Studies on Design and Evaluation of Controlled Release Colon Targeted Formulations of Indomethacin

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LAILA FATIMA ALI ASGHAR

Under the Supervision of

Dr. SAJEEV CHANDRAN

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CERTIFICATE

This is to certify that the thesis entitled, "**Studies on Design and Evaluation of Controlled Release Colon Targeted Formulations of Indomethacin**" and submitted by **Laila Fatima Ali Asghar** ID.No. **2004PHXF419** for award of Ph.D. degree of the Institute embodies original work done by her under my supervision.

Date: / / 2008

(Dr. SAJEEV CHANDRAN)
Assistant Professor of Pharmacy &
Assistant Dean
Engineering Services Division
B.I.T.S., Pilani

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List of Abbreviations / Symbols

μ	Micro
#	Sieve (ASTM) size no
λ_{max}	Wavelength of maximum absorbance
AT_1	Angiotensin
ATC	Accelerated conditions (40 ± 2°C/ 75 ± 5% RH)
ACE	Angiotensin converting enzyme
ANOVA	Analysis of variance
ASA	Amino salicylic acid
AUC	Area under curve
BP	British Pharmacopeia
C_{max}	Maximum serum concentration
CI	Confidence interval
Conc.	Concentration
CP	Carbopol 934P
CRC	Colorectal cancer
CRT	Controlled room conditions (25 ± 2°C / 60 ± 5% RH)
CV	Coefficient of variance
DSC	Differential scanning calorimetry
DTPA	Diethylene triamine pentaacetic acid
EC	Ethyl cellulose
EL100	Eudragit L100
ES100	Eudragit S100
et al.	Co-workers
FAP	Familial adenomatous polyposis
FTIR	Fourier transform infrared
GI	Gastro intestinal
GG	Guar gum
HCl	Hydrochloric acid
HEC	Hydroxy ethyl cellulose
HPC	Hydroxy propyl cellulose
HPMC	Hydroxy propyl methyl cellulose
HPLC	High performance liquid chromatography
HQC	High quality control concentration
IBD	Inflammatory bowel disease
ICH	International conference on harmonization
ICJ	Ileo caecal junction
IP	Indian Pharmacopeia
IR	Infrared
J/g	Joules per gram

K	Release rate constant
K_{deg}	Degradation rate constant
KH_2PO_4	Potassium dihydrogen phosphate
log P	Log of n-octanol water partition coefficient
LQC	Lower quality control concentration
M	Molar
MQC	Medium quality control concentration
MSSR	Mean sum of squares
NaOH	Sodium hydroxide
nm	Nanometer
^oC	Degree centigrade
PCP	Polycarbophil
pH	Negative log to the base 10 of hydrogen ion concentration
r	Correlation coefficient
r^2	Regression coefficient
RH	Relative humidity
RSD	Relative standard deviation (%)
rpm	Revolutions per minute
SD	Standard deviation
$t_{1/2}$	Biological half life
$t_{10\%}$	Time taken for 10% drug release
$t_{90\%}$	Time taken for 90% drug release
$T_{1/2}$	Time taken for 50% gastric emptying
$T_{90\%}$	Time taken for drug concentration to reduce to 90% of its labeled claim (expiry date)
TDW	Triple distilled water
^{99m}Tc	Technetium-99m
T_{max}	Time to reach maximum concentration
USP	United States Pharmacopeia
UV-Vis-NIR	Ultraviolet- visible- near infrared
v/v	Volume by volume
w/w	Weight by weight
XG	Xanthan gum

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ABSTRACT

The aim of the present study was to design and develop matrix-embedded formulations for colon specific delivery of indomethacin for the potential treatment of colorectal cancer. Indomethacin, a non-steroidal anti-inflammatory drug, commonly indicated in the treatment of osteo and rheumatoid arthritis, has shown good potential as an anti cancer agent against colorectal cancer. Therefore, a colon targeted formulation of indomethacin will ensure high local concentrations of drug in colon and reduce upper gastrointestinal and systemic toxicities that arise from the use of its conventional formulations.

For the purpose of formulation design, a novel matrix system that would combine the advantages of pH and transit time controlled systems and overcome the problems associated with coated systems was proposed. It was expected that a dual polymer matrix embedded system comprising of a combination of time or swelling controlled and pH dependent polymers can offer a suitable means of achieving a pH and transit time dependent system that releases the drug in a bimodal (sigmoidal fashion). Therefore, the primary objective of the study was to investigate the effect of pH sensitive polymers in a matrix base individually and in combination with other polymers (hydrophilic and hydrophobic) to develop delayed release pH and time controlled formulations. For this purpose, the formulations were designed as single-unit (tablet) and multi unit (microsphere) based systems. Single matrix embedded tablet formulations were prepared by wet granulation technique and microspheres were prepared by oil/ oil solvent evaporation technique. The prepared tablet formulations were characterized for physical characteristics, in vitro drug release, release kinetics, batch reproducibility and stability on storage while the microparticles were characterized for particle size, physical characteristics, in vitro release, batch reproducibility and stability on storage. Some of the formulations were also investigated for their GI transit and residence studies in rat model and human subjects.

The prepared tablets were found to be of acceptable quality with low weight variation and uniform drug content while the microparticles were spherical, free flowing, distributed over a narrow size with 70-80% entrapment. Preliminary evaluation of formulation in distilled water followed by testing in pH 7.4 revealed that initial release from almost all designed formulations was inhibited to varying extents and was followed by controlled release in buffer (pH 7.4) with gradual increase in release rate with time. Release was complete in a period of 14-18 h in most cases and was dependent on polymer type,

proportion and combination. Selected designed formulations were also evaluated in a medium of simulated gastrointestinal pH. The drug release profile from these formulations in most cases was characterized by an initial lag time period of 4-6 h with low drug release (< 7 -10%) followed by controlled release phase in phosphate buffer media for about 14-16 h and several formulations showed good similarity with target release profile. In case of microparticulate based systems, spherical and discrete microspheres were obtained by means of a solvent evaporation process. The drug release from these formulations was found to be sigmoidal with low initial release followed by complete release in 16-18 h for most formulations. Stability studies as well as drug excipient compatibility studies using thermal analysis and FTIR did not reveal any instability or presence of physical and chemical interaction in both tablet and microsphere based formulations, implying that drug was stable in designed matrices. In vivo studies of selected formulations in healthy Wistar rats showed that drug release from the formulations was dependent on pH and transit time and there was minimum release from the formulations in the initial period. Formulations tested in healthy human subjects for matrix integrity and residence time in different parts of gastrointestinal tract revealed satisfactory matrix strength of the formulations with no adhesion or stagnation in any region during transit. It was concluded that the prepared matrix systems could have potential applications for colon-specific drug delivery.

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Chapter One *Introduction & Literature Survey*

Over the past few decades, with the emerging need for patient acceptability and compliance and the increasing number of new drug entities and biologicals becoming available as therapeutics, drug delivery has turned into core technology to derive market advantage in pharmaceutical industry. It has evolved from development and use of conventional oral and topical systems to sustained and novel drug delivery systems and currently focuses attention on design and development of site specific or targeted delivery systems. Site specific delivery serves to deliver the drug at the site of action so that there is therapeutically effective concentration of drug available locally and thereby avoids the unwanted distribution of drug to other parts of the body. This would also help reduce the dose of the drug as well as prevent the occurrence of adverse effects due to its presence in systemic circulation.

Amongst all the routes of drug delivery, the oral route is the most preferred route on account of high patient acceptability, ease and convenience of administration, flexibility at the time of process scale-up and manufacturing, and availability of advanced packaging technology. However, the oral route becomes a restricted choice in case of delivery of peptides and other sensitive biologicals as well as for drugs that are unstable in the harsh conditions of the upper gastrointestinal (GI) tract or have a poor or erratic absorption through the oral route. It has been reported several decades ago with the discovery of sulfasalazine for the treatment of inflammatory large intestine disease that the large intestine (colon) might serve as a useful alternative site for delivery of drugs and substances that could not otherwise be delivered by the oral route (Sack and Peppercom, 1983). In a sharp contrast to the acidic and weakly acidic conditions and high enzymatic activity in the upper GI tract, the colon, has near neutral to slightly alkaline pH and relatively low enzymatic activity and hence provides the rational basis for site specific delivery of drugs that are susceptible to stability issues in the upper GI tract (Van der Mooter, 2006). Site specific delivery of drug entities to colon is also advantageous in case of local pathologies of colon like irritable bowel disease and colorectal cancer (Kinget et al. 1998). Various techniques that would prevent drug release in other parts of GI tract and facilitate selective delivery into the colon has been investigated in the last 10-15 years.

In the following sections, a brief description of the anatomy and physiology of the colon is presented followed by the importance of colon specific delivery in the light of the

diseases that commonly affect the colon, the biological triggers that can be utilized to ensure colon specificity in drug delivery and recent advances in colon targeting. Methods available for in vitro and in vivo evaluation of colon specific formulations have also been reviewed. Further, advancements reported in the delivery of peptides and proteins, nonsteroidal anti inflammatory drugs (NSAIDs) and anticancer agents to the colon has been highlighted.

1.1. Anatomy and physiology of colon

The upper GI tract comprises of the stomach and small intestine. The major features of the different regions of the GI tract are presented in Table 1.1. The empty stomach has a resting volume (fluid content) of about 25-50 ml. The daily secretion of stomach is around 2 litres and comprises of acid, pepsinogen, gastrin and intrinsic factor. The pH is highly acidic (≈ 1). The bacteria in stomach comprise of acid-resistant species that are Gram positive and aerobic like lactobacilli, streptococci and yeasts. Absorption of drugs and nutrients through the stomach is minimal. The small intestine comprises of duodenum, jejunum and ileum. The small intestine has large surface area for absorption of nutrients and drugs and secretes pancreatic and other enzymes like dissacharidases (α-amylase and carboxypeptidases) and peptidases (trypsin, chymotrypsin) and lipases. The pH varies from 4.5 in duodenum to 7.0-7.5 in distal ileum (Guyton and Hall, 2006). Around 10 litres of fluid pass through the small intestine everyday out of which 90% is absorbed and 10% passes onward to the colon. The bacterial species present comprise of mixture of gram positive and gram negative bacteria like lactobacillus, *Escherichia coli, bacteroides*. The peristaltic movements are slow and regular throughout the stomach and small intestine (Friend, 1991).

The human large intestine is about 1.5 m long and extends from the distal end of the ileum to the anus. The caecum forms the first part of the large intestine and leads to the right colon or the ascending colon followed by the transverse colon, the descending colon, sigmoidal colon, rectum and the anal canal (Fig 1.1). The main functions of the colon are storage of waste like indigestible fibre, absorption of water and electrolytes like sodium, magnesium and chloride from chyme. During its transit, the chyme gets solidified and gets mixed with bacteria and mucus to become feces (Labianca et al., 2004). The two aspects that distinguish the colonic environment from the upper GI tract are the pH and the colonic bacteria known as gut flora.

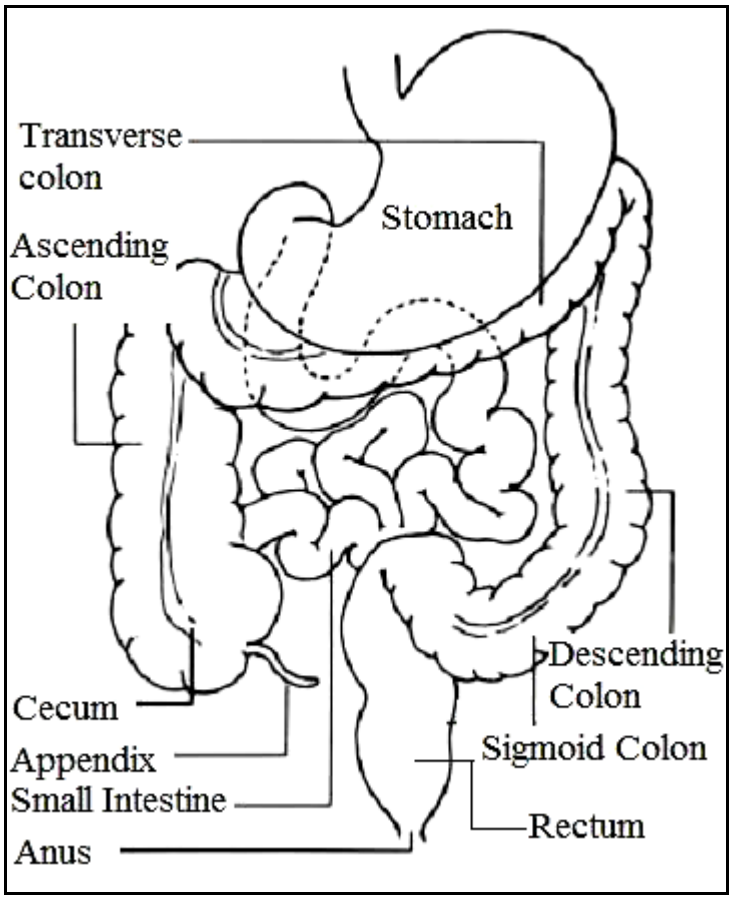


Fig 1.1: Diagram of the human GI tract showing various components of colon

Table 1.1: Characteristics of the human GI tract (Friend, 1991; Sinha and Kumria, 2003)

Characteristic	Stomach	Duodenum	Jejunum	Ileum	Colon
Length (cm)	-	20-30	150-250	200-350	90-150
pH	1-3.5	5.0-7.0	6.0-7.0	7.0-7.5	5.5-7.0
Bacterial count (CFU/ml)	10^3	10^3	10^3	10^4	10^{11-12}
Normal transit time	1-2 h	1 h	1-2 h	1-2 h	10 - 40 h*

* Highly variable (Hinton et al., 1969; Follonier and Doelkar, 1992)

The lumenal pH of the ascending colon is slightly on the acidic side (5.5 - 6.5) due to fermentation of undigested food fibre and carbohydrates that results in the production of organic acids which lower the pH (McDougall et al., 1993). The pH of the transverse and descending colon is neutral to slightly alkaline (7.2 - 7.4). Another important feature is the presence of microflora (10^{11} – 10^{12} CFU per ml) which comprises of anaerobic and facultative anearobic microorganisms that produce a variety of enzymes. The high concentration of bacterial flora in this region is attributed to the neutrality in pH and slow

transit rate of the contents in the large intestine (Sinha and Kumria, 2003). As many as 400 different bacterial species are found in this region, the predominant ones being *bacteroides, bifidobacterium, eubacterium* and *enterobacteriacae* (Hill and Draser 1975, Finegold et al., 1977). The wide range of various enzymes produced by these bacteria includes β-glucuronidase, β-xylosidase, α-arabinosidase, β-galactorolesidase, nitroreductase, azoreductase, deaminase, urea hydroxylase. etc (Simon and Gorbach, 1984). Peristalsis in colon occurs only once or twice in a day and comprises of strong propulsive movements that transport the contents from sigmoid colon to the rectum for secretion. The mean colonic transit time in humans is highly variable and reported to be as high as 33 h in men and 47 h in women (Hinton et al., 1969). In a recent study, the mean transit time of gadolinium-saline solution filled capsules was reported to be 41 ± 9 h in women and 31±10 h in men (Buhmann et al., 2007).

1.2. Pathology of colon

The two major diseases of colon are inflammatory bowel disease (ulcerative colitis and Crohn's disease) and colon carcinoma. An understanding of the disease progression, its pathophysiology and alteration in GI conditions due to these diseases is quite essential for successful design of a colon specific drug delivery system.

1.2.1. Inflammatory bowel disease

Inflammatory bowel disease (IBD) includes both ulcerative colitis and Crohn's disease (Kirsner et al., 1991; Delcò and Sonnenberg, 1999). Ulcerative colitis is an inflammation that occurs in the innermost lining (mucosa) of the colon and rectum and is characterized by tiny open sores, or ulcers, formed on the surface of the lining, which bleed and produce pus or mucus. The inflammation usually begins in the rectum or lower colon and can extend to the entire colon. Clinical symptoms of ulcerative colitis include rectal bleeding, abdominal pain, constipation or diarrhoea, weight loss, fatigue and fever (Shanahan, 1993). Similarly, Crohn's disease, also called ileitis or enteritis, is a deeper inflammation of intestinal wall, with bleeding which if left untreated can get serious and result in anemia (Farrell and Peppercom, 2002). Symptoms of this include cramping, abdominal pain, diarrhoea, fever, weight loss, anal pain, rectal abscess, fissure and joint pain. It can also get complicated by the formation of tunnels or fistulas which can get infected too. The treatment comprises of use of antidiarrhoeal and antispasmodics for symptomatic relief (Järnerot et al., 1998). The bowel inflammation is controlled with 5-amino salicylic acid

(5-ASA) and related compounds. Other drugs include antibiotics and corticosteroids along with immunosuppressive compounds based on intensity and severity of disease and patient requirement (Guslandi, 1998).

Although the pH along the GI tract of healthy subjects is reasonably well characterized (Sasaki et al., 1997), the luminal pH of the distal intestine in patients with IBD is lower than that seen in healthy people. It has been reported that pH in the colon drops by 1-2 to several units in patients with ulcerative colitis (Fallingborg et al., 1993). Similarly, the colonic transit is reported to be less than normal in case of disease conditions like IBD, which is characterized by diarrhoea and disturbances in normal colon function (Rao et al., 1987; Khosla and Davis, 1991; Hardy et al., 1998).

1.2.2. Colon cancer

Colon cancer includes cancerous growth of the colon, rectum and appendix. In the United States, colorectal cancer (CRC) is the third most common cancer diagnosed among men and women. It is the second leading cause of death from cancer. CRC is the second cause of cancer related death in industrialized countries (Montemurro et al., 2008). Both ulcerative colitis and Crohn's disease carry an increased risk of developing colorectal cancer (Zisman and Rubin, 2008). It develops in the large large intestine as benign, non-cancerous adenomatous polyp(s) which eventually increases in size and becomes cancerous. Symptoms include change in large intestine habits, bloody faeces and obstruction. CRC largely can be prevented by the detection and removal of adenomatous polyps, and survival is significantly better when CRC is diagnosed while still localized (Levin et al., 2008). Primary treatment is surgery followed by adjuvant therapy with 5-fluorouracil and levamisole (Connors et al., 1995). Oxaliplatin-based chemotherapy is now considered the standard adjuvant treatment in patients with CRC. Oral fluoropyrimidines play an important role in the management of colorectal cancer and can be currently considered an alternative to 5-fluorouracil (Mano and Duhoux, 2008). Moreover, genetically engineered antibodies like cetuximab and bevacizumab have shown good efficacy in combination with chemotherapy (Montemurro et al., 2008).

1.3. Rationale for colon targeting

A conventional oral dosage form for any ailment of the colon (inflammatory large intestine disease and colon cancer) will result in release of major drug load in stomach and small intestine (the absorption window of most drugs) leading to systemic absorption and drug distribution throughout the body. This would result in non-specific action and

systemic accumulation of drug leading to unwanted side effects. A dosage form that can release the drug directly in the colon will result in more effective and localized action, reduced dose, and also reduce systemic drug load and adverse effects resulting from non-specific action. Colon is also attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability. Additionally, the colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs (Bai et al., 1995). For the treatment of diseases like hypertension and asthma, drug release is usually required to be delayed from the time of administration in order to match with the body's circadian rhythm to maximize efficacy of therapy (Bruguolle and Lemmer, 1993). In such cases, a delayed release system targeting the colon which shall provide a time lag of 4-6 h is suitable. Further, due to a non-hostile environment [low enzyme (peptidase) activity, near neutral pH], the colon has also been investigated as a site for absorption of protein and peptide drugs that otherwise cannot be given by the oral route (Rubinstein, 1995; Basit, 2005; Sinha et al., 2007).

1.4. Mechanisms and approaches to colon specific drug delivery

A variety of approaches have been identified for achieving colon selectivity in drug delivery (Rubinstein, 1995). These include:

i) *pH dependent systems*: These are intended to utilize the relatively high pH gradient between the upper GI tract and the distal ileum /colon as a trigger for drug release.

ii) *Time controlled devices*: These are based on the mechanisms that prevent drug release in upper GI tract such that the drug is released only after a predefined lag time corresponding to the gastric emptying and small intestinal transit time. This lag time is usually 4-5 h and is the time taken by any solid dosage form to reach the colon.

iii) *Bacteria based or enzyme controlled systems*: These employ the selective degradation by colonic microflora as trigger for drug release. These comprise broadly of prodrugs based on linkages that are cleaved by bacterial enzymes in the colon or of certain non-starch polysaccharides that are indigestible in upper GI tract and are susceptible to breakdown and fermentation in the colon.

iv) *Pressure based systems*: These systems utilize the high pressures that are prevalent in the colon due to peristalsis to trigger drug release.

Apart from these conventional approaches, multi- unit formulation approach has also been investigated for colon targeting. A concise description of various techniques is highlighted in subsequent sections.

1.4.1. pH controlled systems

These systems have mostly employed the use of pH sensitive polymeric coatings on dosage forms. One way of protecting the dosage form is by application of thick enteric coats (Marvola et al., 1999). Most commonly, copolymers of methacrylic acid and methyl methacrylate that dissolve at pH 6.0 (Eudragit L100) and pH 7.0 (Eudragit S100) have been investigated (Ashford et al., 1993a). Most studies have utilized mixtures of these polymers in coating in order to achieve drug release in the pH range of 6.0 – 7.0 as the use of single polymer coated systems has been shown to be unsuitable for colonic delivery (Khan et al., 1999). Studies with Eudragit S100 coated tablets in humans have shown that drug release in the colon is not sufficiently reproducible (Leopold, 1999). Eudragit coat dissolution sites can vary from the ileum to the splenic flexure, indicating a lack of site specificity.

Recently, a new polymer Eudragit FS 30 D has been introduced for colonic delivery. It is an ionic co-polymer of methyl acrylate, methyl methaacrylate and methacrylic acid and is pH sensitive and dissolves at pH above 6.5 and therefore, can be used alone for coating purposes. This polymer has a similar threshold dissolution pH as Eudragit S100, but dissolves in a slower and more controlled manner (Basit et al., 2005). A series of in vitro dissolution studies have indicated the usefulness of this polymer in the coating of tablets (Ibekwe et al., 2006a), alginate beads (Iruin et al., 2005), and pellets (Rudolph et al., 2001) for drug delivery to the ileo-colonic region in comparison to the more established Eudragit S100. Another polymer that is intended for drug targeting to distal colon is Eudragit P4135F that dissolves above pH 7.2 (Hu et al., 1999). The advantage of such systems is that it can be easily manufactured on a large scale in a reasonable processing time using conventional powder layering and fluidized bed coating techniques. Examples of pH based systems for colonic delivery are summarized in Table 1.2.

