.51	7.16	12.97	5.75
$t_{30\%}{}^e$ (hours)	3.43	10.04	3.66	8.83
$t_{50\%}{}^f$ (hours)	8.16	22.87	8.05	17.31
$t_{70\%}{}^g$ (hours)	14.45	39.31	13.54	26.98
$t_{90\%}{}^h$ (hours)	22.13	58.92	19.96	37.58

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: Diffusional exponent indicative of the release mechanism
[d]: Release rate constant
[e]: Time for 30% of the drug release
[f]: Time for 50% of the drug release
[g]: Time for 70% of the drug release
[h]: Time for 90% of the drug release

Table 6.27: Release rate parameters based on power equation for designed formulations of flurbiprofen containing 40% w/v PAA and EC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol

Parameters	PAA-40/EC-10	PAA-40/EC-20	EC-40/EC-10	EC-40/EC-20
r[a]	0.9912	0.9904	0.9982	0.9971
MSSR[b]	0.0018	0.0034	0.0011	0.0014
n[c]	0.5919	0.6212	0.7657	0.6803
K[d] ($hour^{-n}$)	12.17	5.51	1.49	1.12
$t_{30\%}$[e] (hours)	4.59	15.28	50.56	125.29
$t_{50\%}$[f] (hours)	10.88	34.78	98.53	265.48
$t_{70\%}$[g] (hours)	19.20	59.78	152.91	435.35
$t_{90\%}$[h] (hours)	29.36	89.58	212.32	629.90

[a]: Correlation coefficient
[b]: Mean sum of square of the residuals of one way ANOVA of the linear regression data
[c]: Diffusional exponent indicative of the release mechanism
[d]: Release rate constant
[e]: Time for 30% of the drug release
[f]: Time for 50% of the drug release
[g]: Time for 70% of the drug release
[h]: Time for 90% of the drug release

Table 6.28: Effect of sterilization method on the drug content in designed formulations of flurbiprofen

Formulation code	Drug content[a] (mg/ disc)		Results of one-way ANOVA of drug content results[b]	
	Before sterilization	After sterilization	F_{calc}	F_{crit}
NaCMC-40	15.0± 0.3	15.1± 0.5	0.039	7.709
PVA-40	15.1± 0.9	15.3± 0.5	0.110	7.709
NaAL-40	14.8± 0.3	15.2± 0.7	0.786	7.709
CMC-40	15.2± 0.7	15.3± 0.5	0.019	7.709
HPMC-40	15.0± 0.3	15.3± 0.5	0.703	7.709
PAA-40	14.6± 0.4	15.4± 0.8	2.400	7.709
EC-40	14.7± 0.4	15.5± 0.6	3.648	7.709
NaCMC-40/EC-20	15.1± 0.2	15.1± 0.9	5.910×10^{-13}	7.709
PVA-40/EC-20	15.3± 0.6	15.3± 0.8	4.600×10^{-13}	7.709
NaAL-40/EC-20	15.3± 1.0	15.1± 0.7	0.273	7.709
CMC-40/EC-20	15.0± 0.4	15.3± 0.7	0.327	7.709
HPMC-40/EC-20	15.3± 0.4	15.4± 0.9	0.034	7.709
PAA-40/EC-20	15.4± 0.7	15.3± 0.7	0.872	7.709
EC-40/EC-10	15.3± 0.9	15.1± 0.8	0.083	7.709
EC-40/EC-20	15.0± 0.5	15.2± 0.8	0.087	7.709

[a]: Average ± S.D. of triplicate determinations
[b]: Based on degree of freedom (1, 4) at 5% level of significance

Table 6.29: Observed and predicted degradation rate constants along with the observed and predicted shelf-life in months for flurbiprofen from designed matrix embedded discs.

Formulation code	Observed degradation rate constant[a] (K_{deg}) × 10^3 ($months^{-1}$)			Shelf-life[c] based on observed K_{CRT} (months)	Predicted values from the Arrhenius plot	
	CRT[b]	40°C/ 75% RH	45°C/ 75% RH		K_{25} × 10^3 ($months^{-1}$)	Predicted Shelf-life[c] (months)
Na CMC-10	2.77	8.25	11.56	37.91	2.83	37.10
Na CMC-20	2.41	8.80	12.32	43.57	2.48	42.27
Na CMC-30	2.39	8.72	12.21	43.93	2.46	42.62
Na CMC-40	2.34	8.54	11.96	44.87	2.38	44.10
Na CMC-50	2.31	8.43	11.80	45.45	2.41	43.53
PVA-10	2.79	10.18	14.26	37.63	2.88	36.51
PVA-20	2.73	9.96	13.95	38.46	2.81	37.31
PVA-30	2.69	9.82	13.75	39.03	2.77	37.87
PVA-40	2.41	8.80	12.32	43.57	2.48	42.27
PVA-50	2.40	8.76	12.26	43.75	2.47	42.45
NaAL-10	3.79	13.83	19.37	27.70	3.91	26.88
NaAL-20	3.61	13.18	18.45	29.09	3.72	28.22
NaAL-30	3.52	12.85	17.99	29.83	3.63	28.94
NaAL-40	3.45	12.59	17.63	30.43	3.56	29.53
NaAL-50	3.12	11.39	15.94	33.65	3.22	32.65
CMC-10	3.56	12.99	18.19	29.49	3.67	28.61
CMC-20	3.39	12.37	17.32	30.97	3.49	30.05
CMC-30	3.11	11.33	15.87	33.82	3.20	32.81
CMC-40	3.03	11.06	15.48	34.65	3.12	33.62
CMC-50	3.02	11.02	15.43	34.77	3.11	33.73
HPMC-10	2.45	8.94	12.52	42.86	2.53	41.58
HPMC-20	2.36	8.61	12.06	44.49	2.43	43.16
HPMC-30	2.28	8.32	11.65	46.05	2.35	44.68
HPMC-40	2.18	7.96	11.14	48.17	2.25	46.73
HPMC-50	2.12	7.74	10.83	49.53	2.19	48.05
PAA-10	3.35	12.23	17.12	31.34	3.45	30.41
PAA-20	3.32	12.12	16.97	31.63	3.42	30.68
PAA-30	3.23	11.79	16.51	32.51	3.33	31.54
PAA-40	3.16	11.53	16.15	33.23	3.26	32.24
PAA-50	3.10	11.32	15.84	33.87	3.20	32.86
EC-10	2.44	8.91	12.47	43.03	2.51	41.75
EC-20	2.36	8.61	12.06	44.49	2.43	43.16
EC-30	2.22	8.10	11.34	47.30	2.29	45.89
EC-40	2.18	7.96	11.14	48.17	2.25	46.73
EC-50	2.13	7.77	10.88	49.30	2.20	47.83

[a] : Based on average of triplicate assay value at six time points with C.V. less than 3.8% in all cases

[b] : CRT= 25 ± 3 °C & 55 ± 15 % RH; [c] : Shelf life in months

Table 6.30: Observed and predicted degradation rate constants along with the observed and predicted shelf-life in months for flurbiprofen from designed polymer coated matrix embedded discs.

Formulation code	Observed degradation rate constant[a] (K_{deg}) × 10^3 ($months^{-1}$)			Shelf-life[c] based on observed K_{CRT} (months)	Predicted values from the Arrhenius plot	
	CRT[b]	40°C/ 75% RH	45°C/ 75% RH		K_{25} × 10^3 ($months^{-1}$)	Predicted Shelf-life[c] (months)
Na CMC-40/EC-10	2.24	6.67	9.33	46.94	2.29	45.94
Na CMC-40/EC-20	2.12	7.74	10.83	49.53	2.19	48.05
PVA-40/EC-10	2.57	9.38	13.13	40.86	2.65	39.64
PVA-40/EC-20	2.47	9.02	12.62	42.51	2.55	41.24
NaAL-40/EC-10	2.34	8.54	11.96	44.87	2.41	43.53
NaAL-40/EC-20	2.02	7.37	10.32	51.98	2.08	50.43
CMC-40/EC-10	2.68	9.78	13.69	39.18	2.76	38.01
CMC-40/EC-20	2.39	8.72	12.21	43.93	2.46	42.62
HPMC-40/EC-10	2.50	9.13	12.78	42.00	2.58	40.75
HPMC-40/EC-20	2.19	7.99	11.19	47.95	2.26	46.52
PAA-40/EC-10	2.55	9.31	13.03	41.18	2.63	39.95
PAA-40/EC-20	2.48	9.05	12.67	42.34	2.56	41.08
EC-40/EC-10	2.06	7.52	10.53	50.97	2.12	49.45
EC-40/EC-20	1.99	7.26	10.17	52.76	2.05	51.19

[a] : Based on average of triplicate assay value at six time points with C.V. less than 3.8% in all cases
[b]: CRT= 25 ± 3 °C & 55 ± 15 % RH;
[c]: Shelf life in months

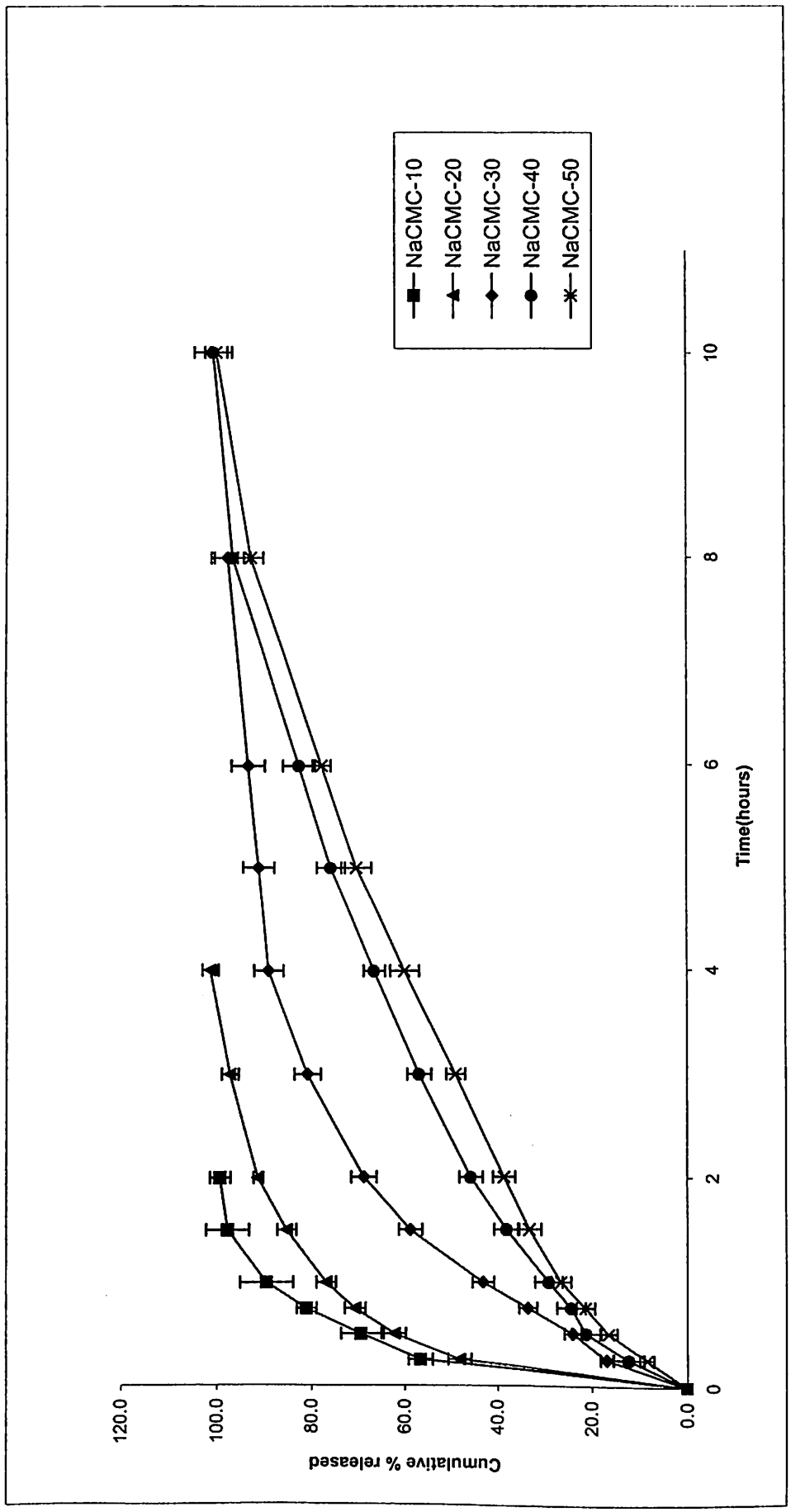


Figure 6.1: Release profile of flurbiprofen from polymeric disc formulations prepared using varying percentage of NaCMC in pH 7.4 phosphate buffer.

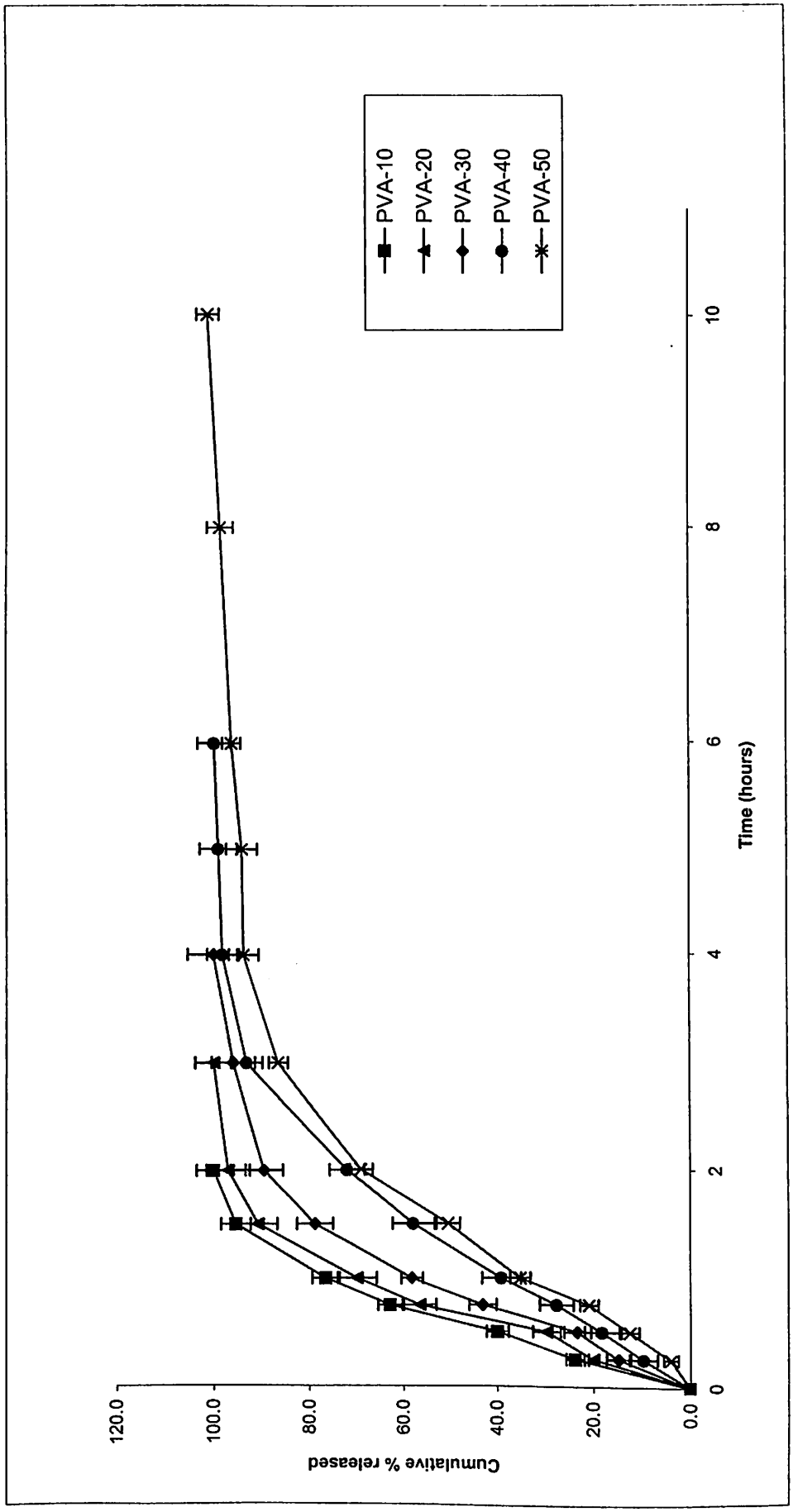


Figure 6.2: Release profile of flurbiprofen from polymeric disc formulations prepared using varying percentage of PVA in pH 7.4 phosphate buffer.

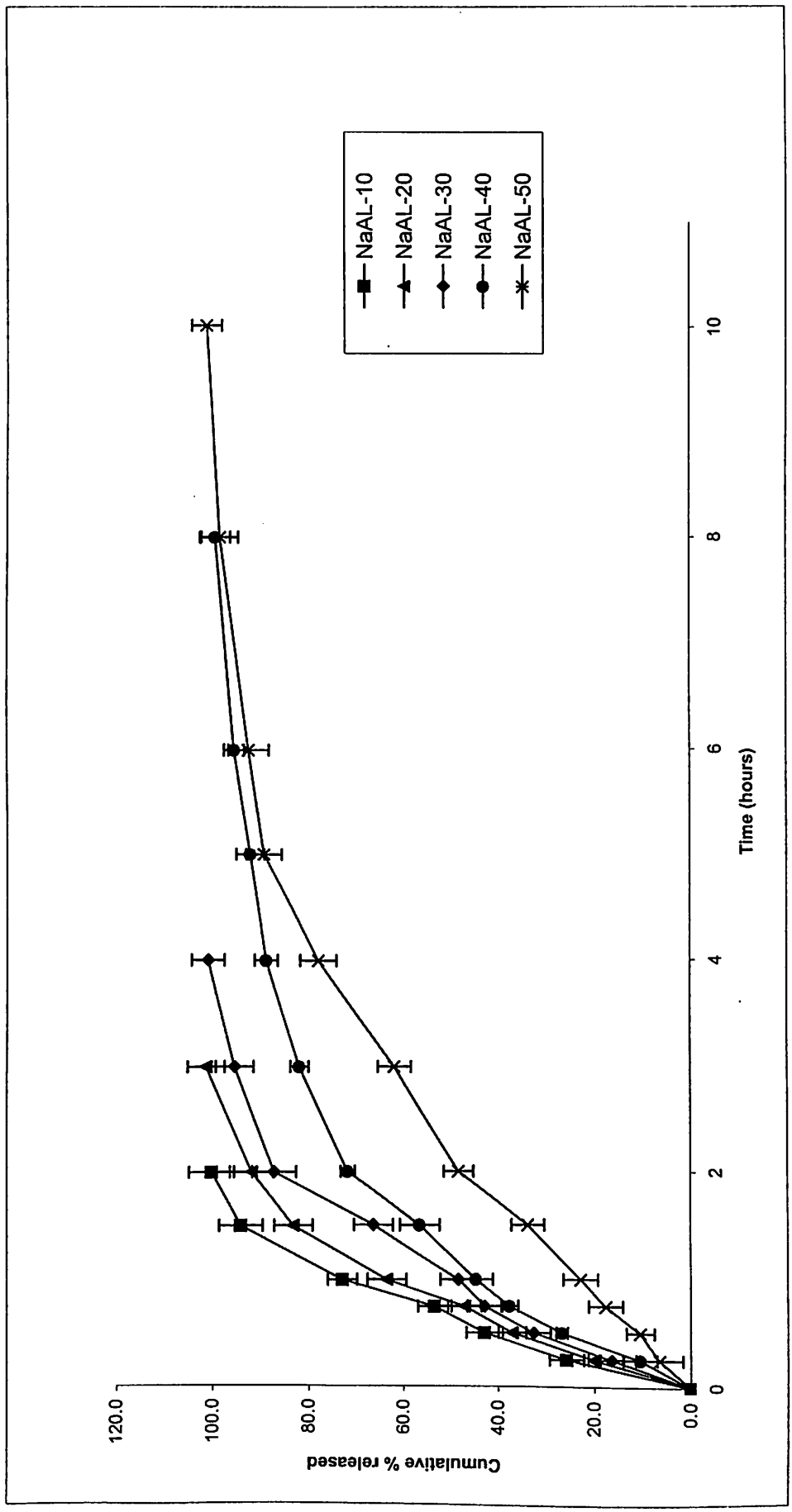


Figure 6.3: Release profile of flurbiprofen from polymeric disc formulations prepared using varying percentage of NaAL in pH 7.4 phosphate buffer.

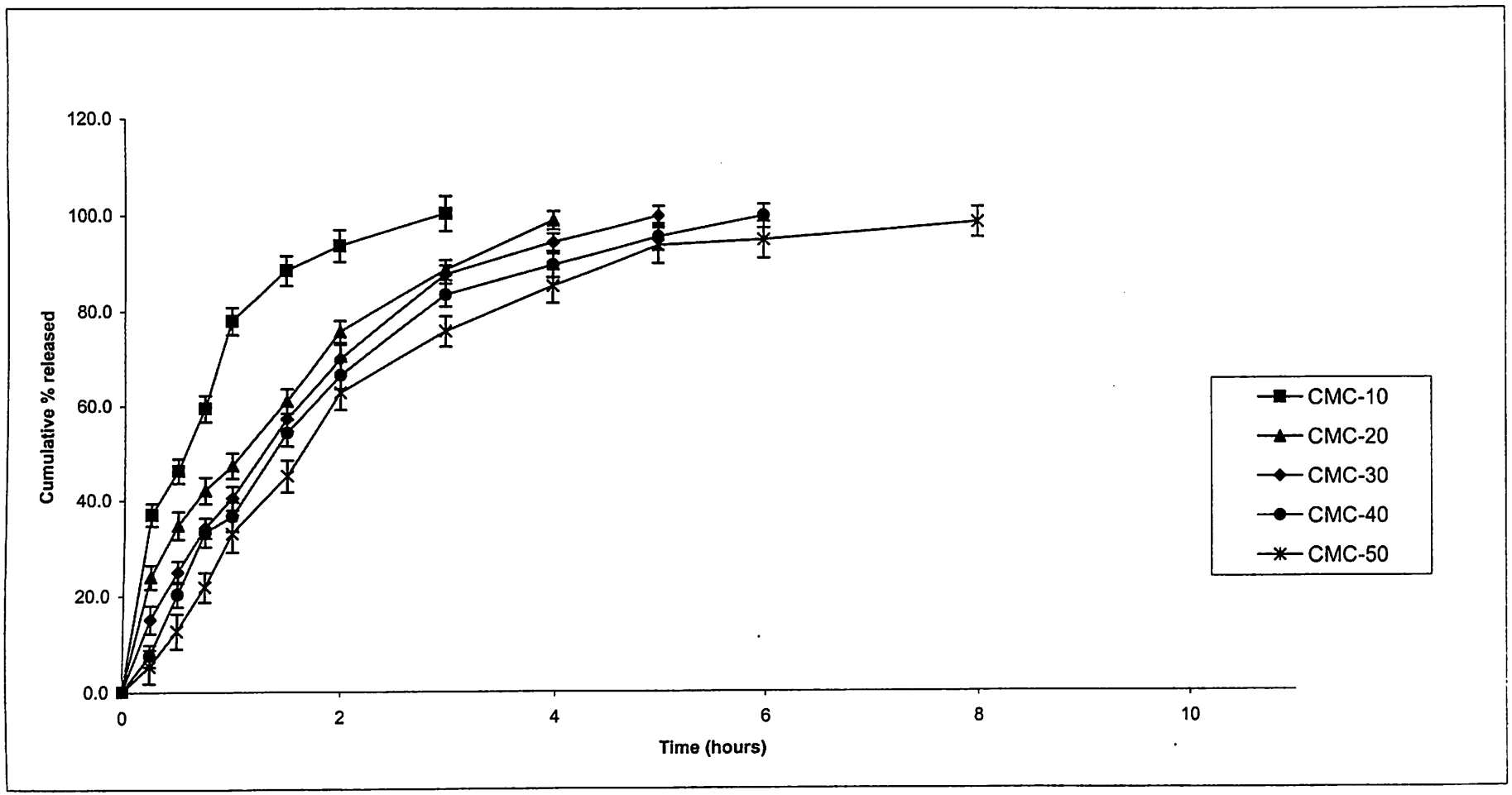


Figure 6.4: Release profile of flurbiprofen from polymeric disc formulations prepared using varying percentage of CMC in pH 7.4 phosphate buffer.

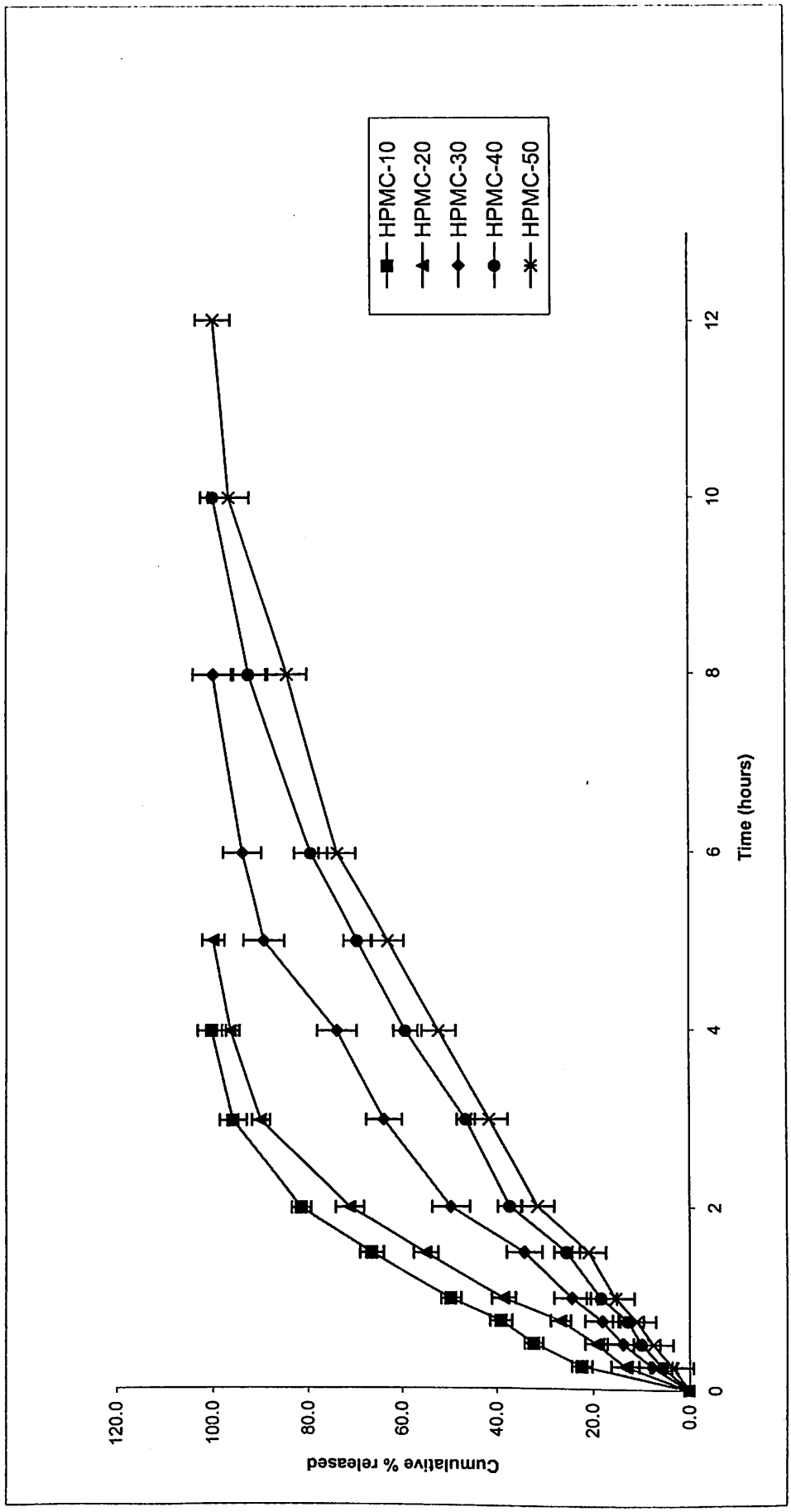


Figure 6.5: Release profile of flurbiprofen from polymeric disc formulations prepared using varying percentage of HPMC in pH 7.4 phosphate buffer.

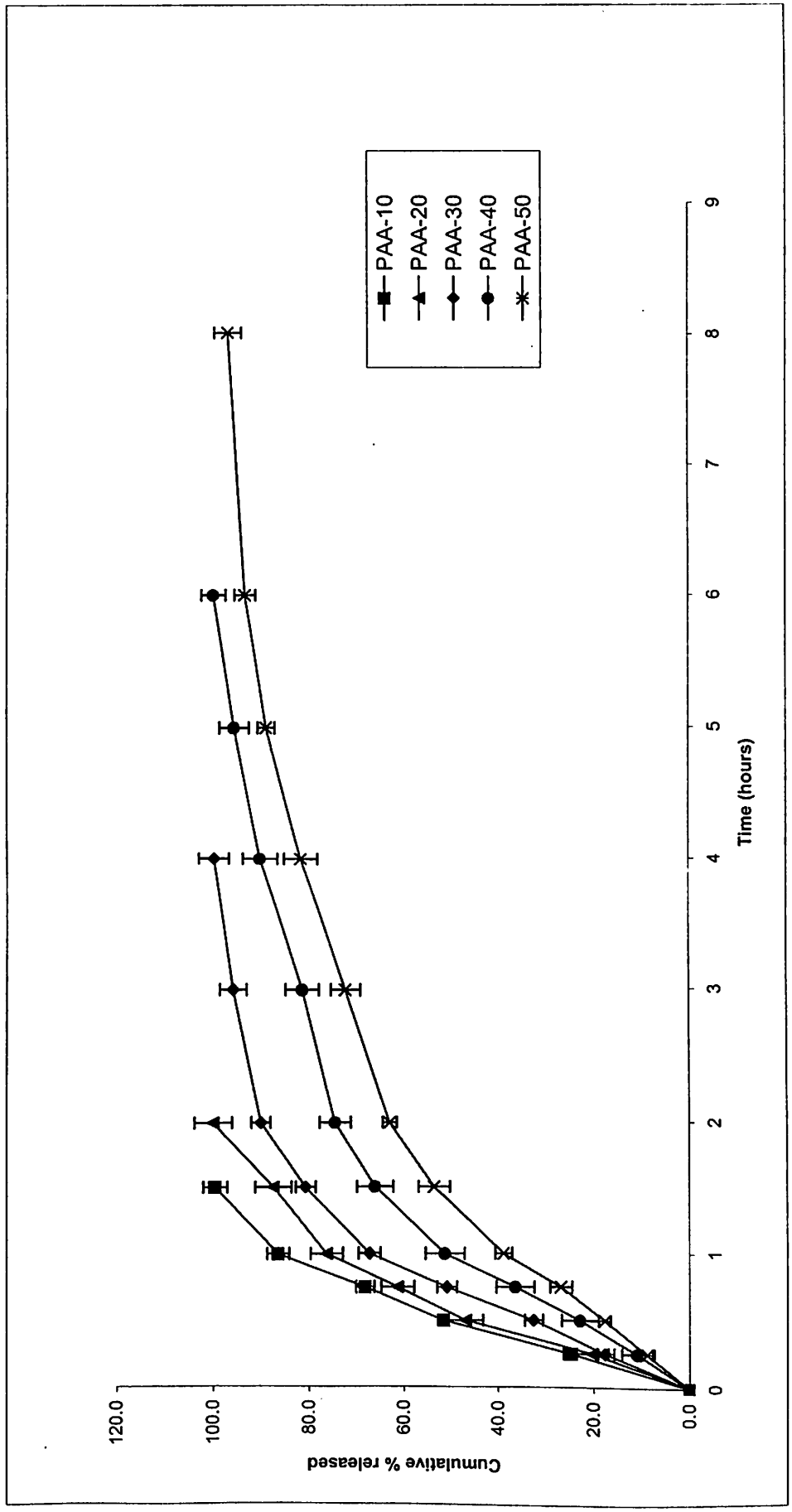


Figure 6.6: Release profile of flurbiprofen from polymeric disc formulations prepared using varying percentage of PAA in pH 7.4 phosphate buffer.

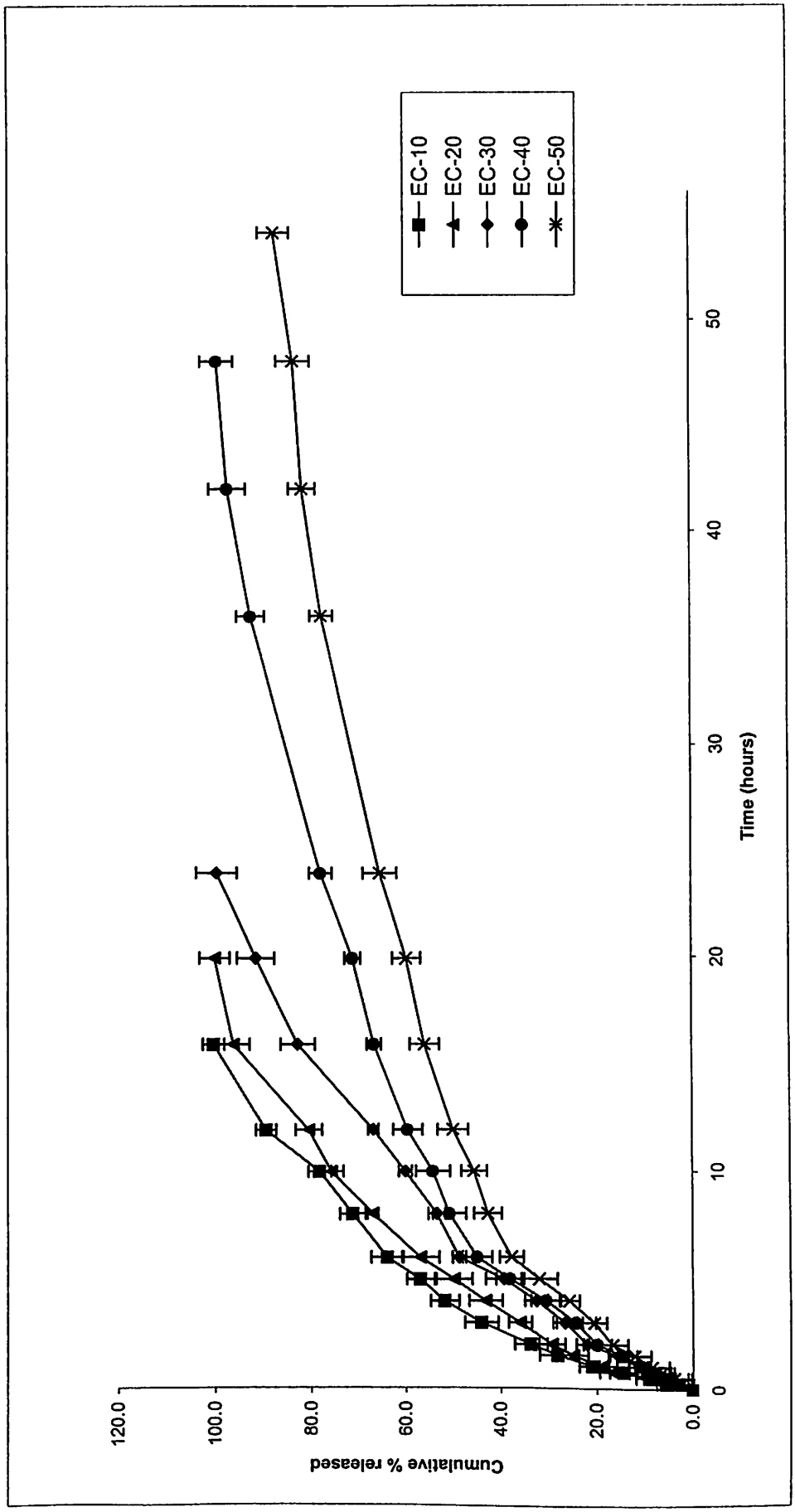


Figure 6.7: Release profile of flurbiprofen from polymeric disc formulations prepared using varying percentage of EC in pH 7.4 phosphate buffer.