In an attempt to develop a pure pH based system, Akhgari et al. (2005) employed a mixture of Eudragit L100 and Eudragit S100 in different proportions for coating indomethacin pellets. Later, the same group reported a coating system in which in addition to Eudragit L100 and Eudragit S100, a controlled release polymer Eudragit RS was also added to the coating solution in order to develop a combined time and pH based formulation. It was found that the pellets released no drug at pH 1.2 and 6.5; release was slow at pH 6.8 and it was fast at pH 7.2. Further, polymethacrylates in combination with inulin were also tried as coating system and were shown to be potentially more colon specific when compared to coating with pH dependent polymers alone (Akhgari et al.,

2006). Recently, Ibekwe et al. (2008a) explored the use of Eudragit S100 with starch in a single matrix film for combined pH-responsive and bacterially-triggered drug delivery. Radiolabeled placebo tablets were coated with this film and the GI transit was monitored in healthy human subjects through gamma scintigraphy. The tablets were shown to resist breakdown in stomach and small intestine. The dosage form was found to disintegrate either at the ileocaecal junction or in the large intestine in all the subjects.

Table 1.2: Examples of use of synthetic pH sensitive polymer based coating systems for colonic delivery of various drugs

Polymer (s) used	Drug used	Formulation	Reference
Eudragit L100 and S100	Mesalazine	Pellets	Khan et al., 1999
Eudragit L100 and S100	Diclofenac sodium and 5-ASA	Coated tablets	Cheng et al., 2004
Eudragit S100, Eudragit FS 30 D, Eudragit P4135 F	Prednisolone	Coated tablets	Ibekwe et al., 2006
Eudragit L 30 D-55 and Eudragit FS 30 D	Paracetamol	Enteric coated capsules	Cole et al., 2002
	Indomethacin	Pellets	Akhgari et al., 2005
Eudragit FS 30 D/ Eudragit RL/RS	5-ASA	Coated beads	Gupta et al., 2001
Eudragit S100	Ibuprofen	Matrix pellets	Krogars et al., 2004
Eudragit FS 30 D	5- Fluorouracil	Matrices	Zambito et al., 2005

The application of double microencapsulation technique to obtain pH dependent microparticles employs a solvent evaporation process to form drug loaded polymeric cores which are encapsulated in pH sensitive polymer coating to yield coated microparticles. An example from a recent study is the formation of budesonide loaded PLGA microparticles which were encapsulated with Eudragit S100 and evaluated for colon specific release. It was shown that this technique could be a promising tool for the site specific and controlled delivery of budesonide in the treatment of Crohn's disease (Krishnamachari et al., 2007). This technique has also been shown to be useful in encapsulation of a low molecular weight heparin in pH sensitive microparticles. The tissue permeability of heparin (enoxaparin) loaded microspheres was shown to be selectively enhanced in the inflamed regions (Meissner et al., 2007; Pellequer et al., 2007).

The inter and intra subject variability in gastrointestinal pH, other intrinsic variables such as electrolyte concentration and transit time can affect the in vivo behaviour of pH responsive systems, ranging from early drug release in the small intestine to no release at

all (Ashford et al., 1993b). This is especially true for patients suffering from ulcerative colitis wherein pH gradient and transit time is different than normal (Fallingborg et al., 1993; Hardy et al., 1998). Therefore, a colonic delivery system which is based only on pH changes of the GI tract may not achieve dependable results. Hence a more suitable system is one that combines the advantages of pH with time or bacteria dependent mechanisms to achieve more reproducible and reliable drug release.

Literature is replete with examples where combination of techniques have been employed to develop colon targeted systems. Of late, a combined pH, time and enzyme controlled system to deliver anti-inflammatory drug 5-ASA to the colon was developed (Nunthanid et al., 2007). Since in patients with Crohn's disease, the luminal pH of the colon is acidic (≈ 5.3), chitosan acetate was used that can swell and dissolve in this acidic medium. In order to confer a time lag in initial drug release, HPMC was added to the compression coat. The degradation of chitosan acetate by microbial enzymes triggered drug release in the colon for patients with Crohn's disease. Similarly, cores of chitosan or guar gum containing diltiazem HCl were coated with inulin (inner layer) for bacterial degradation and shellac (outer layer) for pH and time protection. This resulted in the formation of a combined pH, time and bacteria dependent delivery system (Ravi et al., 2007). Chitosan has also been complexed with succinic acid, adipic acid, and suberic acid to form salts that exhibited pH and bacterial dependent release for colonic delivery of vancomycin (Bigucci et al., 2008a).

In another case, pellets of 5-ASA prepared by extrusion spheronization were coated with three layers- the outer layer comprised of Eudragit L30D-55 to confer protection against gastrointestinal pH, the intermediate layer comprised of ethylcellulose to inhibit drug release during passage through the small intestine, and the inner film was coated with pectin for swelling and enzyme based degradation in the colon (Fude et al., 2007).

On the other hand, in case of severe inflammatory large intestine disease, the average colonic pH of 6.4-7.0 often drops to the range 1.0-5.0, and thus the above formulations are unable to provide adequate drug release. In such cases a formulation that releases the drug at an acidic pH should be used. Such a drug formulation has been developed by Leopold and Eikeler (1998), consisting of a drug core, an acid-soluble basic polymer layer such as aminoalkyl methacrylate copolymer (Eudragit E) or polyvinyl acetal diethylamino acetate and an enteric coating for initial enteric protection. Variability in luminal pH may affect the performance of preparations that deliver 5-ASA in a pH dependent manner, whereas transit time can affect those that depend on bacterial cleavage for release of the active 5-

ASA. pH sensitive delivery systems, in spite of their limitations, are commercially employed for the delivery of mesalazine (Mesacol)®, budesonide (Budenofalk®, and Entocort®,) and 5-ASA (Asacol®, and Salofalk®,) for the treatment of inflammatory large intestine disease.

1.4.2. Time controlled systems

Time controlled oral delivery systems for colon targeting have been reviewed by Gazzaniga et al. (2006). These systems are based on triggering drug release after predetermined lag times. To attain colonic release, the lag time should equate to the time taken for the system to reach the colon. In the case of coated dosage forms designed for time controlled drug release, the lag time usually depends on the coating thickness, and drug release can be triggered either by a change in pH, a change in the osmotic pressure, or by disruption of the coating by swelling of the core (Leopold, 1999). These systems are usually enteric coated to overcome variability in gastric emptying. However, drug release from such systems is not controlled by pH of the surrounding medium. Some examples of swelling controlled delayed release systems are shown in Table 1.3. A delayed release system can be exemplified by a hydrogel compression coated tablet that is able to release the core drug after a certain period of time and has the potential for colon specific drug delivery based on gastrointestinal transit time concept (Wu et al., 2007).

The first time based system was the Pulsincap device which consisted of an impermeable capsule filled with drug and secured at one end with a hydrogel plug (Stevens et al., 2002). On contact with gastrointestinal fluids, the plug gets hydrated and then starts to swell in a time dependent manner and finally getting ejected from the capsule body, to allow drug release. The lag time is controlled by the size and composition of the plug. Another delivery system called the ‘Time Clock’ has also been designed to release the drug in the colon. It is composed of a solid dosage form coated with a thick film comprising of hydrophobic surfactant layer and a water soluble polymer. This outer layer disperses in the aqueous environment in a time dependent manner proportional to the thickness of the film and the core is then available for dispersion in the colon (Steed et al., 1994).

Osmotic pumps are devices that ensure zero order release from drug delivery systems. With incorporation of suitable delay mechanisms, these systems can act as colon targeted systems. A miniature osmotic pump (Osmet®) was developed (ALZA Corporation, USA) which could pass through the stomach and small intestine and deliver its contents (240 μl) over eight hours in the large intestine (Chacko et al., 1990). In vitro studies proved that the

pumps could start to discharge after four to five hours and emptied drug at a reasonably constant rate of 9.6% /h for 9-16 h. In vivo studies using gamma scintigraphy in seven healthy subjects showed that the pumps arrived in the caecum by 6.4 h (range 5-9). Mean start-up time was 5.3 h and the rate of discharge was 15.9 μl/h for pumps studied from 6-12 h and 17.2 μl/h for those studied from 10-20 h. This device was shown to be simple, safe and effective for the delivery of tracer substances to the caecum and colon.

Table 1.3: Examples of delayed release swelling controlled systems for colonic delivery of various drugs

Polymer (s) used	Drug used	Formulation type	Reference
Hydroxy propyl methyl cellulose	Pseudo ephedrine HCl	Coated tablets	Halsas et al., 2001
Hydroxyethyl cellulose, ethyl cellulose, microcrystalline cellulose	Theophylline	Matrix tablets	Alvarez et al., 2004
Lactose and behinic acid	Indomethacin	Dry coated wax matrix tablets	Peerapattana et al., 2004
Hydroxy propyl methyl cellulose	Placebo	Coated tablets	Sangalli et al., 2004

Savastano et al. (1997) described an osmotically controlled device for colonic delivery which comprised of a solid core of the drug coated with a delayed release coat. This was then coated with a semipermeable membrane, which could be drilled to provide a release orifice, which was further optionally coated with an enteric material. It was intended to resist dissolution in 3 h in the intestinal fluids, and about 70 to 80% of its drug in the colon.

Another example of an osmotic device is chitosan based osmotic pump in which the property of chitosan to form gel and dissolve in acidic media (of ascending colon) has been used for producing porosity in the semipermeable membrane as well as creating osmotic pressure inside the device for initiating drug release (Liu et al., 2007).

1.4.3. Bacterial enzyme controlled systems

The resident gastrointestinal bacteria provide a good means of effecting drug release in the colon as mentioned previously. Colonic bacteria are predominantly anaerobic in nature and produce enzymes that are capable of metabolising various substrates, such as carbohydrates that are not digested in the upper gastrointestinal tract. In this context, there are two approaches namely - prodrug and use of naturally occurring polysaccharides in

drug delivery. These are considered resistant to the conditions of the stomach and small intestine, but are susceptible to degradation by bacterial enzymes within the colon.

1.4.3.1. Prodrug approach

For the purpose of colonic delivery, prodrugs are designed in such a way as to undergo minimal absorption and hydrolysis in the upper portion of the GI tract and undergo enzymatic hydrolysis in the colon, thereby releasing the active drug moiety from the carrier (Sinha and Kumria, 2001a). Usually bulky hydrophilic groups are used as carriers in order to bypass absorption in upper GI tract. The metabolism of azo compounds by the azo reductase enzyme secreted by intestinal bacteria is one of the most extensively employed bacterial metabolic processes. Khan et al., in 1977 found that the active moiety in sulphapyridine (effective in IBD) was 5-ASA and sulphapyridine only acted as a prodrug. The azo bond present in sulphapyridine between these two moieties undergoes reduction in the colon by azo reductase enzyme. Prodrugs have been made of 5-ASA which has been linked with various carriers via the azo linkage. Examples are ipsalazide (in which 4-amino benzoyl glycine is the carrier moiety), balsalazide (benzoyl-β-alaninine is the carrier) and olsalazide, where two molecules of 5-ASA are joined together and one acts as a carrier for the other (Chan et al., 1983).

A number of other linkages susceptible to bacterial hydrolysis specifically in the colon have been investigated where the drug is attached to hydrophilic moieties like glycosides, amino acid, glucuronic acid, glucose, galactose, cellulose, etc. (Simpkins et al., 1988). Sugar moieties like glucose, galactose and cellobiose have also been conjugated to drug moieties to form glycosides. The drug-sugar glycosidic linkage was found to be selectively hydrolyzed by glucosidase, galactosidase or cellobiosidase enzymes of bacterial origin in the caecum and colon, thus ensuring site specificity in drug release (Friend et al., 1992). Further, amino acids consisting of polar groups like $–NH_2$ and –COOH have also been reported as carriers for colon specific delivery (Nakamura et al., 1992 a,b,c). The intestinal microflora of the colon release several amidases that could hydrolyze the drug-amino acid linkage and the drug was released free in the lumen of the colon.

The synthesis of azo-linked polymeric prodrugs wherein polymers are linked via an azo linkage to drugs and thereby act as their carriers is another concept utilizing prodrug approach (Brown et al., 1983). A prodrug consisting of a non-absorbable sulphanilamido ethylene polymer was linked to 5-ASA, and was found to be more effective than sulphasalazine in reducing inflammation in guinea pig. Cyclodextrins (alpha, beta and

gamma) have been linked with 5-ASA to form prodrugs with minimum absorption /degradation in upper GI tract and site specific degradation of the conjugate in colon (Zou et al., 2005). Examples of other moieties include nicotinoyl and glycyl groups to form colon specific prodrug of 5-fluorouracil (Lee et al., 2007).

Dextran-budesonide conjugates were prepared with different molecular weights of dextran for site-specific delivery of budesonide in the treatment of ulcerative colitis (Varshosaz et al., 2008). Drug release was less than 10% in the stomach and small intestine of rat GI tract, while about two-fold increase was observed after incubating the conjugates with colonic luminal contents. Prodrugs of some non-steroidal anti-inflammatory drugs (NSAIDs) like naproxen, sulindac and flurbiprofen with alpha or beta-cyclodextrin was developed as colon targeted delivery systems for treatment of inflammatory bowel diseases (El-Kamel et al., 2008). The carboxylic group of those drugs was conjugated onto the amino group of l-aspartic acid or the hydroxyl group of alpha- or beta-cyclodextrin. Prodrug hydrolysis did not occur in buffers of pH range 1.2-7.2 while in colonic fluid that comprised of rat gastrointestinal tract homogenate drug release was significant.

The limitations of synthesizing prodrugs are that their formation depends upon the functional groups available on the drug moiety for chemical linkage. Moreover, they are new chemical entities from regulatory perspective and need a lot of non-clinical and clinical evaluation before being approved for use as drug carriers. So far this approach has been primarily restricted to designing better therapeutic options for the treatment of IBD (Kumar and Mishra, 2008).

1.4.3.2. Azopolymeric systems

The role of azo compounds in colon specific delivery have been reviewed by Roldo et al. (2007). Various azo polymers that selectively degrade in the colon have also been evaluated as coating materials over drug cores. These have been found to be similarly susceptible to cleavage by the azoreductase in the large intestine. The azo polymer is essentially a copolymer of 2-hydroxyl ethyl methacrylate (HEMA) with methyl methacrylate synthesized in the presence of bis(methacryloylamino) azobenzene. The films are resistant to degradation in simulated gastric and intestinal fluids and the presence of azo (-N=N-) group renders them susceptible to reduction by azoreductase secreted by intestinal flora (Van den Mooter et al., 1992).

Few other film coating polymers have also been reported. In one study, a coating system comprising of poly (ether-ester) azopolymer was synthesized and used to coat

capsules of ibuprofen. It was found that the capsules were protected in simulated upper GI tract conditions while increased degradation was observed in presence of rat caecal contents. Similarly, azo containing polyurethane was synthesized and proved effective as a coating polymer for colon targeting (Yamaoka et al., 2006).

1.4.3.3. Hydrogels

They consist of a hydrogel network, which is progressively degraded via the cleavage of the azo cross links in the colon by the azoreductase enzyme produced by the colonic microflora (Shantha et al., 1995). The colonic specificity is achieved due to the presence of pH sensitive monomers and azo-crosslinking agents in the hydrogel structure. During transit through the upper GI tract, the swelling capacity of the hydrogel increases as the pH increases, upto a pH of 7.4. Upon arrival in the colon, the hydrogels reach a degree of swelling that makes the crosslinks accessible to the enzyme azoreductase. Subsequently the hydrogel network is degraded and entrapped drug is released (Chiu et al., 1999). For example, azo polymeric hydrogels were developed for colon specific targeting by copolymerizing methacryloyloxy azobenzene with hydroxyethyl methacrylate. The in vitro release studies of the incorporated 5-fluorouracil were carried out in simulated gastric and intestinal fluids. The in vitro release profiles of the drug showed a zero order pattern in the presence of azoreductase in the culture of intestinal flora. The release was faster and almost followed. This can be attributed to the cleavage of the azo crosslinks in the hydrogel by the azoreductase and thereby affecting release of the entrapped drug at the target colonic site.

An azo-polysaccharide gel- based system has also been proposed that utilizes the principle of dual mechanism of reduction of the azo-groups in the crosslinks as well as enzymatic break down of the polysaccharide backbone for drug release. Azo dextran and azo inulin gel were synthesized and their susceptibility to bacterial degradation was studied in the presence of enzymes (dextranase and inulinase) and rat caecal contents for assessing reduction of azo group (Stubbe et al., 2001). It was found that azo-dextran gels degraded better than azo-inulin gels in the presence of enzymes and rat caecal content

Dextran hydrogels based on the crosslinking between dextran and diisocynate have shown potential for colon targeted delivery due to their insolubility in acidic to weakly acidic pH and selective degradation in the presence of the enzyme dextranase, as well as in rat caecal and human faecal medium (Hovgaard and Bronsted, 1995). More recently, biodegradable dextran hydrogels were synthesized by crosslinking dextran with

epichlorohydrin for the in vitro colon specific delivery of salmon calcitonin and other polypeptides. In vitro release studies indicated that 85% of the loaded salmon calcitonin was released in 17 h in simulated gastrointestinal fluid with dextranase (Basan et al., 2007). In another study, novel hydrogels with polysaccharide–polyaminoacid structure which would be able to undergo an enzymatic hydrolysis by dextranase in the colon were synthesized using methacrylated dextran and methacrylated α, β-poly (N-2-hydroxyethyl)-dl-aspartamide. These were shown to be potentially useful for delivery of beclomethasone dipropionate in the treatment of inflammatory large intestine disease (Pitarresi et al., 2007). A novel hydrogel system based on a polymeric network formed between a natural polymer psyllium and methacrylamide/polyacrylamide was developed to form pH based polysaccharide hydrogels (Singh et al., 2007 a,b).

A composite hydrogel based on a methacrylated and succinic derivative of dextran and a methacrylated and succinic derivative of alpha, beta-poly (N-2-hydroxyethyl)- dl-aspartamide was prepared with the purpose to obtain a colon specific drug delivery system with pH-sensitive behavior and enzymatic degradability (Casadei et al., 2007). The designed hydrogel that showed a pH-responsive swelling in simulated gastrointesinal fluids, and underwent degradation by dextranase and esterase enzymes and therefore, was termed as being potentially colon specific.

1.4.3.4. Polysaccharides

Polysaccharide systems have been shown to be the most promising tools for colon targeting because of their practicality and utilization of abundantly available colonic microflora (Rubinstein, 1990; Basit, 2005). This microflora content is uniquely different from other portion of GI tract. They include naturally occurring polysaccharides obtained like guar gum, inulin, chitosan, chondroitin sulphate, alginates or dextran origin. They are highly stable, safe, non-toxic, and hydrophilic and form excellent gels and in addition are biodegradable (Hovgaard and Brondsted, 1996). These polysaccharides are broken down by the colonic microflora to simple saccharides. However, they are hydrophilic in nature, which renders them either soluble or prone to swelling in an aqueous environment of the upper GI tract and hence unsuitable as singular drug carriers for colonic delivery. Polysaccharides have been used to design into matrix based systems or as compression coats around drug cores (Sinha and Kumria, 2001b; Jain et al. 2007a). In order to render them suitable as carriers for colonic delivery, some form of structural modification and/or formulation strategy is required. For example, in the case of pectin, a methoxylated derivative which is poorly water-soluble was utilized to form a thick compression coating

on tablets (Ashford et al., 1993c). Calcium pectinate has been used as a capsule (Xu et al., 2005) and as multi-unit beads for colonic delivery of drugs (Jain et al., 2007b). The calcium pectinate capsules were prepared by dipping a glass of stainless steel rod successively into pectin and calcium chloride solutions followed by subsequent air-drying and coating. In vitro studies showed that the release of the loaded drug (5- fluorouracil) from capsule increased significantly in the presence of rat caecal contents. It was shown through scintigraphy studies in human subjects that the capsule released the contents selectively in the colon. Calcium pectinate microspheres have been employed to encapsulate methotrexate for colon specific release (Chaurasia et al., 2008). The microspheres were found to degrade selectively in simulated colonic fluid in the presence of pectinase.

A novel water-soluble derivative of chitosan [N-(2-carboxybenzyl) chitosan] with pH sensitive swelling properties was synthesized as a hydrogel based carrier for colonic delivery of 5 -fluorouracil (Lin et al., 2007). The hydrogel was separately tested for in vitro release in two media, pH 1.0 and pH 7.4. Although an initial burst release was observed in both the media, drug release was 40% at the end of 12 h in pH 1.0 and 90% at the end of 10 h in pH 7.4. The polymer was termed as being useful for colon specific release. Similarly, in another study, pectinate gel beads were prepared by ionotropic gelation method with different solutions of cross-linking agents like calcium and zinc (Chambin et al., 2006). Zinc counter ions resulted in the formation of a stronger pectin matrix than calcium. The gel beads were found to release the drug only in colonic fluid with pectinolytic enzymes. Guar gum has been crosslinked with glutaraldehyde to form microspheres (Chaurasia et al., 2006) and hydrogel discs (Das et al., 2006) for better protection of entrapped moiety during transit through upper GI tract. Chemically crosslinked galactomannan was modified by simple enzymatic procedure to form a novel hydrogel matrix that retains the drug until it reaches the colonic environment wherein bacteria secrete enzymes like beta-mannanase to degrade the gel and release the entrapped drug (Burke et al., 2005).

Other naturally occurring polysaccharides have also been modified to render them suitable for colonic delivery. For example, chitosan has been crosslinked with tripoly phosphate to form hydrogel beads with reduced water solubility. Such a system was capable of entrapping protein with minimum loss during upper GI transit (Zhang et al., 2002). Examples of some polysaccharide based matrix and compression coating systems are presented in Table 1.4.

Compression coating is one of the approaches for delaying the release of drugs. Recently, mixtures of xanthan gum, boswellia gum and hydroxypropyl methylcellulose (HPMC) have been reported as coating materials for compression coating of 5-flourouracil core tablets (Sinha et al., 2007). In vitro release studies conducted in simulated gastric and intestinal fluid showed that the tablets released less than 6% drug in initial 5 h and about 80-96% in 24 h. The degradation of tablets enhanced significantly in the presence of 2% rat caecal contents in release media.

Table 1.4: Polysaccharides in matrix systems and as compression coating

Polymer (s) used	Drug used	Formulation type	Reference
Chitosan	Sodium diclofenac	Microspheres	Lorenzo- Lamosa et al., 1998
	Insulin	Enteric coated chitosan capsules	Tozaki et al., 1997a
Chitosan succinate/ phthalate	Sodium diclofenac	Matrices	Aiedeh et al., 1999
Pectin (calcium pectinate)	Indomethacin	Matrix tablets	Rubinstein et al., 1993
Amidated pectin	Ropivacaine	Chitosan coated beads	Ahrabi et al., 2000
	Indomethacin/ sulphamethoxazole	Amidated pectin beads	Munjeri et al., 1997
	Ketoprofen	Microparticles	El- Gibaly et al., 2002
Zinc pectinate	Paracetamol	Coating film	Wakerly et al., 1996
Pectin (as mixed films)	Theophylline	Coating film	Semde et al.,1998, 2000
Guar gum	Dexamethasone	Matrix tablet	Kenyon et al., 1997
	Technecium -^{99m}	Matrix tablet	Wong et al., 1997
	5-ASA	Matrix tablet	Krishnaiah et al., 1998,1999
	Indomethacin	Matrix tablet	Rama Prasad et al., 1998
Borax –crosslinked guar	Indomethacin	Matrix tablet	Rubinstein and Kabir, 1995
Chondroitin sulphate crosslinked	Indomethacin	Matrix tablet	Rubinstein et al. 1992 a,b

Other examples include physical mixtures of pectin with chitosan and /or HPMC (Ugurlu et al., 2007). Since pectin alone is ineffective in preventing drug release in upper GI tract, it was combined with HPMC for conferring a time lag in initial release. In vitro release studies showed good retardation in initial release in pH 1.2 and 3.3 and highest degradation in pH 6.8 in the presence of pectinolytic enzymes. In another study, two gums - khaya and albizia were evaluated as compression coatings for targeted drug delivery to the colon (Odeku et al., 2005). The core tablets were compression coated separately with khaya and albizia gum along with a mixture of both. Drug release studies indicated that both gums were capable of protecting the core tablet in the physiological environment of the stomach and small intestine while they were susceptible to degradation by the colonic

bacterial enzymes when studies were carried out done using rat caecal matter in phosphate buffered saline at pH 6.8 (simulated colonic fluid).