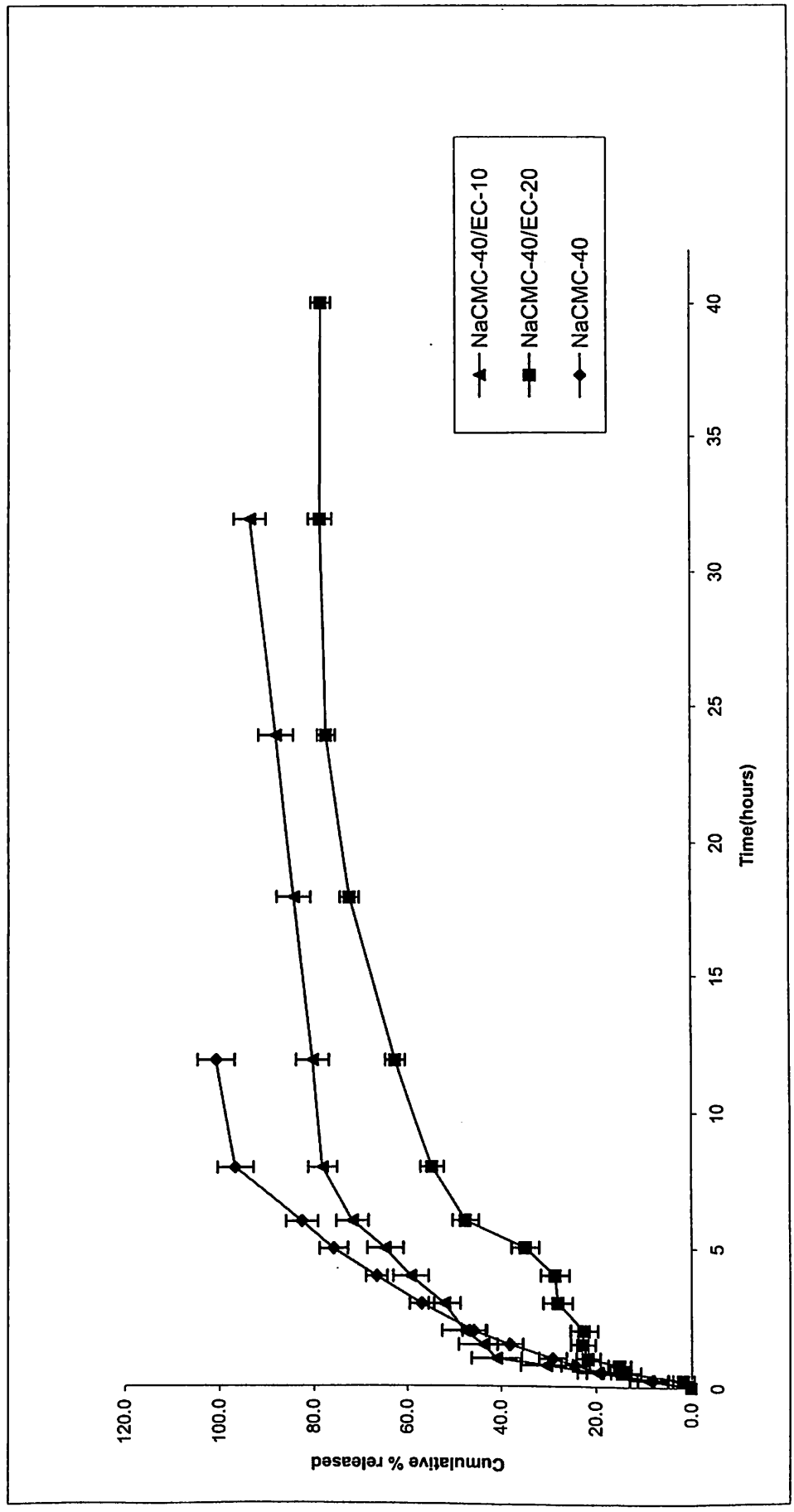


Figure 6.8: Release profile of flurbiprofen from 40% w/v NaCMC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

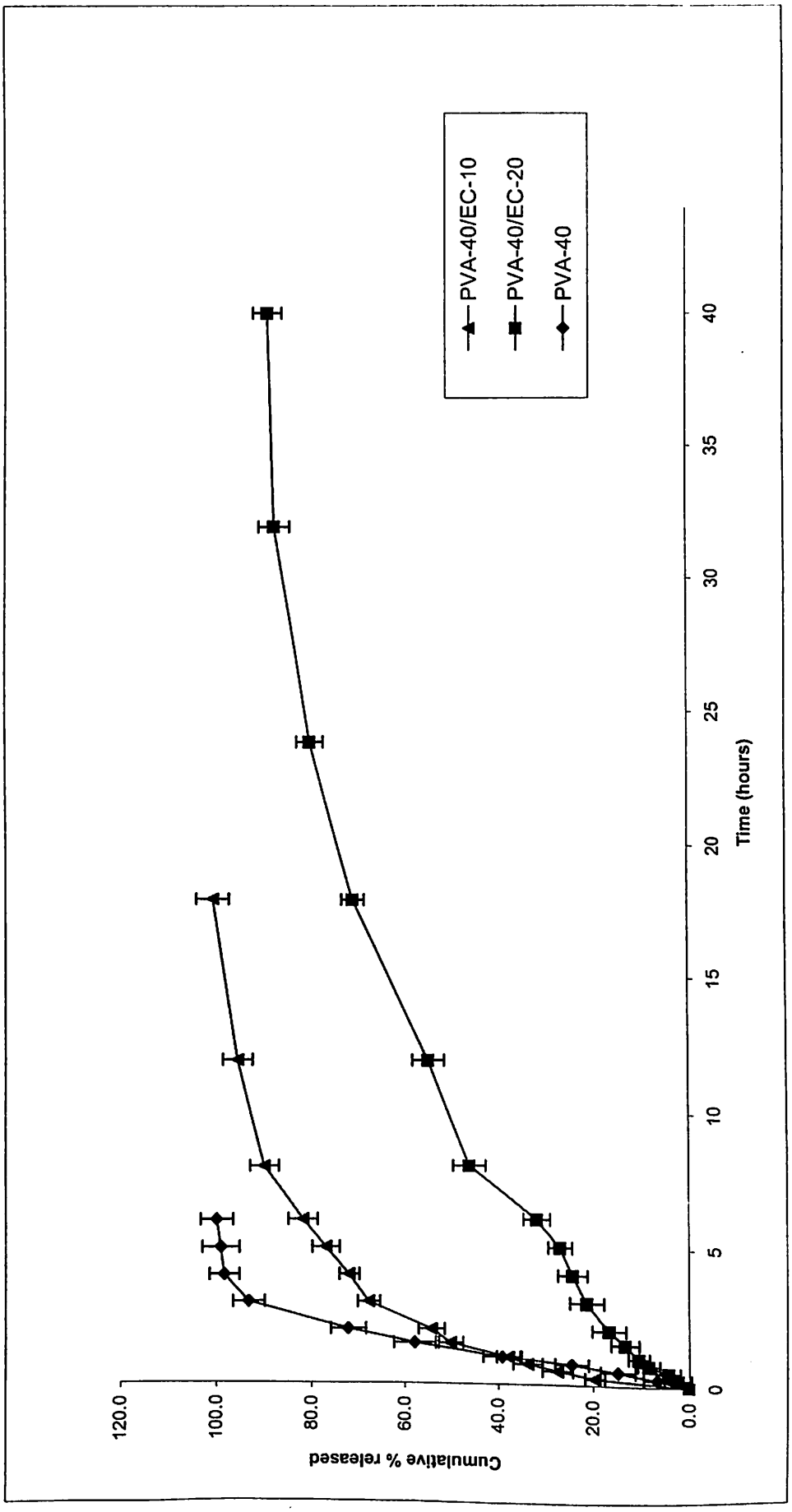


Figure 6.9: Release profile of flurbiprofen from 40% w/v PVA polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

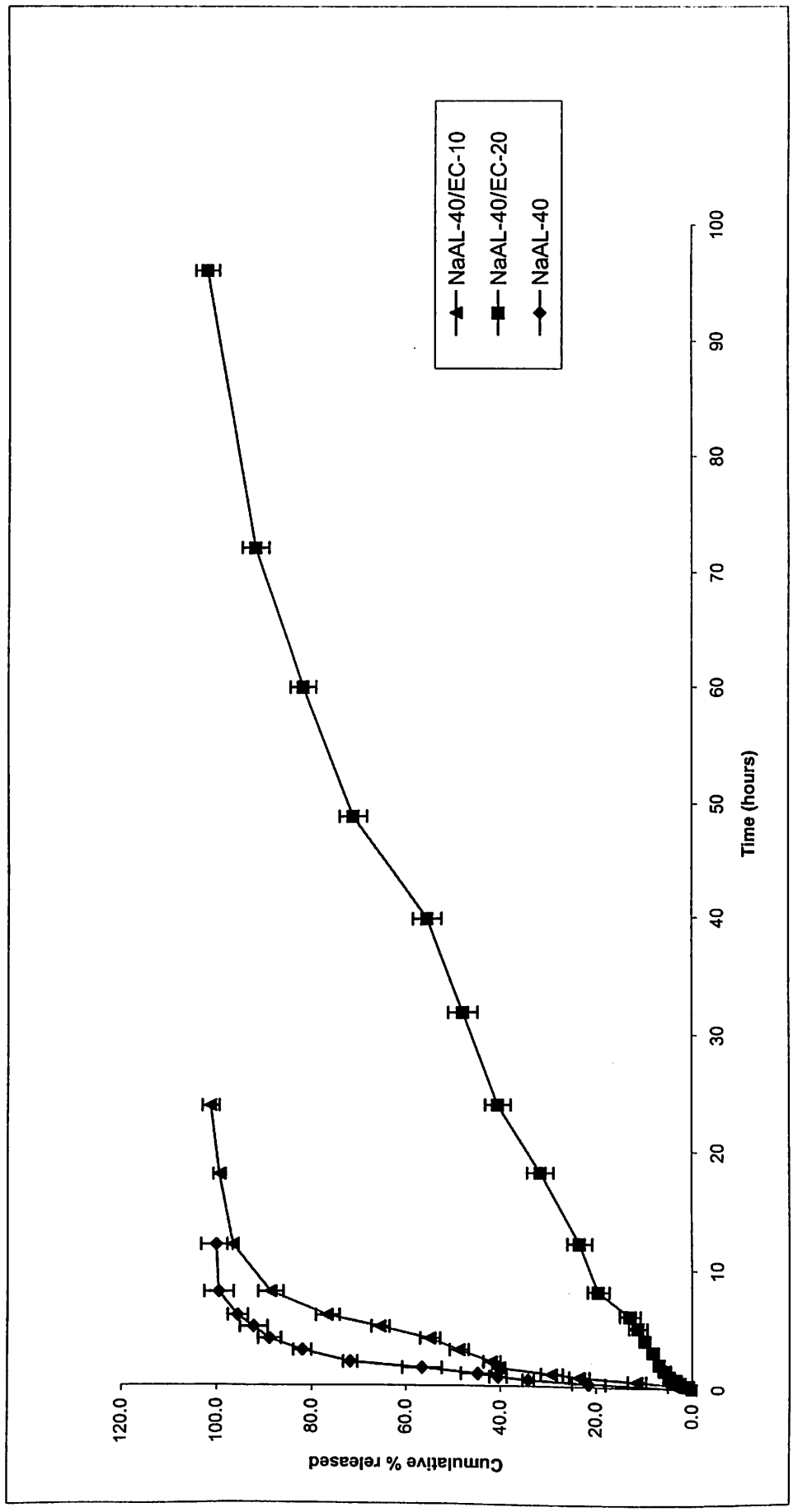


Figure 6.10: Release profile of flurbiprofen from 40% w/v NaAL polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

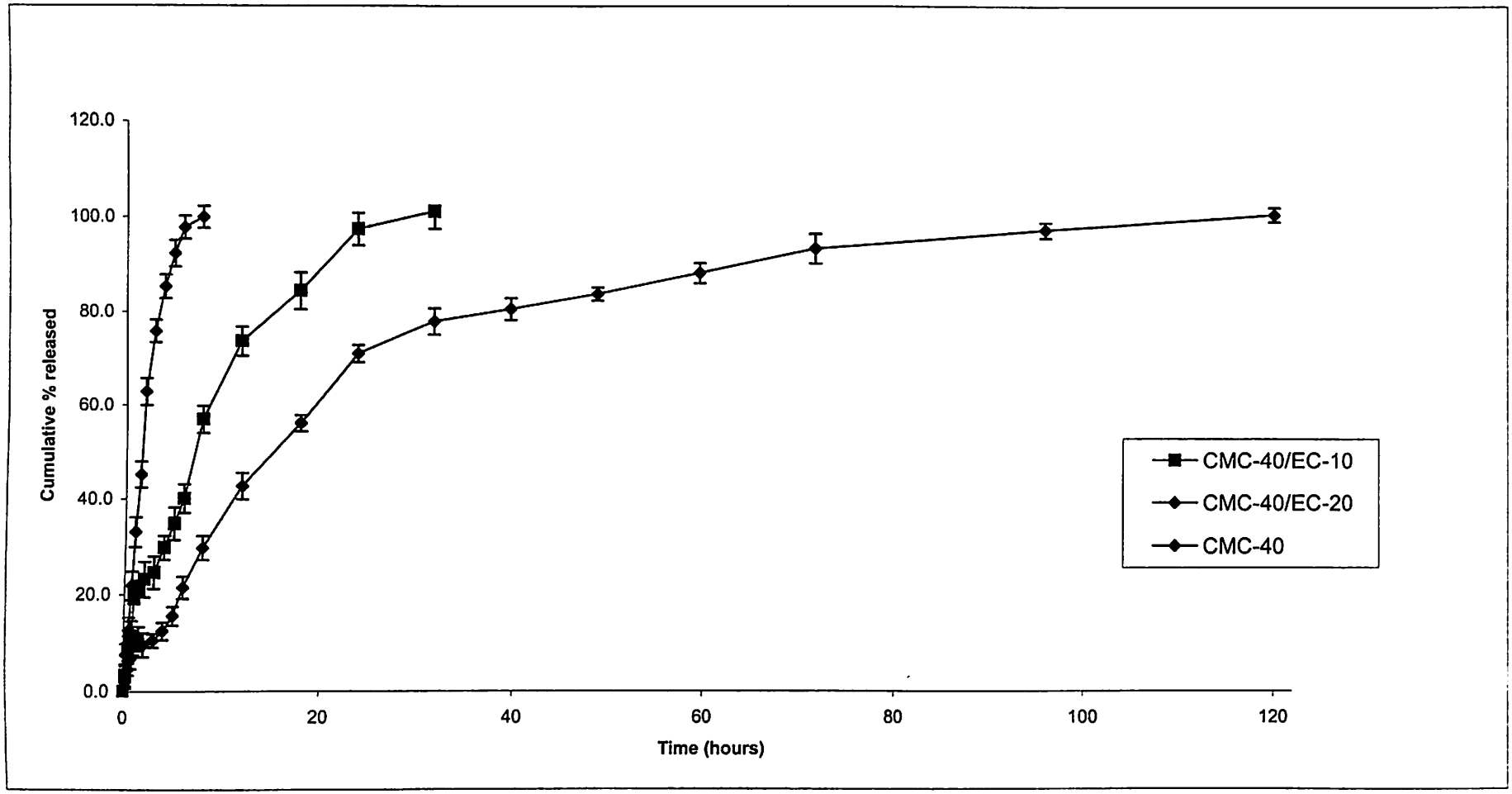


Figure 6.11: Release profile of flurbiprofen from 40% w/v CMC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

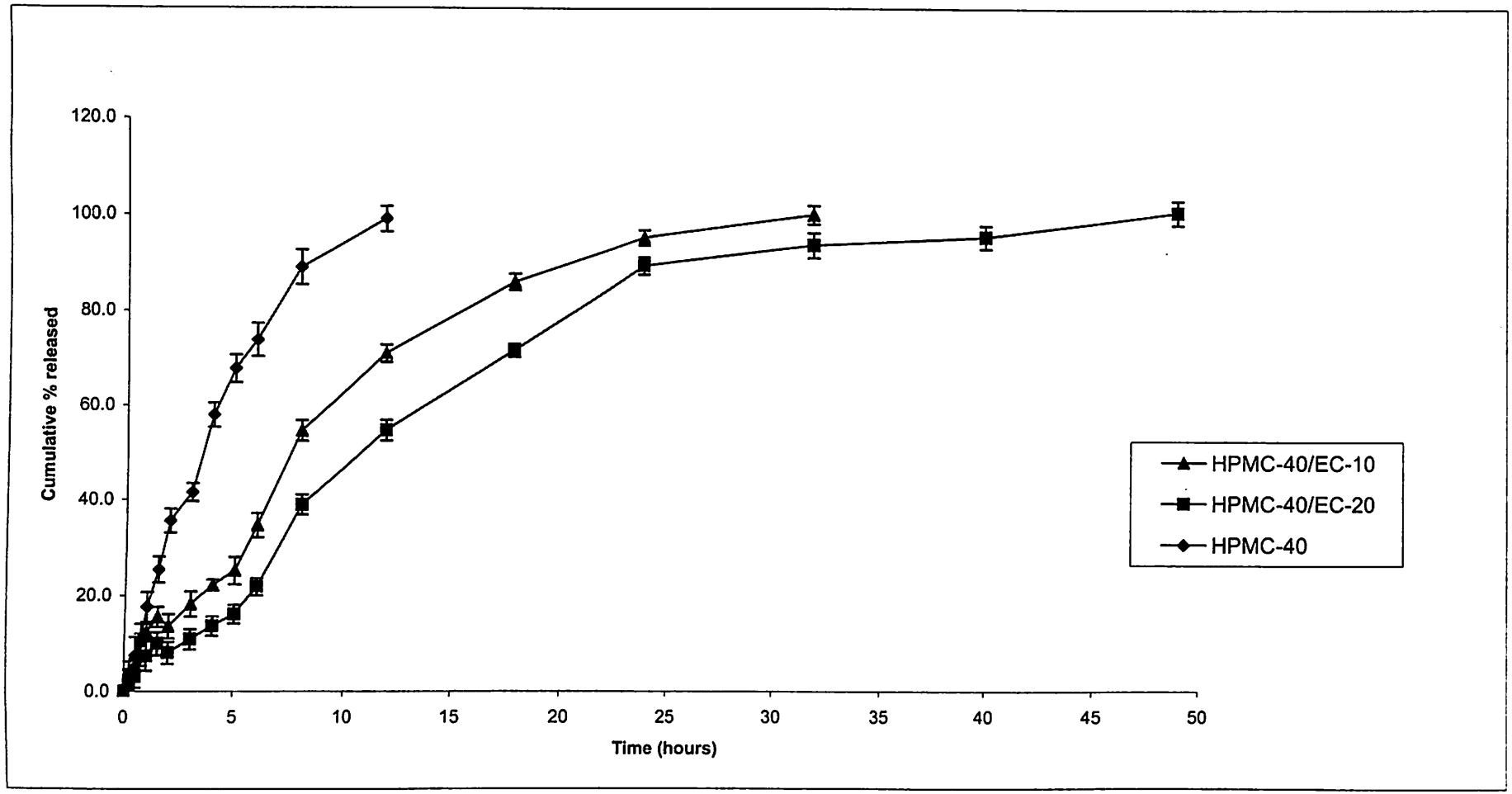


Figure 6.12: Release profile of flurbiprofen from 40% w/v HPMC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

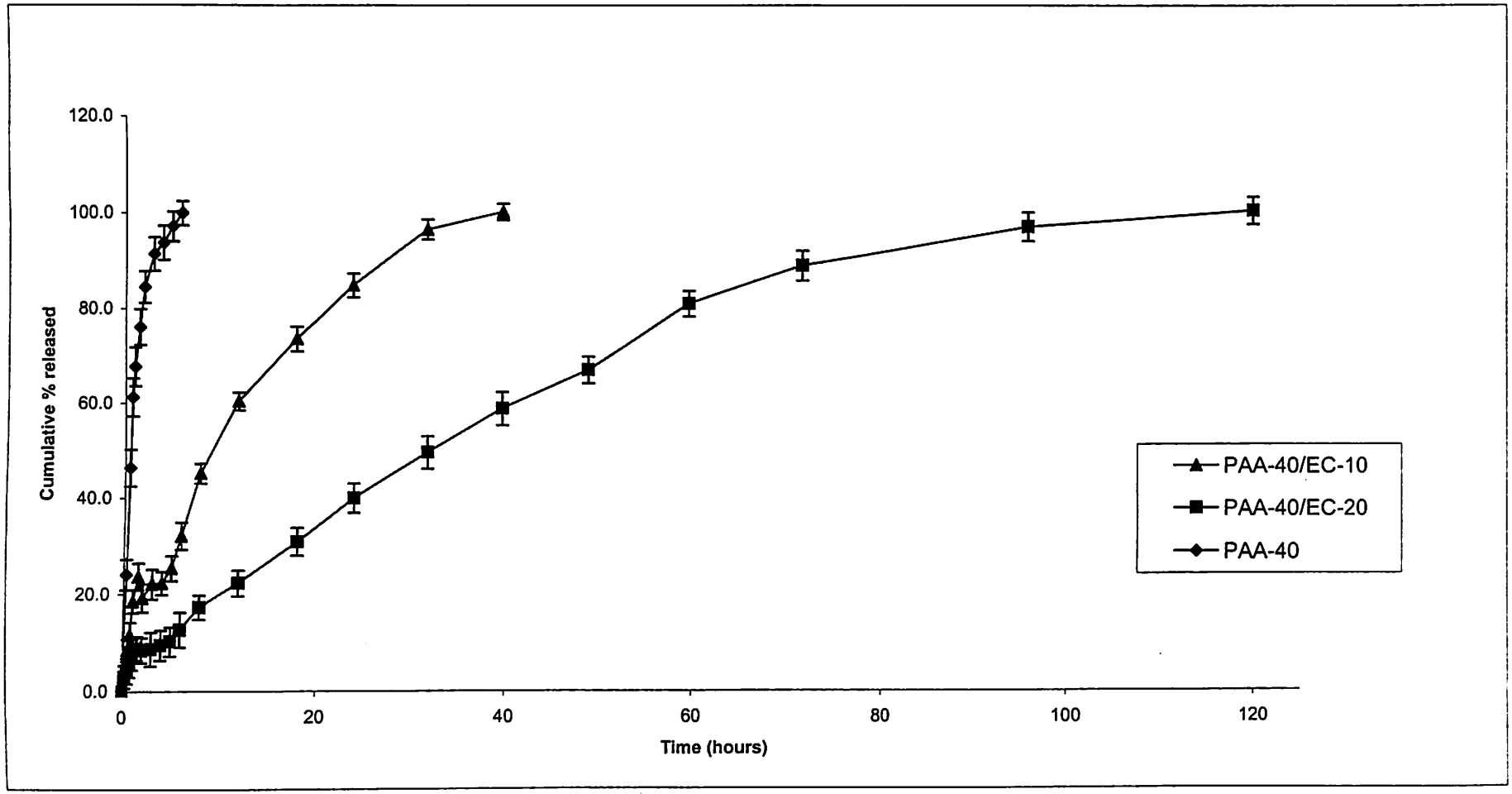


Figure 6.13: Release profile of flurbiprofen from 40% w/v PAA polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

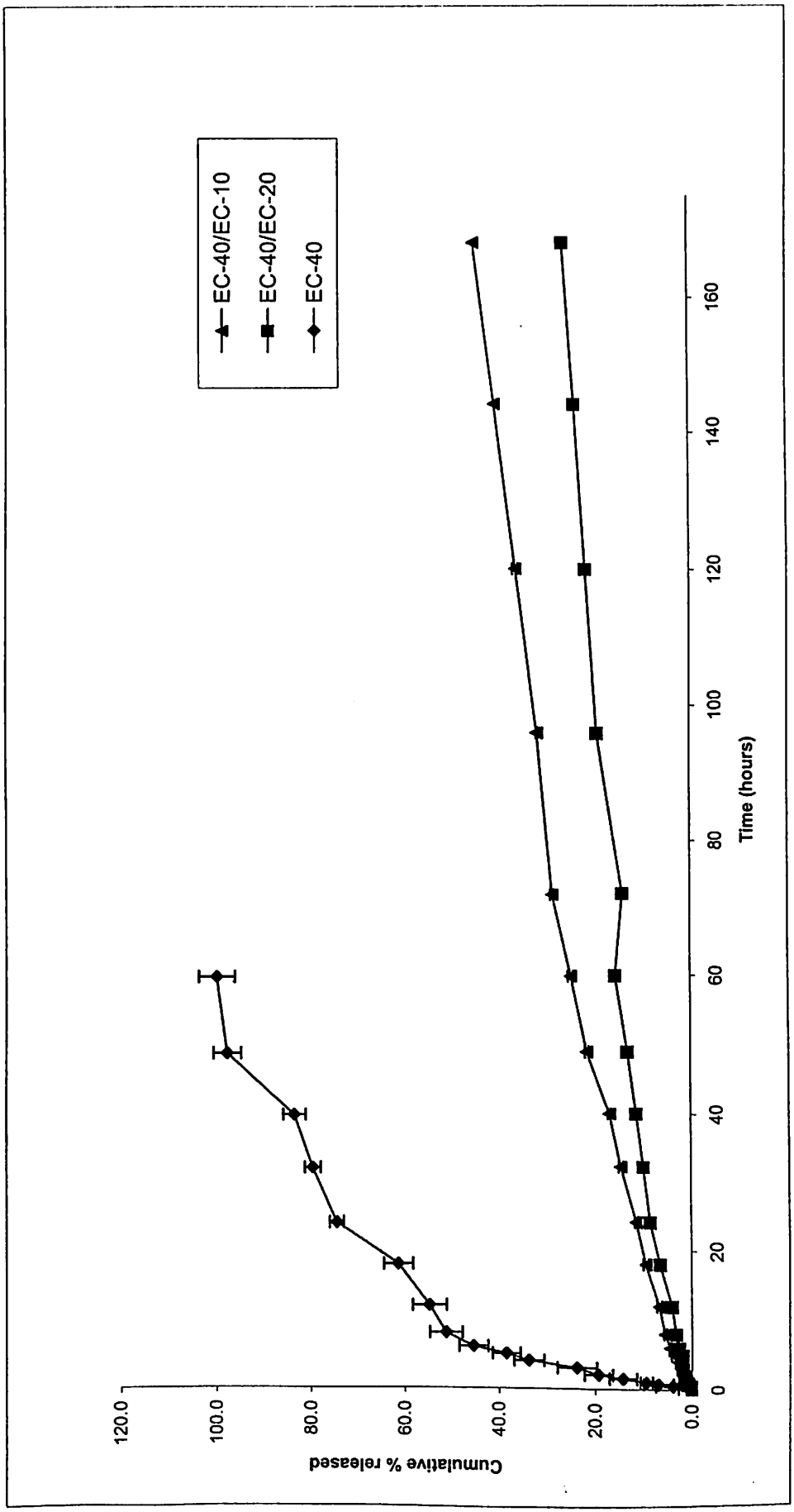


Figure 6.14: Release profile of flurbiprofen from 40% w/v EC polymeric discs coated with 10% w/v or 20% w/v solution of EC in ethyl alcohol in pH 7.4 phosphate buffer.

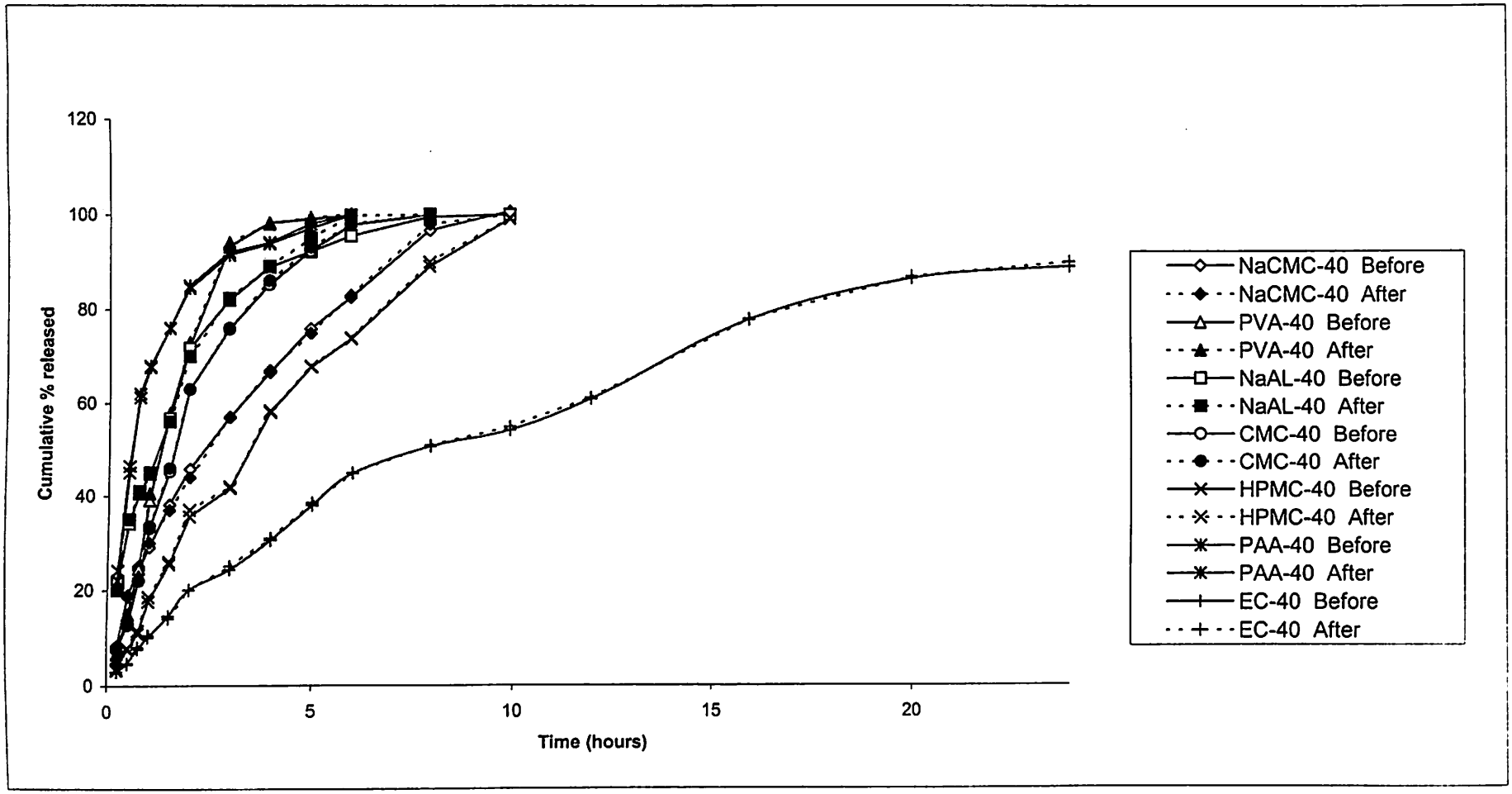


Figure 6.15: Comparative release profile of flurbiprofen from selected matrix embedded polymeric discs formulations before and after sterilization in pH 7.4 phosphate buffer.

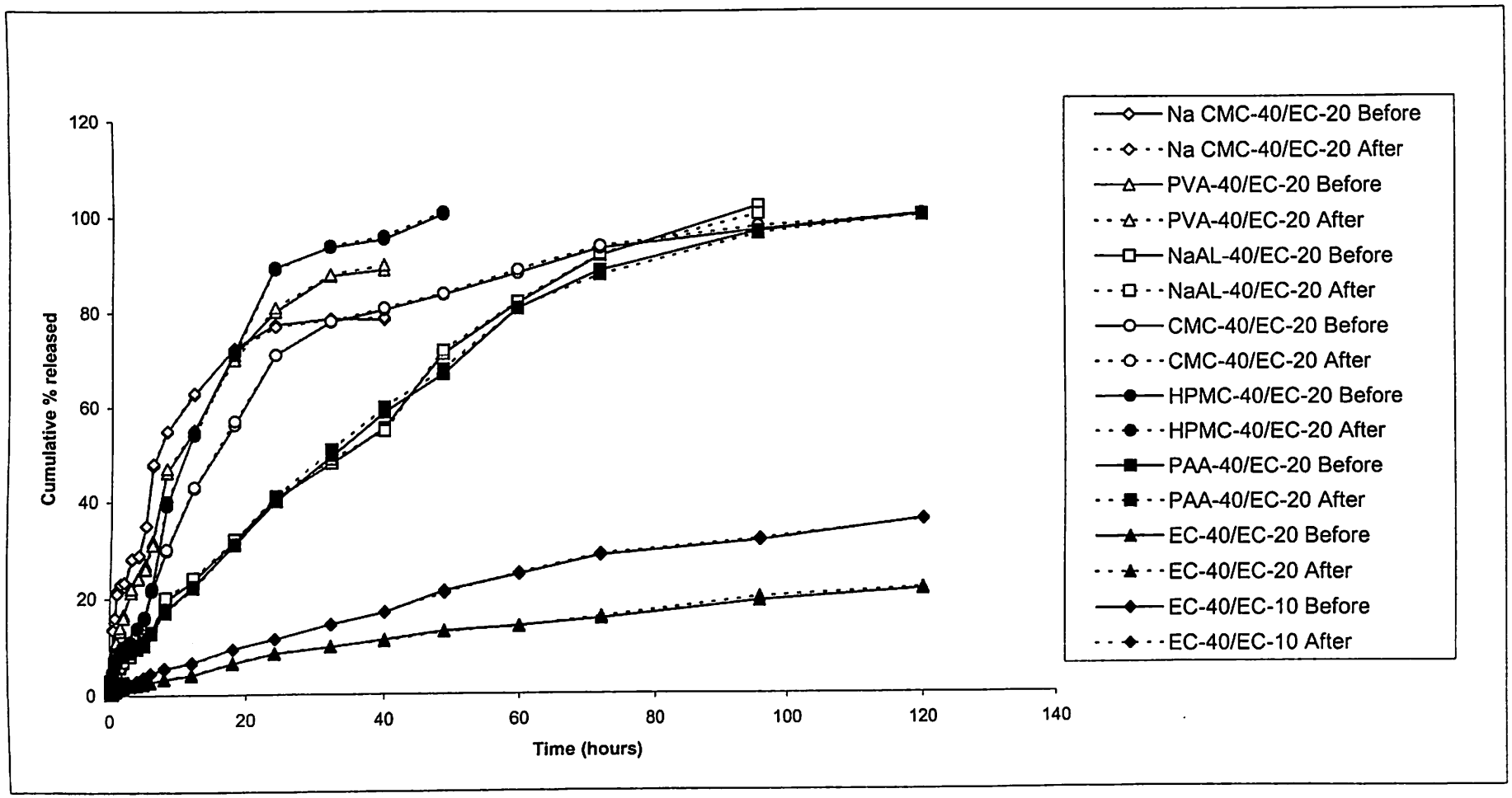


Figure 6.16: Comparative release profile of flurbiprofen from selected polymer coated matrix embedded polymeric discs formulations before and after sterilization in pH 7.4 phosphate buffer.

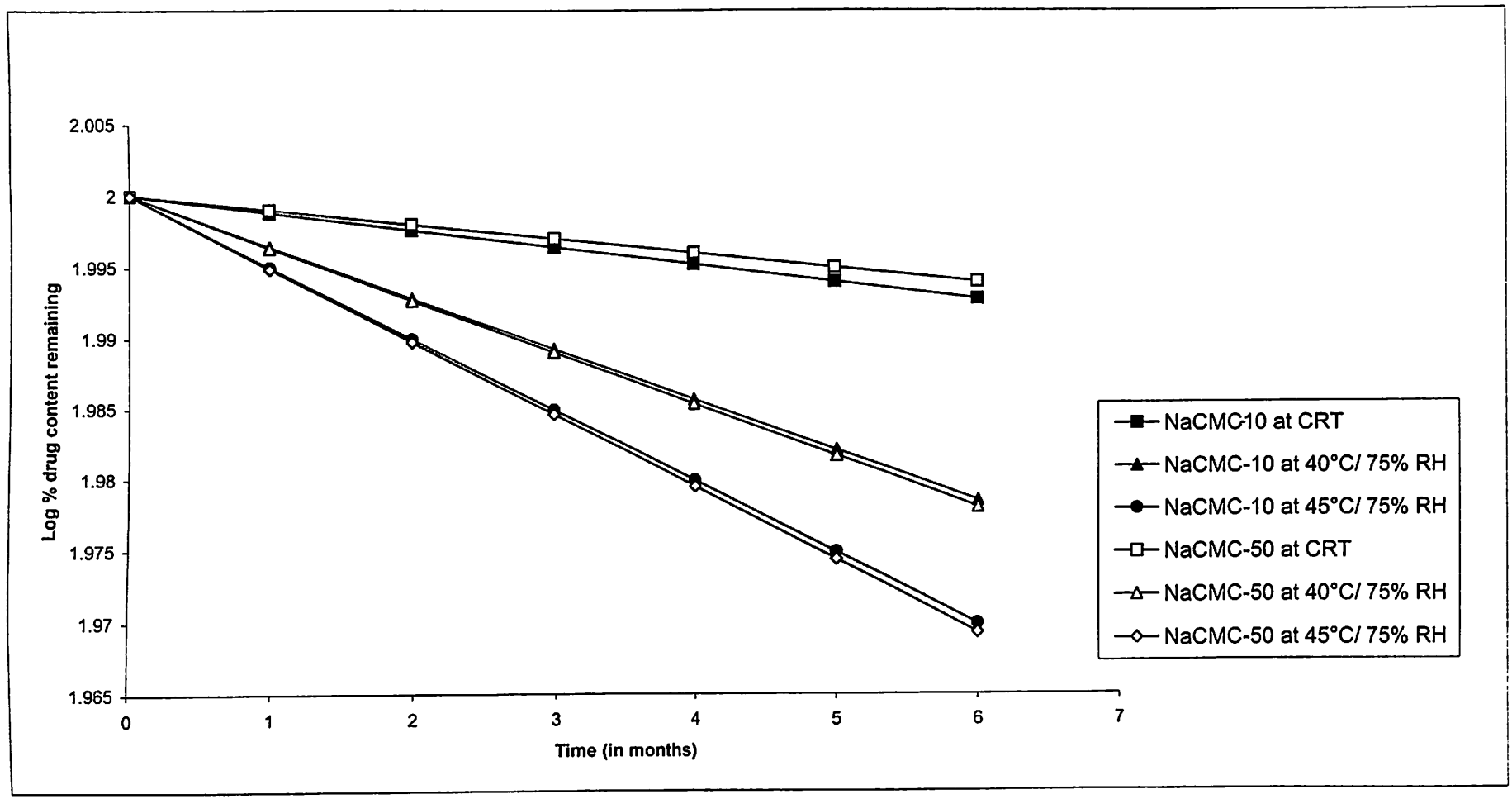


Figure 6.17: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric disc formulations prepared using 10 % and 50 % w/v NaCMC.

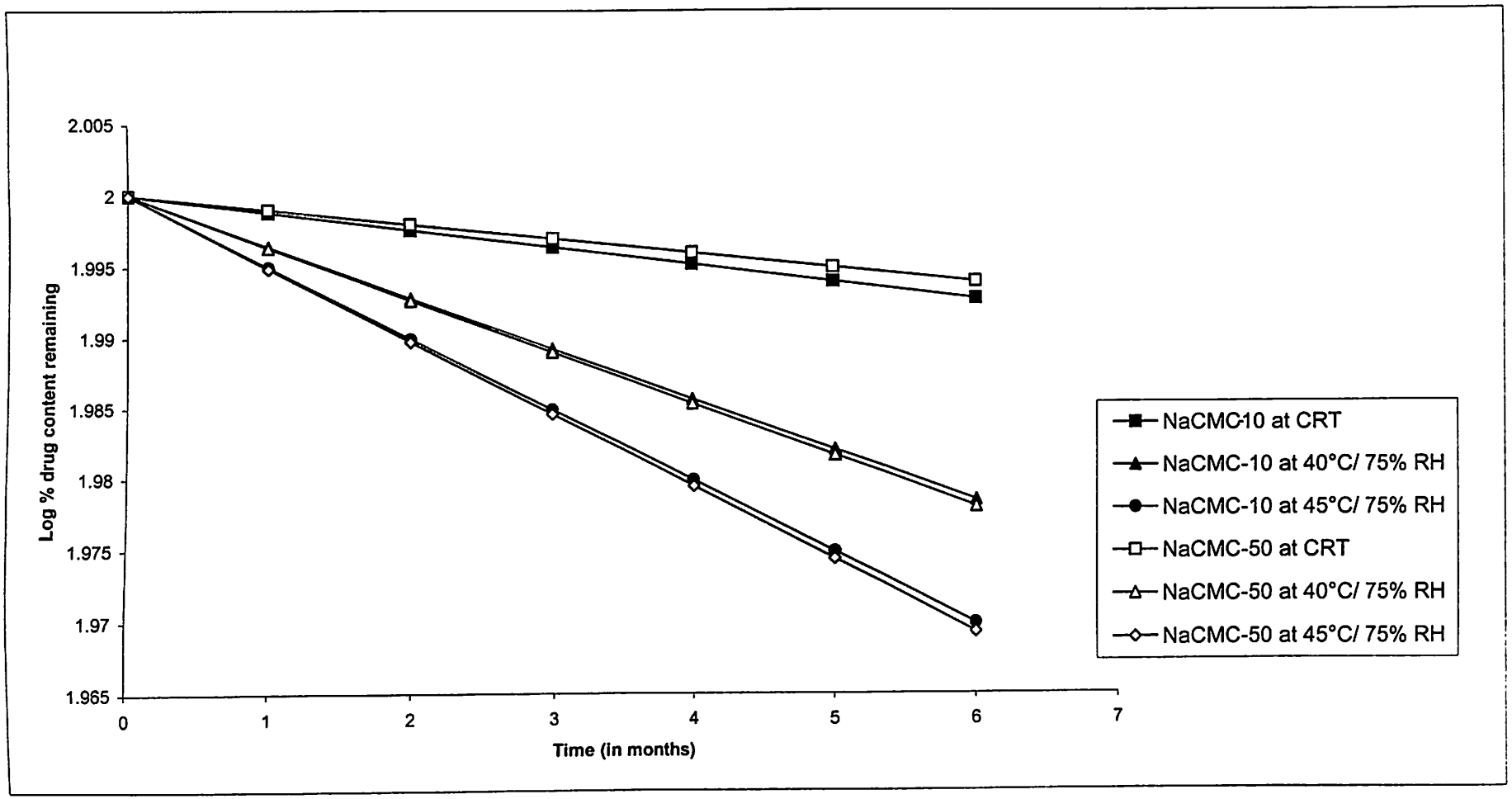


Figure 6.17: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric disc formulations prepared using 10 % and 50 % w/v NaCMC.

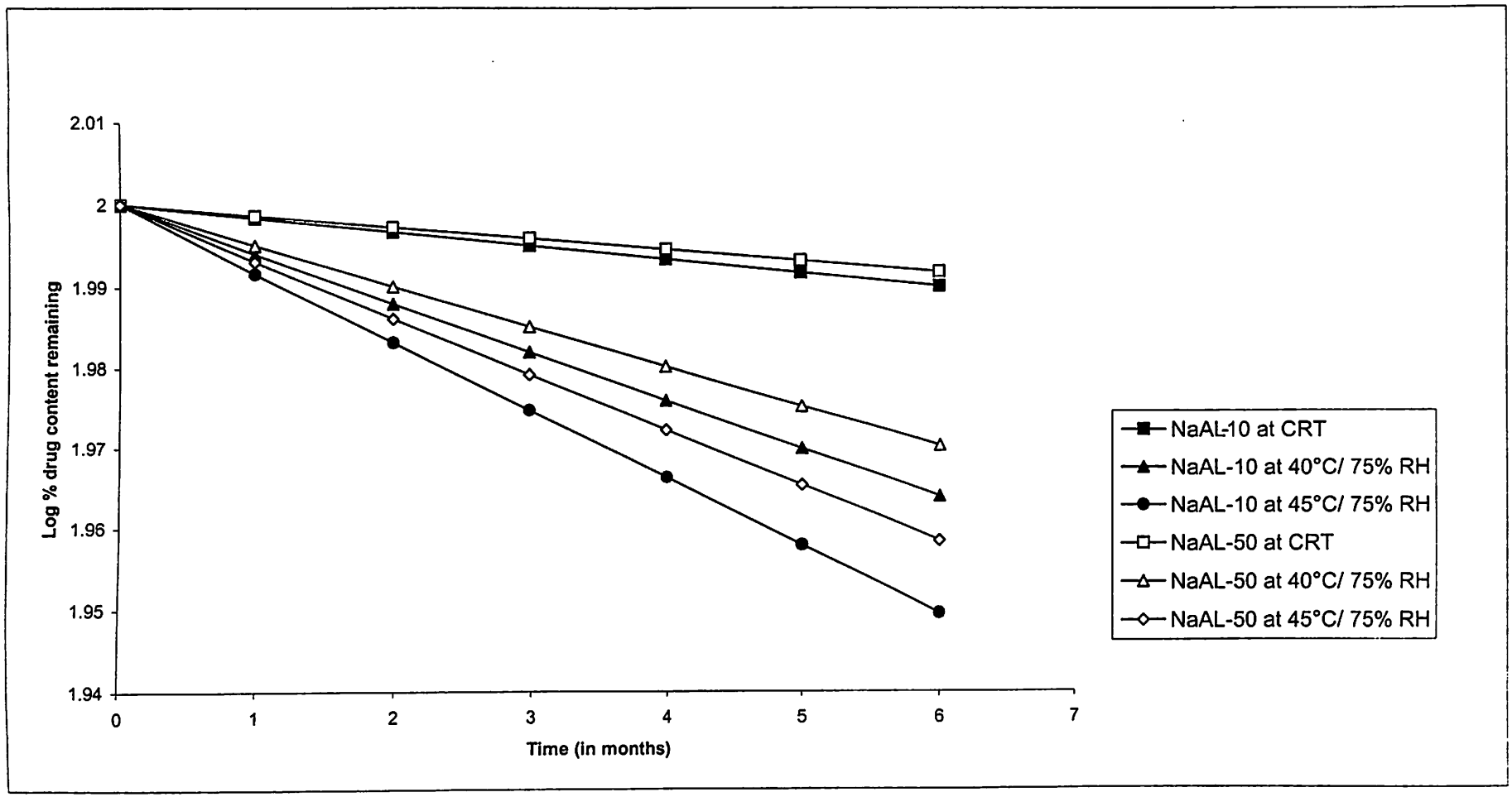


Figure 6.19: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric disc formulations prepared using 10 % and 50 % w/v NaAL.

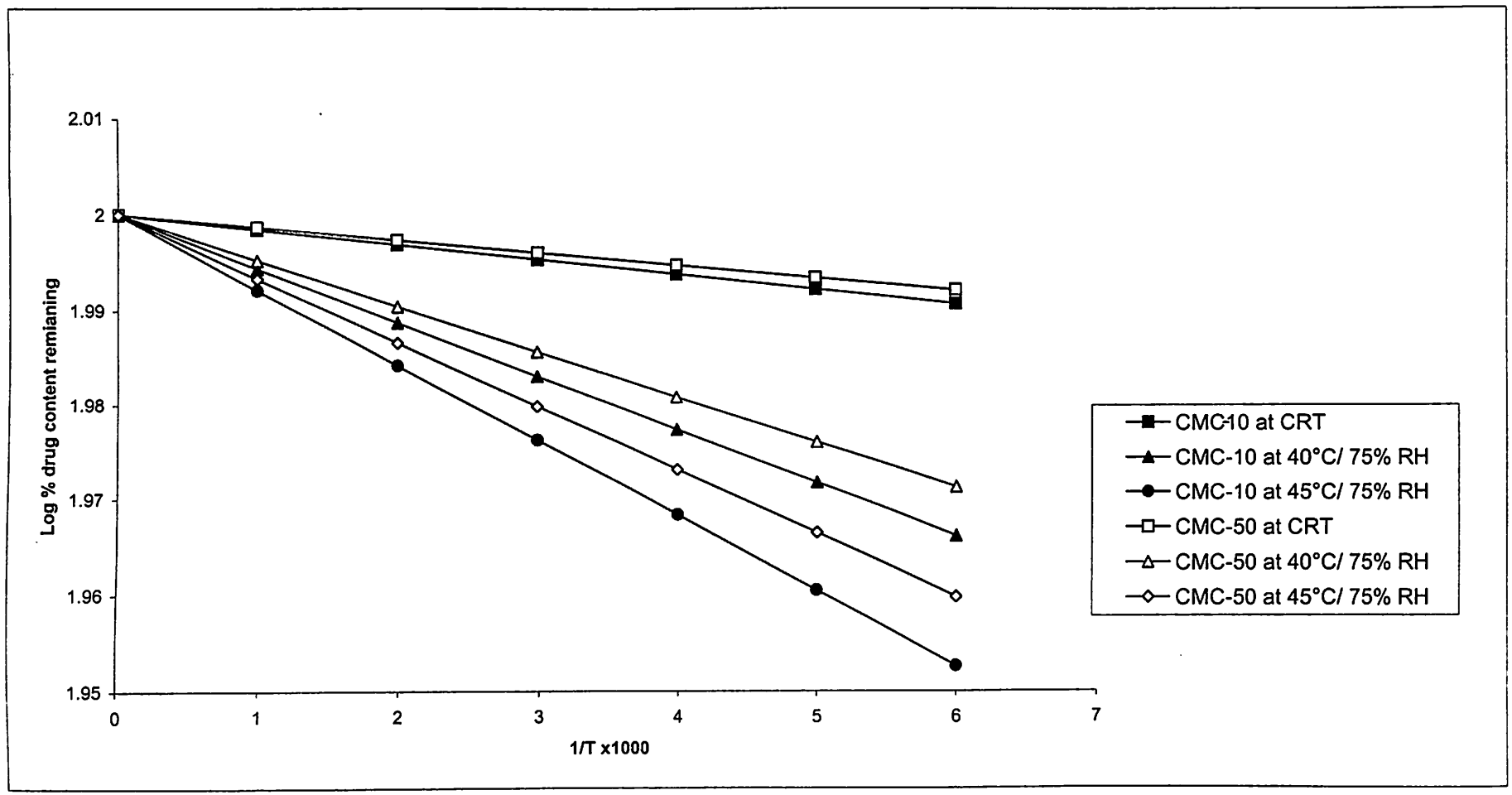


Figure 6.20: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric disc formulations prepared using 10 % and 50 % w/v CMC.

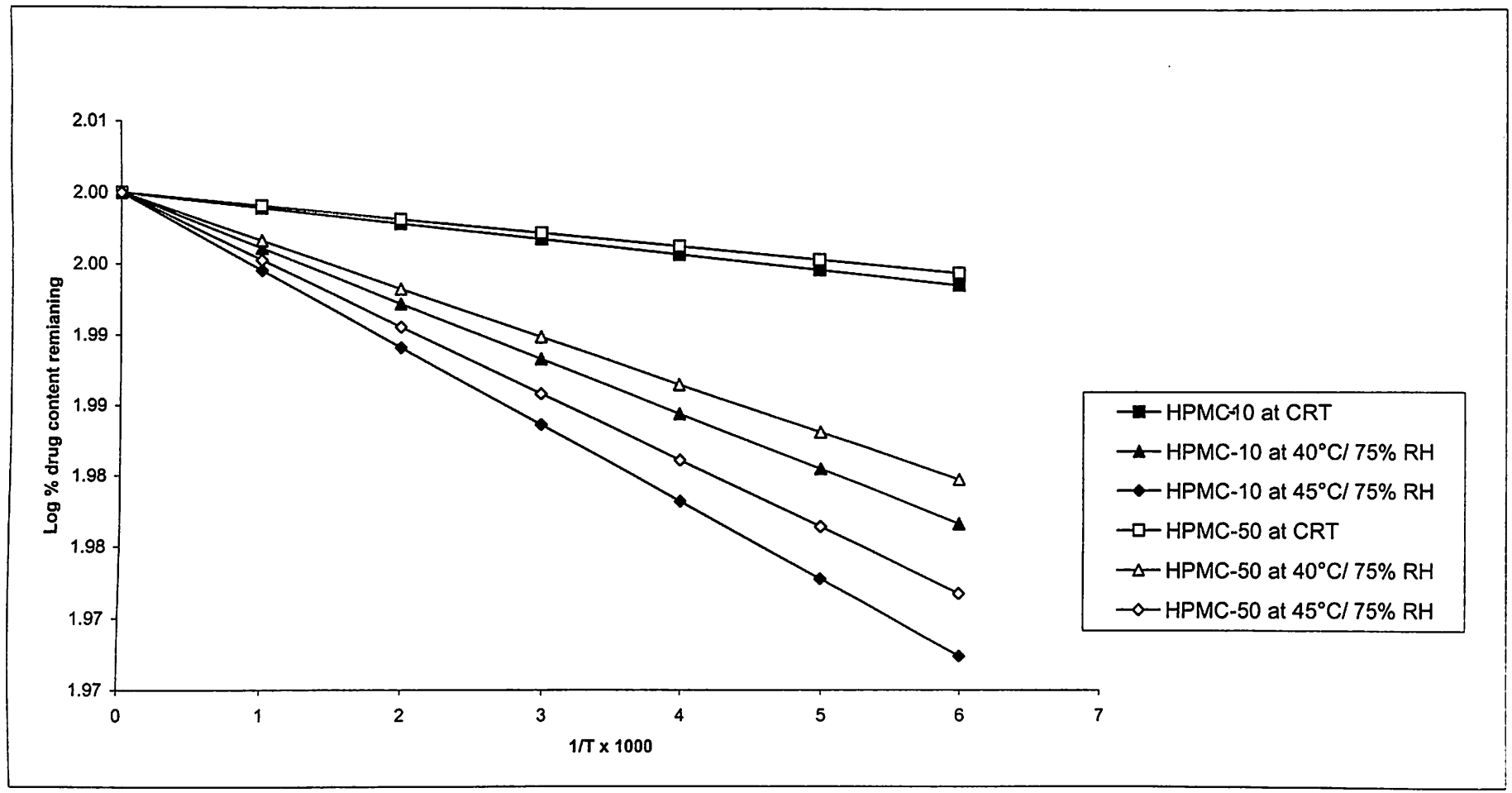


Figure 6.21: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric disc formulations prepared using 10 % and 50 % w/v HPMC.

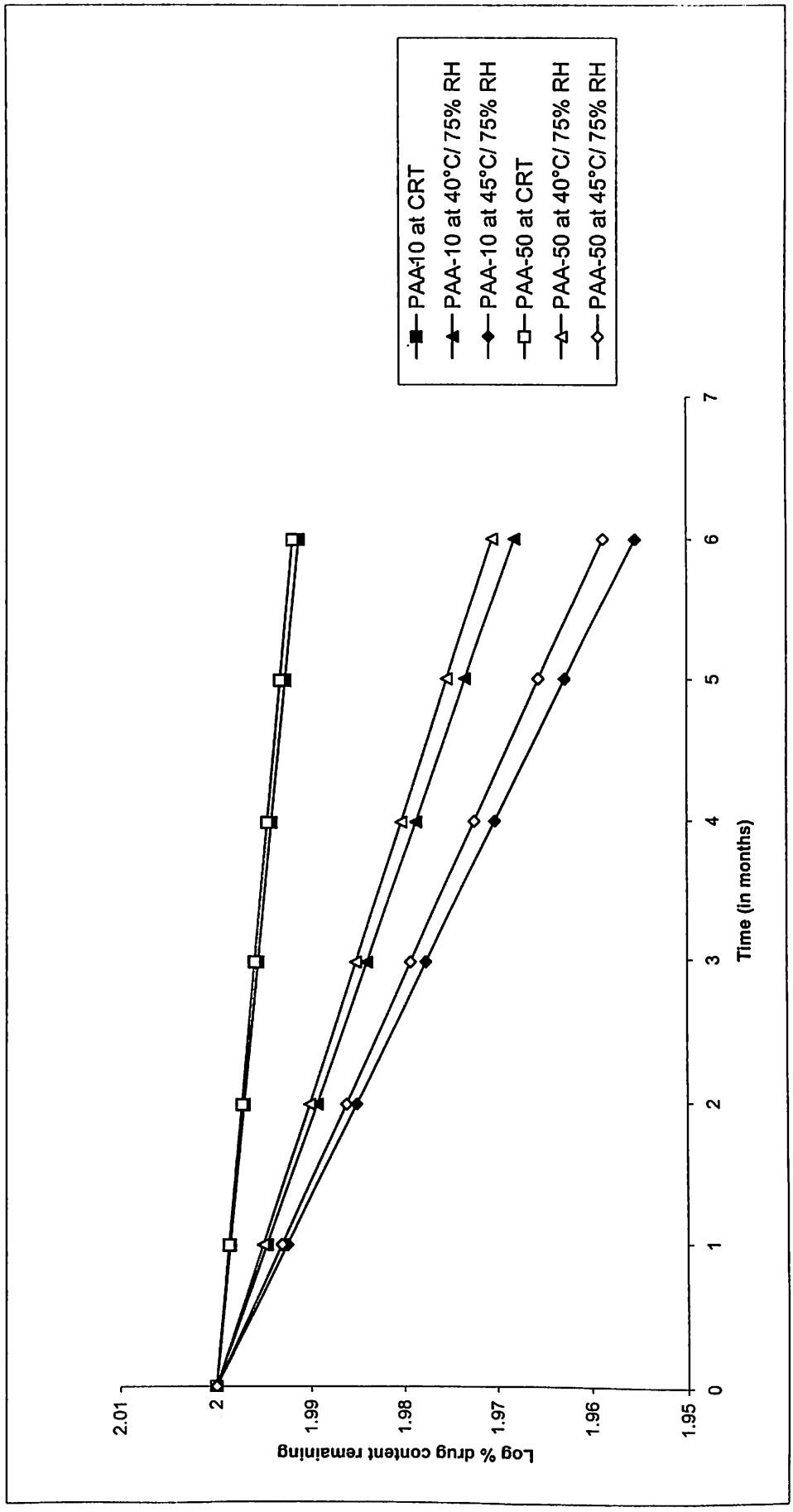


Figure 6.22: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric disc formulations prepared using 10 % and 50 % w/v PAA.

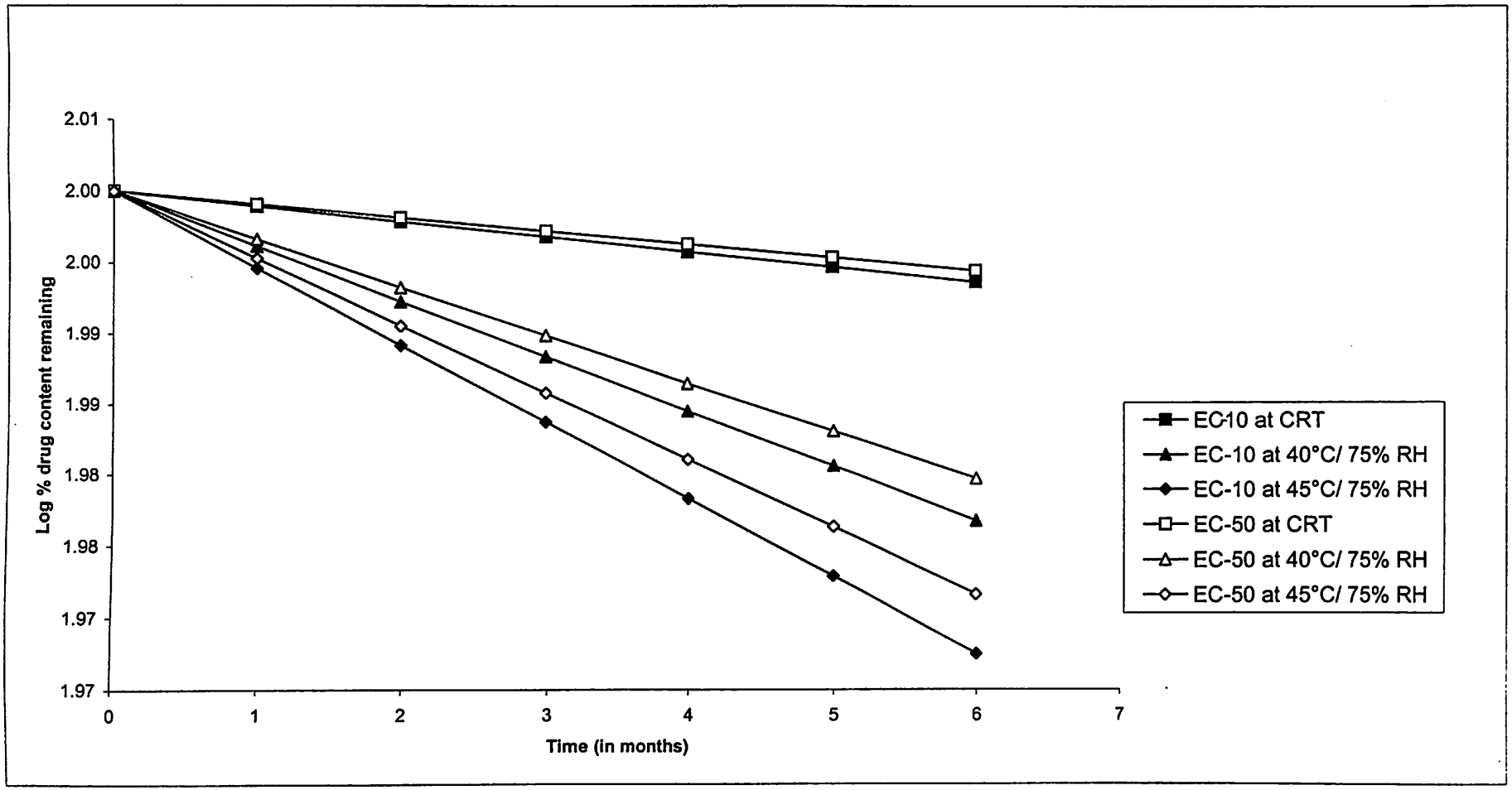


Figure 6.23: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric disc formulations prepared using 10 % and 50 % w/v EC.

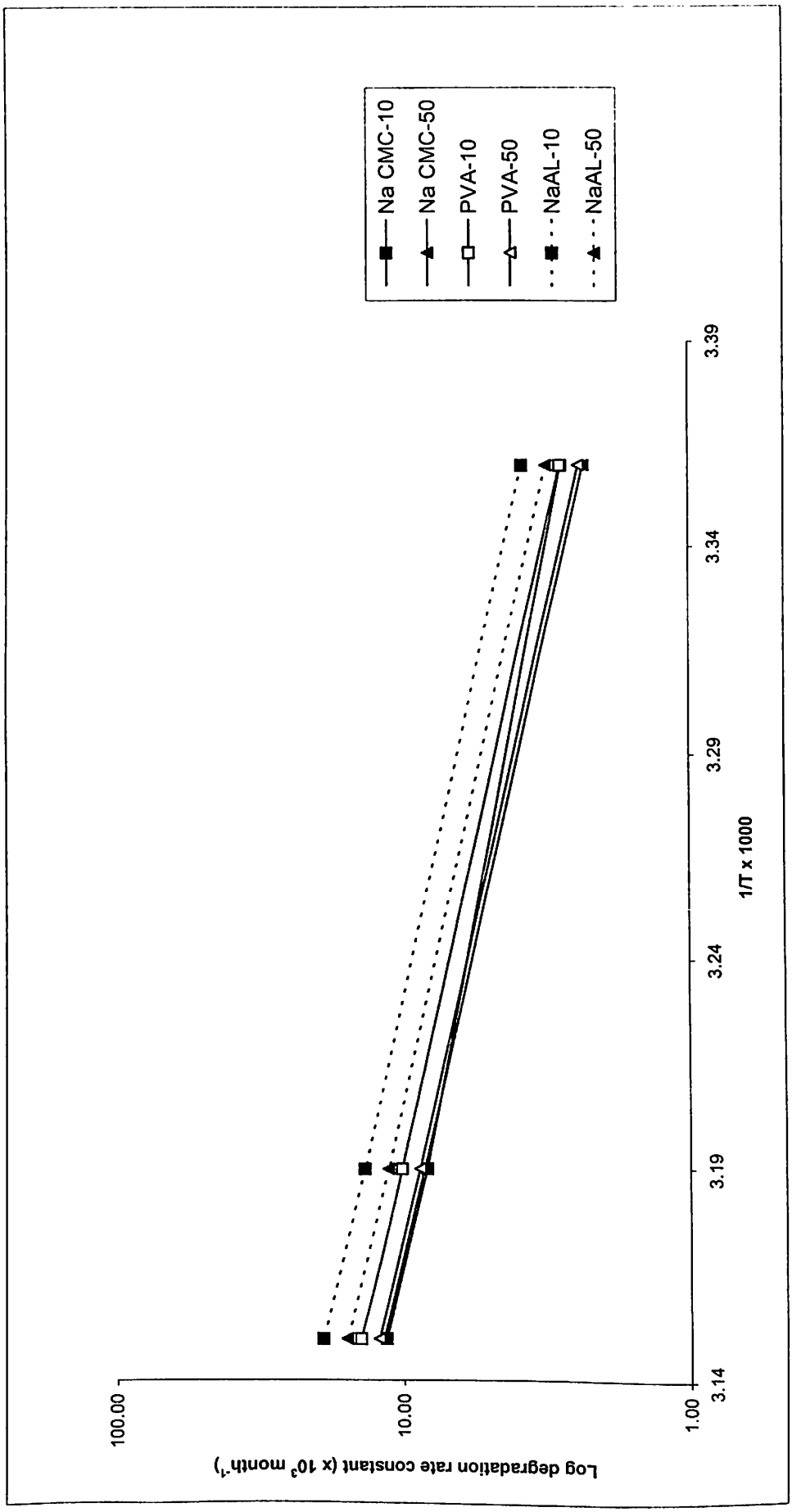


Figure 6.24: Arrhenius plot for degradation of flurbiprofen from NaCMC, PVA and NaAL based matrix embedded polymeric disc formulations.