Polysaccharides are hydrophilic and tend to swell in the GI tract and become porous, resulting in the early release of the drug. One way of protecting a polysaccharide based system is application of an outer pH based polymeric coat which will prevent contact with biological fluids during transit through upper GI tract. For example, tablet matrix comprising of various polysaccharides like guar gum, xanthan gum, pectin, carrageenan, beta-cyclodextrin and indigenously developed graft copolymer of methacrylic acid with guar gum were coated with Eudragit L100-55 and tested in vitro for suitability as colon-specific drug delivery systems (Mundargi et al., 2007).

Uncoated tablets containing xanthan gum or mixture of xanthan gum with graft copolymer showed 30-40% drug release during the initial 4-5 hrs, whereas for tablets containing GG with the graft copolymer, it was 70%. After enteric coating, the release in the initial phase was reduced to 18-24%. However, the other polysaccharides could not prevent drug release under similar conditions. A multiparticulate system of cross-linked chitosan microspheres was prepared by coating with Eudragit L-100 and S-100 as pH-sensitive polymers for combined pH and bacteria dependent release of metronidazole (Chourasia and Jain, 2004).

The use of polysaccharides for coating purposes has gained momentum in the recent past. Most of the non-starch polysaccharides suffer from the drawback of lacking good film forming properties. Chemical modification of some of the polysaccharides has been attempted in the pursuit to confer on them film forming property. In a break through study by Cummings et al. (1996), the ability of amylose as a film forming polymer was investigated. Amylose which is the major fraction of starch, possess the ability to form films through gelation. A particular form of coating, comprising of amorphous amylose is resistant to degradation by pancreatic alpha amylase but is capable of degradation by colonic bacterial enzymes (Milojevic et al., 1996). When arriving in the colonic region, the films are structurally weakened, allowing the swelling and subsequent fermentation of the amylose, which ultimately leads to drug release. In order to confer strength to the films, it has been used in combination with ethylcellulose (Siew et al., 2000 a, b). An ethylcellulose/glassy amylose matrix film is now commercially available as COLAL™ (Alizyme Therapeutics Ltd, Cambridge, UK). Pectin has also been combined with ethyl cellulose to form a film coating polymer for colonic delivery of 5-flourouracil pellets (Wei et al., 2007; Fan et al., 2008). Polyelectrolyte complex (PEC) film between pectin as an

anionic polyelectrolyte and chitosan as a cationic species was prepared by blending the two polymer solutions. Besides pectin/chitosan PEC film, Eudragit RS, pectin/Eudragit RS and pectin/chitosan/Eudragit RS films have also been prepared and characterized for colon specificity (Ghaffari et al., 2007; Bigucci et al., 2008b). Guar gum has also been utilized as a film coating material for colonic delivery of 5- fluorouracil (Ji et al., 2008). The guar gum-based multi-unit pellet system was prepared by coating guar gum and pH-sensitive polymer Eudragit FS30D sequentially around drug-loaded non-pareil cores in a fluid-bed coater. The outer Eudragit FS coating protects the system against gastrointestinal environment while the inner guar gum coating worked as a time-controlled retardant and offers additional protection of the pellets until it is degraded by microbial enzymes at the proximal colon.

The effect of acetylation on the swelling and enzymatic degradation of maize starch has been investigated. Release of bovine serum albumin from its tablets coated with the above polymer was studied. It was shown that the resistant starch content and swelling property of maize starch was increased by acetylation, which retarded its enzymatic degradation in upper GI tract while degradation was complete in the colon. The results suggested that acetylated starch could be used as a potential carrier for targeted delivery of drug to the colon. In another study, resistant starch prepared by pre-gelatinization and cross-linking treatment was used as a carrier in tablet matrix for colon targeting (Chen et al., 2007). Other polysaccharides investigated for colon specific delivery include lauroyl dextran, galactomannan, inulin, psyllium, gellan gum amongst others.

1.4.4. Pressure controlled drug release systems

A pressure controlled drug delivery system relies on the high pressure produced by peristalsis in the distal colon to trigger drug release in the colon The formulation, which consists of a gelatin capsule with an inner ethylcellulose coating, get disrupted and disintegrates by the pressure in the colon due to the destructive force of peristaltic waves. The rate of drug release depends on the thickness of the inner ethylcellulose film. The capsule is filled with a solution of the drug which is considered advantageous due to the presence of only a small amount of fluid in the distal colon, which otherwise could compromise drug dissolution process. Large scale production of such devices has also been attempted (Hu et al., 1998). The capsular shaped suppositories were coated with different levels of ethyl cellulose solution. For the purpose of in vivo evaluation, the empty capsules were filled with flourescein and administered to beagle dogs. The first

appearance of flourescein in plasma was taken to be a parameter for estimating the time at which the pressure controlled capsules burst in the gastrointestinal tract (Hu et al., 1998). Release was found to correlate well with thickness of ethyl cellulose coat. Similar devices containing caffeine have also been tested in human subjects (Muraoka et al., 1998). In another study, two polymer coats were applied. The inner one was a water-insoluble polymer membrane, ethylcellulose. The outer one was an enteric polymer membrane, hydroxypropylmethylcellulose phthalate or hydroxypropylmethylcellulose acetate succinate (Jeong et al., 2001). The formulation was evaluated for colon specificity in vivo in beagle dogs. It was shown that drug was detected in plasma after 3.5 ± 0.7 h of administration which is approximately the colon arrival time (3.5 ± 0.3 h) in beagle dogs. Hence it could be proved that the designed capsules were colon specific.

1.4.5. Multi unit formulation approach to colonic delivery

Studies have shown that multi unit drug delivery systems perform better in vivo than single unit systems, as they spread out throughout the length of the intestine causing less irritation, have a slower transit through the colon and give a more reproducible drug release (Davis et al., 1991; Rudolph et al., 2001). A multi unit system for colon targeting is less susceptible to variations in GI transit time and therefore, is more reliable for colon targeting than single unit systems (Asghar and Chandran, 2006). Thus, multiparticulate and multi unit systems like pellets (Gupta et al., 2001; Krogars et al., 2004), hydrogel beads (Sriamornsalk et al., 1998; El-Gibaly et al., 2002) and mini matrices (Zambito et al., 2005) have been attempted for colonic delivery. Often, pH based coating of these formulations utilizing Eudragit polymers has been attempted to confer on them additional protection during their upper GI transit (Chourasia and Jain, 2004; Mundargi et al., 2007).

pH sensitive natural polymers alone or in combination with other polymers have been employed to design multi particulate systems. For example, there has been increasing interest in the study on alginate/chitosan microparticles as carriers for controlled release of proteins and drugs for their biodegradable and mucoadhesive properties (Wittaya-areekul et al., 2006). Alginates has the property to swell in alkaline pH 7.4 and therefore, chitosan alginate microparticles for colonic delivery of 5-ASA in the treatment of induced colitis in rat model have shown preferential localization of the drug in the colon with reduced systemic absorption (Mladenovska et al., 2007).

It has been shown that drug carrier systems with a size larger than 200µm are subjected to speedy large intestine evacuation due to diarrhoea, resulting in a decreased GI

transit time and decreased efficiency (Watts et al., 1992). Therefore, a multiparticulate system in < 200 μm size range could be a useful alternative in the design of a suitable dosage form for IBD. In this context, pH sensitive microparticles of tacrolimus and 5-fluorouracil (Lamprecht et al., 2003, 2004) were prepared using Eudragit P 4135-F for colonic delivery for the treatment of IBD and colorectal cancer respectively. Nanoparticles have been shown to have better uptake by inflamed mucosal tissue in IBD (Lamprecht et al., 2005a; Meissner et al., 2006). Tacrolimus loaded poly (lactic-co-glycolic acid) nanoparticles were prepared and entrapped into pH-sensitive microspheres comprising of Eudragit P 4135-F in order to avoid premature uptake or degradation of nanoparticles during their passage through the small intestine (Lamprecht et al., 2005b). These findings have led to the development of multi unit systems (micro and nanoparticulate) for drug delivery to colon.

1.4.6. Other approaches for colon targeting

The CODES system was designed to overcome the drawbacks of pH, time and enzyme based technologies (Takemura et al., 2000; Watanabe et al., 2002). This system consisted of a core tablet coated with three layers of polymer coatings, an acid soluble polymer layer (next to the core tablet), a barrier layer of hydrophilic polymer HPMC, and an enteric coating layer of Eudragit L100. The core tablet comprised of the active drug, one or more polysaccharides, and other desirable excipients. The system remains intact in the stomach because of the enteric protection, but the enteric and barrier coatings dissolve in the small intestine, exposing the acid soluble coating layer. Upon entry into the colon, the polysaccharide inside the core tablet dissolves and diffuses out. The bacteria enzymatically degrade the polysaccharide into short-chain fatty acids. This lowers the pH surrounding the system sufficiently to affect the dissolution of the acid soluble coating and subsequent drug release. In this way, site specific delivery to the ascending colon is attained. Utilizing this concept, colon specific drug delivery systems have been developed for other drugs like mebeverine hydrochloride (Omar et al., 2007)

The use of monoclonal antibodies and immunoliposomes has also been reported for colon specific delivery. Immunoliposomes are liposomes which are attached with monoclonal antibodies for targeting tumor cells (Theresa et al., 1999). When antibodies that can recognize several different tumor-associated antigens were coupled to the PEG terminus of liposomes, a significant increase in the in vitro binding of liposomes to the target cells was observed. The binding of immunoliposomes containing entrapped

doxorubicin to their target cell population resulted in increased cytotoxicity compared to liposomes lacking the targeting antibody. Tumor specific immunoliposomes were synthesized by coupling anti-BCG monoclonal antibodies to pH sensitive fusogenic liposomes which could fuse with and be internalized into the tumor cells by endocytosis (Mizoue et al., 2002). The formulation was shown to retain specific antigen-binding ability to target cells and the liposomes were considered to undergo receptor-mediated endocytosis and got fused with the endosomal and/or lysozomal membrane after uptake by tumor cells. In another study, monoclonal antibody against the rat colon carcinoma CC531 was covalently coupled to liposomes for targeting the anti-cancer drug 5-fluoro-2′-deoxyuridine to colon cancer cells (Koning et al., 2002). Specific binding of the immunoliposomes to the tumor cells occured. The mechanism for intracellular delivery of the drug was proposed to be selective transfer of the lipophilic prodrug from the liposomes to the cell membrane with subsequent internalization into the cells.

A study describes the preparation of a novel delivery system called the microsponge for delivering flurbiprofen to colon (Orlu, 2006). The drug was entrapped into a commercial microsponge system. The colon specific formulations were prepared by compression coating of microsponges with pectin - HPMC mixture followed by tableting. This study presents a new approach based on microsponges for colon specific drug delivery.

Recently, it was shown that sialic acid is overexpressed in malignant colonic cells and tissue and hence a cationic polymer could be used as a targeting tool to colonic malignant epithelium. This would ensure site specific binding of the carrier which could be used in drug delivery and diagnosis (Azab et al., 2007).

Colloidal carriers have been shown to improve tumor therapy by increased drug delivery into tumor sites resulting directly from the enhanced permeability and retention effect (Koziara et al., 2006). Paclitaxel entrapped in emulsifying wax nanoparticles was shown to overcome drug resistance in a human colon adenocarcinoma cell line (HCT-15). The in vivo efficacy of these in a HCT-15 mouse xenograft model was demonstrated as significant inhibition in tumor growth for the mice receiving treatment with drug entrapped in nanoparticle. Nanoparticles of 5-ASA have been recently investigated for their therapeutic potential in the treatment of inflammatory large intestine disease (Pertuit et al., 2007). This opens up a new frontier in colonic delivery with a huge potential for exploring the various facets of nanotechnology as a tool for better and advanced drug delivery to the colon.

1.5. In vitro methods of testing colon targeted systems

The strategies currently employed in dissolution testing of microflora activated systems have been recently summarized by Yang (2008). One major challenge in the development of colon specific drug delivery systems is to establish an appropriate dissolution testing method to evaluate the designed system in vitro. Factors that complicate the development of such dissolution testing include the simulation of effect of bacterial flora, inadequate understanding of the colon's hydrodynamics and motility, changes in colonic pH and how they are affected by disease conditions (Siew et al., 2000). A number of conventional and unconventional approaches have been reported for evaluating the performance of colon targeted delivery systems in vitro.

In a conventional design for testing simple pH based and other systems, the dissolution media is intended to mimic or simulate conditions that a dosage form is likely to encounter during its transit through human GI tract. It comprises of initial release testing in acidic medium (to simulate gastric conditions) followed by slightly alkaline pH (to mimic small intestine and colon). In a more elaborate model, the in vitro release studies have been reported to be conducted in media of different pH [1.2 (stomach) for 2 h, 6.5 proximal small intestine) for 1 h, 6.8 (lower part of small intestine) for 2 h and pH 7.2 (terminal ileum) for 1 h based on accepted GI transit time (Akhgari et al., 2005, 2006).

Gao et al. (2006) tested the efficiency of Eudragit FS 30D coated pellets (15% w/w polymer coating) by conducting in vitro release studies in 0.1M HCl for 2 h (for establishing acid resistance) followed by separate testing in pH 6.8, 7.0, 7.2 and 7.4 respectively for 4 h to characterize polymer dissolution. It was shown that drug release was rapid in media of pH above 7.2.

For the purpose of testing bacterial enzyme based colon targeted formulations, in addition to simulation of pH conditions, several researchers have also tried to simulate the influence of bacterial flora or show the effect of necessary enzymes on drug release. For example, in the investigation of guar gum compression coated tablets, the in vitro drug release study was carried out in 0.1 M HCl (2 h) and pH 7.4 Sorensen's phosphate buffer (3 h) to investigate the ability of the tablets to remain intact with respect to the pH conditions prevailing in stomach and small intestine (Krishnaiah et al., 1998). Thereafter, the study was continued in medium containing rat caecal contents. As the caecum is naturally anaerobic, the experiment was carried out with continuous CO_2 supply into the

medium. It was shown that drug release was enhanced in the presence of the rat caecal medium.

Similarly, in vitro drug release studies of systems coated with pectin and pectin mixtures have been studied in the presence of pectinolytic enzymes. For example, microsponges and core tablet formulations of flurbiprofen compression coated with pectin and HPMC, the dissolution was carried out in the presence of changing pH media (0.1 N HCl for 2 h followed by pH 6.8) and in the presence of enzymes. Pectinex Ultra SP-L was added to the dissolution medium at 8^{th} h in order to simulate the enzymatic action of the colonic bacteria (Orlu et al., 2007).

Sometimes, the design of dissolution medium is based on simulation of altered conditions that exist during disease. For instance, the efficiency of the compression coat comprising of mixture of spray dried chitosan acetate and HPMC was evaluated in three stages using USP type III apparatus as follows: At first, the drug dissolution was determined in 0.1 N HCl for 2 h (stage I). Afterwards, each tablet was transferred to pH 6.8, tris–HCl buffer and run for 3 h (stage II) and then in pH 5.0 acetate buffer (mimicking patients with IBD), upto 24 h (stage III). The enzyme effect on the drug release was studied by adding 10 mg of ß-glucosidase into pH 5.0, acetate buffer (250 ml), at the beginning of stage III (Nunthanid et al., 2008). To study the effect of pH of media, the drug release from drug core was studied by using pH 7.0, tris–HCl buffer simulating colonic pH of healthy people instead of acid medium during stage III. Drug release was found to be affected by pH and enzyme with the release rate being higher at pH 5.0 when compared to pH 7.0, indicating suitability of the system for the treatment of IBD.

Siew et al. (2000) proposed enzyme-based fermentation models in order to assess film digestion and drug release from pellets coated with amylose-ethylcellulose films. It was argued that conventional dissolution methodology offer only simple simulations of upper gastrointestinal conditions, e.g. pH, electrolyte concentration, fluid volume and hydrodynamics, and provide only a measure of the delivery system integrity in the stomach and small intestine but no information on actual behaviour or drug release in the real human colon. Therefore, batch culture fermenters, comprising of human feces homogenized in physiological buffer medium of near neutral pH under an atmosphere of nitrogen, total volume of 100 ml, have been proposed as a model of the large intestine (Macfarlane et al., 1993; Silvester et al., 1995; Basit and Lacey, 2001). This model would provide a reasonable simulation of the low-fluid, bacteria-rich, anaerobic environment of the human colon. In an alternate strategy, the film digestibility studies were also carried

out in batch culture fermenter utilizing commercial amylase enzymes obtained from different bacterial and fungal species in an attempt to arrive at an enzyme system which would mimic human faecal activity and thereby serve as an alternative.

1.6. In vivo methods for evaluation of colon targeted systems

For the in vivo evaluation of colon targeted formulations, both pharmacokinetics and in vivo imaging techniques have been employed. Using pharmacokinetic analysis, one can determine if the absorption or appearance of drug in the systemic circulation has been delayed enough to ensure that the dosage form has reached the colon. The suitability of rodent models (mouse and rat) in the evaluation of pH responsive systems has been recently explored by McConnell et al. (2008). The mean intestinal pH values in these animals are lower than in man making them an unsuitable choice for evaluation of enteric coated drug carrier systems. Studies have also been carried out in animal models like the beagle dog which when in fasted conditions has shown similar gastrointestinal transit profiles as humans (Ji et al., 2007).

However, size of the solid oral dosage form is a limiting factor in application of pharmacokinetic studies for in vivo evaluation using rodent models. In vivo imaging technologies are being increasingly used in pharmaceutical development in order to visualize the transit of drug delivery system through GI tract. A review on the different radio nuclide imaging for assessing drug delivery has been discussed by Newman et al. (2003). It has become well established to evaluate the in vivo performance of novel colon specific drug delivery systems in healthy human subjects or patients using gamma scintigraphic technique (Wilding et al., 1991).

In case of gamma scintigraphy, a photographic sequence of images can be obtained to show the passage of the dosage form through the gastrointestinal tract and allow determining the time at which it (1) leaves the stomach, (2) arrives at the colon, (3) begins to release drug and (4) completely releases drug. The information thus generated when combined with results of conventional pharmacokinetic studies (pharmacoscintigraphic study), allows the behavior of the dosage form in the GI tract to be correlated directly with the arrival of drug in the systemic circulation, and can be used to explain inter subject variability (Wilding et al., 2001). The effect of food, disease and size of the formulation on the in vivo performance of the dosage forms can also be elucidated by gamma scintigraphy. It provides human data which is more reliable than either in vitro dissolution

studies or studies in animal models. This technique also helps to clarify the mechanisms by which food can affect drug absorption and formulation performance.

This technique has been used for the evaluation of in vivo behaviour of colonic delivery systems based on pH dependent polymers, pectins and guar gum (Kenyon et al., 1997; Krishnaiah et al., 1998). Technetium-99m (^{99m}Tc) is the most widely used radionuclide in gamma scintigraphy because of its short half-life, low energy and ready availability. Other radionuclides used are indium^{111} (In^{111}), samarium^{153}(Sm^{153}), etc. The radionuclides are usually ligated with different tagging agents such as diethylene triamine pentaacetic acid (DTPA), sulphur colloid (SC), methylene diphosphate and pyrophosphate depending on the human organ that is being imaged. Of all these tagging agents, DTPA and SC are routinely used for imaging in nuclear medicine. In majority of the gamma scintigraphic studies involving the evaluation of in vivo behaviour of oral dosage forms, either technetium^{99m} or indium^{111} are ligated with DTPA (Digenis, 1991). This technique has also been reported to be used for identification and localization of colonic ailments and motility disorders. A few other examples of in vivo studies conducted using gamma scintigraphy are presented in Table 1.5.

Table 1.5: Application of gamma scintigraphy in evaluation of colon specific formulations

S.No	Dosage form	Protocol details	Parameters Investigated	Reference
1	Two-layer-coated tablets of three different pharmaceutical formulations	Samarium oxide mixed with granules and compressed. Irradiated for activation. 9 male and 11 female subjects	I. *Transit profiles of colon-targeted tablets in the GI tract* 1. Gastric emptying 2. Caecal junction arrival 3. Colon arrival 4. Small intestinal transit 5. Caecal junction residence *II. Tablet disintegration site*	Goto, et al., 2004
2	Hydroxyl propyl methyl cellulose capsules	Samarium oxide added to capsule. Irradiated for activation. Six subjects	1. Esophageal transit 2. Gastric residence time 3. Small intestinal transit time 4. Large intestine arrival time 5. Capsule disintegration time and position	Honkanen et al., 2004
3	Pulsincap™ to deliver dofetilide	In^{111}-DTPA Eleven subjects	1. Correlation between position of tablet and pharmacokinetic parameters (AUC)	Stevens et al., 2002
4	Orally administered theophylline solution	^{99m} Tc -DTPA in aqueous solution of drug Two subjects	1. Gastric absorption of theophylline from aqueous solution in fasted and fed states	Haruta et al., 2002

5	Enteric coated HPMC capsules	^{99m} Tc -DTPA added to capsule. Eight subjects	1. Gastric emptying 2. Colon arrival time 3. Initial disintegration time and site 4. Complete disintegration time and site	Cole et al., 2002
6	Intelisite capsule to deliver theophylline	^{99m} Tc -DTPA added to capsule.	1. Anatomical location for radioactive marker release. 2. Time for complete release 3. Correlation between position of tablet and absorption profile (AUC)	Clear et al., 2001
7	Controlled release diclofenac sodium formulation in xanthan gum matrix	^{99m} Tc DTPA adsorbed on Amberlite resin filled into a small hole drilled on the tablet surface. Eight subjects	1. Gastrointestinal transit monitoring 2. Blood sample analysis 3. Correlation between position of tablet and drug absorption (AUC) in fasted and fed state	Billa et al., 2000

1.7. Colonic delivery of proteins and biologicals

Oral delivery of peptides and proteins through colonic absorption remains an attractive alternative to parenteral delivery and has challenged various attempts at formulation development. Advances in protein and peptide delivery over the past few decades have been reviewed by Malik et al. (2007) and via colonic route by Sinha et al. (2007). The colon on account of its low proteolytic activity is a potential route for the systemic delivery of proteins and peptides. The two main practical approaches useful in the delivery of peptides and proteins are either modification of the physicochemical properties of macromolecules or use of improved delivery carriers that ensure that the biological activity of the protein is well preserved (Saffran et al., 1997). The use of microparticulate systems as potential carriers for proteins and peptides has been reviewed by Hillery and co workers (1998).