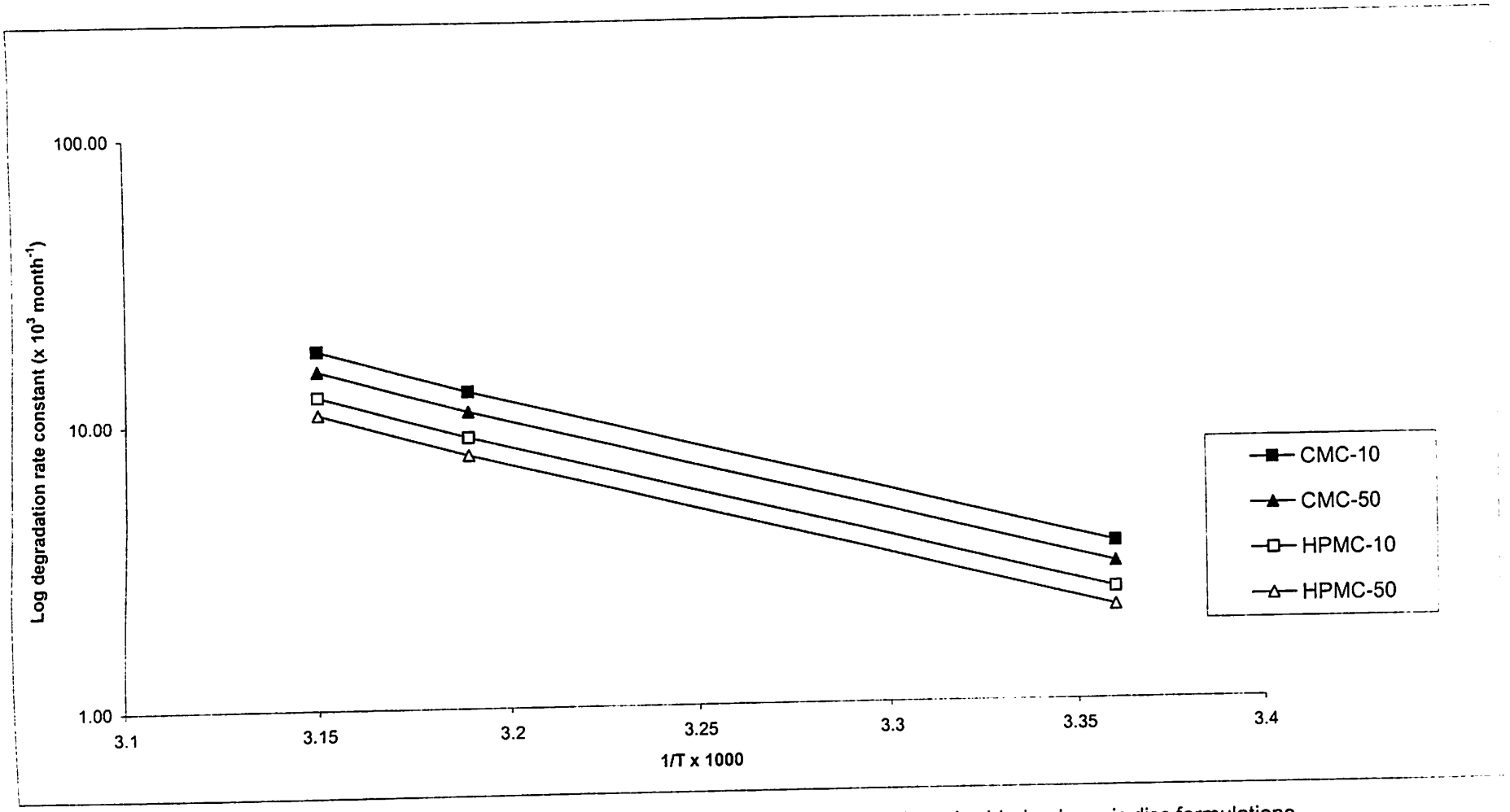


Figure 6.25: Arrhenius plot for degradation of flurbiprofen from CMC and HPMC based matrix embedded polymeric disc formulations.

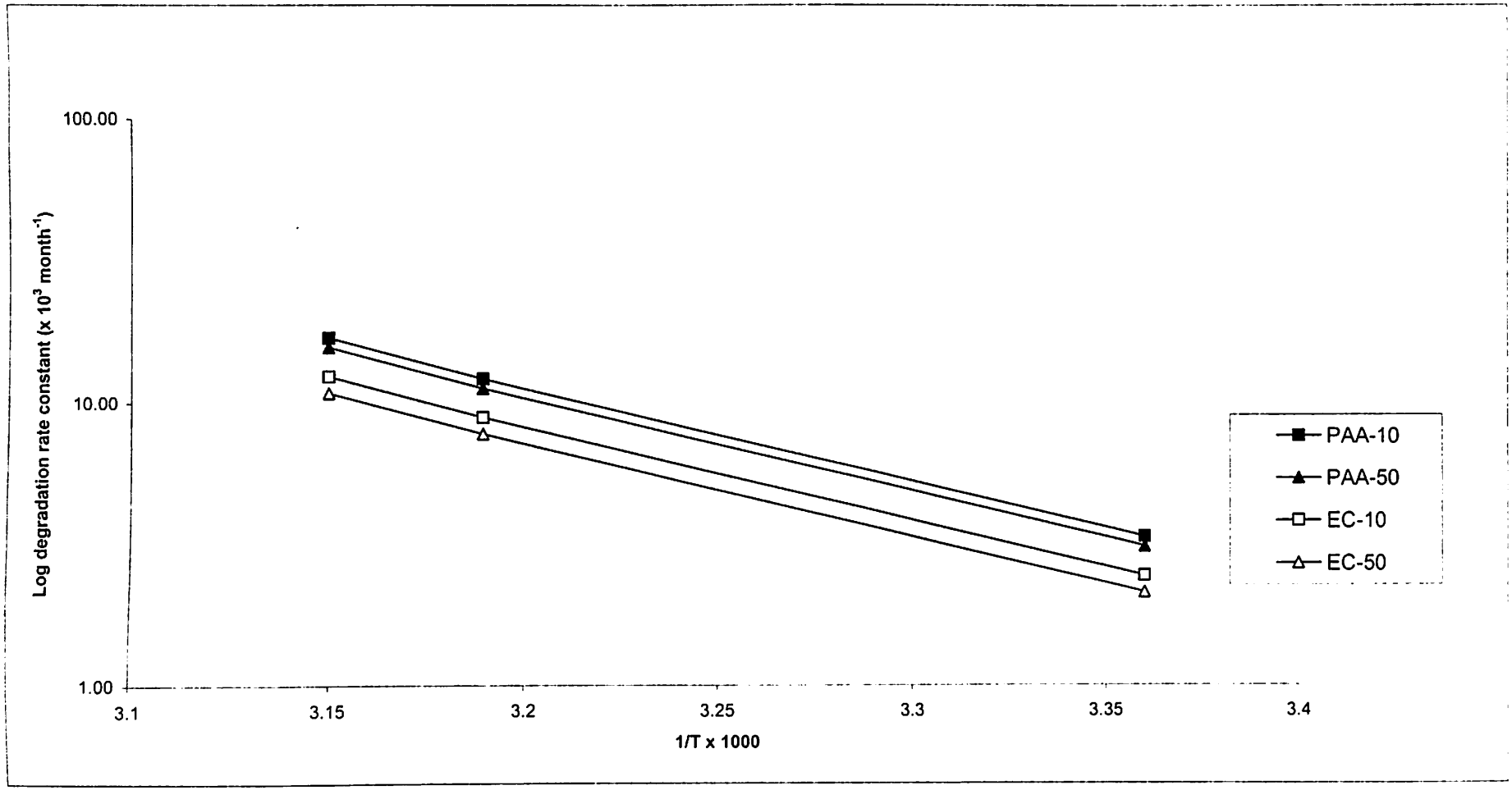


Figure 6.26: Arrhenius plot for degradation of flurbiprofen from PAA and EC based matrix embedded polymeric disc formulations.

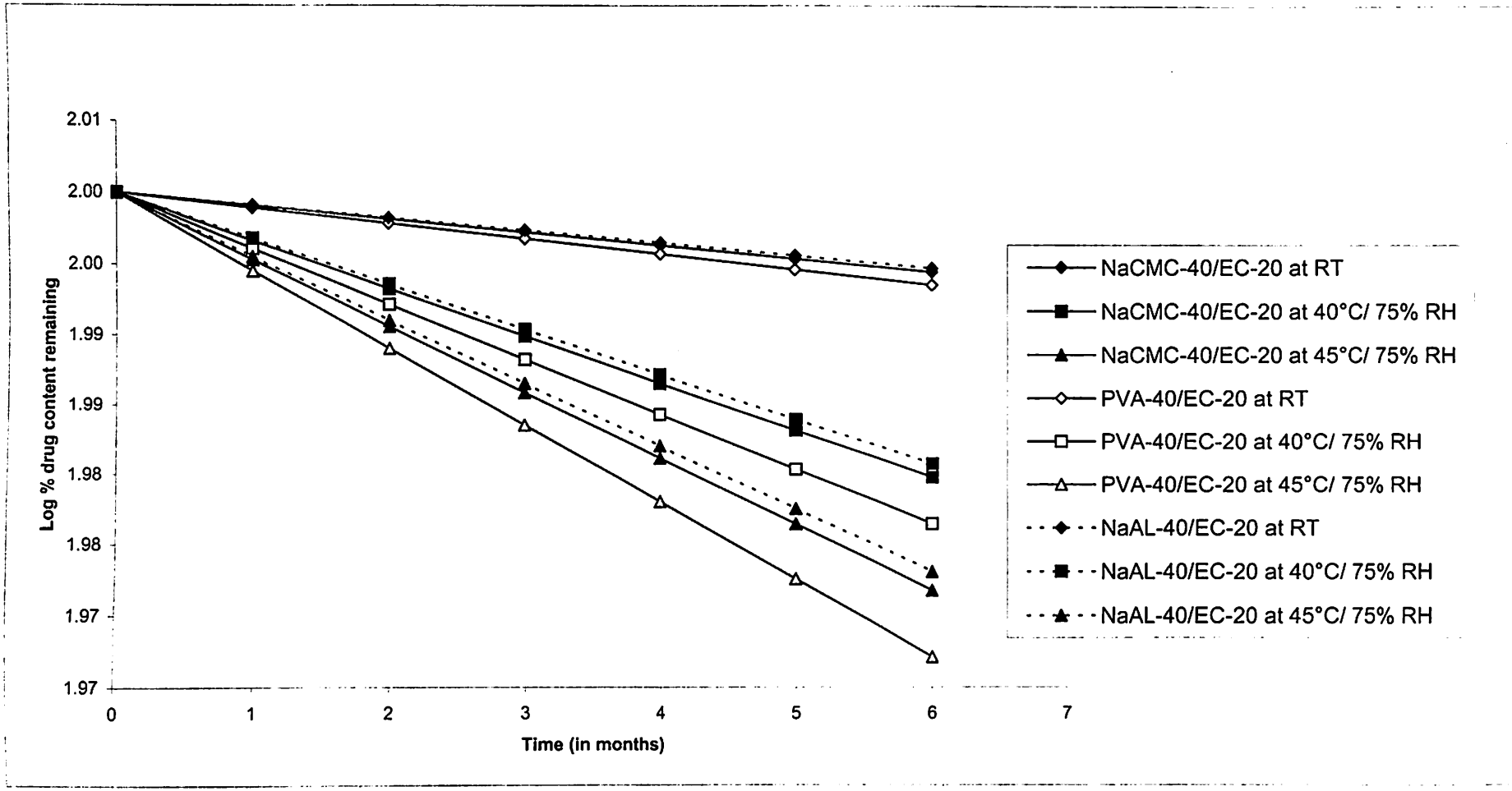


Figure 6.27: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric discs of NaCMC, PVA and NaAL coated with 20% w/v solution of EC.

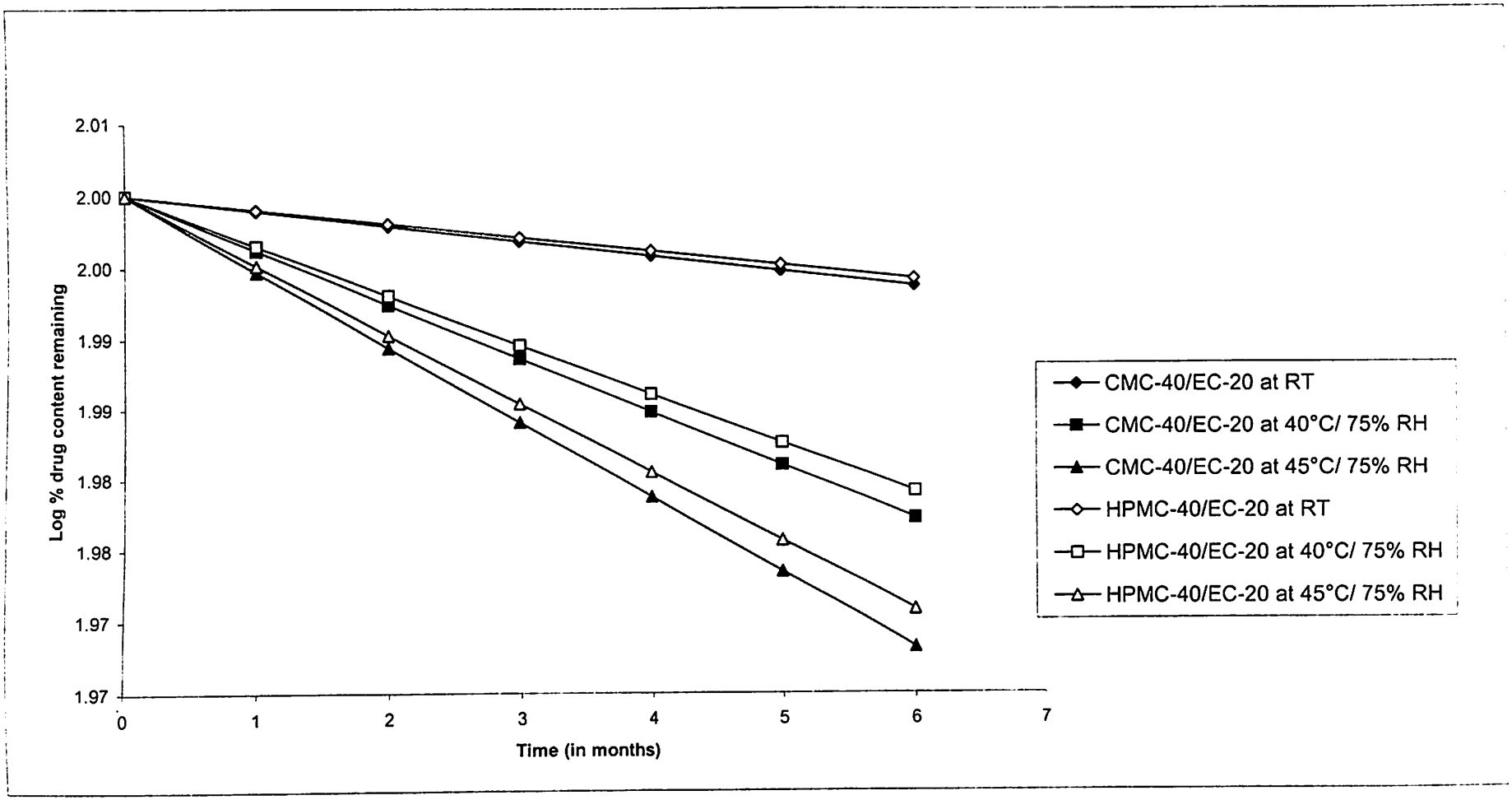
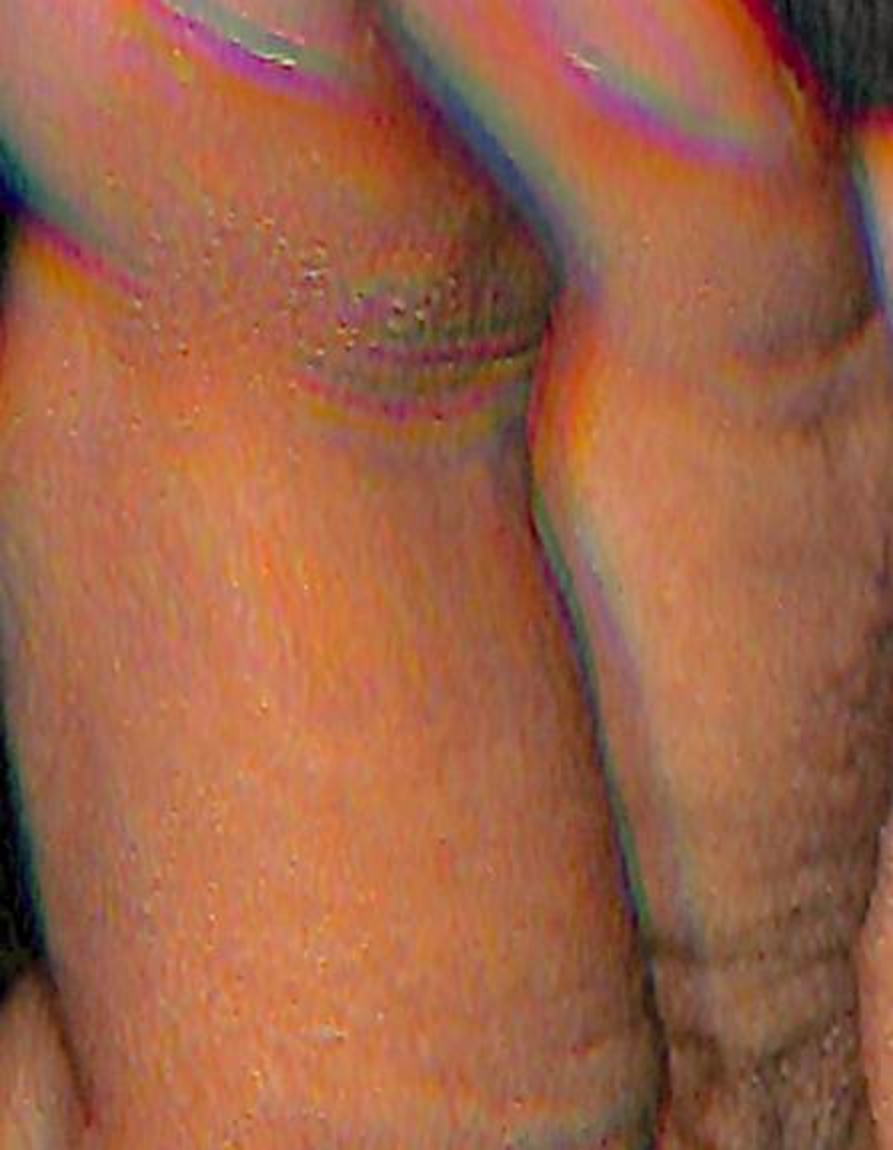


Figure 6.28: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric discs (40% w/v) of CMC and HPMC coated with 20% w/v solution of EC.



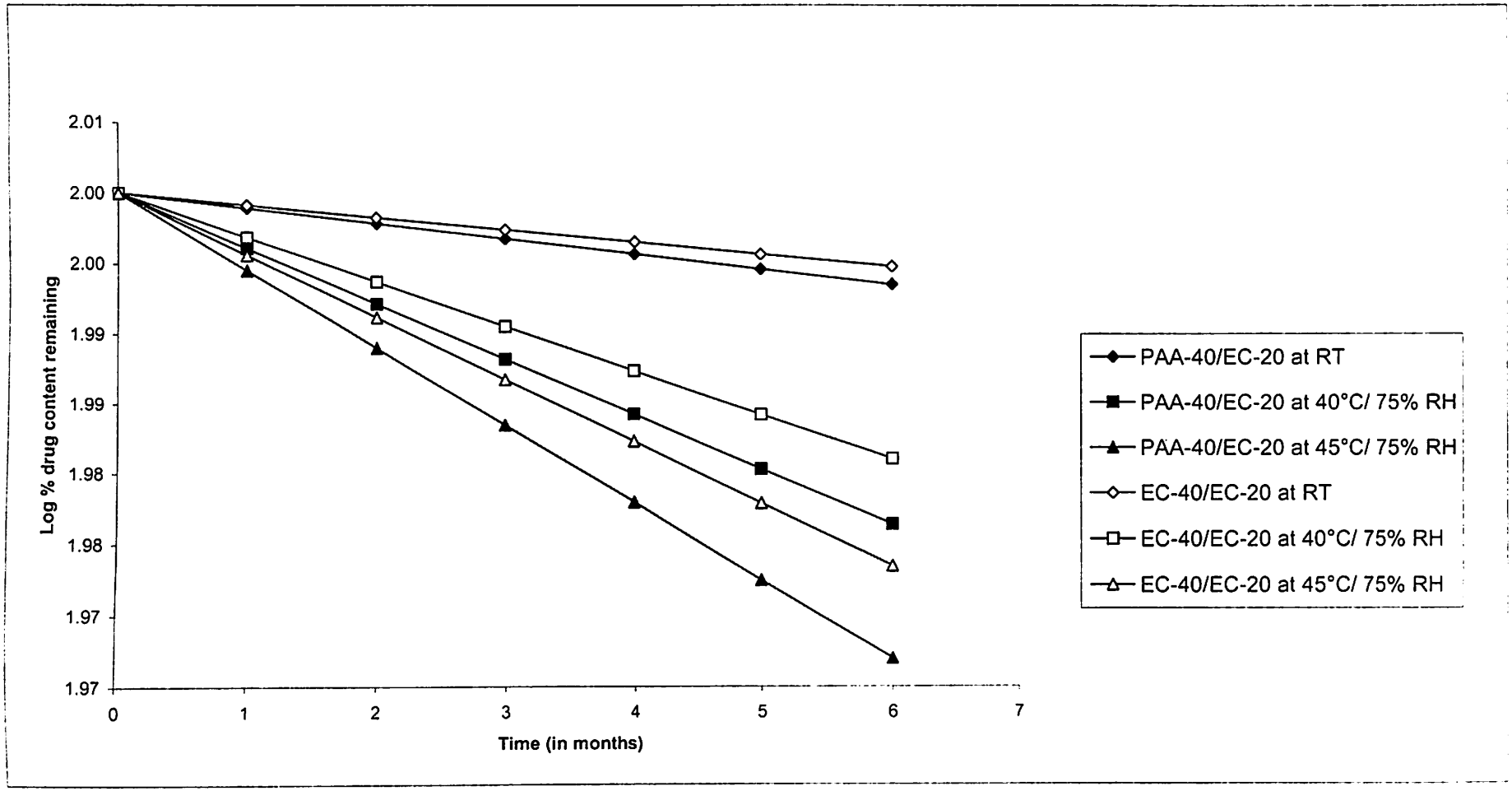


Figure 6.29: Comparative log percentage drug content remaining and time profile at different storage temperature of flurbiprofen from polymeric discs (40% w/v) of PAA and EC coated with 20% w/v solution of EC.

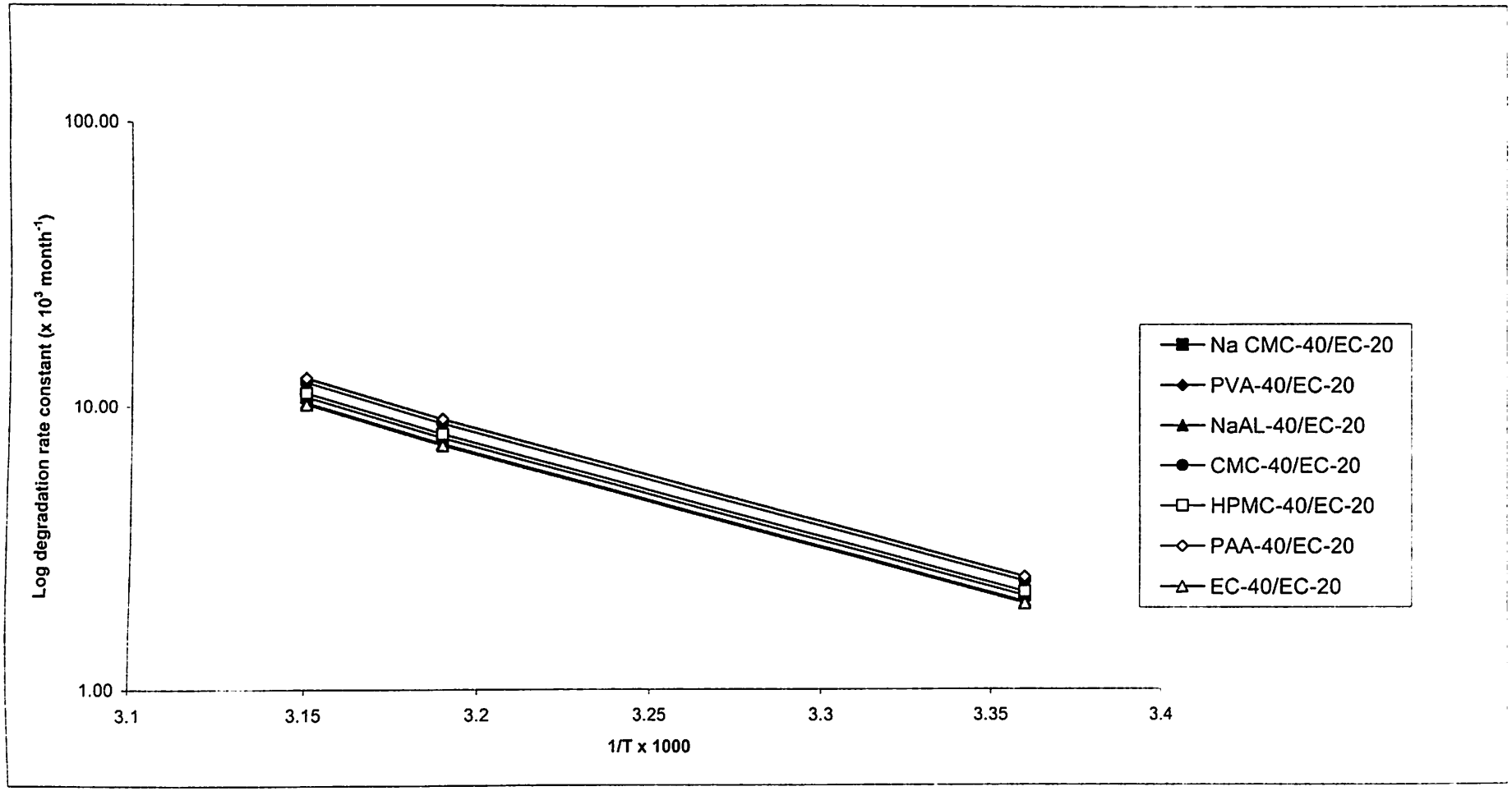


Figure 6.30: Arrhenius plot for degradation of flurbiprofen from polymer coated matrix embedded polymeric discs.

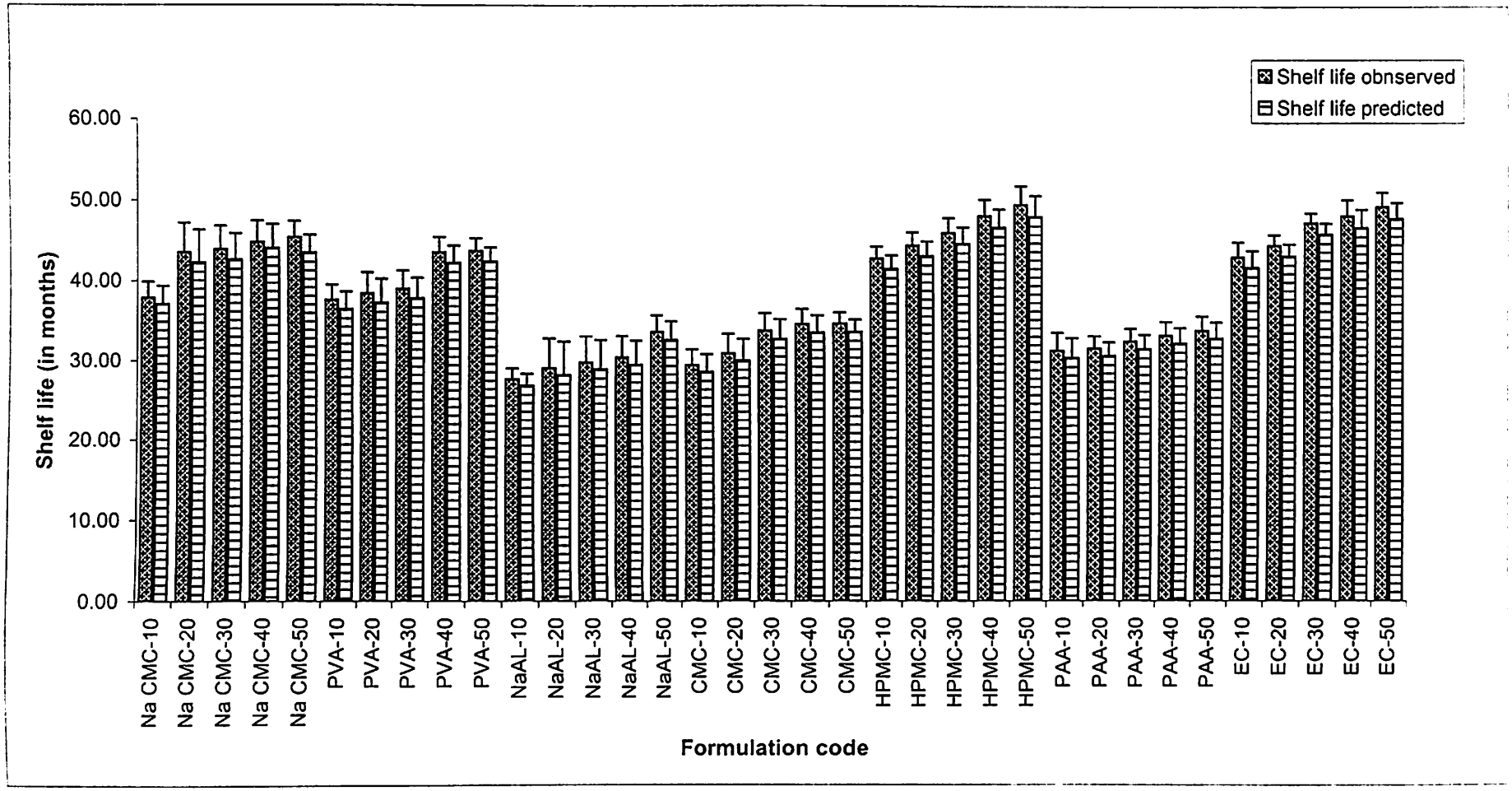


Figure 6.31: Comparative analysis of the predicted and observed shelf life for matrix embedded polymeric disc formulations of flurbiprofen.

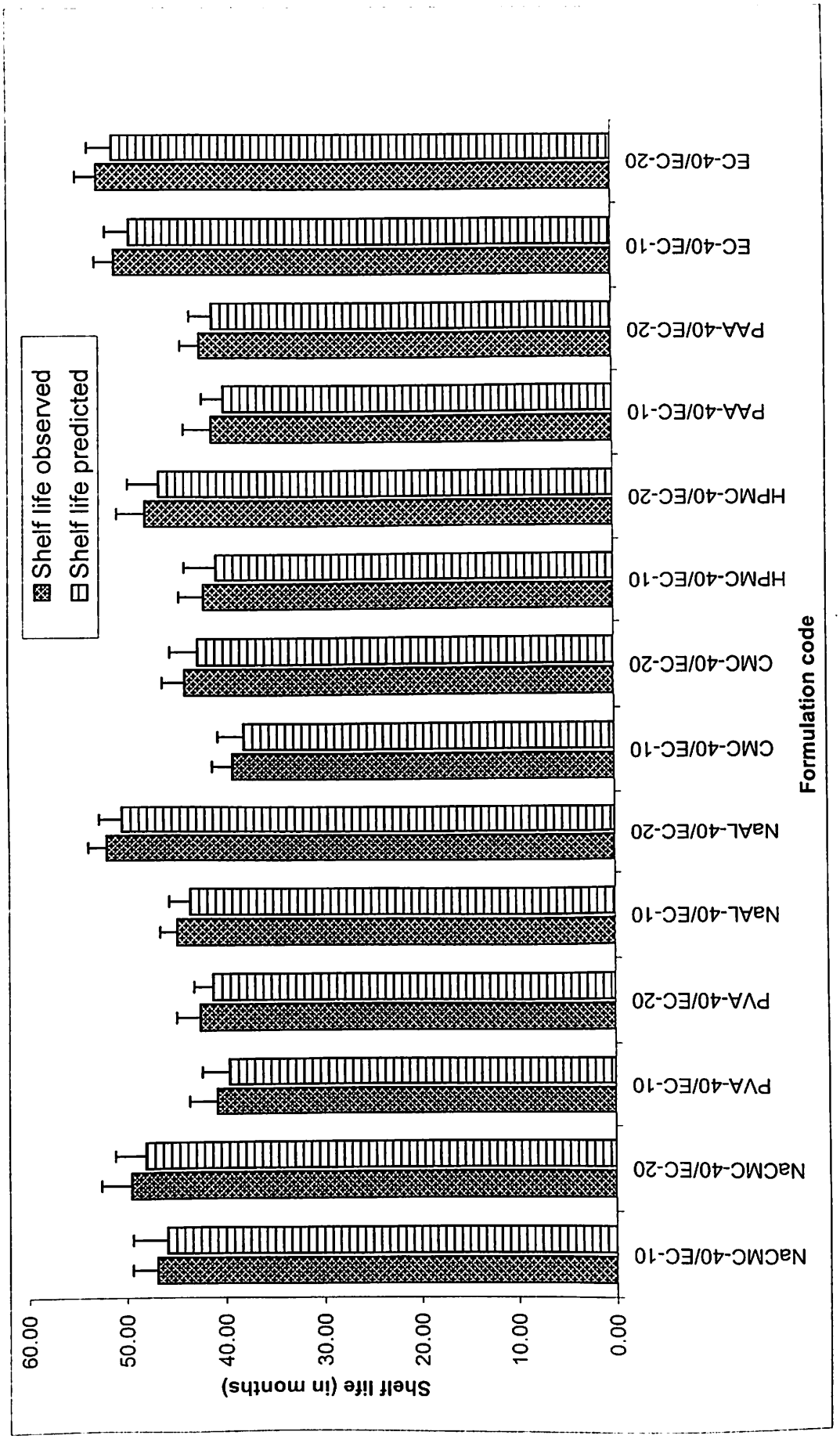


Figure 6.32: Comparative analysis of the predicted and observed shelf life for polymer coated matrix embedded polymeric disc formulations of flurbiprofen.

CHAPTER 7

IN-VITRO TRANSCORNEAL PERMEABILITY, OCULAR SAFETY AND OCULAR BIOAVAILABILITY STUDY

7. IN-VITRO TRANSCORNEAL PERMEABILITY, OCULAR SAFETY AND OCULAR BIOAVAILABILITY STUDY

7.1 Introduction

Most of the topically applied drugs have to enter the eye through the corneal route and the epithelial and stromal layer act as barrier to transcorneal permeability for variety of drugs, both hydrophilic and lipophilic. Paracellular and transcellular pathways are broadly the routes for corneal drug transport. Lipophilic drugs cross the cornea through transcellular pathway while the hydrophilic drugs cross through paracellular pathway. It has been observed from studies on a series of compounds that the fraction absorbed through corneal route varies from less than 1% for hydrophilic drugs to 7 % for lipophilic drugs (Patton and Robinson, 1976; Tang et al, 1984a; Chiang and Schoenwald, 1986). Corneal epithelium and stroma exert varying degree of resistance to penetration with depending on the nature of the drug (Schoenwald and Huang, 1983). A corneal permeability coefficient of the order of 0.1×10^{-5} to 4.0×10^{-5} cm/ sec can be considered a measure of efficient corneal drug permeation (Wang, et al, 1991). Corneal permeability increases when the corneal integrity is compromised by the usage of high concentration of certain formulation excipients like, preservatives and chelating agents.