A novel dosage form based on incorporating insulin into small, soft gelatin capsules coated with polyacrylic polymer (Eudragit RS, L100 and S100) having pH-dependent properties was developed by Touitou et al. (1986). The formulation when administered to rats resulted in a significant hypoglycemic effect. Similarly, insulin which was encapsulated in polyanhydride microspheres was developed by Mathiowitz et al. (1997) which were shown to adhere to the walls of the small intestine and release the insulin upon degradation of the polymeric carrier. Insulin was loaded into polymeric microspheres and administered orally to healthy and diabetic Wistar rats (Lowman et al., 1999). The gel

particles did not swell in the acidic environment of the stomach but rapidly swelled and eroded inside the basic environment of the small intestine, the releasing the entrapped insulin in the terminal ileum. Within 2 h of administration, strong hypoglycemic effects were observed in both healthy and diabetic rats.

In yet another study, stimuli-sensitive (pH/thermal) polymeric beads composed of terpolymers of *N*-isopropylacrylamide (temperature-sensitive), butyl methacrylate and acrylic acid (pH-sensitive) polymers of various molecular weights loaded with insulin were studied (Ramkissoon-Ganorkar et al., 1999). Altering the molecular weight of the polymer modulated the release of insulin. It was found during in vitro release studies in simulated GI conditions, that there was no insulin release at pH 2.0 while at pH 7.4, insulin release was found to occur from all the beads and the release rate was a function of the molecular weight of the polymer. Thus, it was projected that high molecular polymeric beads could be used for delivery of peptide drugs to the colon.

Recently, insulin has been reported to be entrapped in a super porous hydrogel structure which had the property to bind and inactivate ions and enzymes encountered during GI transit such as trypsin (Yin et al., 2007). This hydrogel also had mucoadhesive properties and was reported to adhere well to small intestinal and colonic wall resulting in good permeation of insulin.

The encapsulation of protein within alginate and chitosan beads has been reported to be a promising choice for the delivery of peptides and proteins (George and Abraham, 2006; Xu et al., 2007). By simple covalent modifications of these polymers, their physicochemical properties can be changed and be made suitable for peroral drug delivery purpose. Alginate, being an anionic polymer with carboxyl end groups, can initiate ionic interactions with the negatively charged mucus gel layer making it mucoadhesive. Favourable properties like biocompatibility, biodegradability, pH sensitiveness, mucoadhesiveness, etc. have enabled these polymers to become a favorable option as oral delivery matrices for proteins.

1.8. Non-steroidal anti inflammatory drugs and colon targeting

Numerous experimental and clinical studies propose that nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase-2 (COX-2) inhibitors, hold promise as anticancer agents (Rosenberg et al., 1991). NSAIDs have been shown to restore normal apoptosis in human adenomatous colorectal polyps and in various cancer cell lines (Muscat et al., 1994). The relative risk of colon cancer was

approximately 50% less for people taking NSAIDs compared with non-users. Overall, the protective effects of NSAIDs were shown in several clinical studies. NSAIDs also inhibit angiogenesis in cell culture and rodent models of angiogenesis. Further, epidemiologic studies have found that long-term use of NSAIDs is associated with a lower risk of colorectal cancer, adenomatous polyps, and to some extent other cancers (Suh et al., 1993; Pelleg et al., 1994).

A number of studies have also reported that taking these agents at doses similar to those commonly taken to relieve arthritis pain is associated with a lower rate of colorectal cancer by up to 40% over the long term (Kaza et al., 2002). Two NSAIDs, sulindac and celecoxib, have been found to inhibit the growth of adenomatous polyps and cause regression of existing polyps in randomized trials of patients with familial adenomatous polyposis (FAP) (Kawamori et al., 1998). NSAIDs inhibit the cyclooxygenase (COX) enzyme and prevent the formation of prostaglandin inhibiting proliferation. It has recently been found that NSAIDs also inhibit the APC - β-catenin pathway by inhibiting proliferator peroxisome-activating receptors. In this way, they complement the process of apoptosis. Therefore, NSAIDs inhibit proliferation and augment apoptosis.

The studies in rodents proved conclusively that aspirin, other conventional NSAIDs (such as piroxicam, indomethacin, sulindac, ibuprofen, and ketoprofen), and selective COX-2 inhibitors e.g., celecoxib inhibit chemically induced carcinogenesis in rats and mice (Thun et al., 2002). The highest tolerated dose of nonselective NSAIDs like aspirin, piroxicam, sulindac, indomethacin, ibuprofen, typically reduced the number and size of tumors by 40 - 60%. In another study, it has been reported that nitric oxide-NSAIDs inhibit the growth of cultured cancer cells 10-6000-fold more potently than their parent NSAIDs and prevent colon cancer in animal tumor models (Rigas and Kashfi, 2004). NSAIDs such as sodium salicylate, aspirin, sulindac, ibuprofen and indomethacin cause anti-inflammatory and anti-proliferative effects independent of COX activity and prostaglandin inhibition (Shiff et al. 1996). Of all the NSAIDs that have been explored for their anti cancer potential, indomethacin has been most extensively investigated against both in vitro as well as in vivo models of colon cancer (Hull et al., 2003). The two mechanisms responsible for its anti cancer activity are cyclooxygenase inhibition and peroxisome proliferator-activated receptor -γ- activation (Lehmann et al., 1997).

The therapeutic efficacy of such treatment regimens can be enhanced manifold by increased availability of the drug in colonic tissues and decreased systemic availability by use of colon targeted drug delivery systems.

1.8.1. Colon targeted delivery of indomethacin

One of the most commonly investigated NSAIDs for colon targeting is indomethacin. The potential therapeutic use of indomethacin in colon cancer (details are presented in Chapter Three) has further added weight to its formulation using a colon specific delivery mode. Colon specific formulations of indomethacin will ensure high local concentrations of drug while reduce its systemic burden and toxicities arising out of systemic accumulation. A review of some colon specific formulations of indomethacin is presented in Table 1.6.

Most of the designed colon targeted formulations of indomethacin employ natural polymers to be degraded by colonic microflora (Table 1.6). The bacterial enzyme degradation based systems ensure site-specificity in release, yet suffer from various drawbacks like very slow and unpredictable release behavior in colon (Rubinstein et al., 1990; Krishnaiah et al., 1998). Natural polymers like guar gum, pectin etc., are incapable of preventing premature release in upper GI tract unless they are employed in very high proportions in matrix (Krishnaiah et al., 1998; RamaPrasad et al., 1998; Sinha and Kumria, 2004) or in compression coat (Fernández-Hervás and Fell, 1998; Odeku et al., 2005) or are used in combination with other polymers (Munjeri et al., 1995; Sinha and Kumria, 2002; Wu et al., 2007). Further, drug release from these systems is dependent on individual bacterial enzyme population which may alter in disease states as well as in the presence of other drugs like antiamoebics and antibacterials (Krishnaiah et al., 2001)..

Table 1.6: Colon specific formulations of indomethacin

Strategy employed	Polymers used	Formulation technique	Important findings	Reference
Bacterial enzyme based degradation	Chondroitin sulphate crosslinked	Indomethacin tablets prepared with cross-linked chondroitin sulphate	Indomethacin release from tablets was analyzed in phosphate-buffered saline with and without rat caecal content at 37°C under a CO_2 atmosphere. Prolonged incubation in phosphate buffer saline with rat caecal content increased drug release and by 28 h the released indomethacin levels were significantly higher than those in the buffer only	Rubinstein et al., 1992 a,b
	Amidated pectin	A multiparticulate system comprising of amidated pectin gelled with calcium was developed. Gelation of droplets of amidated pectin solutions in the presence of calcium is the basis of the method of preparation	Significant drug release occurred in simulated upper GI tract conditions. Drug release from the beads was reduced after formation of a chitosan polyelectrolyte complex around the beads. All the preparations released drug in simulated colonic conditions within 135 min	Munjeri et al., 1995
	Guar gum	Indomethacin tablets compression coated with guar gum	In vitro drug release studies indicated that guar gum in the compression coat protected the drug from being released under conditions mimicking mouth to colon transit. Studies in pH 6.8 phosphate buffered saline containing 4% w/v rat caecal contents showed enhanced degradation of guar gum in 24 h	Krishnaiah, et al.,1998
	Pectin and chitosan	Small tablets (multiple units) coated with pectin USP or pectin : chitosan mixture	High amount of pectin (5-10 times the drug weight) was required to prevent premature release in upper GI tract. Pectin/chitosan mixtures achieved better against initial release than pectin alone. Release was enhanced in the presence of pectinolytic enzymes in dissolution medium	Fernández-Hervás and Fell 1998.
	Guar Gum	Indomethacin embedded in guar gum matrix	Drug release from the matrix was prevented in simulated upper GI tract conditions and occurred in the presence of rat caecal contents	Rama Prasad et al., 1998
	Xanthan, Guar gum	Indomethacin tablets using these polymers in matrix	Xanthan gum in combination with guar gum in the tablets could retard initial drug release while guar gum alone could not achieve desired retardation	Sinha and Kumria, 2004

	Khaya and albizia gum	Indomethacin core tablets compression coated with 300 and 400 mg of both gums individually and in combination (1:1)	Tablets coated with khaya and albizia gums did not release the drug in physiological environment of the stomach and small intestine but released in the presence of rat caecal contents	Odeku et al., 2005
	HPMC, pectin, calcium chloride	Matrix tablets comprising of indomethacin with these polymers and varying levels of calcium chloride	The presence of calcium chloride increased crosslinking of the pectin matrix and influenced the initial release. Drug release was enhanced in the presence of pectinolytic enzymes	Wu, et al., 2007
pH based release	Eudragit L100 and Eudragit S100	Indomethacin pellets coated with Eudragit S100: Eudragit L100 (1:4, 1:1 and 1:0) at different level of coating (10%, 15% and 20%, w/w), respectively	Pellets released no indomethacin at pH 1.2 and pH 6.5; drug release was slow at pH 6.8 (simulating lower part of small intestine pH), but it was fast at pH 7.2 (simulating terminal ileum pH)	Akhgari et al.,2005
	Eudragit L100 and Eudragit S100	Indomethacin pellets coated with Eudragit S100 and Eudragit L100 as pH-dependent polymers and Eudragit RS was used as a time-dependent polymer as a single coating formulation	Dissolution studies of pellets in the media with different pH (1.2, 6.5, 6.8 and 7.2) showed that drug release in colon could be controlled by addition of Eudragit RS to the pH-dependent polymers. The lag time prior to drug release could be controlled by coating level	Akhgari et al. 2006
	Eudragit S100, shellac, cellulose acetate pthalate	Indomethacin pellets coated with Eudragit S100/ shellac or CAP	In vitro testing of formulations was done in pH 1.2 (2 h) followed by pH 6.8. 18% drug release in first 3h with 3% Eudragit S100 coat. Shellac (3% coat) was found to give better retardation	Sinha and Kumria, 2003.
	Xanthan, Guar gum, Chitosan and Eudragit E, Eudragit L100	Tablets prepared using these polymers as binders. The prepared tablets were enteric coated with Eudragit L100 to give protection in the stomach	Tablets of indomethacin with guar gum were unable to control initial release (around 50 -60% drug release in the first 5 h) while xanthan gum gave 28% release in upper GI tract conditions. Enteric coated formulations prevented this high initial release in simulated gastric and intestinal fluid	Sinha and Kumria, 2002
Time based	Behenic acid and lactose	Matrix tablets coated with behenic acid and lactose	The lag times of coated indomethacin tablets in pH 7.4 was found to be 50, 162, 94 and 539 mins	Peerpattana et al., 2004
pH and bacteria based	Guar gum, Eudragit FS 30D	Drug loaded pellets coated with both polymers	Presence of Eudragit FS30 D conferred pH protection in stomach and small intestine while guar gum film degraded in the colon and enhanced drug release. Drug release was controlled by pH change as well as degradation by colonic bacteria	Ji et al., 2007

Akhgari et al. (2005, 2006) and Ji et al. (2007) have attempted pH sensitive polymer based coated systems of indomethacin. Although these are commercially feasible systems, there is uncertainty in drug release from these systems in vivo as has already been highlighted in preceding sections (Section 1.4.1). Other formulations include a time dependent release system by Peerpattana et al. (2004). A purely time based system is prone to variations in gastric emptying and may not always reach the colon in the predetermined time frame.

Therefore, the present investigation was aimed at designing a novel matrix based system for potential site specific delivery of indomethacin to colon.

In conclusion, the colon has been explored as a potential site for delivery of therapeutcs for treatment of both local ailments and for diseases that require a time-dependent treatment regimen to match the body's circadian pattern. Also, the colon has also shown to be a useful site for the delivery of peptides and proteins by the oral route. Colon targeted delivery systems have been developed utilizing a variety of techniques that rely on GI pH, transit times, enterobacteria and luminal pressure for site-specific delivery. There are around 54 patents in the area of colonic delivery as reported by Brahma (2007). Around 12 marketed products are commercially available as colon specific drug delivery systems (Patel et al. 2007). Celecoxib has already been approved by USFDA for the treatment of patients suffering from familial adenomatous polyposis (FAP), a precursor disease to colon cancer. With the growing use of NSAIDs in the prophylaxis and chemoprevention of colon carcinoma, the future shall see several colon targeted based formulations entering the market.

The present thesis is therefore devoted to the design, development and characterization of various formulations of indomethacin in the potential treatment of colon cancer. The research objectives and formulation design is discussed in the following chapters.

Chapter Two *Research objectives & Formulation design strategy*

2.1. Background premises

Indomethacin is a non-steroidal anti-inflammatory drug, commonly indicated in the treatment of osteo and rheumatoid arthritis. Recent reports have implicated its use as an anti cancer agent against various in vitro and in vivo models of colorectal cancer (Hull et al., 2003). It has been reported to cause growth inhibition, induction of apoptosis, and reduction in proliferation rates of colon cancer cells. The detailed mechanism of action of indomethacin in the prevention of colon cancer is discussed in Chapter 3 (Section 3.5).

The oral administration of indomethacin has been reported to cause dose dependent systemic and severe local upper gastrointestinal side effects in 35 to 50% patients. Therefore, a formulation of indomethacin with negligible to no release in upper gastrointestinal (GI) tract and controlled release in colonic region would achieve therapeutically effective concentration of drug locally in colon. Thus, apart from maximizing efficacy, it shall also reduce the incidence of GI toxicity and systemic adverse effects associated with the drug.

Several researchers have reported various colon targeted formulations of indomethacin (Chapter 1, Section 1.8.1). These formulations are broadly natural polymer (polysaccharide) based systems that depend on bacterial enzyme degradation to trigger drug release or only pH based or only time based coated systems. pH based coated systems show high intra and inter subject variation in release with release ranging from terminal ileum to no release at all (Ashford et al., 1993; Leopold, 1999; Ibekwe et al., 2006). Similarly, time based formulations are prone to variations in gastric emptying and may or may not release the drug in the colon.

Therefore, in order to overcome the drawback of pH polymer coated systems, a novel matrix design that would combine the advantages of pH and time controlled systems was proposed. It was expected that a dual polymer matrix embedded system comprising of a combination of time or swelling controlled and pH dependent polymers can offer a suitable means of achieving a pH and time dependent system that releases the drug in a bimodal (sigmoidal) fashion. For this purpose, various hydrophilic and hydrophobic polymers were proposed to be used alone and in combination with pH responsive polymers for potentially biphasic (colon specific) release.

It was envisaged that a matrix system, upon exposure to alkaline environment of the colon will result in gradual dissolution of pH responsive polymers and will therefore generate a porous system that will facilitate entry of dissolution medium into the pores of the matrix and affect drug release by diffusion and matrix erosion in high pH region. Such a system will result in a release profile suitable for colonic delivery and may help to reduce the improbability in drug release from a coated system wherein the core is unexposed and drug release can occur only after all the layers of the coat are dissolved.

Matrix systems are easy to manufacture and scale-up with minimum process variables. The only limitation of a matrix system for colon targeting purpose is the probability of high initial release in upper GI tract.

In addition to developing matrix based single unit systems for colonic delivery, it was also envisaged to develop multi unit based formulation as multi unit systems have been shown to perform better than single unit systems in vivo by virtue of reproducible release and prolonged residence in colon (Chapter 1, Section 1.4.5).

2.2. Objectives of the present research endeavour

The primary objective of the present thesis was to design controlled release colon targeted oral matrix formulations of indomethacin so as to overcome the limitations listed in previous sections. As part of the rationale for colon targeted formulation development strategy, following factors were investigated.

i. Effect of polymer type and proportion on indomethacin release from matrix based formulations.
ii. Effect of different hydrophilic and hydrophobic polymers (ionic and nonionic) in combination with pH dependent polymers and their relative proportion on drug release and colonic delivery potential.

Further some of the selected designed formulations were studied for:

iii. Effect of simulated GI fluid pH (without enzymes) on in vitro drug release pattern and to access suitability of designed formulations for colon targeting.
iv. Effect of storage on the stability and release profile of selected formulations as well as for the absence of physical and chemical interactions between the drug and the excipients.

The formulations with approximate colon specific drug release profile were evaluated in vivo to establish proof of concept for:

v. The GI transit pattern and in vivo drug release of selected dosage form in rat model.

vi. The GI transit of selected formulations (for residence time and matrix integrity) in healthy human subjects.

2.3. Overall formulation design strategy

The formulation design comprised of broadly two strategies, namely single unit systems and multi unit (microparticulate based systems). The overall formulation design is shown as follows:

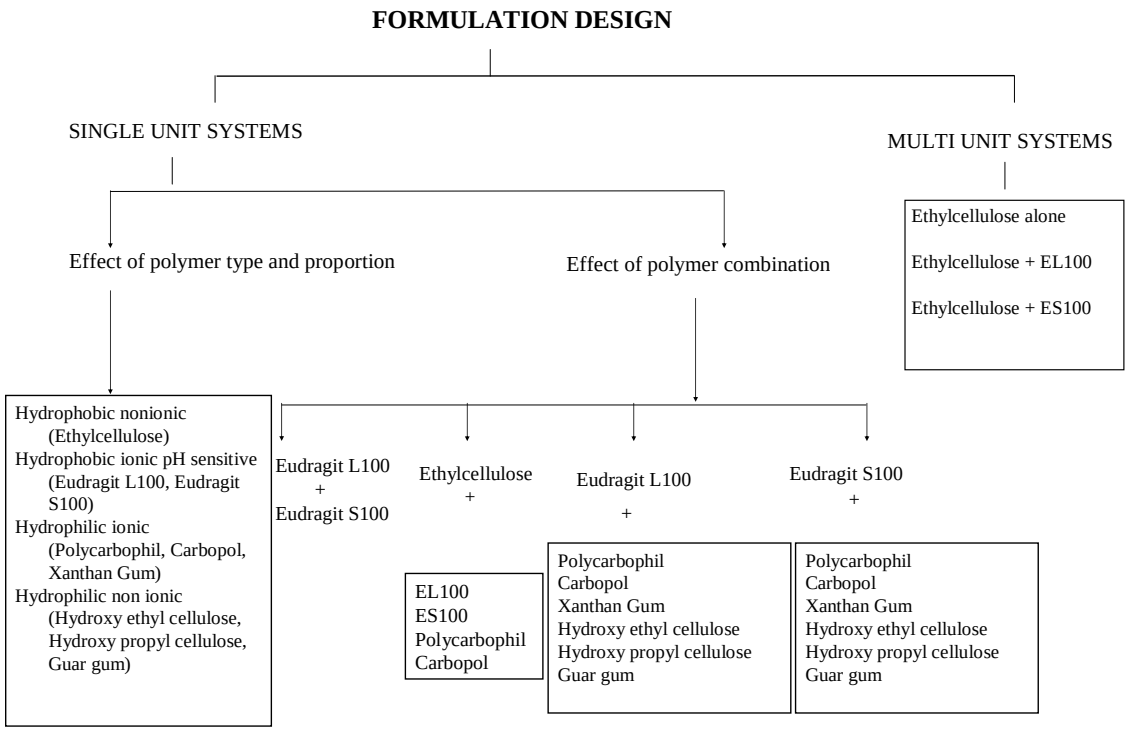


Fig 2.1: Schematic representation of formulation design

Single unit systems:

The formulations were designed to investigate the effect of formulation variables at two levels-

i. Effect of individual polymer type and proportion on drug release.

ii. Effect of polymer combination on sigmoidal release profile.

Five series of matrix embedded formulations were designed for colonic delivery of indomethacin.

i. Formulation of matrix embedded tablets with individual hydrophilic and/ hydrophobic polymers.
ii. Formulation of matrix embedded tablets based on a combination of two pH sensitive polymers EL100 with ES100.
iii. Formulation of matrix embedded tablets based on a combination of ethyl cellulose with pH sensitive polymers EL100, ES100, PCP and CP.
iv. Formulation of matrix embedded tablets based on a combination of EL100 with hydrophilic polymers PCP, CP, XG, HEC, HPC and GG.
v. Formulation of matrix embedded tablets based on a combination of ES100 with hydrophilic polymers PCP, CP, XG, HEC, HPC and GG.

The details pertaining to indomethacin matrix tablet preparation alongwith with the different process and formulation variables are presented in Chapter 4 (Section 4.4.1)

Multi unit system:

For the preparation of microparticulate system, controlled release matrix based microspheres were envisaged utilizing ethyl cellulose as the rate controlling polymer alone and in combination with pH responsive polymers Eudragit L100 or Eudragit S100 for pH and time controlled release. The effect of varying relative polymer proportion as well as other variables like internal: external phase ratio were investigated. The detailed technique of microsphere preparation with the various process and formulation variables are presented in Chapter 4 (Section 4.4.2)

3.1. Introduction

Indomethacin [1 - (4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid] was introduced in 1963 for the treatment of rheumatoid arthritis and related disorders. It is a white to pale yellow, odorless, crystalline powder with its crystals exhibiting polymorphism. In all official compendia, Form I with a melting point of 158-162°C has been reported for use in pharmaceutical preparations. Its molecular weight is 357.79. It is official in U.S., British and Indian Pharmacopoeias.

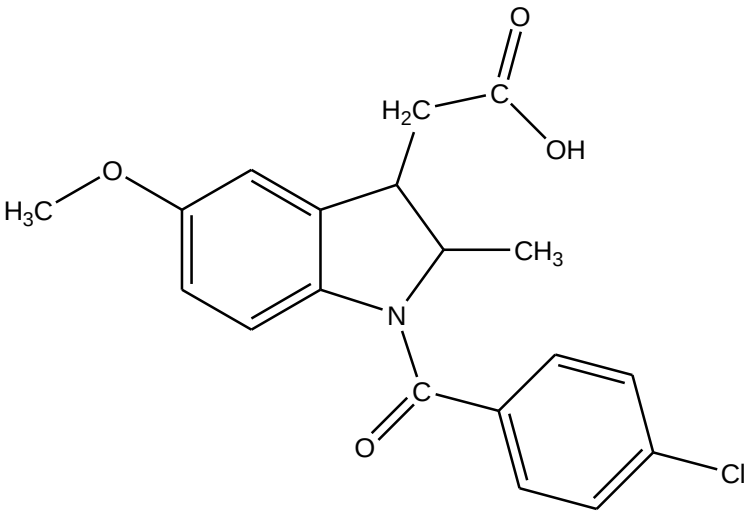


Fig 3.1: Chemical structure of indomethacin

3.2. Physicochemical properties

Indomethacin is practically insoluble in water. It is soluble in chloroform, sparingly soluble in ethanol and ether. It has a pKa of 4.5 for the carboxylic acid group in aqueous media. The octanol/water partition coefficient (log P) is reported to be 3.8 (Beetge et al., 2000). It is soluble at higher pH although it is reported that indomethacin is not stable in alkaline solutions. Solution of indomethacin at pH 7.4 has been reported to be stable up to 24 hours, but shows rapid decomposition in highly alkaline solutions (The Merck Index, 1996).