Rabbit cornea or eye is most commonly used for *in-vitro* and *in-vivo* studies on ocular drug delivery. An investigation on the mammalian corneal epithelium in different species including man, rabbit and pig revealed morphological similarity of corneal cell layers (Ehlers, 1970). Nevertheless dissimilarities do exist between these species in terms of response of the eye to irritation and trauma (Bito, 1984). Rabbit eyes were found to be more sensitive to irritant than other species. Reer, et al (1994), have suggested that rabbit cornea is not always the model of choice and the great morphological uniformity of mammalian cornea allow a different model like goat cornea to be chosen for *in-vitro* permeation studies. Goat corneal membrane has been used for *in-vitro* transcorneal permeability studies of ketrolac tromethamine (Malhotra and Majumdar, 1997a, b). Taking the above reports in view, goat cornea was chosen for *in-vitro* permeation studies. A practical advantage of goat cornea is its easy availability in India at a cheap rate so that larger number of experiments could be performed in a short time period.

The *in-vitro* transcorneal diffusion models are completely devoid of complications in terms of variability in precorneal factors such as blinking, lacrimation, tear turn over and drug washout.

The *in-vivo* evaluation of ocular drug delivery systems entails administration of the dosage form into the precorneal cavity followed by measurement of drug release from the delivery systems and/or the pharmacological response to the drug. Drug release is commonly monitored by measuring the drug concentrations in the ocular tissues and fluids. Previous studies have established albino rabbit as a suitable model for understanding the mechanisms of ocular drug disposition and for predicting the effects of physiochemical properties of drugs on ocular drug disposition (Schoenwald and Boltralik, 1979; Saettone et al, 1982; Urtti and Salminen, 1993). Hence, it was considered necessary to perform *in-vivo* ocular bioavailability of the drug in aqueous humor from the designed formulations in rabbit model.

Another issue in the development of any dosage form for topical delivery to eye is safety. Eye irritancy test model in albino rabbit (Driaze and Kelley, 1952) has been used as the *in-vivo* model for predicting the eye-irritancy potential of the formulations (Klausner, et al, 2000). Inspite of the criticisms of the method, in terms of the pain and trauma induced on the animals, currently no alternative to the Draize eye test has been validated and accepted by any regulatory body (Blazka, et al, 1999). Many study have been undertaken by cosmetic manufacturing companies in an attempt to find a viable *in-vitro* model for testing the eye irritaion potential of hair/ eye care products. A study was conducted using five methods, the isolated rabbit eye (IRE), bovine corneal opacity and permeability (BCOP), EpiOcular^{TM}, fluorescein leakage (FL) and neutral red release (NRR) assays, for predicting the eye irritation potential of hair/eye care formulations (Jones, et al, 2001). The study demonstrated the value of using concurrent benchmarks (reference standards), appropriate to the materials being tested, in interpreting the data obtained from *in-vitro* tests and the predictability was not found to be at par with Draize's eye test. Overall results indicate that further comparisons of the IRE, EpiOcular^{TM} and FL assays are warranted using much larger numbers of test materials. In the present studies Albino rabbit based Draize eye test model was used because of its predictability and non-availability of commercial EpiOcular^{TM} kits. Also care was taken that only a few selected formulations, selected on the basis of *in-vitro* and *in-vivo* studies was used for eye irritancy studies. All the animal experiment protocols (both *in-vitro* and *in-vivo*) were approved by Institutional Animal Ethics Committee, B.I.T.S., Pilani.

The present study was aimed at investigating the *in-vitro* transcorneal permeability of flurbiprofen in excised goat cornea using modified Franz diffusion cell (Figure 7.1) used by

Fu and Lidgate, 1986. Effect of buffer type (phosphate, citrate-phosphate and citrate) and pH (5.4, 6.4, 7.4 and 8.4) of the phosphate buffer on the permeability of flurbiprofen were studied. Also studied was the effect of pH of the unbuffered solution on the permeability of the drug. Effect of formulation excipients like, preservatives and chelating agents on the trans-corneal permeability was also investigated with a view of incorporating them in the designed liquid formulations to increase the stability and/ or corneal permeability of the flurbiprofen in the developed formulations. The formulation additives selected for the purpose include- sodium chloride, thiomersal, benzalkonium chloride, chlorobutanol, chlorohexidine digluconate, phenyl mercuric nitrate, sodium metabisulphite, disodium edetate, methyl hydroxy benzoate and propyl hydroxy benzoate. Also investigated was the transcorneal permeability of flurbiprofen from various designed formulations namely, polymeric gels, oil based, polymeric gels in oil type formulations and polymer coated or non-coated matrix embedded formulations selected on the basis of *in-vitro* dissolution profiles.

Selected formulations were evaluated for ocular safety using Draize's test protocol for their suitability for ophthalmic use in human beings. In this study, the ocular bioavailability of aqueous drop of flurbiprofen was studied using rabbit eye model and compared with market preparation. The ocular bioavailability of the drug from selected designed formulations was determined based on aqueous humor drug concentration upon single topical administration of the drug in the precorneal space in albino rabbit eye model. Various ocular pharmacokinetic parameters and the systemic availability upon topical administration from selected formulations were also determined.

7.2 Experimental

Materials

Pure flurbiprofen was obtained as a gift sample from Optho Remedies Pvt. Ltd., Allahabad. All other the polymers, chemicals and reagents used were pharmaceutical or analytical grade and were used as received. Fresh whole eyeballs of goat (6-7 months old) were obtained from butcher's shop (M/s Dawood Meat Shop, Pilani) within an hour of slaughtering of the animal in cold (4°C) normal saline. Albino rabbits 7-8 months old and weighing 1.5- 2.5 Kgs were

obtained from Animal House, Hissar Agricultural University, Hissar, Haryana. The procured rabbits were quarantined for two weeks and examined for clinically normal eyes. The rabbits were individually housed and provided water and food *ad libitum*.

Equipment

A scanning spectrofluorimeter (Jasco, Tokyo, Japan, model FP-777) with built-in compatible software, link search mode, multiple PMT gain mode, automatic wavelength accuracy of 1.5 nm, range 220-750 nm and 10 mm quartz cells was used for fluorescence intensity measurement of samples obtained from *in-vitro* transcorneal permeability experiments. *In-vitro* transcorneal permeation experiments were carried out in modified Franz diffusion cell (Figure 7.1) (Fu and Lidgate, 1986) fabricated in-house. The receiver chamber had an internal volume of 10 ml and was fitted with a side arm for sampling of receiver fluid. The donor chamber with an internal area of 0.47 cm^2 (for permeation across the cornea) was clamped on top of the receiver chamber with corneal membrane sandwiched between the two. The receiver chamber was provided with water jacket for maintaining dissolution media at 37 ± 1°C and teflon coated magnetic bead kept at the bottom of the receiver chamber to ensure homogenous mixing of the dissolution media. Donor solution (1 ml) or the polymeric discs (of appropriate size and drug content) were placed in the donor chamber on top of its epithelial surface and covered with a glass slip with silicone grease to prevent evaporation. A Jasco model liquid chromatograph equipped with two-pump gradient system (PU-1580), Rheodyne injector (7725i) fitted with a 20 µL loop, UV detector (UV-1575) and BORWIN-I software was used for determining aqueous humor and serum drug concentration.

Analytical method

The spectroflourimetric method (Sajeev et al, 2001) described in Chapter 3 using 1:1 mixture of methanol and 0.1N H_2SO_4 at the λ_{ex} and λ_{em} of 250 nm and 314 nm respectively was employed for analysis of permeability study samples. For the analysis of drug samples at very low concentration the liquid chromatographic method (sajeev et al, 2002a) described in Chapter 3 for the analysis of drug in pure form involving RP-C18 column in 40:20:40 mixture of methanol-acetonitrile-phosphate buffer (pH 5.6) at a flow rate of 0.75 ml/min with UV detection at 248 nm was employed. Aqueous humor and serum drug samples were analyzed by the liquid chromatographic method (Sajeev et al, 2002b) reported in chapter 3 involving RP-

C18 column and using 40:20:40 mixture of methanol-acetonitrile-phosphate buffer (pH 5.6) at a flow rate of 1 ml/min with UV detection at 248 nm.

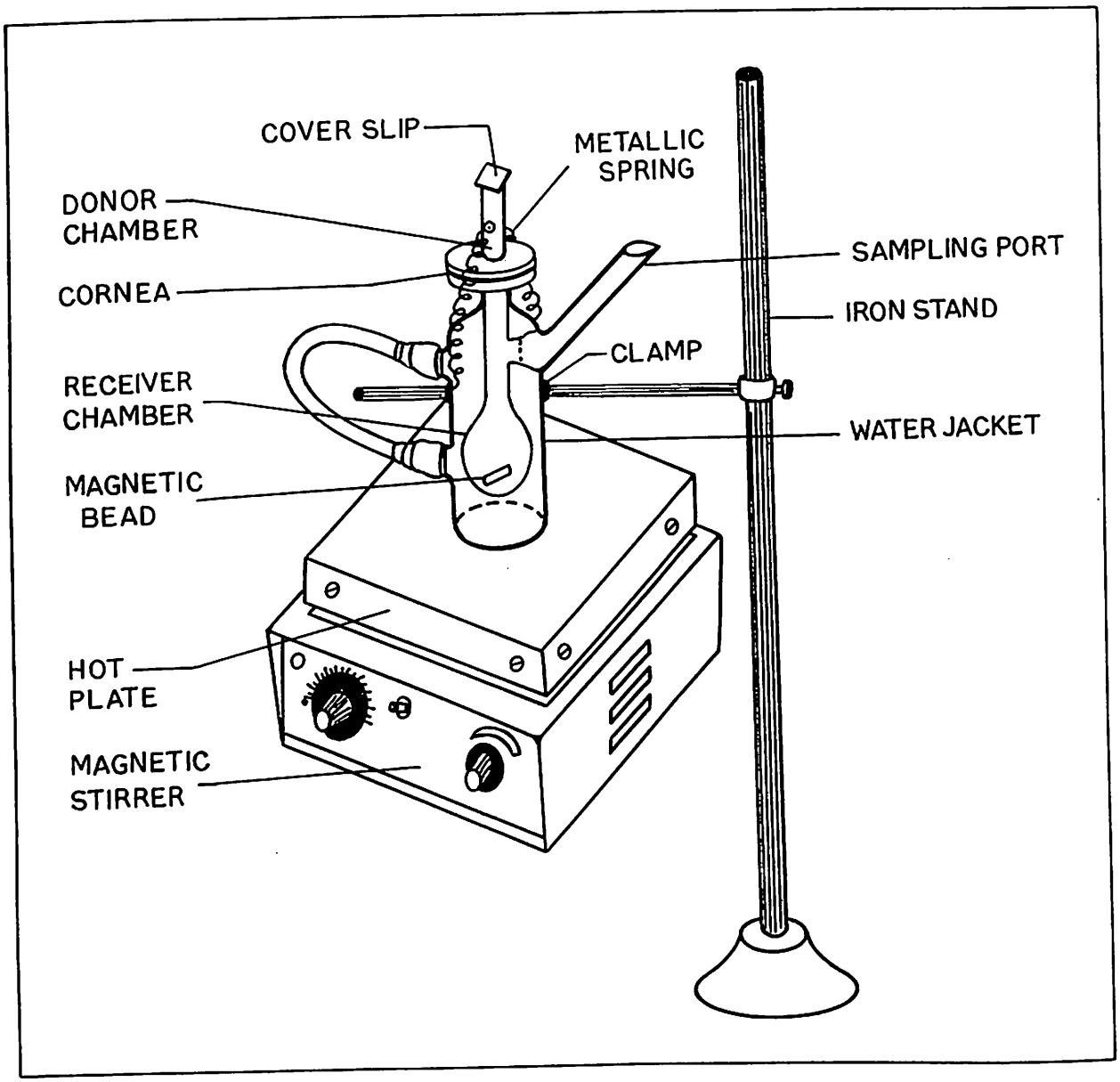


Figure 7.1: Diagrammatic representation of modified Franz diffusion apparatus and the permeation study assembly set-up.

***In-vitro* permeability study**

(a) Corneal preparation: Freshly excised whole eyeballs of goat were transported from butcher's shop to laboratory in cold (4 °C) saline within one hour of slaughtering. The corneas were carefully dissected along with 2-4 mm of surrounding scleral tissue from the eyeball and washed with cold saline to remove any adhering pigments. The washed cornea were preserved in freshly prepared balance base buffer (pH 7.4) with a composition (% w/v) of NaCl - 0.57, $NaHCO_3$ - 0.361, KCl - 0.04, K_2HPO_4 - 0.023, $MgSO_4$ - 0.007 and $CaCl_2$ - 0.08 in glass distilled water (Fu and Lidgate, 1986).

(b) Permeation experiment: Fresh cornea obtained by the previous procedure was mounted on the transcorneal permeability study apparatus by sandwiching the scleral tissues between the clamped donor and the receiver chamber with the epithelial surface facing the donor side. Balance base buffer was filled in receiver chamber after expelling all the air bubbles by inverting the diffusion cell and then allowing the bubbles to travel through the sampling port. The receiver fluid was maintained at 37 ± 1°C with the help of circulating warm water and kept under stirring using a teflon coated magnetic bead. An aliquot of donor solution was placed on the cornea in the donor chamber and the permeation continued for 120-240 mins. A 250 µl sample was withdrawn at predetermined time intervals through the sampling port suitably diluted and analyzed by spectroflourimetric or liquid chromatographic method discussed earlier.

(c) Preparation of test samples: To study the effect of various buffers on the transcorneal permeability of flurbiprofen (750 µg/ml), drug solutions in phosphate buffer pH 7.4, citrate-phosphate buffer pH 7.0 and citrate buffer pH 6.6 (0.01M) were prepared as per the composition given in Chapter 4. To study the effect of pH of phosphate buffer on the transcorneal permeability of flurbiprofen 750 µg/ml solution of the drug was prepared in pH 5.4, 6.4, 7.4 and 8.4. The same protocol was repeated with drug solution in unbuffered (0.01 M) pH (4.5, 5.5, 6.5, 7.5 and 8.5). In all the pH dependent experiments ionic strength was maintained at 0.2 with NaCl. An aqueous drop of the drug in glass triple distilled water with benzyl alcohol (q.s.) served as in-house control in all these studies and a marketed formulation (OCUFLUR, FDC, Aurangabad, India) of flurbiprofen was used as external control.

Effect of formulation excipients like, preservatives and chelating agents on the trans-corneal permeability was also investigated. The formulation additives selected for the purpose include- sodium chloride (NaCl, 0.9 % w/v), thiomersal (THM, 0.004 % w/v), benzalkonium chloride (BAC, 0.01 % w/v), chlorobutanol (CB, 0.5 % w/v), phenyl mercuric nitrate (PMN, 0.002 % w/v), disodium edetate (EDTA, 0.01 % w/v), methyl hydroxy benzoate (MHB, 0.04 % w/v) and propyl hydroxy benzoate (PHB, 0.02 % w/v). Based on earlier studies various polymeric gel and oil-based formulations selected for permeability studies include- CMC-5, MC-5, NaCMC-5, PCB-5, LSO, PCB-LSO, MC-LSO, HPMC-LSO, OO, PCB-OO, MC-OO, HPMC-OO, SO, PCB-SO, MC-SO and HPMC-SO. Representative polymeric coated and non-coated matrix embedded formulations of flurbiprofen taken up for this study were NaCMC-40, NaAL-40, HPMC-40, EC-40, NaAL-40/EC-20, HPMC-40/EC-20, PAA-40/EC-20 and EC-40/EC-10. The above formulations were selected randomly from the lot of formulations with best *in-vitro* dissolution profiles. Formulation EC-40/EC-20 was not selected in this study because of the very slow *in-vitro* dissolution profile. From the slope of the plot between cumulative amount permeated vs. time and the effective corneal area available for permeation, flux was calculated as $\mu g/min/cm^2$.

(d) Determination of corneal hydration: At the end of the experiment, each cornea was freed from adjoining sclera, weighed and soaked overnight in 2 ml of methanol. The soaked corneal membrane on the subsequent day was dried to constant weight at 90 °C and reweighed. From the difference of the two weight corneal hydration percentage was calculated.

Ocular safety study

Three groups of three albino rabbits per formulation were used for the study. A 0.1 ml volume of the formulation was instilled or the polymeric disc inserted into the lower cul-de-sac of one eye and the other eye taken as control (0.1 ml of normal saline). After instillation, the eyes of Group1 animals were left unwashed and of Group 2 and Group 3 animals washed with 20 ml lukewarm water after 2 and 4 sec post instillation/ insertion respectively. The ocular reactions in cornea, iris and conjunctiva were read with a hand slit lamp for seven days or till any residual injury persists. The scoring of the reactions in the ocular tissues due to the formulations was done as per Table 7.1 and safety evaluation done as per Table 7.2.

Ocular biovailability study

Rabbits, fasted overnight, were placed in wooden restraining boxes. To the lower cul-de-sac of each eye 100 µl drop each of aqueous drop or designed polymeric gel or oil-based formulations were instilled. Alternatively the designed polymer coated or non-coated matrix-embedded discs were placed in the lower cul-de-sac. The upper and the lower eyelids were gently held closed for approximately 10 secs immediately after administeration of the dose to enhance drug-corneal contact. At 0.5, 1, 2, 4, 8, 12, 16 hr post dose, eye were anaesthetized using 4% xylocaine solution topically and samples of aqueous humor was collected with a half-inch 30-gauge disposable needle on a 1 ml tuberculin syringe with a transparent hub from the anterior chamber of the rabbit eye by piercing through the corneo-scleral limbus. The procedure was continued at 24, 48, 72-hour time points in case of matrix embedded discs. The collected aqueous humor was mixed with equal volume of methanol and refrigerated at 4 °C for 30 mins and then centrifuged at 3000 rpm for 15 min. The supernatant was collected and stored at –20°C and thawed before usage. Along with the collection of aqueous humor, 100 µl of blood was also collected from the marginal ear vein of the rabbit by vein-puncture and the blood sample collected was left as such for 30 mins at 4°C to allow protein coagulation. The coagulated blood was centrifuged at 4000 rpm for 15 mins and the supernatant serum collected, stored at –20°C and thawed before usage. Eppendorff tubes used in these experiments were freshly sterilized by moist heat sterilization at 121°C for 15 mins. Various formulations selected for this study include aqueous drop (in-house control), market preparation (external control), MC-5, LSO, MC-SO, NaCMC-40, EC-40, PAA-40/EC-20 and EC-40/EC-10.

7.3 Results and discussion

***In-vitro* permeability study**

Results of *in-vitro* trans corneal permeability studies of flurbiprofen from different media and designed formulations are presented in Table 7.3 to 7.12 and Figure 7.2 to 7.11. In all these studies mean amount permeated in 120 mins have been used for comparison. Corneal permeability of flurbiprofen from aqueous drop was very low with only 33.2 ± 2.7 µg of the drug permeating in 120 mins with a calculated flux of only 0.5842 µg/min/cm^2, whereas in case

of market formulation (OCUFLUR) the permeability was considerably enhanced with 98.1 ± 5.4 μg permeating in 120 mins and a flux of 1.7028 μg/min/cm^2 (Table 7.3 and Figure 7.2). The aqueous drop was formulated using triple distilled water and the pH of the water was found to be approximately 6.5. Since flurbiprofen is a weakly acidic drug with a pK_a of 4.27 (Craig, 1990) and will be ionized to a greater extent at pH above 4.27 with the degree of ionization increasing with increase in pH. This increase in ionization of the drug at weakly acidic or neutral pH contributes to increase its solubility and thus decrease in unionized content resulting in decreased permeability. The increase in permeability in case of market formulation can be attributed to the presence of formulation additives like PMN present in the formulation in appreciable concentration that is known to alter corneal membrane permeability by interacting with membrane sulphydryl groups (Burstein and Klyce, et al, 1977; Van Horn, et al, 1977). Replacing the aqueous vehicle with buffer namely, phosphate buffer (pH 7.4), citrate buffer (pH 6.6) and citrate-phosphate buffer (pH 7.0) and resulted in statistically significant (Table 7.3 and Figure 7.2) increase in the corneal permeability of the drug. The cumulative amount permeated in 120 mins was obtained as 122.4 ± 6.6 μg, 105.8 ± 4.2 μg and 100.1 ± 7.6 μg respectively for phosphate buffer (pH 7.4), citrate buffer (pH 6.6) and citrate-phosphate buffer (pH 7.0). The results when correlated with the solubility data (Chapter 4) indicate that with an increase in solubility of the drug in various buffers the partitioning into the corneal membrane decreases thereby decreasing the permeability. Since the solubility of flurbiprofen was found to vary as phosphate buffer (pH 7.4) < citrate buffer (pH 6.6) < citrate-phosphate buffer (pH 7.0), the calculated value of flux for flurbiprofen transcorneal permeability from these vehicles varied as phosphate buffer (pH 7.4) > citrate buffer (pH 6.6) > citrate-phosphate buffer (pH 7.0) (Table 7.3). Malhotra and Majumdar (1997a) have reported similar results for ketorolac tromethamine. Also the variation in permeability profile of flurbiprofen in different buffer types within a narrow range of pH may be probably be due to variation in the ionization profile of the drug in different buffer types.

The vehicle pH under both buffered and unbuffered condition was also found to affect the transcorneal permeability of the drug. In case of phosphate buffer, of varying the pH of the media resulted in statistically significant increase in the corneal permeability for flurbiprofen (Table 7.4) when compared with aqueous drop with the permeability decreasing with increase in pH of the vehicle (Table 7.3). From a cumulative amount permeated (in 120 mins) of only

33.2 ± 2.7 μg in case of aqueous drop, the amount permeated increased to 201.5 ± 1.4 μg, 136.9 ± 3.2 μg, 122.4 ± 6.6 μg and 108.5 ± 3.4 μg in case of phosphate buffer of pH 5.4, pH 6.4, pH 7.4 and pH 8.4 respectively. Again the reason can be that at pH above 4.27 (pK_a of flurbiprofen) the degree of ionization of the drug will increase with increase in pH. This increase in ionization of the drug from weakly acidic to basic pH contributes to decrease in percent unionized fraction of the drug thereby decreasing the permeability of the drug in higher pH. A similar phenomenon was observed with unbuffered solutions of varying pH. Permeability of the drug also increased (statistically significant, Table 7.5 and Figure 7.4) with decrease in the pH of the unbuffered vehicle. The cumulative amount permeated (in 120 mins) increased from 97.7 ± 4.2 μg (pH 8.5) to 179.9 ± 0.9 μg (pH 4.5) (Table 7.5). Also the corneal permeability flux was found to decrease with increase in the pH of the medium. The difference in the permeability profile of the drug from buffered and unbuffered media could be attributed to the difference in ionization capacity of hydrochloride and sodium hydroxide ions (used for adjusting the pH) when compared to mono basic sodium mono phosphate and dibasic sodium mono phosphate ions (used for preparation of phosphate buffer of varying pH). Another reason for pH dependent permeability of the drug (reduced permeation at higher pH) could be because of the fact that corneal membrane has an isoelectric pH of 3.2 and at pH above this cornea attain negative charge. Flurbiprofen being acidic drug would be anionic at higher pH thereby resulting in decreased permeation at higher pH.

Studies on the effect of formulation additives on the transcorneal permeability revealed that compounds like BAC, THM, CB, PMN, EDTA, MHB and PHB which are commonly used as formulation additives in ophthalmic formulations increased the rate of drug corneal permeation by statistically significant amount (Table 7.6 and Figure 7.5). A statistically significant ($p < 0.05$) increase was also observed in case of formulation prepared using 0.9 % w/v NaCl when compared to aqueous drop with cumulative permeation of 81.6 ± 4.7 μg and flux of 1.4115 μg/min/ cm^2 (Table 7.6). Organomercurials like PMN and THM as mentioned earlier interact with the membrane sulphydryl groups (Burstein and Klyce, et al, 1977; Van Horn, et al, 1977) thereby altering the membrane permeability and transport systems. In the present study THM (0.004 % w/v) and PMN (0.002 % w/v) increased the cumulative amount permeated to 189.6 ± 3.5 μg and 105.6 ± 2.1 μg respectively with respective corneal permeability flux of 3.3157 μg/min/cm^2 and 1.7832 μg/min/cm^2. Electrophysiological studies using compounds BAC has

been reported to cause irreversible damage to the corneal cell layer followed with neovascularisation (Gasset, et al, 1974). In our studies also BAC at 0.01 % w/v was found to enhance the corneal permeability of the drug to 211.2 ± 3.1 μg in 120 mins with a flux of 3.5664 μg/min/cm^2 (Table 7.6). Compound CB at 0.5 % w/v concentration in the formulations was found to enhance the corneal permeability of flurbiprofen to the maximum extent to 235.3 ± 2.2 μg in 120 mins with a flux of 3.9247 μg/min/cm^2 (Table 7.6). CB is known to reduce oxygen utilization in the cornea and thereby resulting in loosened epithelial cell adhesion and thus, increased permeation of drugs through cornea (Grant, 1974). It has also been shown to contribute in enhanced corneal drug permeability by producing granularity and small vacuole in epithelial cells (Krejci and Harrison, 1970). EDTA a known calcium-chelating agent is reported to act on epithelial cell junctions by interfering with calcium ions and altering membrane intercellular integrity (Asthon, et al, 1990) and causing increased intercellular permeability (Rojanasakul, et al, 1990; Grass, et al, 1985). EDTA (0.01 % w/v), in case of flurbiprofen, also increased the corneal permeability of the drug to substantial amount (225.4 ± 2.1 μg in 120 mins, with a flux of 3.8053 μg/min/cm^2) (Table 7.6). MHB (0.04 % w/v) and PHB (0.02 % w/v) were also found to significantly increase ($p < 0.05$) the transcorneal permeability of flurbiprofen (Table 7.6).

Transcorneal permeability studies involving selected developed formulations prepared using various mucoadhesive polymers revealed that corneal permeability of flurbiprofen was increased with the use of these polymers to different extent (Table 7.7). Maximum increase in permeability was seen in case of PCB-5 (5 % w/v PCB in the formulation) with a flux of 2.7253 μg/min/cm^2 and a cumulative permeation of 151.4 ± 3.3 μg in 120 mins followed by MC-5 with a flux of 2.3226 μg/min/cm^2 and a cumulative permeation of 145.4 ± 4.7 μg in 120 mins and CMC-5 with a flux of 2.1445 μg/min/cm^2 and a cumulative permeation of 110.5 μg in 120 mins. In case of NaCMC-5 the cumulative amount permeated was 101.3 ± 5.3 μg and a flux of 1.9842 μg/min/cm^2 was observed. The increase in corneal permeation in case of mucoadhesive polymeric gels can be attributed to the increased intimate adherence of the formulation to the corneal epithelium thereby facilitating partitioning and permeation of the drug.

Studies with selected vegetable oil based formulations showed that the permeability results (Table 7.8 to 7.10) correlated well with the solubility data of flurbiprofen presented in Chapter

4. The solubility data showed that flurbiprofen has maximum solubility in sesame oil (SO) followed by olive oil (OO) and linseed oil (LSO). It is expected that oil/water partition coefficient for flurbiprofen will also favour the same order, thereby proportionately decreasing availability of drug in aqueous for permeation. The results of study also reinforced this idea, wherein maximum permeability was seen in case of LSO (123.5 ± 3.1 μg in 120 mins with a flux of 2.0714 g/min/cm^2) (Table 7.8) followed with OO (102.8 ± 2.7 μg in 120 mins with a flux of 1.8635 μg/min/cm^2) (Table 7.9) and SO (97.3 ± 2.3 μg in 120 mins with a flux of 1.7841 μg/min/cm^2) (Table 7.10). Addition of polymer (PCB, MC or HPMC) to these oil based formulations, significantly ($p < 0.001$) decreased the permeability rate of the drug (Table 7.8 to 7.10) probably because of increased viscosity and emulsification resulting in increased barrier to partitioning of the drug to the aqueous layer on the corneal surface. Another reason could be decreased concentration of the drug available in case of viscous/ emulsified preparation on the corneal surface to provide satisfactory flux for corneal drug permeability. The permeability rate of flurbiprofen from selected polymeric discs (Table 7.11 and Figure 7.10) and from polymer coated discs (coated with 20 % w/v EC solution) (Table 7.12 and Figure 7.11) showed statistically insignificant difference in the permeation characteristics compared to aqueous drop, except in case of EC matrix and matrix coated discs. In case of EC matrix based formulations coated with 10 % w/v EC solution (EC-40/EC-10), permeability rate was found to be significantly ($p < 0.05$) less in comparison to aqueous drop. The probable reason being the presence of very low amount of dissolution fluid available for leaching out of the drug through the polymeric matrix and its diffusion across the EC coat. Because of this reason EC-40/EC-20 (coated with 20 % w/v EC solution) discs inspite of very long prolonged *in-vitro* dissolution profile (> 26 days), was not selected for further studies.

A normal intact goat cornea has been reported to have a hydration level of 76 to 80 % (Maurice and Riley, 1970). Corneal damage usually increases the hydration level to 83 to 92 % (Schoenwald and Huang, 1983). Our experiments on freshly excised untreated goat cornea showed a hydration level of 78.9 ± 1.6 %. In all the test samples or the formulations except the one containing BAC, THM, CB and EDTA, the post treatment corneal hydration was found to vary between 76 to 80 % (Table 7.3 and 7.12). But in case of BAC, THM, CB and EDTA, the hydration value varied between 83 to 86 % (Table 7.6) thus confirming the earlier reports (Krejci and Harrison, 1970; Grant, 1974; Burstein and Klyce, et al, 1977; Van Horn, et al,

1977; Grass, et al, 1985; Asthon, et al, 1990; Rojanasakul, et al, 1990) of adverse effect of these agents on the corneal cell structure and its integrity.

Ocular safety study

Ocular safety studies by Draize's test protocol revealed that formulations- aqueous drop, market preparation, polymeric gels (CMC-5, MC-5 and PCB-5), SO based formulations and HPMC-40 disc were non- irritating to the rabbit eye (Table 7.13). Whereas, formulations like OO, LSO and polymeric gels in oil formulations prepared from them, polymeric discs made of NaAL, NaCMC, EC and all polymer coated formulations (NaAL-40/EC-20, HPMC-40/EC-20, PAA-40/EC-20 and EC-40/EC-10) were practically non-irritating to the rabbit eye (Table 7.13). In most cases the non-zero score of these formulations was because of mild redness of the conjunctiva and no apparent effect on the cornea.

Ocular biovailability study

In-vivo ocular bioavailability studies revealed that *in-vitro* extension of the release of the drug from the designed formulations manifested in the form of enhanced area under the aqueous humor drug concentration time profile (AUC) and mean residence time (MRT, obtained from the ratio of AUMC and AUC)) of the drug in the aqueous humor calculated using Win-nonlin software, version 1.5 (Scientific Consulting Inc., USA). Where, AUMC is the area under the moment curve. Table 7.14 presents the cumulative aqueous humor drug concentration of flurbiprofen from various liquid formulations upon in single topical application and the corresponding log aqueous humor drug concentration time profile is shown in Figure 7.12. The aqueous humor drug bioavailability from aqueous drop and market formulation corresponded to earlier reported results (Tang, et al, 1984b). The calculated pharmacokinetic parameters are presented in Table 7.17 for all the topical liquid formulations. In case of aqueous drop the t_{max} and C_{max} was obtained as 2 hours and 1.15 μg/ml respectively with an $AUC_{0\text{-}12\ hr}$ and $AUC_{0\text{-}\infty}$ obtained as 6.61 ± 0.02 μg.hour/ml and 6.82 ± 0.03 μg.hour/ml respectively. In this case the MRT was found to be 4.25 ± 0.14 hours. Whereas, for market formulation, the t_{max} and C_{max} was obtained as 2 hours and 1.42 ± 0.06 μg/ml respectively with an $AUC_{0\text{-}12\ hr}$ and $AUC_{0\text{-}\infty}$ obtained as 8.21 ± 0.24 μg.hour/ml and 8.50 ± 0.27 μg.hour/ml respectively. In this case the MRT was found to be 4.33 ± 0.06 hours. Though there was no statistically significant difference in t_{max}, C_{max} and MRT between aqueous drop and market formulation the

statistically significant difference in the AUC can be attributed to the presence of preservative PMN and viscosity enhancer HPMC present in market formulation. PMN would cause increased rate of permeation and HPMC improved corneal contact, thereby resulting in increased biovailability. In comparison to aqueous drop and market preparation, formulations MC-5 and LSO showed higher t_{max}, lower C_{max} and larger AUC, AUMC and MRT (Table 7.17). The $AUC_{0\text{-}16\ hr}$ for MC-5 and LSO was obtained as 8.73 ± 1.10 μg.hour/ml and 8.50 ± 0.65 μg.hour/ml respectively and $AUC_{0\text{-}\infty}$ was obtained as 8.91 ± 1.12 μg.hour/ml and 9.16 ± 0.72 μg.hour/ml respectively. The t_{max} and C_{max} was obtained as 4 hours and 0.97 ± 0.12 μg/ml in case of MC-5 and as 6 hours and 0.94 ± 0.05 μg/ml in case of LSO. The MRT was obtained as 5.87 ± 0.02 hours and 8.88 ± 0.06 hours in case of MC-5 and LSO respectively. In case of MC-SO, the plot between log aqueous humor drug concentration and time did not give any terminal linear portion. As a result, elimination rate constant, biological half-life, extrapolated AUC, AUMC and MRT could not be calculated, as concentration remained constant till 16 hours. AUC in this case obtained between 0 to 16 hours was found to be 6.21 ± 0.19 μg.hour/ml, which is comparable to that of aqueous drop but the t_{max} was found to be 7 hours with a low C_{max} value of 0.38 ± 0.01 μg/ml. Since the profile between 2 hours to 8 hours was found to be near zero order and from 8 hours to 16 hours was found to be zero order, it can be predicted that drug availability from such formulations will continue for a long period of time (Figure 7.12).