3.3. Official methods of analysis

The methods of analysis reported for estimation of pure indomethacin in I.P. (2007) and B.P. (2007) comprises of titrimetric (acid-base titration) analysis of pure drug in acetone

against carbonate - free 0.1M sodium hydroxide under constant stream of nitrogen. For the assay of indomethacin in capsules, the method described in I.P. 2007 involves extraction of drug in methanol followed by measurement of absorbance at 320 nm after dilution with methanol: phosphate buffer pH 7.2. The U.S.P. XXIII (2007) describes a liquid chromatographic technique for the assay of indomethacin using mobile phase of 0.01M monobasic sodium phosphate and 0.01M dibasic sodium phosphate in acetonile: water (1:1) system. The peak responses of a suitable dilution of indomethacin in the mobile phase are to be compared with the peak responses obtained for USP Indomethacin RS.

Two UV spectrophotometric methods have been reported in literature for measurement of drug in presence of other drugs or degradants. The first method reported in B.P 2007 for estimation of indomethacin in capsules is based on extraction of drug into methylene chloride followed by dilution with phosphate buffer and measurement of UV absorbance. The second method involves quantitation of indomethacin in the presence of other substances by first and second derivative spectrometry (Mahrous et al., 1985). Chromatographic methods reported in literature for estimation of indomethacin in pharmaceutical formulations, in mixtures, or in plasma, serum and other biological fluids include high performance liquid chromatography with UV detection (Novakova et al., 2005; Khuhawar et al., 2005; Al Zaaposabi et al., 2006), fluorescence detection (Bernstein and Evans et al., 1982), mass spectrometry (Abdel-Hamid et al., 2001; Suenami et al., 2006) and in situ electrogenerated Mn(III) chemiluminescence detection (Zhang et al., 2007). Other chromatographic methods for analysis of indomethacin in plasma and serum include electron gas chromatography (Ferry et al., 1974) and thin-layer chromatography with spectrophotometry (Van der Meer et al., 1980).

3.4. Polymorphism

The polymorphic forms of indomethacin have been extensively investigated (Borka, 1974; Spychala et al., 1977; Kaneniwa et al., 1985). The crystal structures of both stable and metastable forms of indomethacin have been determined using X-ray crystallographic analysis (Kistenmacher and Marsh, 1972; Chen et al. 2002). The (158-161ºC), form II (152-154°C), form III (148-149°C), form IV (134°C), pseudo polymorphic form V (95ºC) and an amorphic form (55-57°C). Form I with the highest point of fusion and the least solubility is thermodynamically the most stable form (Yamamoto et al. 1968; Borka et al. 1974).

3.5. General Pharmacology

3.5.1. Mechanism of action

Indomethacin has prominent antiinflammatory and analgesic-antipyretic properties similar to those of salicylates (Shen et al. 1963; Winter et al., 1963). It is a nonselective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes that participate in prostaglandin synthesis from arachidonic acid. It is a more potent inhibitor of the cyclooxygenases than aspirin, but patient intolerance generally limits its use to short-term dosing (Smyth, 1970). Indomethacin also inhibits the motility of polymorphonuclear leukocytes and depresses the biosynthesis of mucopolysaccharides. It also may have a direct cyclooxygenase independent vasoconstrictor effect. The inhibitory activity on cyclooxygenase is relevant to cancer chemoprevention because cyclooxygenase catalyzes the conversion of arachidonic acid to proinflammatory substances such as prostaglandins which can stimulate tumor cell growth and suppress immune surveillance (Goodman and Gilman, 2006).

3.5.2. Therapeutic uses

Indomethacin is effective for relieving joint pain, swelling, and tenderness; it can increase grip strength, and decreases the duration of morning stiffness (Smyth et al.1963). It is estimated to be approximately 20 times more potent than aspirin for the above indications. When tolerated, indomethacin often is more effective than aspirin in the treatment of ankylosing spondylitis and osteoarthritis (Smyth et al. 1964, 1965). It also is very effective in the treatment of acute gout, although it is not uricosuric. Indomethacin is also USFDA approved for closure of persistent patent ductus arteriosus (Martindale, 1993).

3.5.3. Dosage and administration

In the treatment of rheumatoid arthritis, the recommended dosage is 25 mg three times a day while for osteoarthritis, the recommended dosage is 75-100 mg at night. It is also administered as 100 mg tablet at night time for treatment of morning stiffness (Martindale, 1993).

3.5.4. Pharmacokinetic parameters

A brief summary of its pharmacokinetic parameters of indomethacin is given in Table 3.1. Oral indomethacin has excellent bioavailability (Baer et al, 1974). Peak concentrations occur 1 to 2 h after dosing. Indomethacin is 99% bound to plasma proteins and tissues. Between 10% and 20% of indomethacin is excreted unchanged in the urine, partly by tubular secretion. The majority of the systemically absorbed dose is converted to inactive

metabolites, including those formed by *O*-demethylation (about 50%), conjugation with glucuronic acid (about 10%), and *N*-deacylation (Duggan et al, 1972). Free and conjugated metabolites are eliminated in the urine, bile, and feces. There is enterohepatic recycling of the conjugates and probably of indomethacin itself (Duggan et al, 1975). The half-life in plasma is variable, perhaps because of enterohepatic cycling, but averages about 2.5 h to 11 h (Kwan et al, 1976).

Table 3.1. Pharmacokinetic parameters of indomethacin (Goodman and Gilman, 2006)

PK parameters	**Reported values**
Bioavailability	100% (oral), 80–90% (rectal)
C_{max}	1-2 hours
Protein binding	99%
Metabolism	Hepatic
Metabolites	*O*-demethylation (50%); unchanged (20%)
Half-life	$2^1/_2$ - 11 hours
Excretion	Renal 60%, fecal 33%
Dosing	25 mg 2-3 times/day for rheumatoid arthritis ; 75-100 mg at night for osteoarthritis

3.5.5. Drug interactions

The concomitant administration of probenecid increased the blood concentration of indomethacin, so an enhanced anti-inflammatory effect can be expected when these two drugs are combined (Duggan et al. 1977; Brouwers and de Smet, 1994). Indomethacin, like other NSAIDs can induce an increase in blood pressure and may potentially reduce the efficacy of several antihypertensive drugs (Polónia, 1997). It does not directly modify the effect of warfarin, but platelet inhibition and gastric irritation increase the risk of bleeding and therefore, concurrent administration is not recommended. It antagonizes the natriuretic and antihypertensive effects of furosemide and thiazide diuretics and blunts the antihypertensive effect of β receptor antagonists like metoprolol, Angiotensin (AT_1) receptor antagonists like losartan, and ACE inhibitors like enalapril (Johnson et al. 1994).

3.5.6. Adverse effects

A very high percentage (35% to 50%) of patients receiving usual therapeutic doses of indomethacin experience untoward symptoms, and about 20% must discontinue its use

because of the side effects. Most of the adverse effects are dose related. Gastrointestinal complaints are common and can range from nausea, vomiting, dyspepsia, gastrointestinal lesions to serious reactions like bleeding, ulceration, and perforation (Rainsford, 2007). Diarrhea may occur and sometimes is associated with ulcerative lesions of the bowel. Underlying peptic ulcer is a contraindication to indomethacin use. Dizziness, vertigo, light-headedness, and mental confusion may occur. Seizures, severe depression, psychosis, hallucinations, and suicide have also been reported (Martindale, 1993). Hematopoietic reactions reported with use of indomethacin include neutropenia, thrombocytopenia, and rarely aplastic anemia. Generally, overdose in humans causes drowsiness, dizziness, severe headache, mental confusion, paraesthesia, and numbness of limbs, nausea and vomiting (Cuthbert, 1974).

3.6. Indomethacin in colorectal cancer

The anti colorectal cancer (CRC) activity of indomethacin has been reviewed by Hull et al. (2003). In 1981, it was shown that indomethacin has the ability to prevent the development of carcinomas from the microscopic nascent lesions of rat large bowel carcinomas induced by intrarectal doses of N- methyl nitrosourea (Pollard et al., 1980; Narisawa et al., 1981). The effect of indomethacin against colon cancer was proposed to be through suppression of prostaglandin biosynthesis (Lynch et al., 1978, 1979; Shiff and Rigas, 1999). However, withdrawal of indomethacin treatment led to a rapid regrowth of the tumors, giving an increase in the incidence and number comparable to that seen in the untreated control rats. This observation suggests that indomethacin may have a carcinostatic but not a cancerocidal effect against N-methyl nitroso urea-induced primary large bowel carcinomas in rats.

The anti CRC activity of indomethacin has also been described in several rodent models of colorectal carcinogenesis (Siemer et al., 1995). Brown et al. (2000, 2001) have also demonstrated that indomethacin treatment (2 mg/kg daily) was associated with around 83.5% and 95% reduction in tumor multiplicity and size respectively. Data from a subcutaneous mouse colon 26 adenocarcinoma cell xenograft model suggests that both anti-inflammatory and direct anti-tumor mechanisms of indomethacin may contribute to increased survival in mice bearing a large tumor burden (Tanaka et al., 1989; Gob et al., 2001). Further, studies have shown that indomethacin treatment was effective in causing a regression of malignant tumors or decreasing their growth rate and also in increasing the survival time of tumor bearing animals (Narisawa et al., 1985). However, any residual tumors resumed growth rapidly after withdrawal of the drug.

Indomethacin treatment has also been associated with regression of lymphoma malignant melanoma, head, neck cancer and desmoid tumours (Wadell et al., 1980, 1983; Hirota et al., 1996). The majority of reports of the anti-neoplastic properties of indomethacin in humans have been uncontrolled case reports or case series (Rodriguez and Alvarez, 2000). There are several mechanisms whereby COX inhibition could mediate the anti-CRC activity of indomethacin including inhibition of synthesis of pro-tumorigenic prostaglandins (Frenkian et al., 2001), decreased production of the DNA mutagen malondialdehyde and increased arachidonic acid substrate levels leading to an increase in cellular ceramide concentration (Tanaka et al., 1989). Indomethacin has shown impairment of the growth of human colon cancer cells, resulting in decreased ornithine decarboxylase (ODC) activity, increased intracellular spermidine/ spermine N^1-acetyltransferase (SSAT) enzyme activity and enhanced polyamine acetylation and efflux from colon cancer cells (Vujcic et al., 2000).

More recently, the effect of indomethacin on growth inhibition, induction of apoptosis, and alterations in the expression of several genes involved in various signal transduction pathways (*Wnt* signaling) in adenocarcinoma (HT-29) cells and is associated with expression of proteins involved in embryogenesis and cancer (Kapitanovic et al., 2006). It was shown that indomethacin reduces the proliferation rate of HT-29 colon cancer cells and induces apoptosis. Concentrations of indomethacin from 10^{-4} to 10^{-3} M strongly inhibited the growth of HT-29 cells. The inhibition of growth, as well as induction of apoptosis was found to be dose and time dependent. The treatment of cells with 4×10^{-4} M indomethacin caused strong inhibition of cell growth (about 70%), enhanced expression of APC, decreased expression of beta-catenin (Veeramachaneni et al., 2003) and induced expression of E-cadherin proteins. It was suggested that the anti proliferative effect of indomethacin may contribute to enhanced cell adhesion through increased expression of E-cadherin and translocation of beta-catenin from the nucleus to the cell membrane. Recently, it was elucidated that indomethacin decreases EP2 prostanoid receptor expression in colon cancer cells (Fujino et al., 2007).

Materials

Indomethacin was obtained as a gift sample from Ajanta Pharma Ltd, Mumbai, India; Eudragit (both L100 and S100) were obtained as gift samples from Rohm Pharma, Germany; Polycarbophil (PCP) (*Noveon AA1*) was obtained from B.F. Goodrich Co., and Carbopol 934P (CP) and Guar gum (GG) were purchased from S.D. Fine Chemicals, India. Ethylcellulose (EC) (*Aqualon N-22 cps, 18-24% standard ethoxyl substitution*), Hydroxy ethyl cellulose (HEC) (*Natrosol HHX, viscosity 3500-5500 mPa of 1% aqueous solution*) and Hydroxy propyl cellulose (HPC) (*Klucel LF, viscosity 75-150 mPa of 5% aqueous solution*), and Xanthan (XG) (*Xantural*) were purchased from Signet Chem, Mumbai, India. All other chemicals, excipients and solvents used were of either analytical or pharmaceutical grade.

Equipment/instruments

A five digit analytical balance Mettler Toledo (*AG135, Mettler, GMBH, Greifensee, Switzerland*) was used for all weighing purposes. A constant temperature water bath shaker (*MAC instruments, New Delhi, India*) was used for solubility studies. All pH measurements were performed using Toshvin pH meter (*Toshwin, Ajmer, India*) which was equipped with glass electrode filled with potassium chloride gel and temperature probe. A humidity and temperature control cabinet (*MAC instruments, India*) was used to maintain accelerated stability conditions (40 ± 2°C and 75 ± 5% RH). Thermal analysis was performed using differential scanning calorimeter (*Shimadzu Corporation, Tokyo, Japan, model: DSC-60*) equipped with TA-60WS thermal analyzer and TA60 analysis software. A UV-Visible-NIR spectrophotometer (*Jasco, Tokyo, Japan, model: V-570,*) with spectra manager software was used for estimation of drug in formulation and dissolution samples. Chemical compatibility studies were carried out using Fourier Transform Infrared (FTIR) Spectrophotometer (*Shimadzu Corporation, Tokyo, Japan, model: IR Prestige-21*). The software IR solutions, version 1.0, was used for IR data processing.

A 16 station rotary tablet compression machine (*Cadmach, Ahmedabad, India*) was used for compression of prepared granules. The tablet hardness was measured using tablet hardness tester (*Monsanto* standard type). Friability was determined in a Campbell

Electronic Friabilator (*Campbell Electronics, Mumbai, India*). In vitro dissolution studies were carried out using USP Type II (paddle method) apparatus with Autosampling unit (*Electrolab* TDT-08L, *Mumbai, India*). The images of microparticles were taken using microscope (*Olympus BX-41, Olympus America Inc, Pennsylvania, USA)* equipped with digital camera. Radioactivity for in vivo studies was measured using radioisotope calibrator beta-counter (*Capintec Inc, CRC-15 beta, Pittsburg, USA*) located in hot lab of Nuclear Medicine Dept of M. N. Budhrani Cancer Institute, Pune, India. Gamma scintigraphy was done using dual headed gamma camera (*Siemens, E.CAM, Germany*) fitted with low energy high resolution collimators and data obtained was processed using Icon software.

Methods

4.1. Characterization of bulk drug

Bulk drug was characterized by various official tests of identification as per I.P 1996 and infrared spectrum of pure drug was obtained and compared with that given in official compendia (I.P. 1996). The infrared spectrum of pure indomethacin is shown in Chapter 5 (Section 5.1).

4.2. Analytical method development and validation

A simple, sensitive, precise and accurate analytical method was developed for the estimation of indomethacin in designed pharmaceutical formulations, and for analysis of samples from solubility, in vitro dissolution and stability studies. Analytical method was developed in phosphate buffer pH 7.4. The developed method was validated as per ICH guidelines. Following are the detailed procedures employed for development and validation of UV method for estimation of indomethacin.

4.2.1. Selection of solvent system

Different pH media alone and in combination with different organic solvents, in various proportions, were tried. For selection of media the criteria employed was sensitivity of the method, ease of sample preparation, solubility of the drug, cost and applicability of the method for various purposes.

4.2.2. Preparation of standard curve

A stock solution of the drug was prepared by dissolving 10 mg of drug in methanol: phosphate buffer pH 7.4 (10:90 v/v) mixture and volume made up to 100 ml to get a final concentration of 100 µg/ml. Six different concentrations were made in 10 ml volumetric

flasks from the stock solution in phosphate buffer pH 7.4 so as to obtain solutions of 5, 10, 20, 30, 40 and 50 μg/ml for calibration curve and absorbance was measured against blank (phosphate buffer pH 7.4) at 320 nm.

Linearity: To establish linearity of the proposed method, ten separate series of solutions of the drug in selected medium were prepared from the stock solution and analyzed. Least square regression analysis was done for the obtained data. One-way ANOVA test was performed based on the absorbance values observed for each pure drug concentration during the replicate measurement of the standard solutions to establish linearity of the proposed method.

4.2.3. Method validation

Selectivity of the method: Indomethacin solutions (10 μg/ml) were prepared in the selected medium with and without common excipients (lactose, microcrystalline cellulose, magnesium stearate and talc) as well as polymeric excipients which were proposed to be used in tablet matrix (ethyl cellulose, polycarbophil, carbopol, hydroxy ethyl cellulose, hydroxy propyl cellulose, xanthan gum, guar gum and eudragits). All the solutions were scanned at 200 nm/min from 200 nm to 400 nm and compared against UV spectrum of pure drug for the change in absorbance pattern.

Accuracy: For determining accuracy of the proposed method, different levels of drug concentrations (low quality control concentration (LQC): 5 μg/ml, medium quality control concentration (MQC): 20 μg/ml, and high quality control concentration (HQC): 50 μg/ml) were prepared independently from stock solution and analyzed (n = 18). Accuracy was assessed as the mean percentage recovery from six triplicate determinations. To give additional support to accuracy of the developed assay method, recovery studies were done. A known amount of drug was added to preanalyzed sample of pure drug solution (n = 3), tablet formulation prepared in-house and commercial dosage form. The percent analytical recovery of the added pure drug was calculated as, % recovery = [(Cv - Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard addition; Cu, drug concentration in the formulation; Ca, drug concentration added to the pre-analyzed sample.

Precision: Precision was determined through repeatability and intermediate precision. Repeatability was determined by using different levels of drug concentrations (as mentioned in accuracy), prepared from independent stock solution and analyzed (n = 18). Inter- and intra-day variation was studied to determine intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared three

different times in a day and studied for intraday variation. For interday variation, the same protocol was followed for three different days (n = 18). The relative standard deviation (%) or % CV of the calculated concentrations from the regression equation was taken as measure of precision.

DL and QL: The detection limit (DL) and quantitation limit (QL) of indomethacin by the proposed method was determined using calibration standards. DL and QL were calculated as 3.3 σ/S and 10 σ /S respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.

Robustness: Robustness of the proposed method was determined by changing pH of the media by ± 0.2 units and repeating the analysis.

Recovery studies: In order to keep an additional check on the accuracy of the developed assay method and to study the possible interference of formulation additives, analytical recovery studies were performed by adding known amount of pure drug solution (10 µg/ml) to pre analyzed samples of the commercial dosage form. The percent analytical recovery values (n = 3) were calculated by comparing concentration obtained from the spiked samples with the actual added concentrations.

4.2.4. Estimation of drug from marketed/ in house preparation

Ten tablets were weighed and pulverized. Amount of the powder equivalent to 10 mg of indomethacin was taken and extracted with methanol: phosphate buffer pH 7.4 (10:90 v/v) mixture for 30 min. The solution was diluted suitably with phosphate buffer pH 7.4 medium to prepare a 100 µg/ml concentration. This primary stock solution was filtered through Whatman filter paper No 41 and the filtrate was further diluted suitably to prepare a secondary stock solution of 20 µg/ml concentration and the samples were analyzed using proposed method. Based on absorbance values, the drug content was calculated on average weight basis. The results of analytical method development and validation are presented in Chapter 5 (Section 5.2).

4.3. Preformulation Studies

4.3.1. Determination of solubility profile

In order to assess in-house solubility for the obtained drug sample, solubility measurements were performed according to the shake flask method. In brief, excess amount of indomethacin was added into eppendorf tubes to which 10 ml of aqueous buffer medium (pH 1.2, 4.5, 6.8, 7.4, 8.0 and TDW) was added. These suspensions were left for shaking on a platform shaker for 48 h at 25 ± 2^{o} C. Solutions were then filtered through

Whatman filter paper No 41 and analyzed after suitable dilution with phosphate buffer pH 7.4 at 12, 24 and 48 h using the developed UV analytical method. The results of solubility studies are presented in Chapter 5 (Section 5.3.1).

4.3.2. Determination of physical form of indomethacin

The physical form of drug was determined by thermal analysis using Differential Scanning Calorimeter (DSC). Pure drug (2 mg) was accurately weighed onto a standard aluminium pan and the lid was crimped using Shimadzu SSC-30 sample sealer-crimper. Temperature program consisted of heating rate of 10°C/ min starting at 25° C and ending at 200° C. Nitrogen gas was continuously purged into the sample chamber at a flow rate of 30ml/min in order to provide an inert atmosphere and to prevent oxidative degradation of the drug. The DSC thermogram was recorded and endothermic peak was analyzed for melt temperature and enthalpy of fusion and is presented in Chapter 5 (Section 5.3.2).

4.3.3. Solution state stability studies

Rate kinetic studies for estimation of drug stability in media of varying pH in both buffered as well as unbuffered solvent systems were done to give an idea about degradation profile of the drug. This also helps in selection and design of suitable dissolution medium for in vitro drug release studies. A stock solution of indomethacin was prepared in methanol (100 µg/ml). It was added to buffered and unbuffered solutions of varying pH and the volume was made upto 50 ml. The final drug concentration was kept at 20 µg/ ml. Buffered solutions of varying pH (1.2, 4.5, 6.8, 7.4 and 8.0) were prepared as per IP 1996. For prepared unbuffered solutions, the pH of distilled water was adjusted with 0.2M HCl and 0.2M NaOH to get a pH range between 1.2 and 12.5. In all the above solutions, the ionic strength (µ) was kept constant at 0.2 with 0.2 M NaCl. Solutions were maintained at controlled room (CRT) (25 ± 2^{o} C and 60% ± 5% RH) and at accelerated test conditions (ATC) (40 ± 2^{o} C and 75% ± 5% RH). Samples were withdrawn at various time points (0, 1, 2, 5, 7, 14 and 21 day) and analyzed after suitable dilution at 320 nm. The degradation profiles of the various solutions were obtained as the plot of log % drug remaining vs. time. The rate constant of degradation was computed from the slope of the curve(s) and time taken to reach to 90% of the potency or labeled claim ($T_{90\%}$) was calculated. The plot of degradation rate constant (K deg) vs. time for both buffered and unbuffered systems is shown in Chapter 5 (Section 5.3.3).

4.3.4. Solid state drug excipient compatibility study

Physical mixtures of drug along with different excipients were prepared in the ratio of 1:5. The drug along with the each excipient was thoroughly blended and the mixture was passed through 80 mesh sieve to ensure uniform and intimate mixing. Pure drug alone and the blends of drug with excipients were transferred to storage glass vials with screw caps and kept at controlled room temperature (CRT) (25 ± 2°C and 60 ± 5% RH) and accelerated conditions (40 ± 2°C and 75 ± 5% RH). Samples in duplicate were drawn at predetermined time intervals (0, 1, 3 and 6 months) and analyzed by the developed UV method described earlier.

Further, in order to confirm absence of physical and chemical interaction between drug and the excipients, samples stored at CRT conditions were analyzed for change in their DSC thermograms and FTIR spectra with respect to the pure drug. For FTIR, the samples were appropriately diluted with dried potassium bromide and IR spectra were acquired in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The obtained spectrums were processed using Kubelka Munk conversion method before interpretation. The results of the different preformulation studies carried out are presented in Chapter 5 (Section 5.3.4).

4.4. Formulation design and development

The formulation design comprised of broadly two strategies, namely single unit systems and multi unit (microsphere based systems). The overall experimental design for single-unit and multi unit systems has been presented in Chapter 2. The preparation of matrix embedded formulations is explained in Section 4.4.1. The composition of prepared batches is given in Table 4.1 to 4.5. The technique for preparation of microsphere based systems is explained in Section 4.4.2 and the composition of the different prepared batches is shown in Table 4.6 and 4.7.

4.4.1. Matrix embedded formulation preparation

Batch quantities of drug and polymer(s) pre-sieved through # 120 mesh (ASTM) and dried at 55°C were mixed. The dry blend was granulated with ethyl alcohol (q.s.) and passed through # 40 mesh and dried at 55°C in a hot air oven. The dried granules were passed through # 60 mesh and the passings blended with 1% w/w talc and 0.5% w/w magnesium stearate and compressed using 7 mm punches on a 16 station rotary tablet compression machine (Cadmach, Ahmedabad, India). Three batches of tablets were prepared for each formulation. Each tablet comprised of 75 mg of indomethacin.

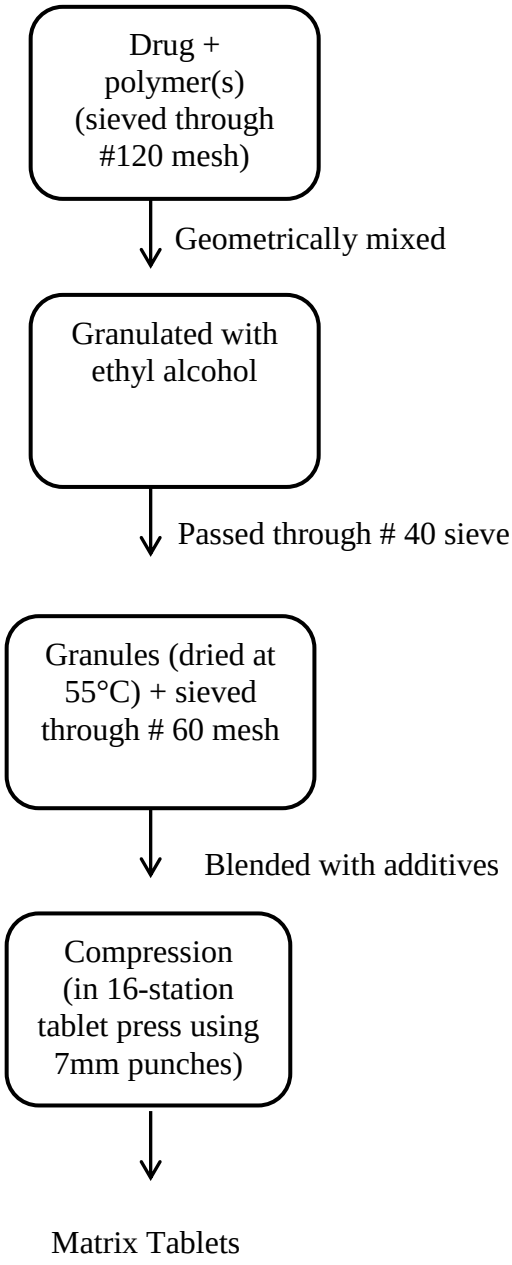


Fig 4.1: Flowchart for tablet manufacturing process

The formulation codes and composition of the prepared colon specific controlled release matrix tablets of indomethacin using single polymer is presented in Table 4.1. Composition of indomethacin controlled release colon targeted tablets prepared using combination of various polymer types is given in Table 4.2 (combination of EL100 and ES100), Table 4.3 (combination of EC with EL100, ES100, PCP and CP), Table 4.4 (combination of EL100 with PCP, CP, XG, HEC, HPC and GG) and Table 4.5 (combination of ES100 with PCP, CP, XG, HEC, HPC and GG).

Table 4.1: Composition of single polymer based tablet formulations

Formulation code	Composition								
	EC (mg)	**EL100 (mg)**	**ES100 (mg)**	**PCP (mg)**	**CP (mg)**	**XG (mg)**	**HEC (mg)**	**HPC (mg)**	**GG (mg)**
(a) EC									
IEC5	3.75	-	-	-	-	-	-	-	-
IEC10	7.5	-	-	-	-	-	-	-	-
IEC20	15	-	-	-	-	-	-	-	-
(b) EL100									
IEL25	-	18.75	-	-	-	-	-	-	-
IEL50	-	37.5	-	-	-	-	-	-	-
(c) ES100									
IES25	-	-	18.75	-	-	-	-	-	-
IES50	-	-	37.5	-	-	-	-	-	-
(d) PCP									
IPCP5	-	-	-	3.75	-	-	-	-	-
IPCP10	-	-	-	7.5	-	-	-	-	-
IPCP20	-	-	-	15	-	-	-	-	-
(e) CP									
ICP5	-	-	-	-	3.75	-	-	-	-
ICP10	-	-	-	-	7.5	-	-	-	-
ICP20	-	-	-	-	15	-	-	-	-
(f) XG									
IXG5	-	-	-	-	-	3.75	-	-	-
IXG10	-	-	-	-	-	7.5	-	-	-
IXG20	-	-	-	-	-	15	-	-	-
(g) HEC									
IHEC5	-	-	-	-	-	-	3.75	-	-
IHEC10	-	-	-	-	-	-	7.5	-	-
IHEC20	-	-	-	-	-	-	15	-	-
(h) HPC									
IHPC5	-	-	-	-	-	-	-	3.75	-
IHPC10	-	-	-	-	-	-	-	7.5	-
IHPC20	-	-	-	-	-	-	-	15	-
(i) GG									
IGG5	-	-	-	-	-	-	-	-	3.75
IGG10	-	-	-	-	-	-	-	-	7.5
IGG20	-	-	-	-	-	-	-	-	15

Each tablet contains 75 mg of indomethacin. Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives.

Table 4.2: Composition of indomethacin matrix tablets containing combination of EL100 and ES100

Formulation code	EL100 (mg)	ES100 (mg)
IEL15ES10	11.25	7.5
IEL12.5ES12.5	9.37	9.37
IEL10ES15	7.5	11.25
IEL30ES20	22.5	15
IEL25ES25	18.75	18.75
IEL20ES30	15	22.5

Each tablet contains 75 mg of indomethacin. Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives.

Table 4.3: Composition of indomethacin matrix tablet formulations containing combination of EC with other polymers

Formulation code	EC (mg)	EL100 (mg)	ES100 (mg)	PCP (mg)	CP (mg)
(a) EC + EL100					
IEL15EC10	11.25	7.5	-	-	-
IEL20EC5	15	3.75	-	-	-
IEL30EC20	22.5	15	-	-	-
IEL40EC10	30	7.5	-	-	-
(b) EC + ES100					
IES15EC10	11.25	-	7.5	-	-
IES20EC5	15	-	3.75	-	-
IES30EC20	22.5	-	15	-	-
IES40EC10	30	-	7.5	-	-
(c) EC + PCP					
IPCP10EC5	3.75	-	-	7.5	-
IPCP10EC10	7.5	-	-	7.5	-
IPCP10EC20	15	-	-	7.5	
IPCP10EC40	30	-	-	7.5	-
IPCP20EC5	3.75	-	-	15	-
IPCP20EC10	7.5	-	-	15	-
IPCP20EC20	15	-	-	15	-
IPCP20EC40	30	-	-	15	-
(d) EC + CP					
ICP10EC5	3.75	-	-	-	7.5
ICP10EC10	7.5	-	-	-	7.5
ICP10EC20	15	-	-	-	7.5
ICP10EC40	30	-	-	-	7.5
ICP20EC5	3.75	-	-	-	15
ICP20EC10	7.5	-	-	-	15
ICP20EC20	15	-	-	-	15
ICP20EC40	30	-	-	-	15

Each tablet contains 75 mg of indomethacin-. Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives.

Table 4.4: Composition of indomethacin matrix tablet formulations containing combination of EL100 with other polymers

Formulation code	EL100 (mg)	PCP (mg)	CP (mg)	XG (mg)	HEC (mg)	HPC (mg)	GG (mg)
(a) EL100 + PCP							
IPCP5EL10	7.5	3.75	-	-	-	-	-
IPCP5EL20	15	3.75	-	-	-	-	-
IPCP10EL20	15	7.5	-	-	-	-	-
IPCP10EL40	30	7.5	-	-	-	-	-
IPCP20EL40	30	15	-	-	-	-	-
(b) EL100 + CP							
ICP5EL10	7.5	-	3.75	-	-	-	-
ICP5EL20	15	-	3.75	-	-	-	-
ICP10EL20	15	-	7.5	-	-	-	-
ICP10EL40	30	-	7.5	-	-	-	-
ICP20EL40	30	-	15	-	-	-	-
(c) EL100 +XG							
IXG5EL5	3.75	-	-	3.75	-	-	-
IXG5EL10	7.5	-	-	3.75	-	-	-
IXG5EL20	15	-	-	3.75	-	-	-
IXG5EL40	30	-	-	3.75	-	-	-
IXG10EL10	7.5	-	-	7.5	-	-	-
IXG10EL20	15	-	-	7.5	-	-	-
(d) EL100 +HEC							
IHEC5EL10	7.5	-	-	-	3.75	-	-
IHEC5EL20	15	-	-	-	3.75	-	-
IHEC10EL20	15	-	-	-	7.5	-	-
IHEC10EL40	30	-	-	-	7.5	-	-
IHEC20EL40	30	-	-	-	15	-	-
(e) EL100 + HPC							
IHPC5EL10	7.5	-	-	-	-	3.75	-
IHPC5EL20	15	-	-	-	-	3.75	-
IHPC10EL20	15	-	-	-	-	7.5	-
IHPC10EL40	30	-	-	-	-	7.5	-
IHPC20EL40	30	-	-	-	-	15	-
(f) EL100 + GG							
IGG5EL5	3.75	-	-	-	-	-	3.75
IGG5EL10	7.5	-	-	-	-	-	3.75
IGG5EL20	15	-	-	-	-	-	3.75
IGG5EL40	30	-	-	-	-	-	3.75
IGG10EL10	7.5	-	-	-	-	-	7.5
IGG10EL20	15	-	-		-	-	7.5

Each tablet contains 75 mg of indomethacin. Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives.

Table 4.5: Composition of indomethacin matrix tablet formulations containing combination of ES100 with other polymers

Formulation code	ES100 (mg)	PCP (mg)	CP (mg)	XG (mg)	HEC (mg)	HPC (mg)	GG (mg)
(a) ES100 + PCP							
IPCP5ES10	7.5	3.75	-	-	-	-	-
IPCP5ES20	15	3.75	-	-	-	-	-
IPCP10ES20	15	7.5	-	-	-	-	-
IPCP10ES40	30	7.5	-	-	-	-	-
IPCP20ES40	30	15	-	-	-	-	-
(b) **ES100 + CP**							
ICP5ES10	7.5	-	3.75	-	-	-	-
ICP5ES20	15	-	3.75	-	-	-	-
ICP10ES20	15	-	7.5	-	-	-	-
ICP10ES40	30	-	7.5	-	-	--	-
ICP20ES40	30	-	15	-	-	-	-
(c) ES100 + XG							
IXG5ES5	3.75	-	-	3.75	-	-	-
IXG5ES10	7.5	-	-	3.75	-	-	-
IXG5ES20	15	-	-	3.75	-	-	-
IXG5ES40	30	-	-	3.75	-	-	-
IXG10ES10	7.5	-	-	7.5	-	-	-
IXG10ES20	15	-	-	7.5	-	-	-
(d) ES100 + HEC							
IHEC5ES10	7.5	-	-	-	3.75	-	-
IHEC5ES20	15	-	-	-	3.75	-	-
IHEC10ES20	15	-	-	-	7.5	-	-
IHEC10ES40	30	-	-	-	7.5	-	-
IHEC20ES40	30	-	-	-	15	-	-
(e) ES100 + HPC							
IHPC5ES10	7.5	-	-	-	-	3.75	-
IHPC5ES20	15	-	-	-	-	3.75	-
IHPC10ES20	15	-	-	-	-	7.5	-
IHPC10ES40	30	-	-	-	-	7.5	-
IHPC20ES40	30	-	-	-	-	15	-
(f) ES100 + GG							
IGG5ES10	7.5	-	-	-	-	-	3.75
IGG5ES20	15	-	-	-	-	-	3.75
IGG5ES40	30	-	-	-	-	-	3.75
IGG10ES10	7.5	-	-	-	-	-	7.5
IGG10ES20	15	-	-		-	-	7.5

Each tablet contains 75 mg of indomethacin. Also contains 1% w/w talc and 0.5% w/w magnesium stearate as formulation additives.

4.4.2. Preparation of microspheres

Phase separation-coacervation method induced by solvent evaporation was employed to formulate matrix- based microspheres of indomethacin using different ratios of indomethacin with either EC alone or in combination with EL100 (or ES100). Initial batch of microspheres were prepared to optimize internal phase (acetone) to external phase (light

liquid paraffin) ratio. Finely pulverized drug (50 mg) was dissolved in acetone to which EC alone and in combination with EL100 or ES100 were added in varying proportions. This internal phase was added to liquid paraffin containing 2% Span 80 to form an oil/ oil emulsion. This was stirred at constant speed (600 rpm) on a magnetic stirrer for 3-4 h under ambient conditions till the organic solvent evaporated, precipitating out the microspheres. The product obtained was washed with petroleum ether followed by air drying.

The composition of indomethacin microsphere at fixed proportion of EC (5% w/w of drug) with varying internal to external phase ratios (1:1 to 1:9) is presented in Table 4.6. The composition of formulations with EC in combination with EL100/ES100 is shown in Table 4.7.

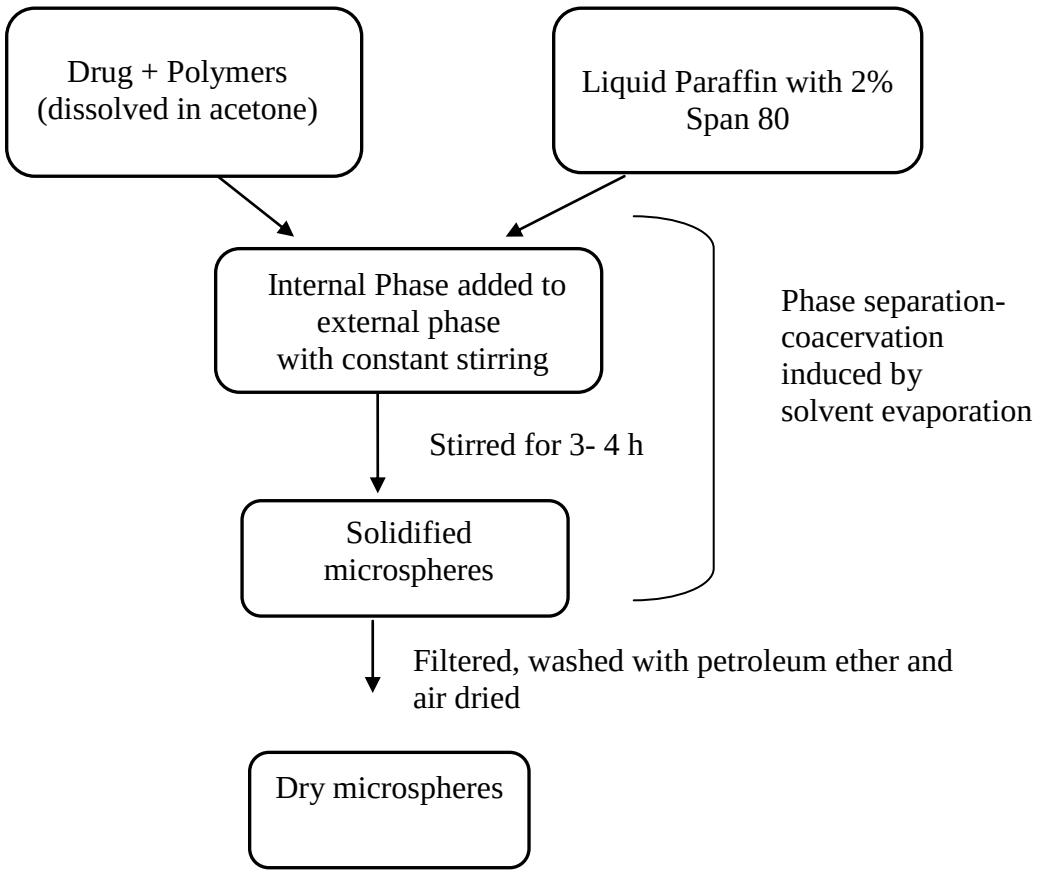


Fig 4.2: Flowchart for microsphere preparation

Table 4.6: Composition of EC based microparticulate formulations

Formulation code	EC (%)*	Int : ext phase ratio	Stabilizer (%) #
a) Microspheres prepared with fixed EC concentration with different internal to external phase ratio			
M1	5	1:1	2%
M2	5	1:3	2%
M3	5	1:5	2%
M4	5	1:7	2%
M5	5	1:9	2%
b) Microspheres with varying EC concentration			
M1EC1	1.25	1:1	2%
M1EC2	2.5	1:1	2%
M1EC3	5	1:1	2%
M5EC1	1.25	1:9	2%
M5EC2	2.5	1:9	2%
M5EC3	5	1:9	2%

* %w/v of internal phase; # %v/v of external phase. Each batch contains 50 mg of indomethacin.

Table 4.7: Composition of EC based microparticulate formulations in combination with EL100/ES100

Formulation code	EC (%)*	EL100 (%)*	ES100 (%)*	Int : ext phase ratio	Stabilizer (%) #
M1EC1	1.25	-	-	1:1	2%
M1EC1EL1	1.25	0.5	-	1:1	2%
M1EC1EL2	1.25	0.75	-	1:1	2%
M1EC1EL3	1.25	1.25	-	1:1	2%
M1EC1ES1	1.25	-	0.5	1:1	2%
M1EC1ES2	1.25	-	0.75	1:1	2%
M1EC1ES3	1.25	-	1.25	1:1	2%
M5EC1	1.25	-	-	1:9	2%
M5EC1EL1	1.25	1.25	-	1:9	2%
M5EC1ES1	1.25	-	1.25	1:9	2%

* %w/v of internal phase ; # %v/v of external phase. Each batch contains 50 mg of indomethacin.

4.5. Physical characterization of designed formulations

The designed tablet formulations were studied for their physicochemical properties like weight variation, thickness, crushing strength, friability and drug content uniformity. For estimating weight variation, 20 tablets of each formulation were weighed using a Mettler Toledo balance. The crushing strength of 10 tablets was measured using Monsanto

(standard type) tablet crushing strength tester. Friability was determined on 10 tablets in a Campbell Electronic Friabilator for 4 mins at 25 rpm. For estimation of drug content, 10 tablets were crushed and the aliquot of powder equivalent to 10 mg of drug was extracted in methanol: phosphate buffer pH 7.4 (1:9), suitably diluted using phosphate buffer pH 7.4 and analyzed spectrophotometrically at 320 nm. The physical characteristics of the various formulations are shown in Chapter 5 (Section 5.5).

The prepared microspheres were studied for appearance and size distribution using optical microscopy and images of batches (at 100X and 400X magnification) were acquired using fluorescent microscope (Olympus BX-41) equipped with digital camera. The particle size was measured using optical microscopy using calibrated eye piece micrometer (1 division = 14.28 μm). The percentage yield value of microspheres was determined from the ratio of amount of solidified total microspheres to total solid material used in the inner phase, multiplied by 100. The drug content in each batch of microparticle was determined by dissolving accurately weighed aliquot of the formulation equivalent to 10 mg of drug in 10 ml of methanol (to dissolve the polymer coat) and phosphate buffer pH 7.4 was added to make up the volume to 100 ml. The solution was filtered, suitably diluted and analyzed using developed UV method at 320 nm. The encapsulation efficiency of microspheres was determined by taking the ratio of the actual drug content to the theoretical drug content expressed in percentage. The physical properties of the designed microspheres are presented in Chapter 5 (Section 5.8).

4.6. In vitro release studies of tablet formulations

In vitro dissolution studies for the tablets were carried out using USP Type II (paddle method) apparatus at 75 rpm at 37 ± 0.5°C. The dissolution was carried out for the first 2 h in distilled water (500 ml). Then, 200 ml of phosphate buffer concentrate (4.75 g of KH_2PO_4 and 1.07 g of NaOH in distilled water) was added to raise the total media volume to 700 ml and pH to 7.4 for the remaining period. At predetermined time intervals, a 10 ml sample was withdrawn and replaced with fresh dissolution media. The samples were filtered, suitably diluted and analyzed using the UV method discussed earlier. The release studies were conducted in duplicate and the mean values along with the SD were plotted against time (Chapter 5, Section 5.6).

4.6.1. Effect of simulated GI fluid pH (without enzymes) on release

The release profile of selected tablet formulations was further studied in a medium of changing pH using USP Type II (paddle method) apparatus at 75 rpm at 37 ± 0.5°C. The initial condition was 350 ml of 0.1N HCl (pH 1.2) for 0-2 h. At the end of 2nd h, the pH of the media was raised to 4.5 (by addition of 3.75 g of KH_2PO_4 in 190 ml and 60 ml of 0.5M NaOH in distilled water). At the end of 4th h, pH was raised to 7.4 by adding 300 ml phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water). The study was further continued till the end in 900 ml volume. At predetermined time intervals, a 10 ml sample was withdrawn and replaced with fresh dissolution media. After appropriate dilutions, the samples were analyzed by the UV method discussed earlier. The corresponding release profiles for the different formulation series are presented in Chapter 5 (Section 5.7).

4.6.2. In vitro release studies of microsphere formulations

For the microspheres, in vitro release rate studies were carried out in USP dissolution apparatus Type I (basket method) in simulated gastric fluid pH (described below) media at 37 ± 0.5°C at 75 rpm. Microspheres equivalent to 10 mg of drug were placed in the basket. Samples were withdrawn at predetermined time intervals and replaced with fresh dissolution media up to 16 h. Cumulative percentage drug released at various time intervals was calculated and the mean of three different batches was used in data analysis. The corresponding release profiles are presented in Chapter 5 (Section 5.8).

4.7. Characterization of release kinetics

In order to understand the mechanism of drug release from these formulations, the cumulative percentage drug release data (post 2 h) was fitted into the power law equation given by Korsemeyer et al. (1983) and Ritger and Peppas (1987)

$$M_t / M_\infty = Kt^n \qquad \ldots\ldots \qquad (1)$$

Where, M_t / M_∞ is percentage of drug released at any time ‘t’; ‘K’ is release rate constant incorporating the structural and geometric characteristics of the polymeric system and the drug and ‘n’ is the diffusion exponent indicative of the release mechanism of the drug. The details of model analysis is presented in Appendix-I.