In case of *in-vivo* study involving polymeric discs (EC-40, EC-40/EC-10 and PAA-40/EC-20), the log aqueous humor drug concentration profile was extended beyond 72 hours with sustained continuous availability except in case of NaCMC-40 where it extended marginally beyond 24 hours (Table 7.15 and Figure 7.14). In case of NACMC-40 discs the t_{max} and C_{max} was obtained as 8 hours and 0.65 ± 0.08 μg/ml respectively with an $AUC_{0\text{-}24\ hr}$ and $AUC_{0\text{-}\infty}$ obtained as 12.55 ± 0.24 μg.hour/ml and 12.75 ± 0.27 μg.hour/ml respectively. In case of EC-40, the t_{max} and C_{max} was obtained as 24 hours and 0.55 ± 0.01 μg/ml respectively with an $AUC_{0\text{-}72\ hr}$ and $AUC_{0\text{-}\infty}$ obtained as 27.62 ± 0.06 μg.hour/ml and 38.81 ± 0.95 μg.hour/ml respectively (Table 7.18). In case of PAA-40/EC-20, the $AUC_{0\text{-}72\ hr}$ and $AUC_{0\text{-}\infty}$ were obtained as 30.97 ± 2.69 μg.hour/ml and 44.01 ± 14.65 μg.hour/ml respectively. The t_{max} and C_{max} in this case was obtained as 24 hours and 0.73 ± 0.08 μg/ml respectively. The MRT in case of

NaCMC-40, EC-40 and PAA-40/EC-20 was found to be 4.87 ± 0.50 hours, 60.67 ± 2.62 hours and 61.63 ±7.81 hours respectively. The pharmaokinetic parameters obtained for all the designed formulations were found to be related to *in-vitro* release profile. For EC-40/EC-10, the terminal slope could not be calculated, as concentration remained constant till 72 hours. Therefore elimination rate constant, biological half-life, extrapolated AUC, AUMC and MRT could not be determined. The $AUC_{0\text{-}72\ hr}$ in case of EC-40/EC-10 was obtained as 16.76 ± 6.42 μg.hour/ml with t_{max} and C_{max} of 60 hours and 0.28 ± 0.10 μg/ml respectively (Table 7.18).

The corresponding serum drug concentration vs. time profile from liquid formulations discussed above is shown in Figure 7.13. The serum drug concentration time profile obtained in case of market preparation and aqueous drop upon single dose topical application was comparable to the reported work of Tang and co workers (1984b). The detectable drug level was found at 0.5 hours in case of aqueous drop and market formulations whereas in case of MC-5, LSO and MC-SO at 1 hours (Table 7.16). And the detectable levels were observed up to 6 hours in case of aqueous drop, market preparation and MC-5 and up to 12 hours and 16 hours in case of LSO and MC-SO respectively. The duration of presence of detectable levels of the drug in serum were found to be directly related to the time for 90 % of the drug release *in-vitro*. In case of *in-vivo* studies involving polymer discs (both coated and uncoated), drug levels in serum were not detected. There can be two reasons for this, firstly because of the slow release of drug from these matrices, very negligible amount of drug drainage would have occurred resulting in absorption from naso-lachrymal passage. Alternatively, low levels of drug in serum from these formulations could be present, but were not detected because the developed method for estimation of flurbiprofen in serum involves protein precipitation using acetonitrile thereby diluting the sample. Instead, a method involving extraction of the drug from the bio-matrices could be more useful in detecting such levels.

In-vitro and *in-vivo* correlation

A good inverse correlation was obtained between time for 90 % of the drug released *in-vitro* ($t_{90\%}$) and $AUC_{0\text{-}24hr}$ *in-vivo* for developed liquid topical preparations (MC-5, LSO and MC-SO) with a correlation coefficient value (r) of 0.9853 but in case of polymeric discs type formulations only a moderate correlation was obtained with a 'r' value of 0.7653. The results

indicate that the dissolution process from the formulation is the rate limiting step during drug absorption (Cheng, et al, 1995). Similar results were obtained when time for 50 % of the drug released *in-vitro* ($t_{50\%}$) and time for 70 % of the drug released *in-vitro* ($t_{70\%}$) was correlated with $AUC_{0\text{-}24}$. A 'r' value of 0.7555 was obtained between $t_{90\%}$ *in-vitro* and MRT *in-vivo*. In this case, for discs alone the value of 'r' was 0.6903 and for liquid formulations alone it was 0.8171. A very good correlation with a 'r' value of 0.9537 and 0.9552 were obtained between t_{max} and $t_{50\%}$ and between t_{max} and $t_{70\%}$ respectively.

7.4 Conclusions

Corneal permeability of flurbiprofen from aqueous drop was very low with only 33.2 ± 2.7 μg of the drug permeating in 120 mins with a calculated flux of only 0.5842 μg/min/cm^2, whereas in case of market formulation (OCUFLUR) the permeability was considerably enhanced due to the presence of formulation additives like, PMN.

The results of permeability studies in different buffers indicate that with increase in solubility of the drug in various buffers the partitioning into the corneal membrane decreases thereby decreasing the permeability. In case of buffered and unbuffered vehicles of varying pH the permeability decreased with increase in pH of the vehicle. Studies on the effect of formulation additives on the trans corneal revealed that compounds like BAC, THM, CB, PMN, EDTA, MHB and PHB which are commonly used as formulation additives in ophthalmic formulations increased the rate of drug corneal permeation by statistically significant amount.

Trans corneal permeability studies involving selected developed formulations prepared using various mucoadhesive polymers revealed that corneal permeability of flurbiprofen was increased with the use of these polymers to different extend. The increase in the extent of corneal permeation in case of mucoadhesive polymeric gels can be attributed to the increased adherence of the formulation to the corneal epithelium thereby facilitating partitioning and permeation of the drug. Studies with selected vegetable oil based formulations showed that the permeability results correlates well with the solubility data of flurbiprofen with permeability decreasing with increase in solubility of the drug in the oil. Permeability rate of flurbiprofen from selected polymeric discs and from polymer coated discs showed statistically insignificant

difference in the permeation characteristics compared to aqueous drop except in case EC matrix and matrix coated discs. In case of BAC, THM, CB and EDTA the corneal hydration value varied between 83 to 86 % suggesting adverse effect of these agents on the corneal cell structure and its integrity thereby increasing the drug permeability.

Ocular safety studies by Draize's test protocol revealed that aqueous drop, market preparations, polymeric gels (CMC-5, MC-5, PCB-5), SO based formulations and HPMC discs were non-irritating to the rabbit eye.

The aqueous humor drug bioavailability from aqueous drop and market formulations corresponded to earlier reported results (Tang, et al, 1984b). *In-vivo* ocular bioavailability studies revealed that *in-vitro* extension of the release of the drug from the designed formulations manifested in the form of enhanced area under the aqueous humor drug concentration time profile (AUC) and prolonged MRT of the drug in the aqueous humor. In case of MC-SO and EC-40/EC-10 log aqueous humor drug concentration profile in the later period was found to be constant therefore it can be predicted that drug availability from such formulations will continue for a long period of time. In case of *in-vivo* study involving polymeric discs (EC-40, EC-40/EC-10 and PAA-40/EC-20) the aqueous humor drug concentration profile was extended beyond 72 hours with sustained continuous availability except in case of NaCMC-40 where it extended marginally beyond 24 hours.

A good inverse correlation was obtained between time for 90 % of the drug released *in-vitro* ($t_{90\%}$) and $AUC_{0\text{-}24hr}$ *in-vivo* for developed liquid topical preparations but in case of polymeric discs type formulations only a moderate correlation was seen. Moderate correlation were obtained when $t_{50\%}$ and $t_{70\%}$ was correlated with $AUC_{0\text{-}24}$. Good correlation was obtained between $t_{90\%}$ *in-vitro* and MRT *in-vivo*. A very good correlation with high 'r' value was obtained between t_{max} and $t_{50\%}$ and between t_{max} and $t_{70\%}$ respectively.

7.5 References

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Table 7.1: Safety scoring for Draize's test protocol.

Ocular tissue	Scoring scale	Calculation	Maximum total
Cornea:			
Opacity (O)	0, 1, 2, 3, 4	O × A × 5	80
Area involved (A)	0, 1, 2, 3, 4		
Iris:			
Values for congestion and haemmorhage (I)	0, 1, 2	I × 5	10
Conjunctiva:			
Redness (R)	0, 1, 2, 3	(R + C + D) × 2	20
Chemosis (C)	0, 1, 2, 3, 4		
Discharge (D)	0, 1, 2, 3		
Grand maximum total score			110

Table 7.2: Safety evaluation chart for Draize's test protocol.

Rating	Score range
Non-irritating	0.0 – 0.5
Practically non-irritating	0.5 – 2.5
Minimally irritating	2.5 – 15.0
Mildly irritating	15.0 – 25.0
Moderately irritating	25.0 – 50.0
Severely irritating	50.0 – 80.0
Extremely irritating	80.0 – 110.0

Table 7.3: Cumulative amount of flurbiprofen permeated across goat corneal membrane from different buffers (0.01 M and ionic strength of 0.2).

Time (mins)	Cumulative amount permeated[a] (μg)				
	Aqueous Drop	Market preparation*	Phosphate buffer (pH 7.4)**	Citrate buffer (pH 6.6)*	Citrate-phosphate buffer (pH 7.0)*
15	5.0 ± 2.6	27.4 ± 4.3	18.4 ± 8.1	31.3 ± 3.3	28.8 ± 1.7
30	18.5 ± 5.2	39.6 ± 2.2	27.6 ± 7.0	43.7 ± 4.3	39.1 ± 7.0
45	21.1 ± 7.6	51.7 ± 4.0	38.4 ± 8.0	65.7 ± 7.2	59.4 ± 1.4
60	27.8 ± 4.5	61.7 ± 4.5	60.8 ± 3.1	79.0 ± 8.5	73.8 ± 1.2
90	29.8 ± 2.6	91.3 ± 9.7	103.3 ± 3.0	94.6 ± 7.3	91.8 ± 5.2
120	33.2 ± 2.7	98.1 ± 5.4	122.4 ± 6.6	105.8 ± 4.2	100.1 ± 7.6
Flux[b] (μg/min/cm^2)	0.5842	1.7028	2.2573	1.8003	1.7385
Corneal Hydration (%)	79.8 ± 0.3	78.3 ± 0.5	78.8 ± 0.6	79.9 ± 0.7	76.8 ± 1.1

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group
*: Statistically significant difference at $p < 0.05$ from the control
**: Statistically significant difference at $p < 0.005$ from the control
Control - Aqueous drop without any additives

Table 7.4: Cumulative amount of flurbiprofen permeated across goat corneal membrane from different pH phosphate buffer (0.01 M and ionic strength of 0.2).

Time (mins)	Cumulative amount permeated[a] (μg)			
	pH 5.4**	**pH 6.4****	**pH 7.4****	**pH 8.4***
15	32.8 ± 7.0	20.2 ± 2.7	18.4 ± 8.1	16.6 ± 4.5
30	49.1 ± 5.2	33.6 ± 1.8	27.6 ± 7.0	25.6 ± 6.5
45	72.6 ± 6.6	51.3 ± 1.9	38.4 ± 8.0	36.0 ± 5.8
60	108.3 ± 3.1	72.1 ± 2.6	60.8 ± 3.1	57.3 ± 4.9
90	158.9 ± 3.0	108.3 ± 3.8	103.3 ± 3.0	86.4 ± 3.7
120	201.5 ± 1.4	136.9 ± 3.2	122.4 ± 6.6	108.5 ± 3.4
Flux[b] (μg/min/cm^2)	3.5996	2.4566	2.2573	1.9582
Corneal Hydration (%)	77.9 ± 0.3	77.7 ± 0.4	76.4 ± 0.3	78.6 ± 0.5

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group
*: Statistically significant difference at $p < 0.05$ from the control
**: Statistically significant difference at $p < 0.005$ from the control
Control - Aqueous drop without any additives

Table 7.5: Cumulative amount of flurbiprofen permeated across goat corneal membrane from unbuffered drops of varying pH (0.01 M and ionic strength of 0.2).

Time (mins)	Cumulative amount permeated[a] (μg)				
	pH 4.5**	pH 5.5**	pH 6.5**	pH 7.5**	pH 8.5*
15	29.2 ± 4.6	23.6 ± 1.8	18.7 ± 6.6	17.5 ± 7.4	14.9 ± 5.5
30	46.3 ± 3.4	35.3 ± 1.2	31.1 ± 5.7	26.2 ± 6.4	23.0 ± 8.0
45	68.9 ± 4.3	52.2 ± 1.2	47.4 ± 6.6	42.3 ± 7.3	32.4 ± 7.1
60	96.7 ± 2.0	77.9 ± 1.7	66.6 ± 2.5	63.5 ± 2.8	51.6 ± 6.0
90	141.9 ± 2.1	114.3 ± 2.5	100.1 ± 2.5	95.6 ± 2.7	77.8 ± 4.6
120	179.9 ± 0.9	145.0 ± 2.1	126.5 ± 5.4	116.5 ± 6.0	97.7 ± 4.2
Flux[b] (μg/min/cm^2)	3.1982	2.5897	2.2705	2.1305	1.7623
Corneal Hydration (%)	78.9 ± 0.6	80.1 ± 0.4	78.2 ± 0.2	77.9 ± 0.8	78.3 ± 0.5

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group
*: Statistically significant difference at $p < 0.05$ from the control
**: Statistically significant difference at $p < 0.005$ from the control
Control - Aqueous drop without any additives

Table 7.6: Cumulative amount of flurbiprofen permeated across goat corneal membrane in the presence of various formulation excipients at pH 7.4.

Time (mins)	Cumulative amount permeated[a] (μg)							
	NaCl* (0.9 % w/v)	BAC*** (0.01 % w/v)	THM** (0.004 % w/v)	CB*** (0.5 % w/v)	PMN* (0.002 % w/v)	EDTA*** (0.01 % w/v)	MHB* (0.04 % w/v)	PHB* (0.02 % w/v)
15	24.5 ± 3.4	53.3 ± 2.8	24.1 ± 3.1	75.1 ± 4.2	26.7 ± 3.1	56.9 ± 1.6	20.3 ± 1.1	28.1 ± 2.1
30	37.7 ± 4.5	79.6 ± 3.5	48.3 ± 3.4	109.2 ± 4.6	39.8 ± 2.6	84.9 ± 1.8	37.5 ± 1.5	49.8 ± 2.3
45	51.8 ± 5.6	103.6 ± 3.4	72.0 ± 3.9	142.3 ± 4.3	51.8 ± 2.5	110.5 ± 1.9	63.2 ± 1.8	56.3 ± 2.4
60	65.4 ± 5.1	132.3 ± 3.8	105.7 ± 2.8	179.6 ± 3.2	66.2 ± 2.7	141.2 ± 1.4	66.8 ± 2.3	64.1 ± 2.9
90	76.9 ± 4.8	173.5 ± 4.1	134.8 ± 3.7	210.5 ± 3.9	86.8 ± 2.8	185.1 ± 1.7	70.8 ± 2.4	72.3 ± 3.2
120	81.6 ± 4.7	211.2 ± 3.1	189.6 ± 3.5	235.3 ± 2.2	105.6 ± 2.1	225.4 ± 2.1	74.8 ± 1.8	80.1 ± 2.2
Flux[b] (μg/min/cm^2)	1.4115	3.5664	3.3157	3.9247	1.7832	3.8053	1.2853	1.2640
Corneal Hydration (%)	79.3 ± 0.6	84.6 ± 0.7	83.9 ± 0.4	85.3 ± 0.3	78.6 ± 0.1	85.7 ± 0.6	78.6 ± 0.5	79.2 ± 0.2

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group
*: Statistically significant difference at $p < 0.05$ from the control
**: Statistically significant difference at $p < 0.005$ from the control
***: Statistically significant difference at $p < 0.001$ from the control
Control - Aqueous drop without any additives

Table 7.7: Cumulative amount of flurbiprofen permeated across goat corneal membrane from selected polymeric gel type formulations containing 5% w/v polymer.

Time (mins)	Cumulative amount permeated[a] (μg)			
	CMC-5**	MC-5**	NaCMC-5*	PCB-5**
15	9.6 ± 5.1	45.2 ± 4.2	5.2 ± 4.7	12.1 ± 6.3
30	23.7 ± 6.8	64.4 ± 5.3	21.5 ± 5.1	27.1 ± 6.9
45	50.1 ± 8.4	76.9 ± 5.1	45.7 ± 5.9	57.8 ± 6.5
60	74.6 ± 7.7	92.3 ± 5.7	66.3 ± 4.2	78.5 ± 4.8
90	98.2 ± 7.2	116.1 ± 6.2	89.1 ± 5.6	106.1 ± 5.9
120	110.5 ± 7.1	145.4 ± 4.7	101.3 ± 5.3	151.4 ± 3.3
Flux[b] (μg/min/cm^2)	2.1445	2.3226	1.9842	2.7253
Corneal Hydration (%)	78.6 ± 0.3	76.9 ± 0.4	78.5 ± 0.2	79.6 ± 0.4

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group
*: Statistically significant difference at $p < 0.05$ from the control
**: Statistically significant difference at $p < 0.005$ from the control
Control - Aqueous drop without any additives

Table 7.8: Cumulative amount of flurbiprofen permeated across goat corneal membrane from 5% w/v PCB, MC and HPMC with linseed oil based formulations.

Time (mins)	Cumulative amount permeated[a] (μg)			
	LSO*	PCB-LSO[#, ϕ]	MC-LSO*, [ϕ]	HPMC-LSO [ϕ]
15	30.9 ± 2.1	7.6 ± 2.7	8.7 ± 2.5	1.2 ± 2.1
30	46.1 ± 3.6	16.1 ± 2.8	16.9 ± 2.8	4.7 ± 2.0
45	59.2 ± 3.5	26.1 ± 3.1	26.7 ± 2.4	11.5 ± 1.9
60	76.4 ± 3.7	34.5 ± 3.2	35.2 ± 2.5	19.5 ± 2.3
90	99.1 ± 3.8	49.7 ± 3.3	56.3 ± 3.9	31.6 ± 2.8
120	123.5 ± 3.1	66.3 ± 3.0	86.9 ± 4.0	40.1 ± 3.8
Flux[b] (μg/min/cm^2)	2.0714	1.1830	1.5025	0.7764
Corneal Hydration (%)	78.6 ± 0.7	79.6 ± 0.3	80.7 ± 0.5	78.3 ± 0.2

[a]: Mean and S.D. of two cornea (experiments) per group

[b]: Mean of two cornea (experiments) per group

*: Statistically significant difference at $p < 0.05$ from the control

#:Statistically significant difference at $p < 0.10$ from the control

ϕ: Statistically significant difference at $p < 0.001$ from the formulation containing only LSO

Control - Aqueous drop without any additives

Table 7.9: Cumulative amount of flurbiprofen permeated across goat corneal membrane from 5% w/v PCB, MC and HPMC with olive oil based formulations.

Time (mins)	Cumulative amount permeated[a] (μg)			
	OO*	PCB-OO$^{\phi}$	MC-OO$^{\phi}$	HPMC-OO$^{\phi}$
15	7.3 ± 2.2	6.9 ± 1.7	5.6 ± 2.2	0.0 ± 2.1
30	18.9 ± 2.7	14.6 ± 3.1	11.3 ± 2.3	3.2 ± 2.5
45	29.3 ± 3.1	29.7 ± 3.9	21.9 ± 2.1	7.5 ± 2.6
60	40.5 ± 3.5	39.3 ± 3.2	32.3 ± 2.7	11.3 ± 3.1
90	74.3 ± 3.8	45.2 ± 3.6	42.6 ± 3.2	23.6 ± 3.8
120	102.8 ± 2.7	60.3 ± 3.3	49.8 ± 1.7	33.6 ± 3.4
Flux[b] (μg/min/cm^2)	1.8635	0.0821	0.8990	0.6331
Corneal Hydration (%)	80.2 ± 0.5	80.6 ± 0.1	81.1 ± 0.3	80.2 ± 0.5

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group
*: Statistically significant difference at $p < 0.05$ from the control
$^{\phi}$: Statistically significant difference at $p < 0.001$ from the formulation containing only OO
Control - Aqueous drop without any additives

Table 7.10: Cumulative amount of flurbiprofen permeated across goat corneal membrane from PCB, MC and HPMC with sesame oil based formulations.

Time (mins)	Cumulative amount permeated[a] (μg)			
	SO**	PCB-SO*, $^{\phi}$	MC-SO$^{\phi}$	HPMC-SO$^{\#, \phi}$
15	7.7 ± 2.3	9.4 ± 1.2	6.9 ± 2.2	7.9 ± 2.1
30	20.3 ± 2.9	20.3 ± 1.9	14.6 ± 2.3	17.2 ± 2.5
45	36.2 ± 2.5	33.6 ± 2.2	25.3 ± 2.1	30.6 ± 2.6
60	51.3 ± 2.8	48.1 ± 2.3	33.2 ± 2.7	41.3 ± 3.1
90	73.6 ± 2.9	62.3 ± 2.5	44.1 ± 3.2	49.6 ± 3.8
120	97.3 ± 2.3	83.4 ± 2.6	60.3 ± 1.7	69.2 ± 3.4
Flux[b] (μg/min/cm^2)	1.7841	1.4945	1.0689	1.2207
Corneal Hydration (%)	77.6 ± 0.7	82.6 ± 0.1	82.4 ± 0.6	81.9 ± 0.8

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group
$^{\#}$: Statistically significant difference at $p < 0.10$ from the control
*: Statistically significant difference at $p < 0.05$ from the control
**: Statistically significant difference at $p < 0.005$ from the control
$^{\phi}$: Statistically significant difference at $p < 0.001$ from the formulation containing only SO
Control - Aqueous drop without any additives

Table 7.11: Cumulative amount of flurbiprofen permeated across goat corneal membrane from selected non-coated matrix embedded formulations.

Time (mins)	Cumulative amount permeated[a] (μg)			
	NaCMC-40	NaAL-40	HPMC-40	EC-40
15	2.2 ± 1.4	4.0 ± 0.8	4.6 ± 1.1	1.3 ± 1.2
30	7.2 ± 1.5	8.1 ± 0.7	9.2 ± 1.4	5.4 ± 1.6
45	10.7 ± 0.6	13.1 ± 0.6	13.8 ± 0.9	8.7 ± 1.3
60	14.3 ± 0.9	16.2 ± 0.8	18.5 ± 0.8	11.8 ± 1.2
90	21.5 ± 0.8	25.3 ± 1.1	27.7 ± 0.7	15.6 ± 1.9
120	28.6 ± 0.7	32.3 ± 1.0	36.9 ± 0.5	19.9 ± 1.2
Flux[b] (μg/min/cm^2)	0.5172	0.5795	0.6548	0.3663
Corneal Hydration (%)	76.3 ± 0.5	77.6 ± 0.4	78.4 ± 0.3	77.9 ± 0.7

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group

Table 7.12: Cumulative amount of flurbiprofen permeated across goat corneal membrane from selected coated matrix embedded formulations.

Time (mins)	Cumulative amount permeated[a] (μg)			
	NaAL-40/EC-20	HPMC-40/EC-20	PAA-40/EC-20	EC-40/EC-10
15	2.0 ± 1.1	4.3 ± 0.9	4.1 ± 1.1	0.0 ± 1.1
30	5.1 ± 1.6	8.6 ± 1.1	8.2 ± 0.9	2.3 ± 1.3
45	7.3 ± 1.5	14.0 ± 0.7	12.2 ± 1.0	4.2 ± 1.4
60	10.5 ± 1.0	17.2 ± 0.4	16.3 ± 1.3	7.8 ± 1.3
90	16.3 ± 0.8	24.8 ± 0.7	24.5 ± 1.4	10.7 ± 1.2
120	23.6 ± 1.1	29.6 ± 1.1	32.6 ± 0.8	14.5 ± 1.0
Flux[b] (μg/min/cm^2)	0.4176	0.5355	0.5780	0.2770
Corneal Hydration (%)	79.3 ± 0.8	77.1 ± 0.5	79.2 ± 0.4	78.8 ± 0.2

[a]: Mean and S.D. of two cornea (experiments) per group
[b]: Mean of two cornea (experiments) per group

Table 7.13: Results of ocular safety evaluation of designed formulations

Formulation code	Total score	Rating
Aqueous drop	0.00	Non-irritating
Market preparation	0.00	Non-irritating
PCB-5	0.00	Non-irritating
CMC-5	0.00	Non-irritating
MC-5	0.00	Non-irritating
NaCMC-5	0.67	Practically non-irritating
LSO	1.00	Practically non-irritating
OO	0.67	Practically non-irritating
SO	0.00	Non-irritating
PCB-LSO	0.67	Practically non-irritating
MC-LSO	0.67	Practically non-irritating
HPMC-LSO	0.67	Practically non-irritating
PCB-OO	0.67	Practically non-irritating
MC-OO	0.67	Practically non-irritating
HPMC-OO	0.67	Practically non-irritating
PCB-SO	0.00	Non-irritating
MC-SO	0.00	Non-irritating
HPMC-SO	0.00	Non-irritating
NaCMC-40	1.00	Practically non-irritating
NaAL-40	1.33	Practically non-irritating
HPMC-40	0.00	Non-irritating
EC-40	0.67	Practically non-irritating
NaAL-40/EC-20	0.67	Practically non-irritating
HPMC-40/EC-20	0.67	Practically non-irritating
PAA-40/EC-20	0.67	Practically non-irritating
EC-40/EC-10	0.67	Practically non-irritating

[a]: Mean of nine eyes from three groups

Table 7.14: Aqueous humor concentration of flurbiprofen from different formulations prepared using polymers and vegetable oils with time from single topical administration. Also shown are data pertaining to controls (market preparation and aqueous drop).

Time (Hours)	Aqueous humor drug concentration (μg/ml)[a]				
	Aqueous drop	Market preparation	MC-5	LSO	MC-SO
0.25	0.238 ± 0.004	0.652 ± 0.001	0.314 ± 0.040	0.026 ± 0.012	0.049 ± 0.010
0.5	0.846 ± 0.002	1.122 ± 0.020	0.771 ± 0.098	0.057 ± 0.030	0.180 ± 0.011
1	1.065 ± 0.011	1.294 ± 0.064	0.828 ± 0.105	0.091 ± 0.009	0.225 ± 0.015
2	1.148 ± 0.021	1.424 ± 0.065	0.922 ± 0.117	0.218 ± 0.069	0.279 ± 0.032
4	1.026 ± 0.037	1.086 ± 0.070	0.969 ± 0.123	0.691 ± 0.157	0.307 ± 0.020
6	0.473 ± 0.019	0.686 ± 0.103	0.847 ± 0.107	0.941 ± 0.049	0.352 ± 0.021
8	0.250 ± 0.007	0.326 ± 0.001	0.673 ± 0.086	0.887 ± 0.012	0.364 ± 0.032
12	0.069 ± 0.009	0.095 ± 0.010	0.210 ± 0.027	0.517 ± 0.065	0.353 ± 0.046
16			0.057 ± 0.005	0.149 ± 0.010	0.309 ± 0.023

[a]: Mean and S.D. of two eyes per treatment

Table 7.14: Aqueous humor concentration of flurbiprofen from different formulations prepared using polymers and vegetable oils with time from single topical administration. Also shown are data pertaining to controls (market preparation and aqueous drop).

Time (Hours)	Aqueous humor drug concentration (μg/ml)[a]				
	Aqueous drop	Market preparation	MC-5	LSO	MC-SO
0.25	0.238 ± 0.004	0.652 ± 0.001	0.314 ± 0.040	0.026 ± 0.012	0.049 ± 0.010
0.5	0.846 ± 0.002	1.122 ± 0.020	0.771 ± 0.098	0.057 ± 0.030	0.180 ± 0.011
1	1.065 ± 0.011	1.294 ± 0.064	0.828 ± 0.105	0.091 ± 0.009	0.225 ± 0.015
2	1.148 ± 0.021	1.424 ± 0.065	0.922 ± 0.117	0.218 ± 0.069	0.279 ± 0.032
4	1.026 ± 0.037	1.086 ± 0.070	0.969 ± 0.123	0.691 ± 0.157	0.307 ± 0.020
6	0.473 ± 0.019	0.686 ± 0.103	0.847 ± 0.107	0.941 ± 0.049	0.352 ± 0.021
8	0.250 ± 0.007	0.326 ± 0.001	0.673 ± 0.086	0.887 ± 0.012	0.364 ± 0.032
12	0.069 ± 0.009	0.095 ± 0.010	0.210 ± 0.027	0.517 ± 0.065	0.353 ± 0.046
16			0.057 ± 0.005	0.149 ± 0.010	0.309 ± 0.023

[a]: Mean and S.D. of two eyes per treatment

Table 7.15: Aqueous humor concentration of flurbiprofen from different matrix embedded and polymer coated formulations with time from single topical administration. Also shown are data pertaining to control (aqueous drop).

Time (Hours)	Aqueous humor drug concentration (μg/ml)[a]			
	NaCMC-40	**EC-40**	**EC-40/EC-20**	**PAA-40/EC-20**
0.25	0.047 ± 0.005	0.039 ± 0.006	0.023 ± 0.001	0.026 ± 0.005
0.5	0.109 ± 0.015	0.067 ± 0.003	0.037 ± 0.001	0.036 ± 0.008
1	0.153 ± 0.022	0.105 ± 0.001	0.045 ± 0.008	0.055 ± 0.004
2	0.239 ± 0.035	0.135 ± 0.002	0.055 ± 0.015	0.073 ± 0.001
4	0.339 ± 0.052	0.189 ± 0.003	0.074 ± 0.014	0.099 ± 0.006
6	0.471 ± 0.072	0.262 ± 0.009	0.104 ± 0.025	0.147 ± 0.029
8	0.653 ± 0.084	0.303 ± 0.010	0.135 ± 0.038	0.195 ± 0.032
12	0.422 ± 0.054	0.379 ± 0.008	0.176 ± 0.069	0.379 ± 0.174
16	0.137 ± 0.018	0.467 ± 0.006	0.209 ± 0.095	0.548 ± 0.169
24	0.038 ± 0.005	0.551 ± 0.005	0.244 ± 0.091	0.677 ± 0.152
48		0.420 ± 0.001	0.275 ± 0.111	0.507 ± 0.230
72		0.217 ± 0.008	0.281 ± 0.097	0.225 ± 0.099

[a]: Mean and S.D. of two eyes per treatment

Table 7.16: Serum concentration of flurbiprofen from different formulations prepared using polymers and vegetable oils with time from single topical administration. Also shown are data pertaining to controls (market preparation and aqueous drop).

Time (Hours)	Serum drug concentration (μg/ml)[a]				
	Aqueous drop	Market preparation	MC-5	LSO	MC-SO
0.5	0.239 ± 0.009	0.319 ± 0.009			
1	0.154 ± 0.007	0.233 ± 0.003	0.212 ± 0.008	0.062 ± 0.003	0.053 ± 0.002
2	0.103 ± 0.025	0.100 ± 0.007	0.156 ± 0.003	0.085 ± 0.001	0.072 ± 0.015
4	0.046 ± 0.005	0.048 ± 0.001	0.091 ± 0.006	0.184 ± 0.016	0.177 ± 0.007
6	0.035 ± 0.002	0.034 ±0.001	0.044 ± 0.001	0.126 ± 0.004	0.137 ± 0.002
8				0.106 ± 0.022	0.124 ± 0.001
12				0.056 ± 0.020	0.097 ± 0.002
16					0.051 ± 0.002

[a]: Mean and S.D. of two eyes per treatment

Table 7.17: Pharmacokinetic parameters obtained from aqueous humor concentration vs. time profile upon single topical administration of flurbiprofen from different formulations prepared using polymers and vegetable oils. Also shown are data pertaining to controls (market preparation and aqueous drops).

Pharmacokinetic Parameters	Treatment[a]				
	Aqueous drop	Market preparation	MC-5	LSO	MC-SO
t_{max} (hours)	2.00 ± 0.00	2.00 ± 0.00	4.00 ± 0.00	6.00 ± 0.00	7.00 ± 1.41
C_{max} (μg/ml)	1.15 ± 0.02	1.42 ± 0.06	0.97 ± 0.12	0.94 ± 0.05	0.38 ± 0.01
Elimination rate constant ($hour^{-1}$)	0.32 ± 0.03	0.33 ± 0.00	0.31 ± 0.01	0.22 ± 0.01	ϕ
$t_{1/2}$ (hours)	2.15 ± 0.17	2.12 ± 0.01	2.26 ± 0.04	3.11 ± 0.09	ϕ
AUC[b] (μg.hour/ml)	6.61 ± 0.02	8.21 ± 0.24	8.73 ± 1.10	8.50 ± 0.65	6.21 ± 0.19
$AUC_{(0-\infty)}$ (μg.hour/ml)	6.82 ± 0.03	8.50 ± 0.27	8.91 ± 1.12	9.16 ± 0.72	ϕ
AUMC[b] ($\mu g.hour^2/ml$)	25.75 ± 0.34	32.41 ± 1.24	48.67 ± 6.10	67.68 ± 4.41	64.24 ± 3.41
$AUMC_{(0-\infty)}$ ($\mu g.hour^2/ml$)	28.98 ± 1.07	36.79 ± 1.69	52.28 ± 6.33	81.38 ± 5.81	ϕ
MRT[b] (hours)	3.90 ± 0.06	3.95 ± 0.03	5.58 ± 0.01	7.97 ± 0.09	10.34 ± 0.24
$MRT_{(0-\infty)}$ (hours)	4.25 ± 0.14	4.33 ±0.06	5.87 ± 0.02	8.88 ± 0.06	ϕ

[a]: Mean and S.D. of two eyes per treatment

[b]: Based on 0 hour to last time point of data collection

ϕ: Parameter cannot be calculated, as the terminal linear portion of the log drug concentration vs. time profile was not attained

Table 7.18: Pharmacokinetic parameters obtained from aqueous humor concentration vs. time profile upon single topical administration of flurbiprofen from different matrix embedded and polymer coated formulations. Also shown are data pertaining to controls (market preparation and aqueous drops).