The $t_{10\%}$ (time required for 10% drug release) was determined directly from the plot of cumulative percentage drug released vs. time while the $t_{90\%}$ (time required for 90% drug release) was calculated as

$$t_{90\%} = anti\ \log\ \{(0.9 - \log\ K)/n\} \quad \ldots\ldots\ (2)$$

The values of K, n, $t_{10\%}$ and $t_{90\%}$, 'r' (correlation coefficient of the regression analysis) and MSSR (Mean sum of squared residuals), as obtained from the dissolution data of designed formulations are given in Chapter 5.

The release data for formulations in simulated GI fluid pH was compared with the ideal theoretical release profile using dissimilarity (f_1) and similarity (f_2) factor analysis. The details of similarity and dissimilarity factor analysis is presented in Appendix - I.

4.8. Batch reproducibility and stability on storage

To study batch reproducibility, three batches of each formulation were prepared and evaluated for drug content and release profile of indomethacin. In order to assess the long term stability of the various formulations prepared, selected formulations from each batch were stored at 40 ± 2°C and 75 ± 5% RH for 6 months. At the end of the study period, the formulations were observed for change in physical appearance, drug content and in vitro drug release characteristics. The initial (zero time) results were compared with post stability testing period results for statistical differences. The powdered samples of indomethacin matrix tablets and microspheres were also subjected to DSC and FTIR studies.

(i) DSC studies: The possibility of any interaction between indomethacin and other polymeric excipients during tablet processing was assessed by carrying out thermal analysis on pure drug and excipients and powdered samples of formulation matrix before and after storage using Differential Scanning Calorimeter. Pure drug and formulation each equal to 2.5 mg of drug were accurately weighed onto standard aluminium pans and thermograms were obtained after crimping as mentioned previously.

(ii) FTIR studies: For FTIR, the samples were appropriately diluted with dried potassium bromide and IR spectra were acquired as described previously. The DSC thermograms and FTIR spectrum of pure indomethacin and formulations are shown in Chapter 5 (Section 5.9)

4.9. In vivo evaluation of selected formulations in animal model (Wistar rat)

Preliminary screening of selected formulations was carried out in vivo in rats. The protocol was previously approved by the Institutional Animal Ethics Committee (Approval No- IAEC-/RES/10-5). Healthy Wistar rats (350-400g), both male and female were selected for the study. Prior to tablet administration, the animals were kept on overnight fast. Six different formulations were taken for GI transit studies. The tablet was

placed in the throat of the animal with a pair of forceps and about 1.5-2 ml of water was flushed down the throat slowly to facilitate entry of tablet into oesophagus with the help of syringe. The animals were sacrificed at fixed time intervals (2, 4, 6 and 8 h) and the position of tablet was located. The recovered tablets at various time points were analyzed for residual drug content to estimate the amount of drug released at each time point.

For evaluation of GI transit of microspheres, formulations were colored using a water-insoluble dye (Oil Red) by a method reported previously (Roy, 2008). In vitro release studies were done to check if adsorption of dye affects the in vitro release. Microspheres were suspended in water containing 0.5% w/v sodium carboxy methyl cellulose and administered (50mg/2ml) via oral feeding tube to rats. This was followed by animal sacrifice at regular intervals as mentioned above to examine the fate of these microspheres. The results of GI transit analysis in rats is presented in Chapter 5 (Section 5.10)

4.10. In vivo evaluation of selected formulations in human subjects

All the processing involving radionuclide Technetium-99m (^{99m}Tc) was done in hot lab of Nuclear Medicine Dept. of M. N. Budhrani Cancer Institute, Pune, India. Subsequent studies involving human subjects were carried out in the same department. Healthy male human subjects, aged 22-25 years were selected for the study. The subjects were non-smokers and refrained from medication and alcohol two weeks prior to the commencement of study. An informed written consent was obtained prior to the start of the study which was conducted in accordance with the guidelines laid down by the Helsinki Declaration, 1964 and was approved by the Institutional Human Ethics Committee of both M. N. Budhrani Cancer Institute, Pune, India and BITS, Pilani, India (Approval No: IHEC-14/06-07).

The radionuclide ^{99m}Tc was eluted from automatic ^{99m}Tc sterile generator in saline (0.9% NaCl) and DTPA (diethylene triamine pentaacetic acid) was labeled with ^{99m}Tc using a standard test kit. The eluent was checked for initial radioactivity using radioisotope calibrator beta-counter (Capintec Inc, USA). Around 10 MBq (1MBq = 0.027 mCi) was drawn into 28 gauge needle and was slowly delivered onto tablet surface and dried using hot air blower. Gamma scintigraphy was done using dual headed Siemens gamma camera fitted with low energy, high resolution collimators and data obtained was processed using Icon software. The subjects were prescreened for gastric emptying parameters by measuring clearance of 100 ml radiolabelled (1MBq) water from the

stomach and those with normal gastric emptying rate corresponding to ≥ 60% clearance in 1 h were only included to participate in the study. The results are presented in Chapter 5 (Section 5.11).

Each treatment (tablet) was administered to subject who was fasted overnight for 12 h with 250 ml of water. Images were acquired intermittently at 30 mins and 1 h intervals in 128 x 128 matrix. The subjects were allowed light breakfast comprising of one sandwich and a glass of juice after the tablet had passed through stomach. Lunch was provided after the tablet passed the small intestine. In order to compute colon residence time, the time of defecation was noted and image was taken at 24^{th} h to confirm absence of tablet in colon. The results are presented in Chapter 5 (Section 5.11).

5.1. Characterization of bulk drug

Indomethacin was found to comply with the various tests of identification as per Indian Pharmacopeia (IP) 1996. The IR spectrum of the drug revealed peak bands at 1060 cm^{-1} due to stretching of ether group (-C-O-). The peak corresponding to the tertiary amide (-CON-) was observed at 1650 cm^{-1} while carbonyl stretching of aliphatic COOH was observed at 1720 cm^{-1}. The aromatic nature of the compound was confirmed by peaks observed in the region 800-650 cm^{-1} and 3000-3080 cm^{-1} due to aromatic H bending and stretching respectively. The IR spectrum of pure indomethacin is shown in Fig 5.1.

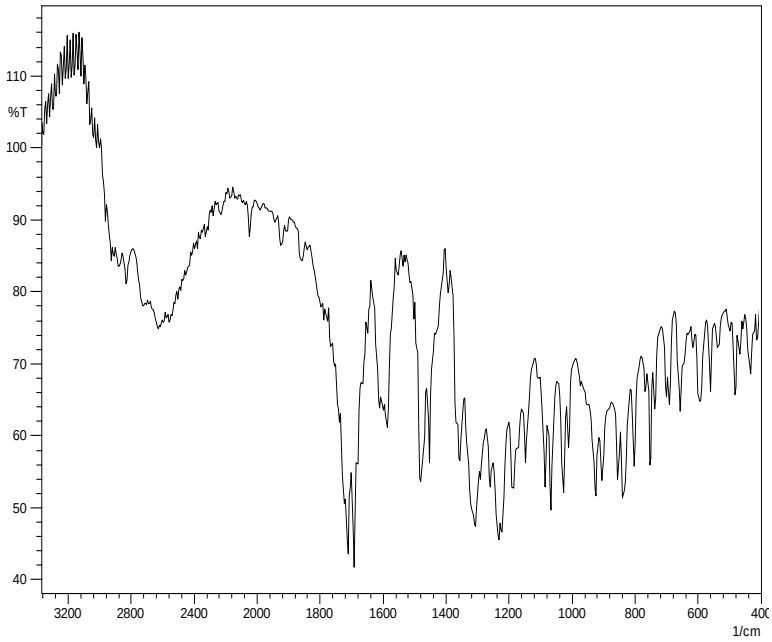


Fig 5.1: IR spectrum of pure indomethacin

5.2. Analytical method development and validation

Several official and non - official methods of analysis for estimation of indomethacin have been reported in literature (Chapter 3, Section 3.3). A detailed survey of literature revealed two UV spectrophotometric methods for estimation of indomethacin (Mahrous et al., 1985; B.P. 2007). Both methods involve the determination of drug in the presence of degradants and involve either an extraction step or use a derivative mode to quantify the drug. None of these reported methods were found to be suitable for estimation of

indomethacin for routine analysis like drug content estimation of prepared formulations, in vitro dissolution sample analysis and preliminary studies like solubility and bench-top stability studies. A UV spectrophotometric method would offer the advantage of a simple and speedy analysis in such cases.

Therefore, the first objective of the present study was to develop simple, sensitive, precise, accurate, and cost-effective analytical method for the estimation of indomethacin in in-house prepared pharmaceutical formulations, and for analysis of samples from in vitro dissolution studies. Analytical method was developed in phosphate buffer pH 7.4. The developed method was validated as per ICH guidelines (2005).

5.2.1. Selection of solvent system

For media optimization, various solvent systems comprising of different pH media alone and in combination with different organic solvents and in various proportions were tried. The final selection of a solvent system was based on certain criteria like: sensitivity, solubility of the drug at this pH, ease of preparation and analysis time. For the preparation of stock solution, methanol: phosphate buffer (pH 7.4) in the ratio of 10: 90% v/v was considered optimum as a medium. Further dilutions were made in phosphate buffer pH 7.4. Effect of various formulation additives on the absorbance of indomethacin was studied and no interference was observed. The corresponding UV spectra of drug (10 µg/ml) in phosphate buffer pH 7.4 are shown in Fig 5.2 (a). The spectrum of drug was unchanged after analysis in the selected medium at 24 h indicating stability of the drug and suitability of the solvent system for UV analysis of the drug (Fig 5.2 b).

The λ_{max} of indomethacin in phosphate buffer (pH 7.4) was found to be at 220 nm with a second peak at 266 nm and third peak at 320 nm. The wavelength selected for estimation purpose was 320 nm as there was no interference from excipients at this wavelength (Fig 5.2 c).

5.2.2. Calibration curve and regression analysis

For the developed UV method for estimation of indomethacin, the calibration curve data is presented in Table 5.1. The linearity range was found to be 5-50 µg/ml. The linear regression equation was obtained as Y = 0.0221 X + (–0.0001) where, Y is the absorbance and X is the concentration (in µg/ml) of pure indomethacin solution (Table 5.2). Goodness of fit of regression equation was supported by highly significant value of ‘r’ (0.9999).

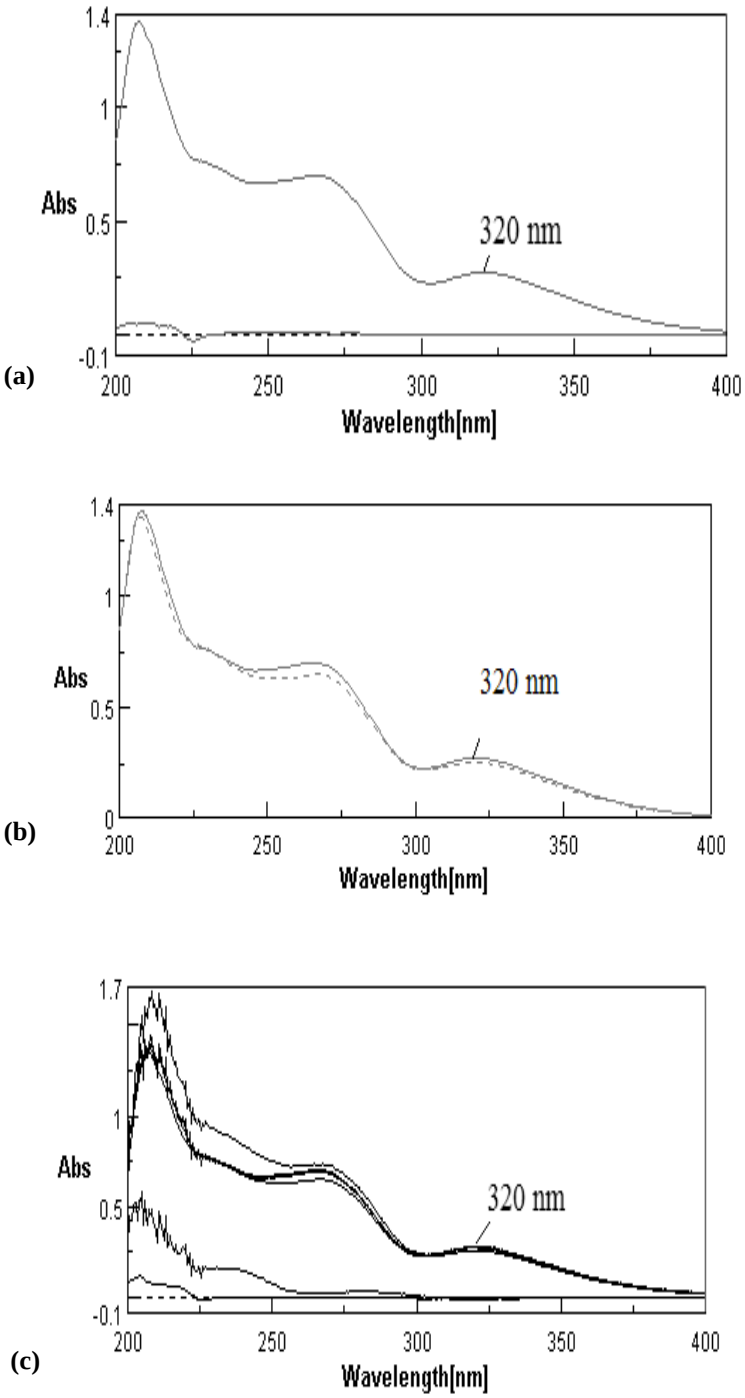


Fig 5.2: UV Absorbance Spectra of indomethacin in phosphate buffer pH 7.4 over (a) blank (b) after 24 h (10 μg/ml), (c) in presence of polymeric excipients.

Further, a one way ANOVA test performed between the replicate samples established good linearity of the proposed method as evident from low calculated *F*-value (0.0015) at 5% level of significance (Table 5.3). Lower values of parameters like standard error of slope, intercept, and estimate indicated high precision of the proposed method (Table 5.2). The regression line can be assumed to be passing through origin as the slope value without

intercept fell within the 95% CI of the slope. The absorbance spectrum of indomethacin was not changed in the presence of common excipients in selected medium. Since the interference of excipients was insignificant in the estimation of drug, it was therefore concluded that proposed method was specific and selective for the drug (Fig 5.2 c).

Table 5.1: Calibration curve data points for estimation of pure indomethacin solution in phosphate buffer pH 7.4 at 320 nm

Concentration of solution (µg/ml)	Absorbance*	% RSD
5	0.1113 ± 0.0041	3.69
10	0.2200 ± 0.0049	2.23
20	0.4411 ± 0.0154	3.49
30	0.6622 ± 0.0096	1.45
40	0.8833 ± 0.0228	2.58
50	1.1044 ± 0.0378	3.42

*Values are presented as mean ± SD of ten determinations.

Table 5.2: Regression analysis parameters for estimation of pure indomethacin solution in phosphate buffer pH 7.4 at 320 nm

Statistical parameter	Value
Regression equation	Y = 0.0221X + (– 0.0001)
Correlation coefficient (r)	0.9999
Standard error of slope	4.0×10^{-4}
Standard error of intercept on ordinate	1.31×10^{-2}
Standard error of estimate	1.69×10^{-2}
95% confidence interval limits of slope	2.09×10^{-2} to 2.33×10^{-2}
95% confidence interval limits of intercept	-3.76×10^{-2} to 3.53×10^{-2}
Slope without intercept	2.21×10^{-2}

Y = absorbance; X = concentration

5.2.3. Method validation

Accuracy is reported in terms of % recovery values that were obtained at three concentration levels (LQC: 5 µg/ml, MQC: 20 µg/ml and HQC: 50 µg/ml), each determined for six samples in triplicate (*n* = 18). The mean percentage analytical recoveries (± SD) at different concentrations were found to be 101.7 ± 2.7 % (LQC: 5 µg/ml), 100.7 ± 2.5% (MQC: 20 µg/ml) and 101.9 ± 2.5% (HQC: 50 µg/ml) respectively. The high (nearly 100%) mean % recovery values with low standard deviation represented accuracy of the method (Table 5.4).

Table 5.3: One – way ANOVA* test for linearity of the analytical method.

Source of variation	Degree of freedom (DF)	Sum of squares(SS)	Mean sum of squares (MS)	F- value	
				F_{calc}	F_{crit}
Between group	9	2.232×10^{-3}	2.48×10^{-4}	1.5×10^{-3}	2.095
Within group	45	7.425	1.65×10^{-1}		
Total	54	7.427			

* at 5% level of significance

Precision was determined in terms of repeatability and intermediate precision. In repeatability study (n = 18), the mean RSD was found to be 2.5%. At all three concentration levels, the low values of coefficient of variance indicate good level of precision (Table 5.4). Intermediate precision for within day and between day variations showed low mean RSD of 2.6% for intraday precision and 2.5% for interday precision indicating that this method has excellent repeatability and intermediate precision.

The detection limit (DL) was found to be 1.8 µg/ml, and quantitation limit (QL) was determined as 4.8 µg/ml respectively. Robustness was found to be very good as variation of pH of the selected media by ± 0.2 did not have any significant effect on the absorbance of the drug. The mean percentage recovery from robustness study was found to be 99.94 ± 0.826%. The drug was found to be stable in selected solvent system for 24 h.

Table 5.4: Accuracy and precision data for the developed method

Level	Accuracy (Recovery)				Precision (% RSD)	
	Actual concentration (ug/ml)	Mean ± SD[a]	% RSD	Recovery (%)	Intraday [b]	Interday[c]
LQC	5	5.1 ± 0.2	2.6	101.7 ± 2.7	3.2	2.8
MQC	20	20.1 ± 0.7	2.5	100.7 ± 2.5	2.1	2.7
HQC	50	50.9 ± 1.7	2.4	101.9 ± 2.5	2.5	2.0

[a] Mean in µg/ml and SD for six triplicate determinations.

[b] Based on three triplicate determinations per day.

[c] Based on three triplicate determinations for 3 days.

The proposed method was evaluated by estimation of indomethacin in pharmaceutical formulations. The assay value of indomethacin for the marketed formulation was found to be 73.5 ± 1.05 mg/cap and for an in-house prepared formulation (tablet) was found to be 73.97 ± 2.01 mg/ tablet. Assay values of formulations were close to the label claim. Further, analytical recovery studies were performed by adding known amount of pure drug

solution (10 µg/ml) to preanalyzed samples of the commercial dosage form and the percent analytical recovery values were calculated by comparing concentration obtained from the spiked samples with the actual added concentrations. The high values of percent analytical recovery were found indicating that the interference of excipient matrix is insignificant in estimation of indomethacin by the proposed method (Table 5.5).

Table 5.5: Application of developed method in drug recovery studies.

Sample	Label claim (mg/ capsule)	Amount recovered as per label claim #	Analytical recovery# (%)
Pure drug solution	10 mg in 100 ml	10.01 ± 0.21 mg in 100 ml	100.1 ± 1.2
Tablet (in-house formulation)	75 mg/tab	73.97 ± 2.01 mg/tab	101.0 ± 1.3
Marketed sample (Indocap)	75 mg/cap	73.5 ± 1.05 mg/cap	100.2 ± 1.6

#Values are presented as mean ± SD (n = 3) of labeled claim

In summary, the proposed method was sensitive, simple, rapid, accurate and precise and can be used for routine analysis of indomethacin in pure form, in quality control of formulations and for analysis of samples obtained from drug release studies.

5.3. Preformulation Studies

The main goals of a preformulation process are determination of necessary physicochemical properties of a new drug substance, its kinetic rate profile, and its compatibility with common excipients (Chassagneux, 2004). Preformulation studies generally include physicochemical characterization of drug like determination of solubility, dependence solubility on pH, pH stability, dissociation constant and partition coefficient of the drug. A thorough understanding of stability of drug in pure form and in physical mixture with excipients under varying conditions of temperature, light and humidity is important for identification of potential drug stability and in drug excipient compatibility problems.

5.3.1. Determination of solubility profile

For poorly soluble, highly permeable (Class II) drugs, the rate of oral absorption is often controlled by the dissolution rate in the gastrointestinal tract. Therefore, together with permeability, the solubility and dissolution behavior of a drug are key determinants of its oral bioavailability. The reported value for solubility of indomethacin (Form I) in water is 4.0 µg/ml (Borka, 1974) at 25°C and around 3.66 µg/ml and 1975 µg/ml respectively in

pH 1.2 and pH 7.2 buffered aqueous medium at 37°C (Valizadeh et al., 2004). Our studies revealed that the drug that was present in a micronized form had a solubility of 53.5 ± 0.15 µg/ml at 25°C in distilled water. The drug was shown to have pH dependent solubility with solubility increasing from very low value of 2.3 ± 0.01 µg/ml (pH 1.2) and 4.1 ± 0.02 µg/ml (pH 4.5) to 487.6 ± 4.84 µg/ml at pH 6.8 and 1044.7 ± 4.19 µg/ml at pH 7.4 (Table 5.6, Fig 5.3). With increase in pH, increase in the protonation (ionization) of the carboxylic acid moiety resulted in abrupt increase in solubility of indomethacin in alkaline pH. Further, increase in pH to 8.0 was not found to increase solubility. Therefore, according to the USP solubility definition, indomethacin has been shown as a practically insoluble drug at pH 1.2 and slightly soluble at pH 7.4.

Table 5.6: Solubility of indomethacin in various buffered solutions at 25°C.

Media	Maximum solubility* (µg/ml)
pH 1.2	2.3 ± 0.01
pH 4.5	4.1 ± 0.02
pH 6.8	487.6 ± 4.84
pH 7.4	1044.7 ± 4.19
pH 8.0	1039.3 ± 3.14
TDW	53.5 ± 0.15

*Values given as mean ± SD of triplicate determinations.