Pharmacokinetic Parameters	Treatment[a]			
	NaCMC-40	**EC-40**	**EC-40/EC-20**	**PAA-40/EC-20**
t_{max} (hours)	8.00 ± 0.00	24.00 ± 0.00	60.00 ± 16.97	24.00 ± 16.97
C_{max} (μg/ml)	0.65 ± 0.08	0.55 ± 0.01	0.28 ± 0.10	0.73 ± 0.08
Elimination rate constant ($hour^{-1}$)	0.19 ± 0.00	0.02 ± 0.00	ϕ	0.02 ± 0.01
$t_{1/2}$ (hours)	3.58 ± 0.00	35.70 ± 1.84	ϕ	35.51 ± 21.22
AUC[b] (μg.hour/ml)	12.55 ± 0.24	27.62 ± 0.06	16.76 ± 6.42	30.97 ± 2.69
$AUC_{(0-\infty)}$ (μg.hour/ml)	12.75 ± 0.27	38.81 ± 0.95	ϕ	44.01 ± 14.65
AUMC[b] ($\mu g.hour^2/ml$)	61.10 ± 5.27	972.21 ± 4.56	688.49 ± 262.29	1125.91 ± 253.92
$AUMC_{(0-\infty)}$ ($\mu g.hour^2/ml$)	66.89 ± 6.02	2355.47 ± 159.39	ϕ	2916.13 ± 126.78
MRT[b] (hours)	5.24 ± 0.46	35.20 ± 0.24	41.10 ± 0.09	36.14 ± 5.06
$MRT_{(0-\infty)}$ (hours)	4.87 ± 0.50	60.67 ± 2.62	ϕ	61.63 ± 7.81

[a]: Mean and S.D. of two eyes per treatment

[b]: Based on 0 hour to last time point of data collection

ϕ: Parameter cannot be calculated, as the terminal linear portion of the log drug concentration vs. time profile was not attained

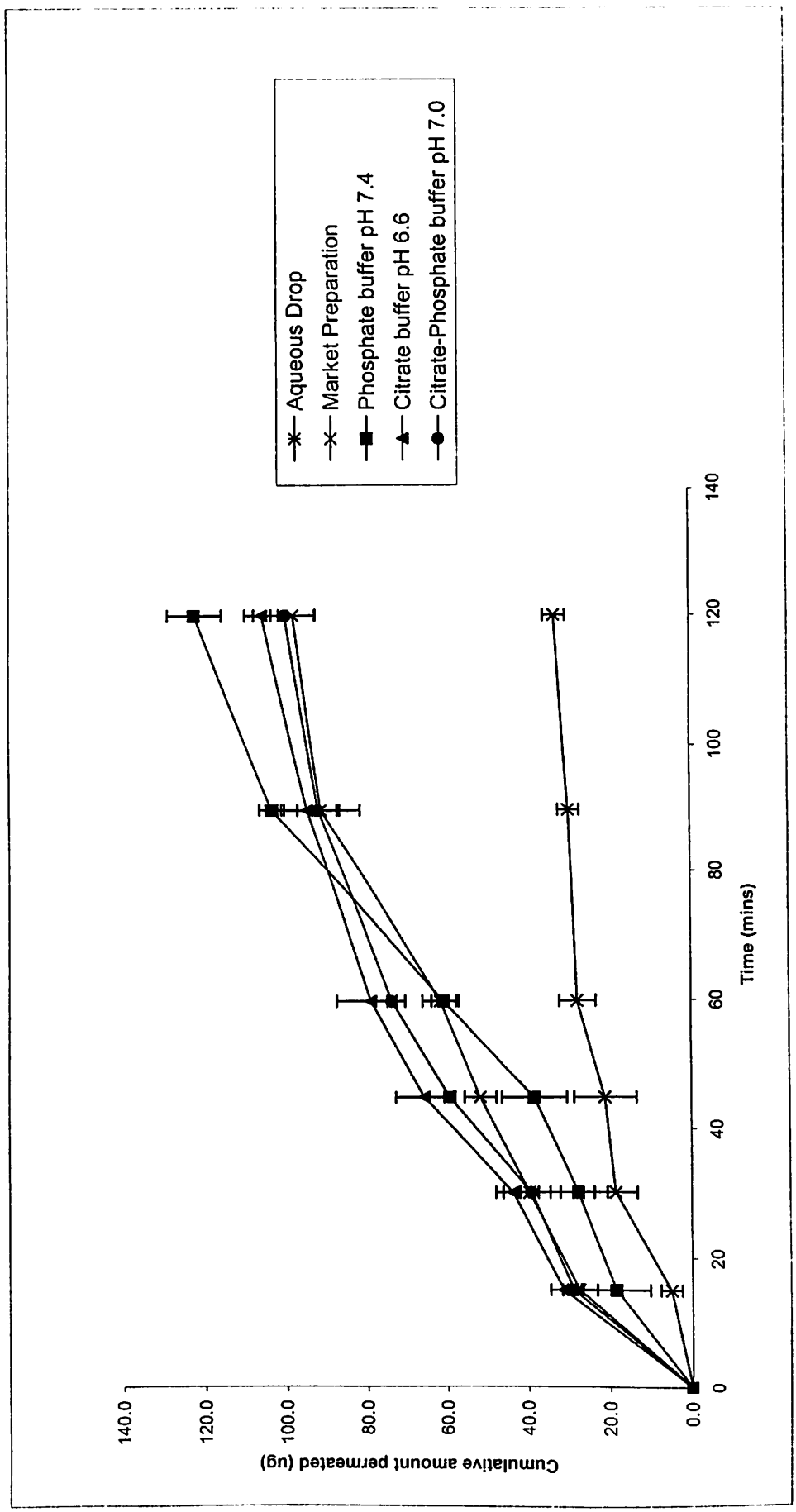

Figure 7.2: Transcorneal permeation profile of flurbiprofen from different buffers (0.01M and ionic strength of 0.2).

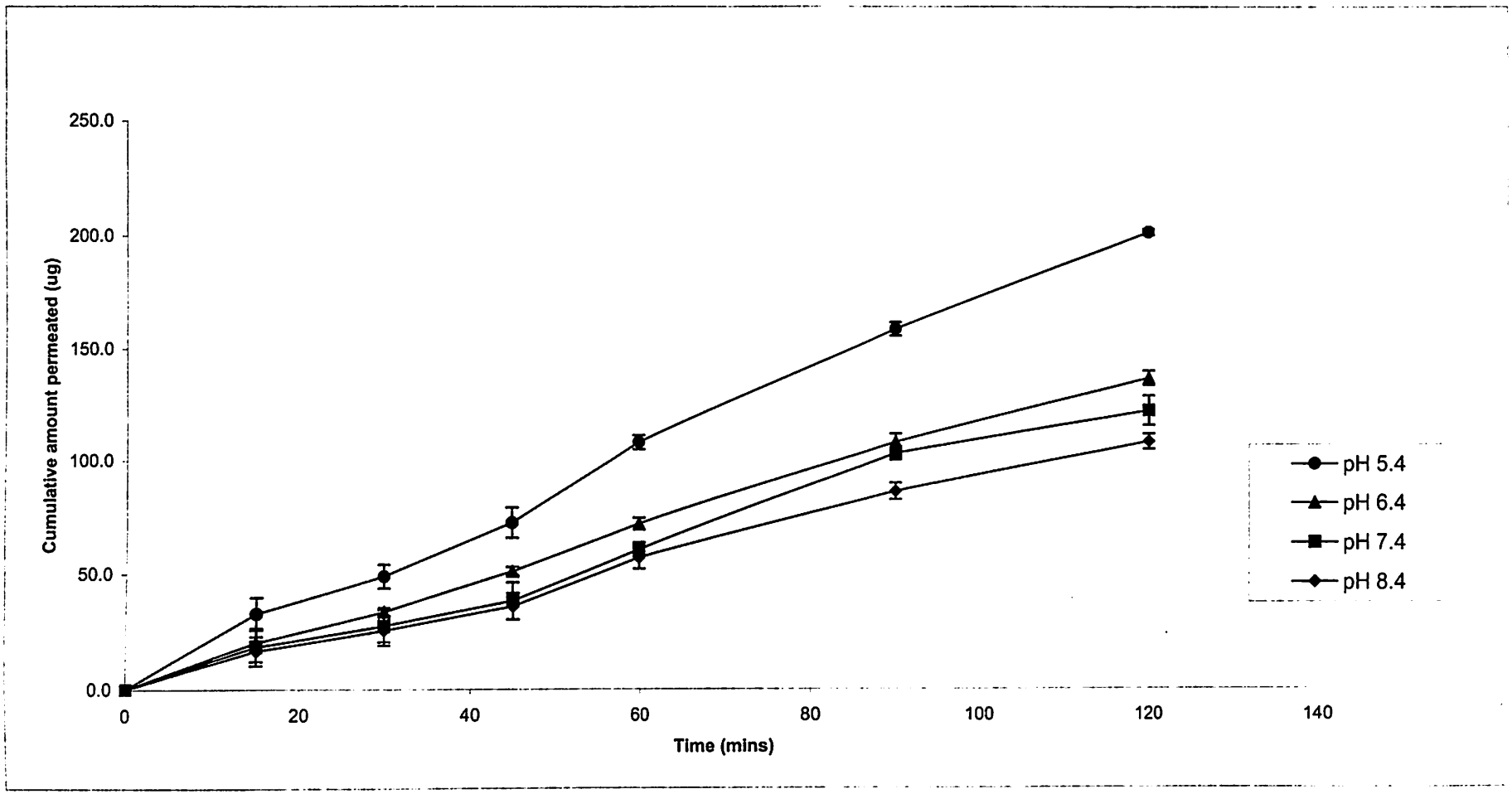

Figure 7.3: Transcorneal permeation profile of flurbiprofen from different pH phosphate buffer (0.01M and ionic strength of 0.2).

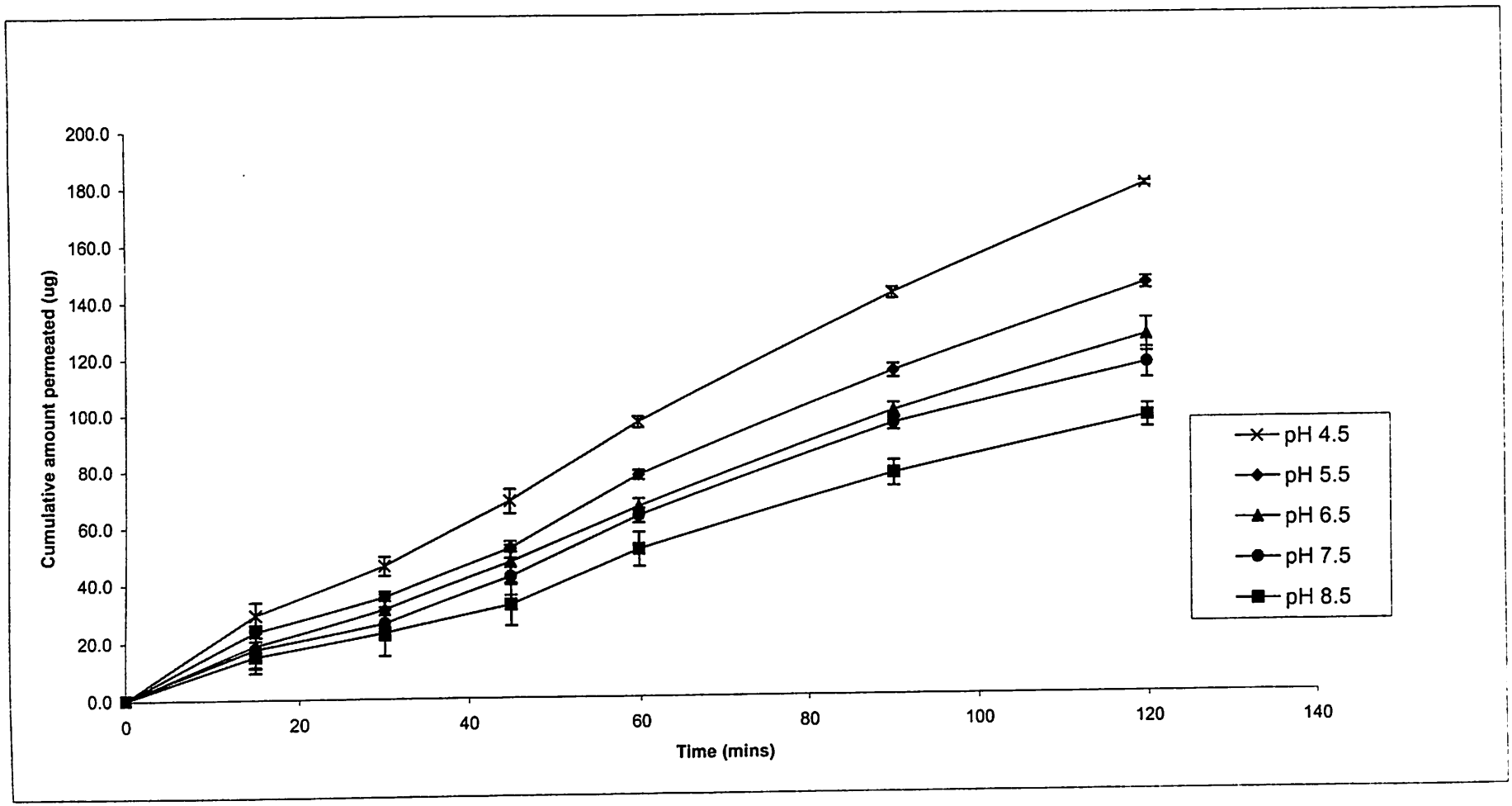

Figure 7.4: Transcorneal permeation profile of flurbiprofen from unbuffered drops of varying pH (0.01M and ionic strength of 0.2).

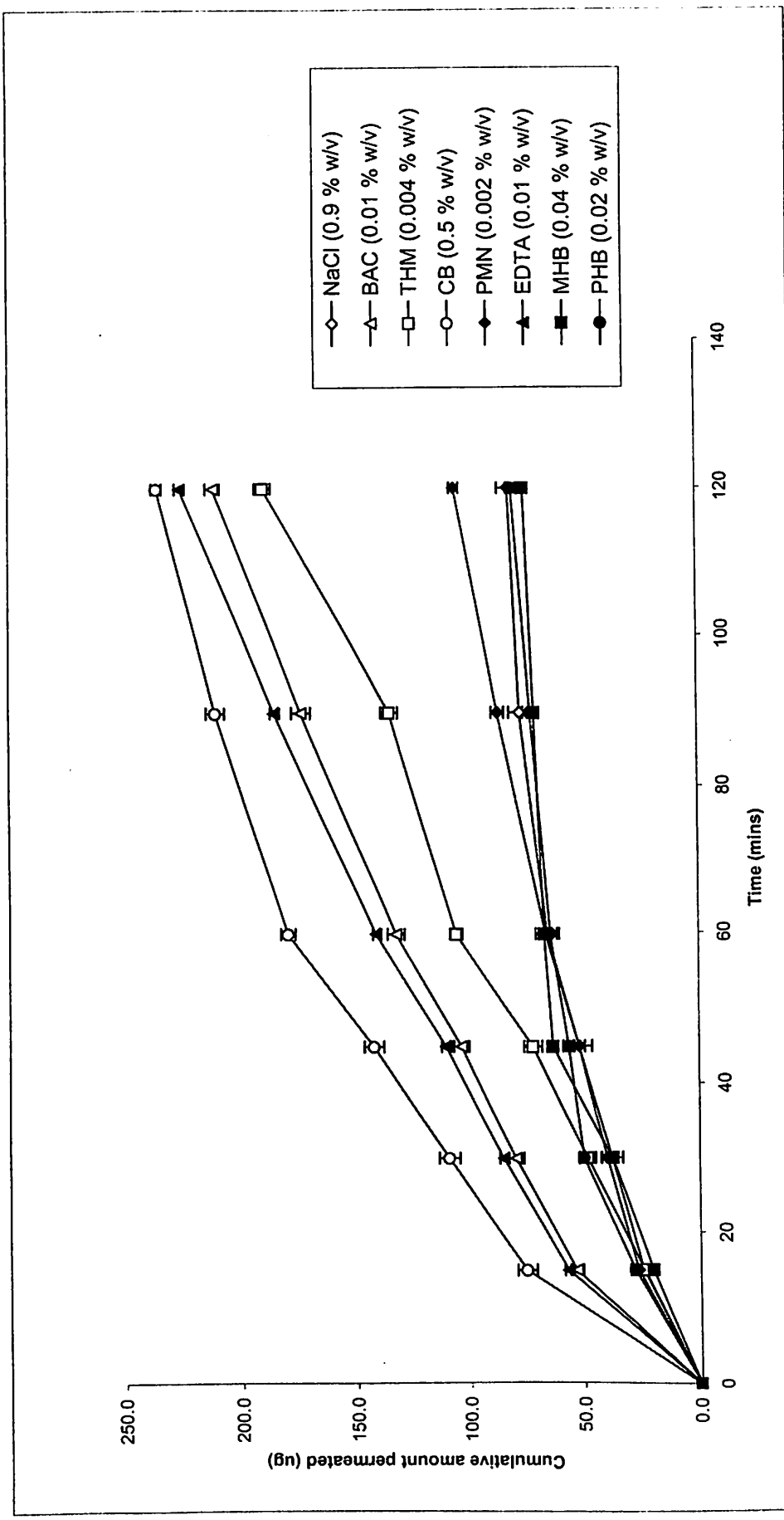

Figure 7.5: Transcorneal permeation profile of flurbiprofen in the presence of various formulation excipients at pH 7.4.

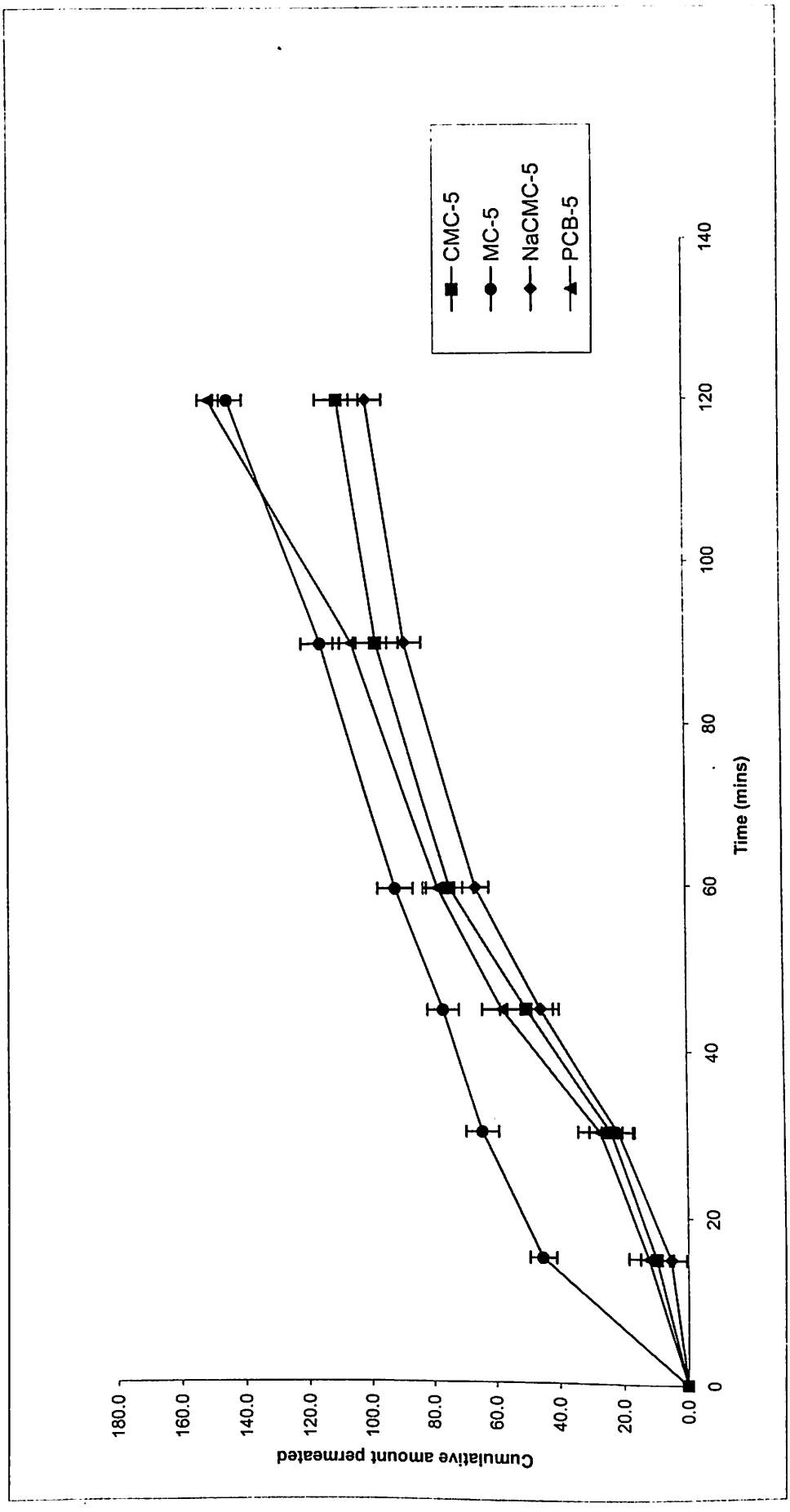

Figure 7.6: Transcorneal permeation profile of flurbiprofen from selected polymeric gel type formulations containing 5% w/v polymer.

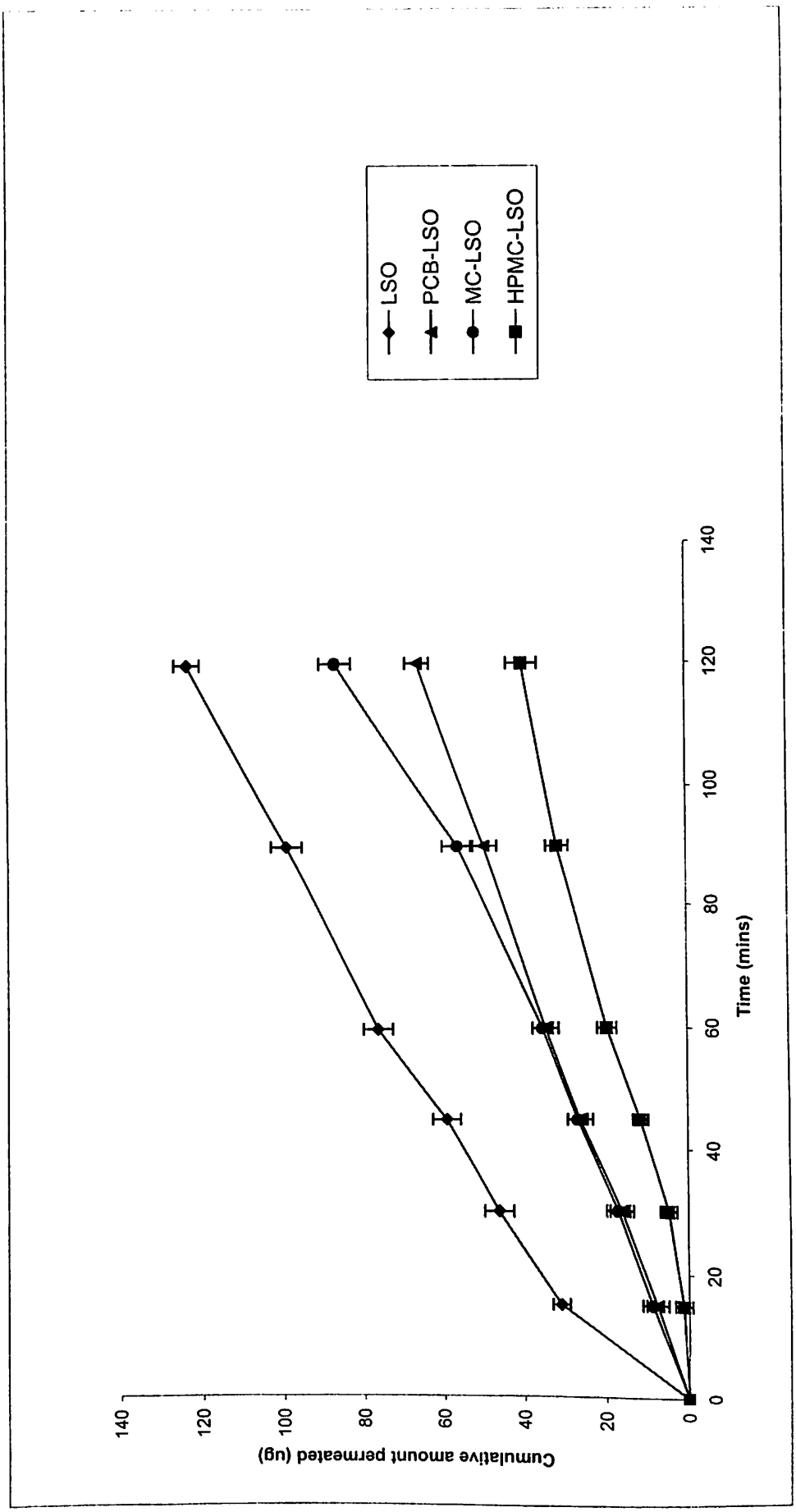

Figure 7.7: Transcorneal permeation profile of flurbiprofen from LSO based and polymer in LSO based formulations containing 5% w/v polymer.

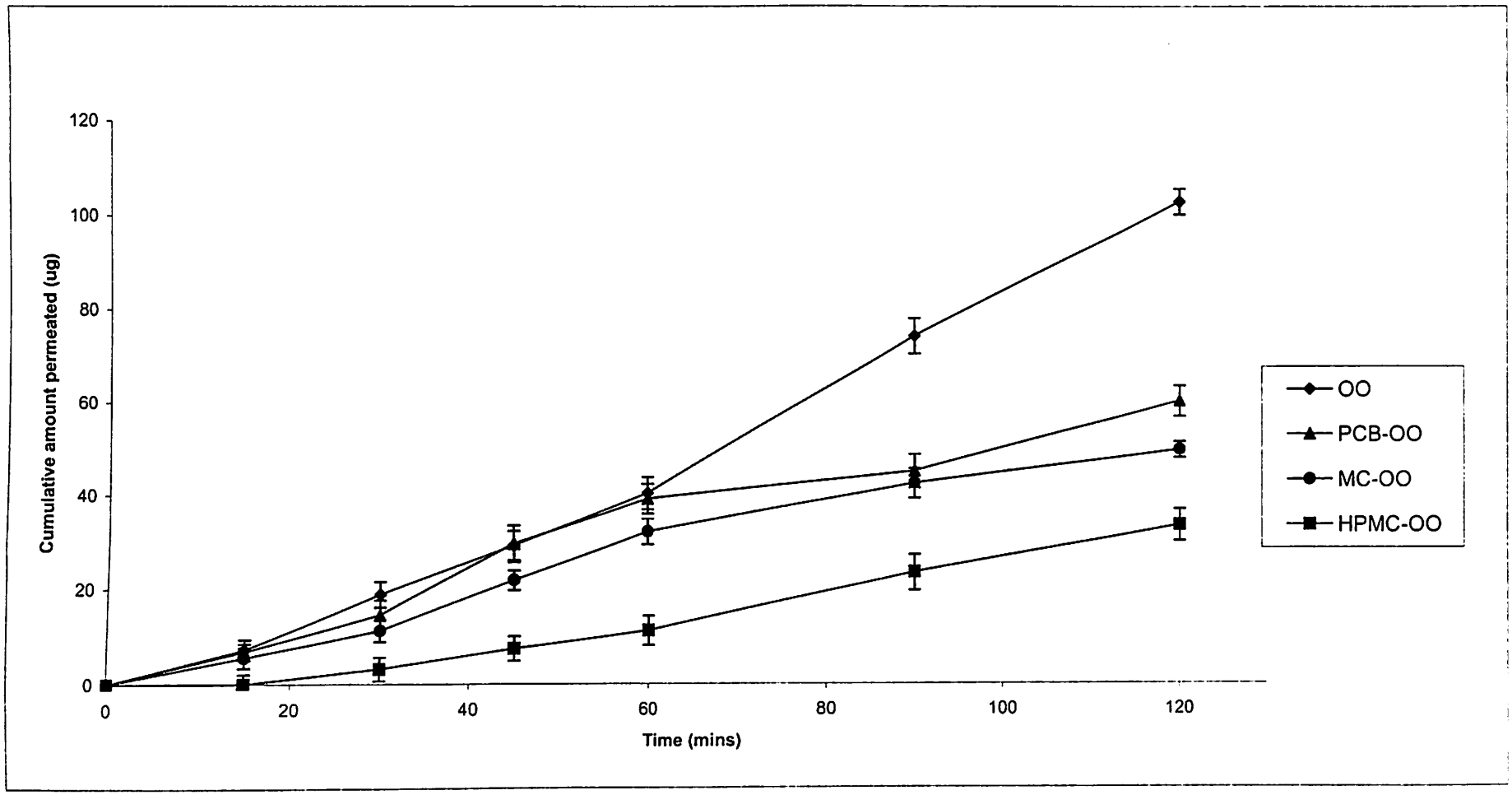

Figure 7.8: Transcorneal permeation profile of flurbiprofen from OO based and polymer in OO based formulations containing 5% w/v polymer.

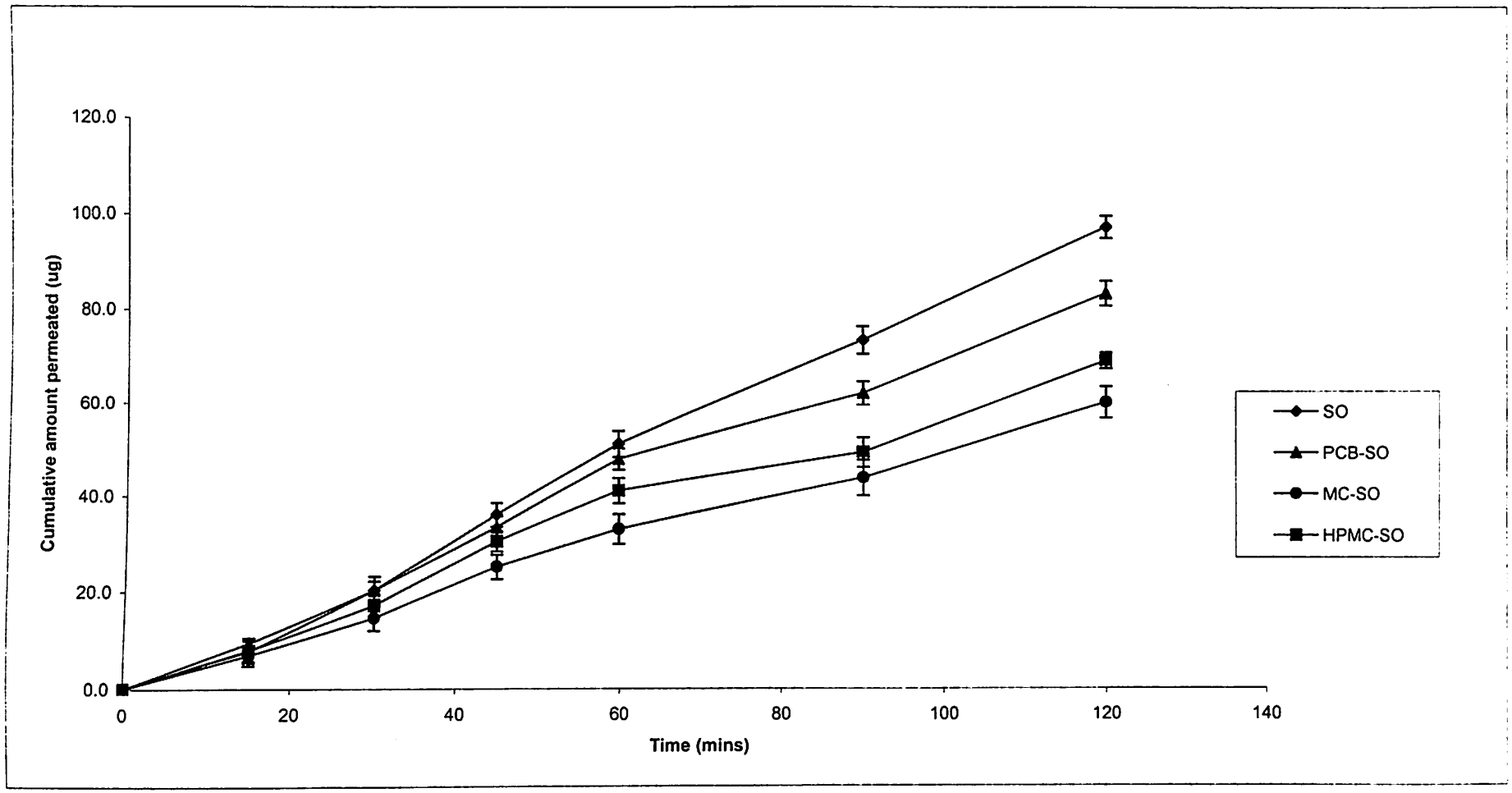

Figure 7.9: Transcorneal permeation profile of flurbiprofen from SO based and polymer in SO based formulations containing 5% w/v polymer.

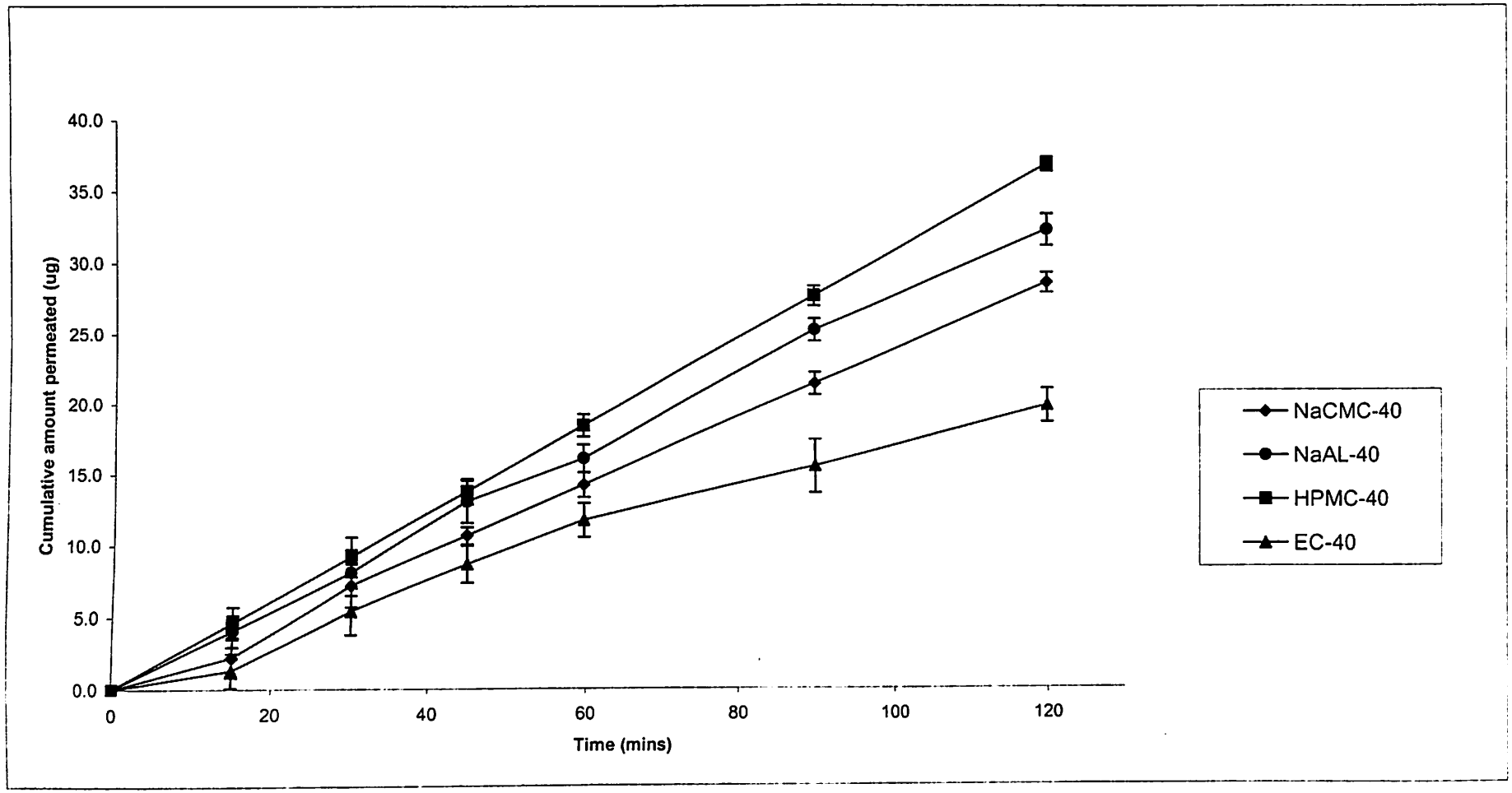

Figure 7.10: Transcorneal permeation profile of flurbiprofen from selected non-coated matrix embedded formulations.

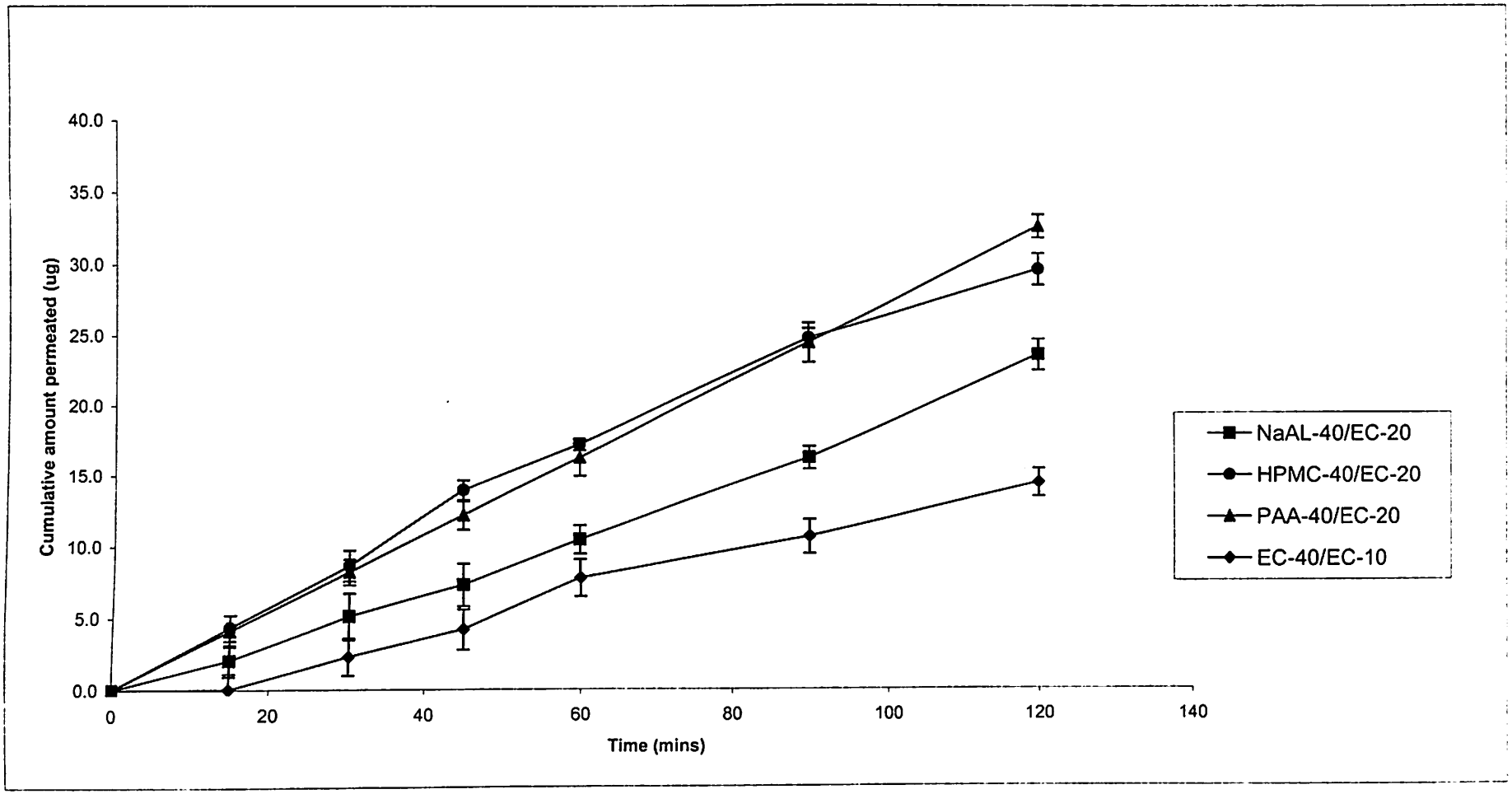

Figure 7.11: Transcorneal permeation profile of flurbiprofen from selected coated matrix embedded formulations.

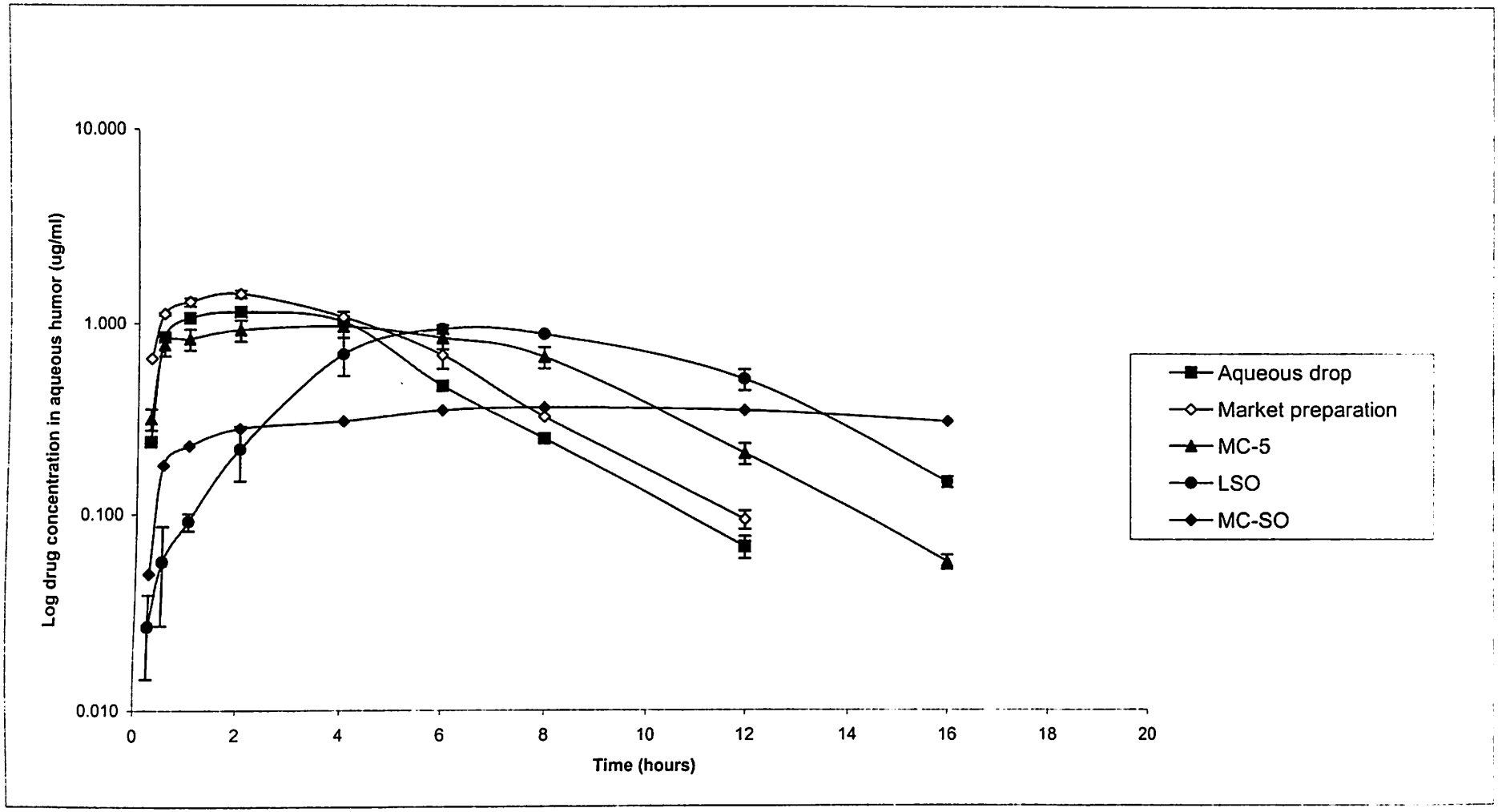

Figure 7.12: Aqueous humor concentration-time profile of flurbiprofen after topical administration of designed liquid formulations.

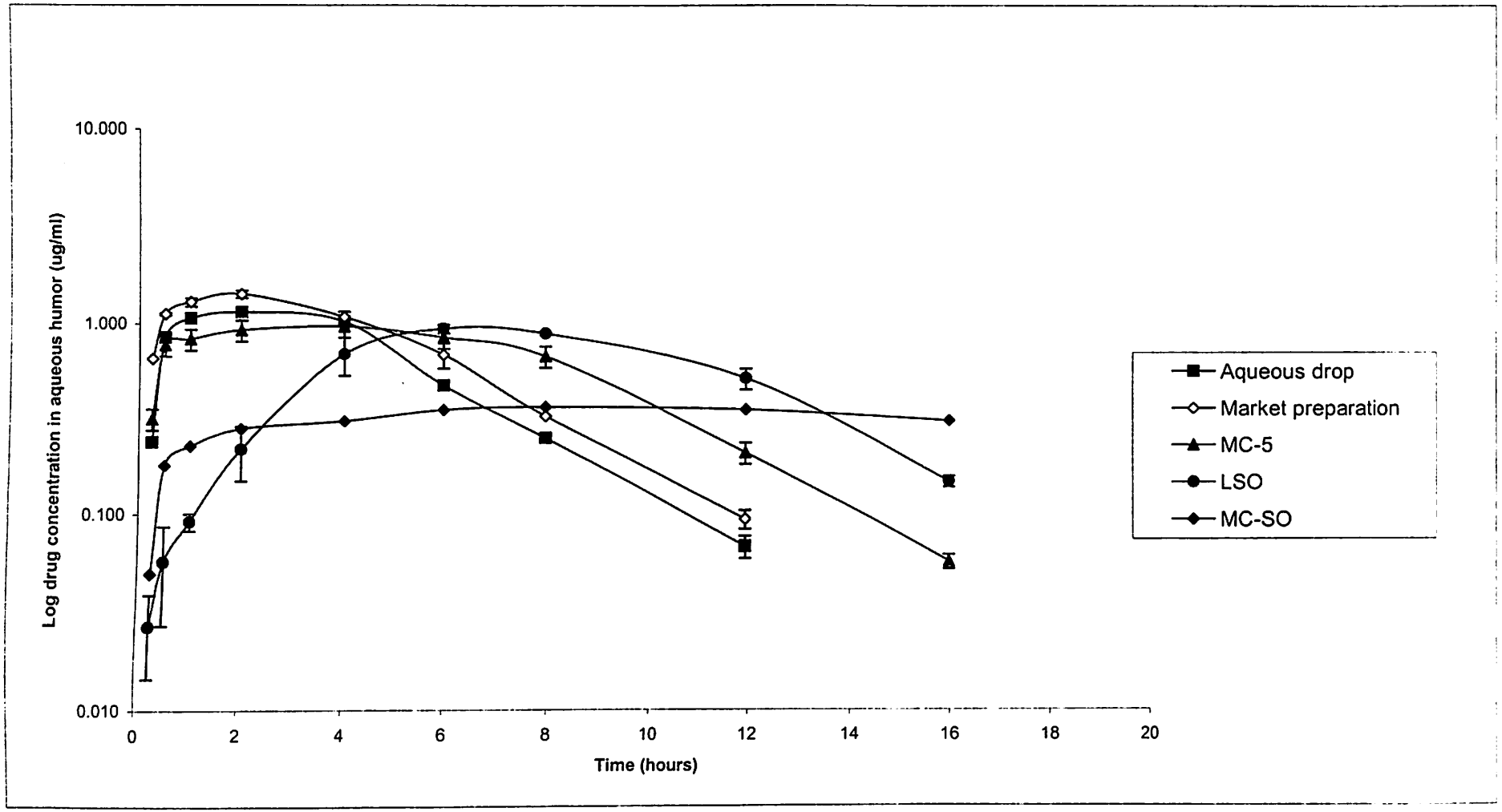

Figure 7.12: Aqueous humor concentration-time profile of flurbiprofen after topical administration of designed liquid formulations.

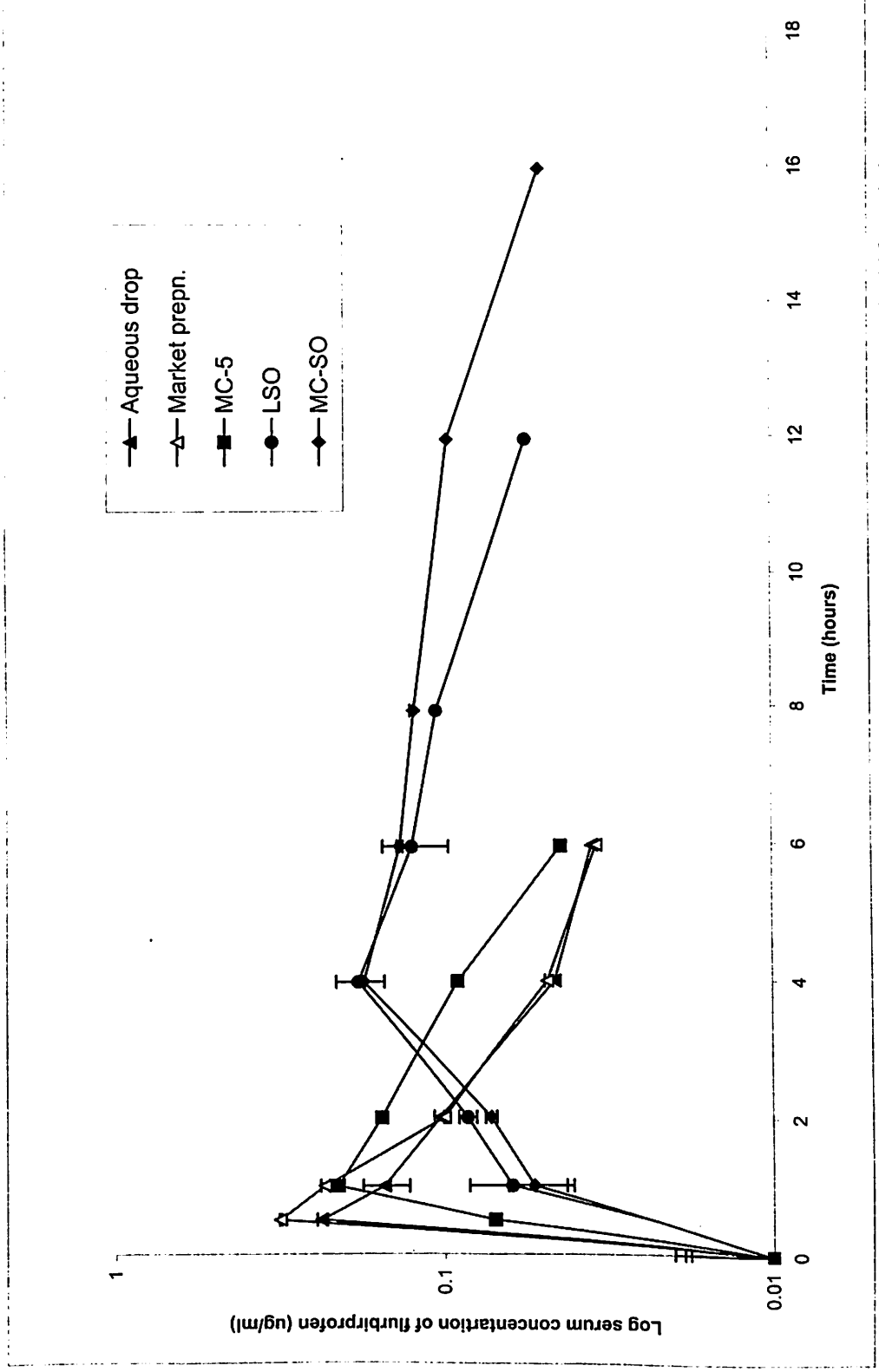

Figure 7.13: Serum concentration-time profile of flurbiprofen after topical administration of designed liquid formulations.

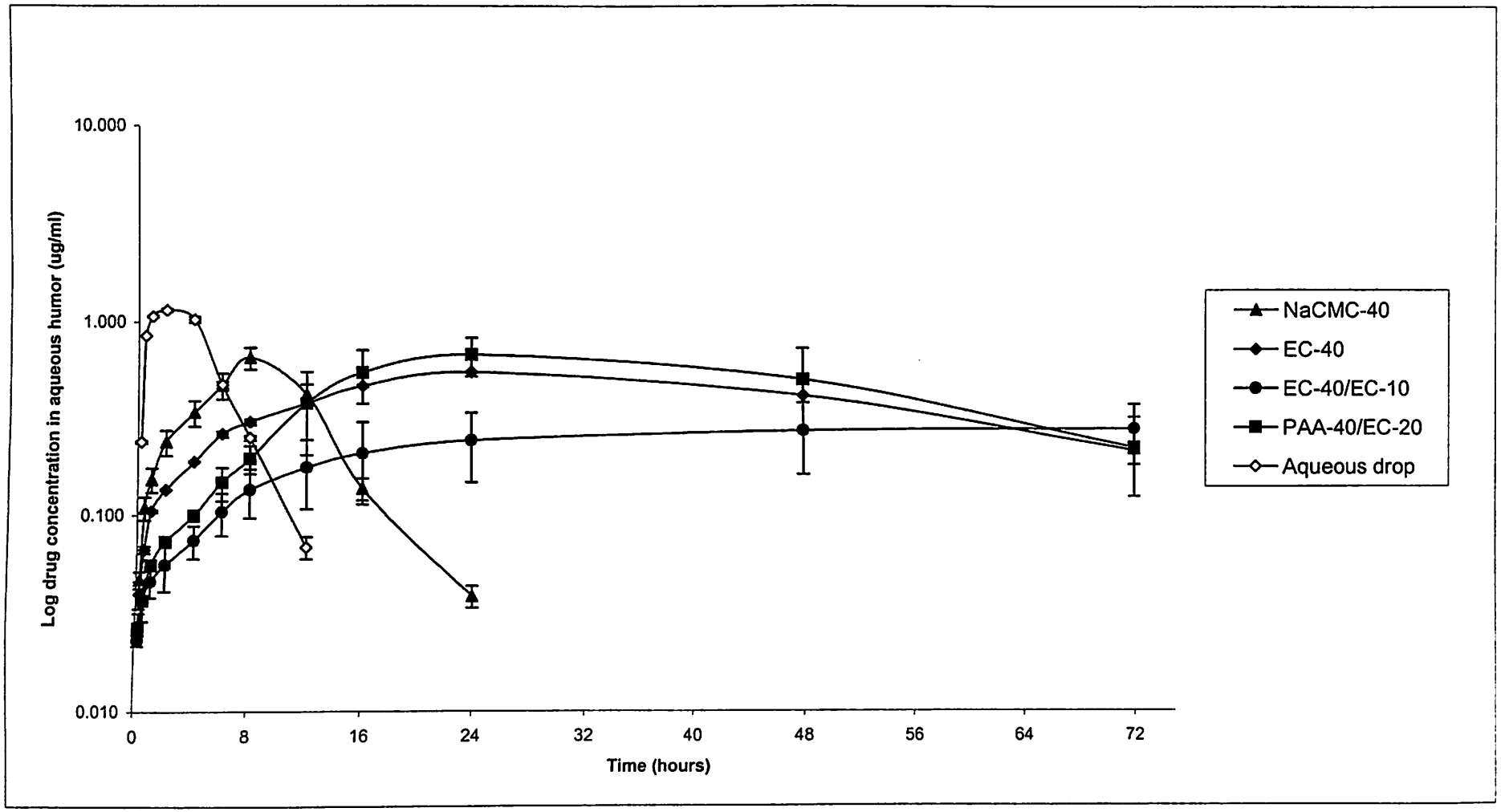

Figure 7.14: Aqueous humor concentration-time profile of flurbiprofen after topical administration of designed uncoated/ coated polymeric discs.

CHAPTER 8

CONCLUSIONS

8. CONCLUSIONS

The aim of this study was to design and study better ocular drug delivery systems for flurbiprofen by extended or controlled release formulations. This has been achieved by viscosity enhancement of the formulation vehicle and by formulating polymeric matrix based drug delivery discs using different polymers.

As a necessity, to support the whole work, new analytical methods were developed and validated for the estimation of flurbiprofen in pure form, in formulations and in biological samples like, blood serum and aqueous humor. Developed UV spectrophotometric, spectroflourimetric and liquid chromatographic methods for estimation of flurbiprofen in formulations and liquid chromatographic method in biological samples were found to be accurate, precise, rugged, reproducible and simple. The methods were validated as per USP 2000, ICH guidelines and by recovery studies.

Based on the preformulation studies done, it can be concluded that in solid drug-excipient mixture, flurbiprofen was found to be fairly stable with moderate to negligible degradation. Increased degradation was observed at higher humidity conditions because of the tendency of the hydrophilic excipients to absorb moisture. Flurbiprofen was found to be compatible with phosphate and borate buffer and incompatible with citrate and citrate-phosphate buffer. Stability of flurbiprofen increased with increase in pH of the buffered and unbuffered media, with lower rate of degradation in buffered media in comparison to unbuffered media. Acid catalysis seemed to be the mechanism of degradation of flurbiprofen in buffered and unbuffered solutions. Among different vegetable oils studied, the drug was found to be most stable in sesame oil and least stable in olive oil. Flurbiprofen was found to have a poor solubility in triple distilled water. Highest solubility was observed in borate buffer and lowest in citrate buffer. Solubility was found to increase with increase in pH in case of both buffered and unbuffered medium. Amongst the vegetable oils studied, maximum flurbiprofen solubility was observed in sesame oil and minimum in castor oil. The log P value of the drug decreased with increase in pH of the aqueous phase in both buffered and unbuffered system.

The developed polymeric gel based and vegetable oil based formulations of flurbiprofen were found to possess good physical properties with no or insignificant batch-to-batch variation in formulation consistency and quality. Viscosity and mucoahesiveness of the gel was found to increase with increase in the proportion of the polymer in the formulation. The duration of release was extended with the increase in the percentage of polymer in the gel (from 1 % w/v

to 5 % w/v) in case of all the polymers with MC-5 (5 % w/v methyl cellulose) extending the duration of release to beyond 7 hours. Release of flurbiprofen from different vegetable oil based formulations was extended up to 4–5 hours probably because of higher solubility of the drug in the selected oils, thereby decreasing the partitioning of the drug into the dissolution medium. Polymeric gels and vegetable oil combination were found to control the release of the drug better than either polymer or vegetable oil alone, with release extended from 3 hours to 40 hours. This was probably due to the emulsification of drug loaded oil globules in polymeric gel, which would have retarded the release further. The release profile was found to follow first order kinetics in case of all the designed formulations.

The designed and developed matrix embedded discs, with or without EC coat, possessed good physical properties and release character without any batch-to-batch variation. The duration of release was extended with the increase in the percentage of polymer in the matrix embedded discs. EC matrix (50% w/w EC) was found to prolong the duration of flurbiprofen release beyond 36 hours and PAA (50 % w/w PAA) and CMC (50 % w/w CMC) extended release only up to 5 hours. Since matrix embedded formulations containing 40 % w/w of the polymer gave satisfactory initial release in the first 15 mins, except in case of EC discs, separate initial dose is not required. Duration of release was extended to several days to weeks by providing a hydrophobic polymeric coat of EC to polymeric discs. In this case also, coated EC matrix discs extended the release up to 10 days (EC-40/EC-10) and beyond 4 weeks (EC-40/EC-20). The release from polymeric matrix discs was found to follow non-Fickian kinetics in most cases and Fickian kinetics in few.

In all the designed formulations, flurbiprofen was found to be fairly stable with commercially viable predicted shelf life at 25 °C with insignificant difference between predicted and observed shelf life. The products were found to be stable to respective sterilization procedures employed.

Corneal permeability of flurbiprofen from aqueous drops was very low, whereas in case of market formulation (OCUFLUR^{TM}), the permeability was considerably enhanced probably due to the presence of formulation additives like, PMN. The results of various transcorneal permeability studies indicated that an increase in pH of the media decreased the permeability of the drug. Compounds like, BAC, THM, CB, PMN and EDTA significantly increased the rate of drug corneal permeation but with a high corneal hydration between 83 to 86 %, indicating the adverse effects of these agents on the corneal cell structure and its integrity. Transcorneal permeability studies involving mucoadhesive polymers revealed that increased

adherence of the formulation to the corneal epithelium facilitated increased partitioning and permeation of the drug. Permeability results of selected vegetable oil based formulations correlated well with the solubility data of flurbiprofen with permeability decreasing with increase in solubility of the drug in the oil. Permeability character of flurbiprofen from selected polymeric discs as well as from polymer coated discs showed statistically insignificant difference in the permeation characteristics compared to aqueous drop.

Ocular safety studies by Draize's test protocol revealed that most of the formulations were non- irritating and few were found to be practically non-irritating to the rabbit eye. *In-vivo* ocular bioavailability studies revealed that *in-vitro* extension of the release of the drug from the designed formulations manifested in the form of enhanced area under the aqueous humor drug concentration time profile and mean residence time of the drug in the aqueous humor. In case of MC-SO and EC-40/EC-10, log aqueous humor drug concentration profile in the later period was found to be constant, therefore predicting that drug availability from such formulations will continue to be constant for a long period of time.

A good inverse correlation was obtained between time for 90 % of the drug released *in-vitro* ($t_{90\%}$) and $AUC_{0\text{-}24hr}$ *in-vivo* for developed liquid topical preparations, but in case of polymeric discs type formulations only a moderate correlation was seen. Good correlation was obtained between $t_{90\%}$ *in-vitro* and MRT *in-vivo*. A very good correlation with high 'r' value was obtained between t_{max} and $t_{50\%}$ and between t_{max} and $t_{70\%}$ respectively.

The developed products of flurbiprofen need to be studied on human volunteers for prolonged pharmacodynamic effect of the drug. Further studies can be carried out using combination of hydrophilic and hydrophobic polymers as matrix base. Also, polymer coated matrix embedded discs may be considered for implantable intraocular devices for NSAIDs, antibiotics and anti-viral drugs used in ocular cytopathologies.

APPENDIX A-1

LIST OF SYMBOLS AND ABBREVIATIONS

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/ Abbreviation	Meaning
#	Number
%	Percentage
% RSD	Percentage relative standard deviation
% v/v	Percentage volume by volume
% w/v	Percentage weight by volume
% w/w	Percentage weight by weight
%/min	Percentage per minute
~	Approximately
α	Alpha
β	Beta
ω	Omega
λ_{em}	Emission wavelength
λ_{ex}	Excitation wavelength
λ_{max}	Wavelength of maximum absorption
°A	Degrees Armstrong
°C	Degrees centigrade
µg.min/ml	Micrograms.minute per milliliter
µg/hr(s)	Micrograms per hour(s)
µg/min/cm^2	Micrograms per minute per square centimeters
µg/ml	Micrograms per milliliters
µl	Microliters
µl/min	Microliters per minute
µm(s)	Micrometer(s)
µV.sec	Microvolts.second
<	Less than
>	Greater than

®	Registered trademark
ANOVA	Analysis of variance
AO	Arachis oil
AUC	Area under the aqueous humor drug concentration time profile
$AUC_{0-\infty}$	Area under the aqueous humor drug concentration time profile from zero time to infinity
$AUC_{0-t\ hr}$	Area under the aqueous humor drug concentration time profile from zero time to t hr
AUMC	Area under the moment curve
b.i.d.	Two times daily
BAC	Benzalkonium chloride
BCOP	Bovine corneal opacity and permeability
BP	British Pharmacoepia
C.V.	Coefficient of variation
C-18	Octa decyl silane column packing
$CaCl_2$	Calcium chloride
CB	Chlorobutanol
cm	Centimeters
cm/sec	Centimeters per second
cm^2	Square centimeters
cm^2/min	Square centimeters per minute
C_{max}	Maximum concentration
CMC	Carboxy methylcellulose
CNS	Central nervous system
CO	Castor oil
COX	Cyclo oxygenase
cps	Centipoise
CRT	Controlled room temperature (25 ± 3 °C and 55 ± 15 % RH)
CSO	Cottonseed oil
D	Diffusion coefficient
day^{-1}	Per day

DF	Degrees of freedom
DNA	Deoxyribose nucleic acid
e.g.	Example
EC	Ethyl cellulose
EDTA	Ethylene diamine tetra acetic acid
EGF	Epidermal growth factor
et al	And co-workers
F-value	The measurement of distance between individual distributions
F_{Calc}	Calculated F-value (Ratio of mean square of X and mean square of error)
F_{Crit}	Critical F-value from F-statistics table
US FDA	Food and drug administration, United States of America
FGF	Fibroblast growth factor
FL	Fluorescein leakage
FT	Refrigerated condition (4 ± 3 °C)
g	Grams
GF	Growth factors
$H_2PO_4^-$	Monobasic phosphoric acid
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
$hour^{-n}$	Per hour raised to the power 'n', where 'n' is the diffusional exponent
HPLC	High performance liquid chromatography
HPMC	Hydroxy propyl methyl cellulose
HPO_4^{2-}	Dibasic phosphoric acid
hr(s)	Hour(s)
HSV	Herpes simplex virus
i.e.	That is
ICH	International Conference on Harmonization
IL-1	Interlukin-1
IP	Indian Pharmacoepia
IR	Infra red
IRE	Isolated rabbit eye

K	Calculated release rate constant ($time^{-1}$)
K_2HPO_4	Potassium dihydrogen phosphate
K_{30}	First order degradation rate constant at 30 °C
KCl	Potassium chloride
K_{CRT}	First order degradation rate constant at CRT
K_{deg}	First order degradation rate constant
kg(s)	Kilogram(s)
kg/cm^2	Kilograms per square centimeters
L1	Octa decyl column packing as per USP 2000
LC	Liquid chromatography
LC1	Octa decyl column packing as per IP 1996
LOD	Limit of quantitation
log P	Log partition coefficient (measure of lipophilicity)
LOQ	Limit of detection
LPS	Lipopolysaccharides
LSO	Linseed oil
M	Molar (e.g., 0.1 M NaOH is 0.1 molar NaOH solution)
MC	Methyl cellulose
mEq/l	Miliequivalents per liter
mg	Milligrams
mg/kg/day	Milligrams per kilogram body weight per day
$MgSO_4$	Magnesium sulphate
MHB	Methyl hydroxy benzoate
min(s)	Minute(s)
min^{-1}	Per minute
ml	Milliliters
ml/min	Milliliters per minute
mm Hg	Millimeters of mercury
mm	Millimeters
mm^2	Square millimeters

mOsm/l	Milliosmolars per liter
mPa	Millipascals
mRNA	Messenger RNA
MRT	Mean residence time
MS	Mean sum of squares
MSSR	Mean sum of squared residuals
M_t/M_∞	Fraction of drug released at any time 't'
MW	Molecular weight
n	Diffusional exponent, indicative of the release mechanism
N	Normal (e.g., 0.1 N NaOH is 0.1 normal NaOH solution)
$Na_2HPO_4.7H_2O$	Disodium hydrogen phosphate with seven water molecules of crystallization
$NaH_2PO_4.H_2O$	Sodium dihydrogen phosphate with one water molecule of crystallization
NaAL	Sodium alginate
NaCl	Sodium chloride
NaCMC	Sodium carboxymethyl cellulose
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
ng/ml	Nanograms per milliliters
NIR	Near infra red region
nm	Nanometers
NODS	New ophthalmic delivery system
NRR	Neutral red release
NSAID	Non steroidal anti-inflammatory drug
OO	Olive oil
OTS	Ocular therapeutic system
p or p-value	Probability of observing a test statistic that is as extreme or more extreme than currently observed, assuming that the null hypothesis is true
PAA	Polyacrylamide
PCB	Polycarbophil 934 NF
PDGF	Platelet derived growth factor

PG	Prostaglandin
PGH-2 S-2	Prostaglandin H-2 synthase-2
pH	Negative logarithm of hydrogen ion concentration
PHB	Propyl hydroxy benzoate
pK_a	Negative logarithm of acid dissociation constant
PMN	Phenyl mercuric nitrate
PMT	Photo multiplier tube
PVA	Polyvinyl alcohol
PVP	Polyvinyl pyrolidone
q.i.d.	Four times daily
q.s.	Quantity sufficient
QC	Quality control
R	Correlation coefficient
RCS	Rabbit aorta contracting substance
RH	Relative humidity
RNA	Ribonucleic acid
RP	Reverse phase
rpm	Revolutions per minute
S.D.	Standard deviation
sec(s)	Second(s)
SO	Sesame oil
SODI	Soluble ocular drug inserts
SS	Sum of squares
T	Absolute temperature, in Kelvin
$t_{1/2}$	Half life
$t_{30\%}$	Time for 30 % of drug release
$t_{50\%}$	Time for 50 % of drug release
$t_{70\%}$	Time for 70 % of drug release
t_{90}	Time for drug concentration to fall to 90% level (shelf life)
$t_{90\%}$	Time for 90 % of drug release
TDW	Triple distilled water

TGFb	Transforming growth factor-b
THM	Thiomersal
t_{max}	Time to reach maximum concentration
TNF	Tumor necrosis factor
TS	Test solution
USP	United States Pharmacoepia
UV	Ultra violet
VS	Volumetric solution
vs.	Versus