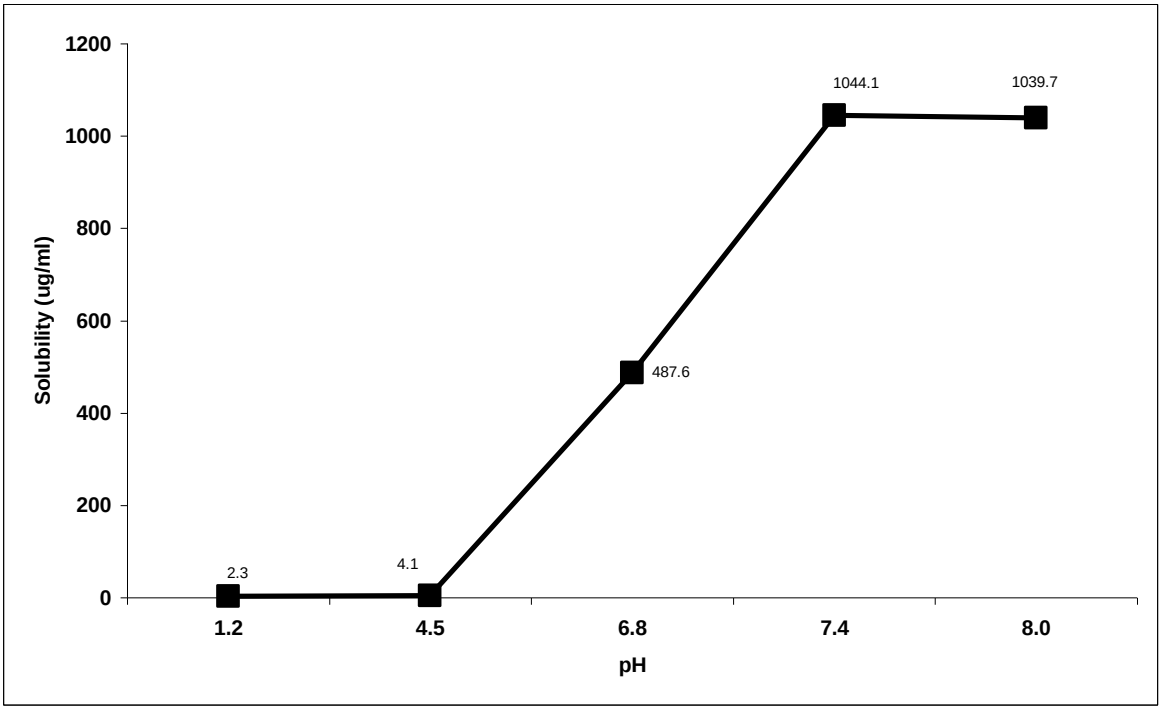


Fig 5.3. Solubility profile of indomethacin in buffered systems of varying pH and TDW

5.3.2. Determination of physical form of indomethacin

The physical form of indomethacin was confirmed by estimation of melting point and DSC thermal analysis. The melting point determined using Buchi apparatus was found to be 158-161°C. The DSC thermogram of pure indomethacin (Form I) revealed a sharp endothermic peak of the drug at 161°C with onset at 157°C confirming the polymorphic form of the drug as well as indicating absence of impurities (Fig 5.4). .

In order to ascertain the stability of drug during tableting process as well as to ensure the absence of polymorphic conversions, the pure drug was subjected to grinding with commonly used tablet excipients, granulated with ethyl alcohol, dried at 50°C and then compressed to yield compacts which were then subsequently powdered and thermal analysis repeated (Fig 5.4). It was observed that the thermogram of processed drug was identical to the unprocessed form indicating stability of drug during manufacturing process (Del Rio, 2002).

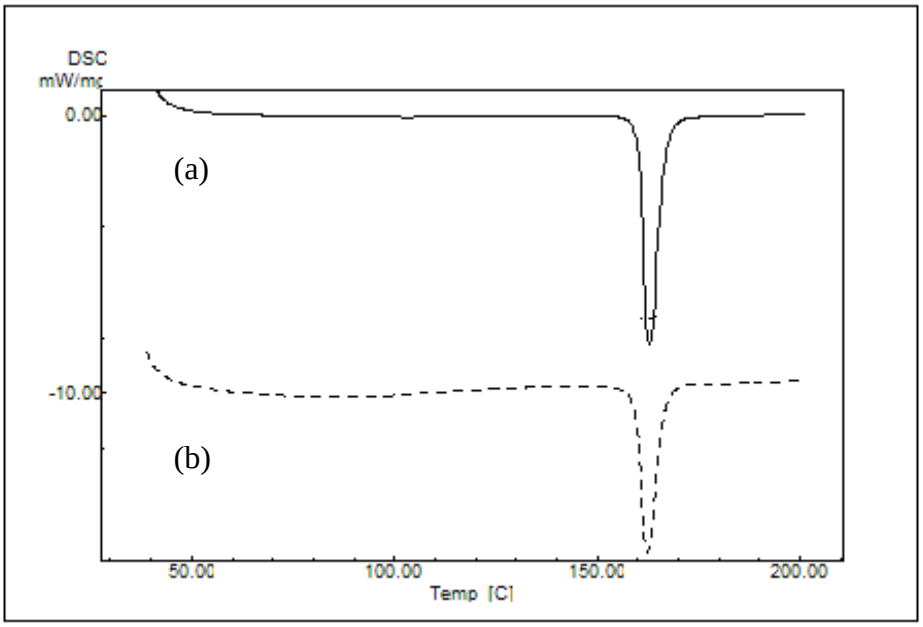


Fig 5.4: DSC thermogram of indomethacin (a) before processing and (b) after processing

5.3.3. Solution state stability studies

The solution state stability of indomethacin was determined in media of varying pH in the range of 1.2 to 8.0 in order to determine stability of drug in conditions it is likely to encounter during GI transit (Table 5.7). Further, this information will help in selection of media for dissolution study. It was observed that at both CRT and accelerated conditions the drug degradation followed first order kinetics at all pH conditions in buffered systems.

The degradation rate constant was found to decrease from pH 1.2 to 4.5 and then further increase as the pH increases at both CRT and accelerated condition (Table 5.7 and Fig 5.5). In TDW with a pH of 6.8-7.0, drug degradation rate values were close to that observed for pH 7.4. The drug was found to show rapid degradation at CRT in extreme acidic (K_{deg} of 5.30 x 10^2 day^{-1}; $T_{90\%}$ of ~ 2 days) and alkaline pH (K_{deg} of 14.25 x 10^2 day^{-1}; $T_{90\%}$ of ~ 0.74 days). This was attributed to formation of two products of hydrolysis 4-chlorobenzoic acid and 5-methoxy-2-methylindoleacetic acid as reported previously (Novakova et al., 2005). Similar results were observed for degradation of drug in buffered systems at accelerated conditions (Table 5.7). It has been shown previously that indomethacin degradation was rapid and followed first order kinetics in alkaline aqueous medium (Hajratwala and Dawson 1976; Singla et al., 1991). Further, maximum stability of drug was observed in intermediate pH range of 4.5 and 6.8 (Table 5.7, Fig 5.5). This observation was in agreement with a previous study that indicated that indomethacin showed maximal stability at pH 4.9 and 4.7 at 25°C (Kahns et al., 1989).

Table 5.7: Degradation kinetics of indomethacin in buffered solutions of varying pH at controlled (CRT) and accelerated conditions (ATC)

pH	CRT			ATC		
	r	K_{deg} x 10^2 (day^{-1})	$T_{90\%}$ (days)	r	K_{deg} x 10^2 (day^{-1})	$T_{90\%}$ (days)
1.2	0.9623	5.30	1.99	0.9573	12.86	0.82
4.5	0.9105	0.53	19.90	0.9873	2.09	5.06
6.8	0.9919	3.43	3.08	0.9866	9.01	1.17
7.4	0.9950	3.68	2.87	0.9588	15.07	0.70
8.0	0.9123	14.25	0.74	0.9436	17.29	0.61
TDW	0.9906	3.60	2.93	0.9490	10.24	1.03

r- first order correlation coefficient; K_{deg} - first order degradation rate constant; $T_{90\%}$ - time taken for drug to degrade to 90% of the labeled claim

Solution state stability stability studies were also carried out in unbuffered pH range of 1.2 to 12.5 to check the possible influence of buffer salts on degradation. In case of solution state stability studies performed in unbuffered solutions of varying pH under controlled conditions, the drug showed slower rate of degradation upto pH 8.5 (Table 5.8, Fig 5.6). This was attributed to the fact that in case of buffered systems, the phosphate bases (HPO_4^{2-}) in the buffer probably react with the carboxylate moiety present in indomethacin, resulting in dissociation and enhanced degradation. As in case of buffered systems, minimum degradation was observed in intermediate pH range of 4.5. The drug

degradation was rapid in alkaline conditions above pH 8.0 due to hydrolysis which conforms to earlier reports (Archontaki, 1995).

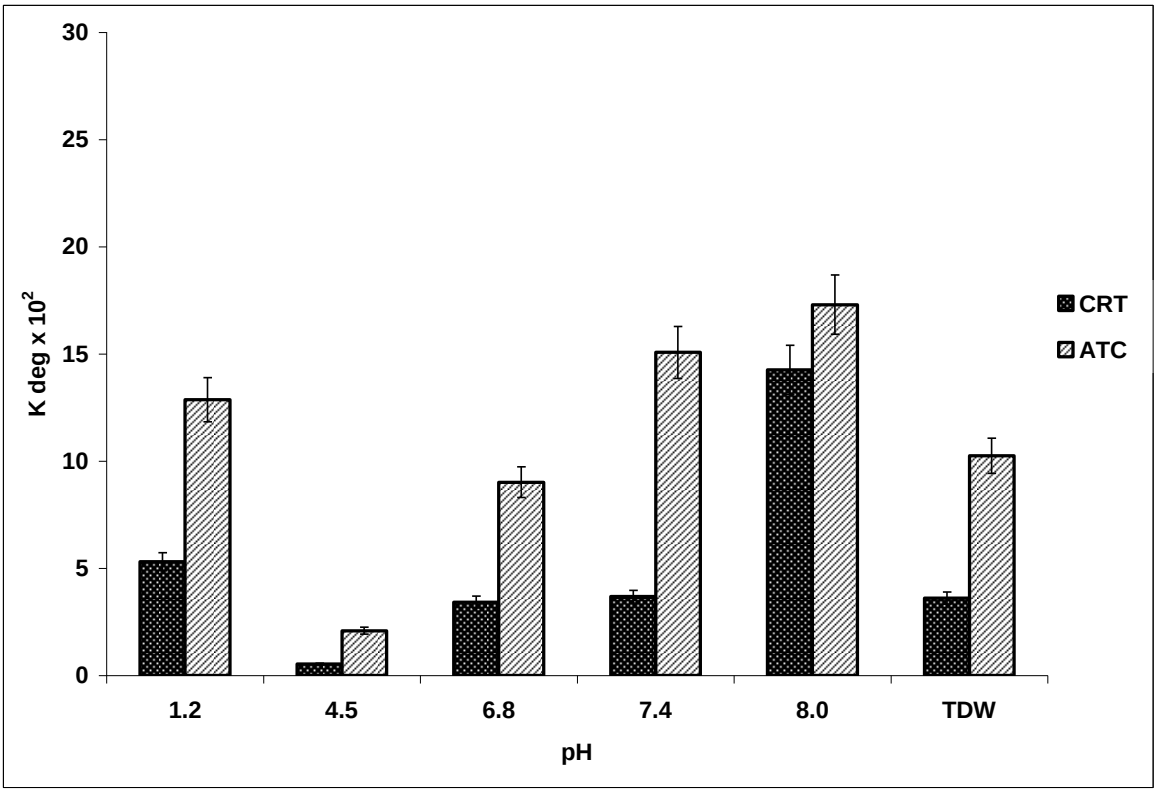


Fig 5.5: Degradation profile of indomethacin in buffered medium of varying pH at CRT and ATC

Table 5.8: Degradation kinetics of indomethacin in various unbuffered solutions of varying pH at controlled (CRT) and accelerated conditions (ATC)

pH	CRT			ATC		
	r	K_{deg} x10^2 (day $^{-1}$)	$T_{90\%}$ (days)	r	K_{deg} x10^2 (day $^{-1}$)	$T_{90\%}$ (days)
1.2	0.9113	10.76	0.98	0.9264	12.41	0.85
2.5	0.9658	5.38	1.96	0.9232	10.04	1.05
3.5	0.9112	8.86	1.19	0.9484	8.94	1.18
4.5	0.9839	2.99	3.52	0.9732	5.07	2.08
5.5	0.9513	1.95	5.40	0.9667	3.22	3.27
6.5	0.9820	1.55	6.79	0.9686	1.97	5.35
7.5	0.8823	1.57	6.70	0.9415	3.47	3.04
8.5	0.9794	2.06	5.11	0.9746	4.63	2.28
9.5	0.9138	16.48	0.64	0.8546	20.68	0.51
10.5	0.9589	5.86	1.80	0.9823	13.18	0.80
12.5	0.9863	12.26	0.86	0.9141	25.73	0.41

r- first order correlation coefficient; K_{deg} - first order degradation rate constant; $T_{90\%}$ - time taken for drug to degrade to 90% of the labeled claim

When solution state stability study in unbuffered pH systems was carried out at accelerated conditions, overall degradation rate constants were relatively higher than those at controlled temperature conditions. One interesting observation made in unbuffered systems was the relatively higher stability of drug in pH range 6.5 -7.5 ($T_{90\%}$ ~ 6-7 days) in controlled temperature and ($T_{90\%}$ ~ 3-6 days) in accelerated conditions. This was attributed to negligible dissociation of indomethacin in neutral pH.

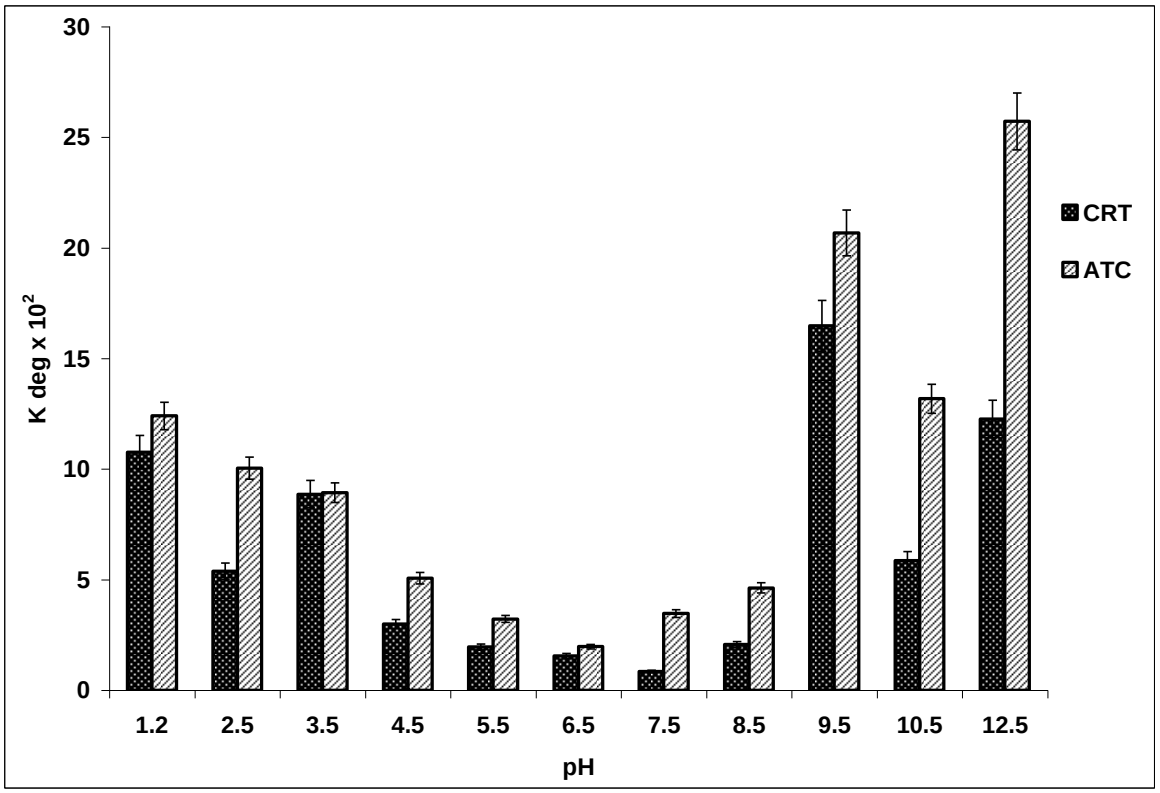


Fig 5.6: Degradation profile of indomethacin in unbuffered medium of varying pH at CRT and ATC

5.3.4. Solid state drug excipient compatibility study

Solid state drug stability determined at controlled and accelerated conditions revealed excellent stability for pure indomethacin (Table 5.9), as indicated by very low values for K_{deg} (0.87×10^{-3} $month^{-1}$ at CRT and 2.37×10^{-3} $month^{-1}$ at accelerated conditions) and high values of $T_{90\%}$ (120.7 and 44.5 months respectively). The predicted $T_{90\%}$ for all the solid admixtures at CRT conditions show that the drug is stable in the presence of the various polymers and excipients to be used in the different formulations. It may be observed that K_{deg} values were slightly higher for certain polymeric excipients due to their ability to pick moisture. A relatively higher value of K_{deg} was obtained at accelerated conditions in the presence of certain heat and moisture sensitive polymers like polycarbophil, carbopol, xanthan gum and hydroxy propyl cellulose. Drug stability under

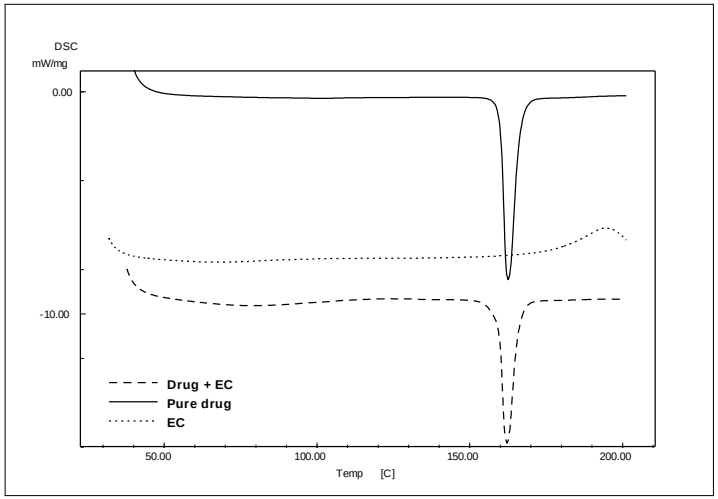
accelerated condition was found to be lowest for its physical mixture with PCP + EC. The packaging and storage of indomethacin formulations prepared using such polymers will also require special attention.

Stability of formulations was further ascertained by DSC and FTIR studies. Representative DSC thermograms of drug in different physical mixtures with polymeric excipients obtained for the physical mixture stored at CRT conditions for six months are shown in Fig 5.7 and 5.8. It was observed that the melting endotherm and enthalpy of fusion of drug was preserved in all the samples. Further, FTIR studies showed that the spectrum of drug was unchanged with respect to the major functional groups of the pure drug (Fig 5.9). Thus, it may be concluded that indomethacin is stable in the presence of selected excipients and polymers that were proposed to be used for further studies.

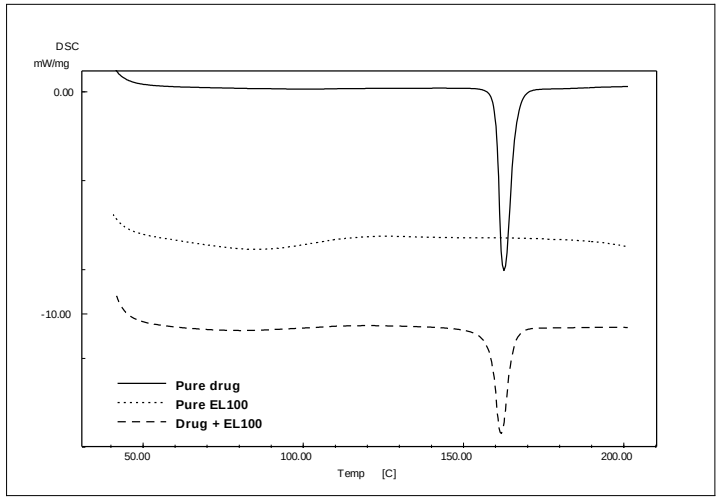
Table 5.9: Degradation kinetics of indomethacin in solid admixtures at controlled (CRT) and accelerated conditions (ATC)

Physical admixture Drug/ Drug + Excipient	CRT		ATC	
	K_{deg} x 10^3 ($month^{-1}$)	$T_{90\%}$ (months)	K_{deg} x 10^3 ($month^{-1}$)	$T_{90\%}$ (months)
Indomethacin	0.87	120.7	2.37	44.5
I + EC	4.30	24.5	4.46	23.6
I + EL	2.98	35.4	4.16	25.3
I + ES	2.95	35.7	3.83	27.5
I + PCP	4.41	23.9	14.25	7.4
I + CP	5.22	20.2	16.48	6.4
I+ HPC	4.38	24.1	12.12	8.7
I+ XG	5.38	19.6	16.22	6.5
I + GG	5.67	18.6	14.44	7.3
I + T	2.34	45.0	2.41	43.8
I + M	1.96	53.7	2.07	50.9
I + PCP+EC	4.04	26.1	32.96	3.2
I + EL+ES	3.64	28.9	4.11	25.6
I + ES+EC	3.57	29.5	4.20	25.1
I + EL+EC	3.68	28.6	3.72	28.3
I+ HPC+ EL	3.49	30.2	5.35	19.7
I + T + M	0.88	119	2.33	45.2

K_{deg} - first order degradation rate constant; $T_{90\%}$ - time taken for drug to degrade to 90% of the labeled claim; I- Indomethacin; EC- ethyl cellulose; EL- Eudragit L100; ES-Eudragit S100; PCP-polycarbophil; CP-Carbopol; HPC-hydroxy propyl cellulose; XG- xanthan gum; GG- guar gum; T- talc; M- magnesium stearate



(a)



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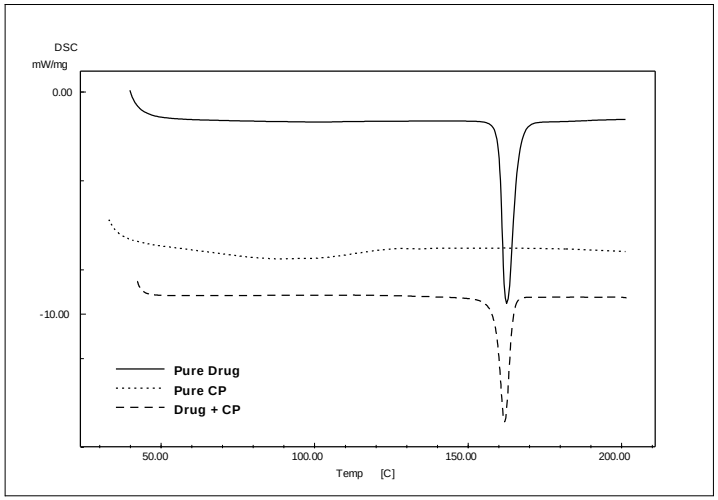

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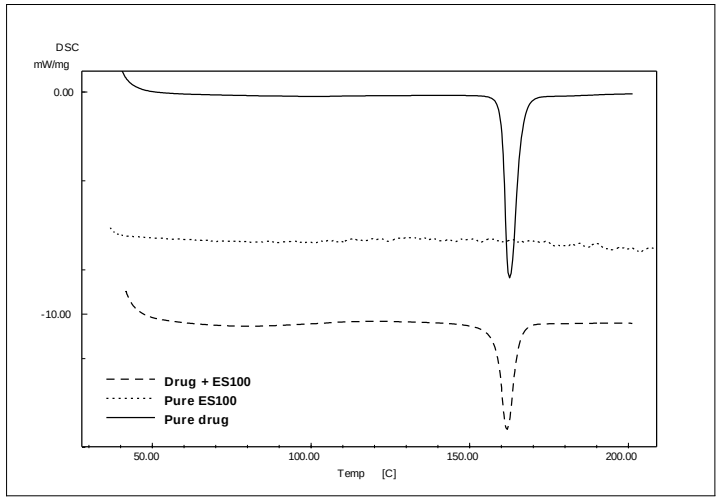
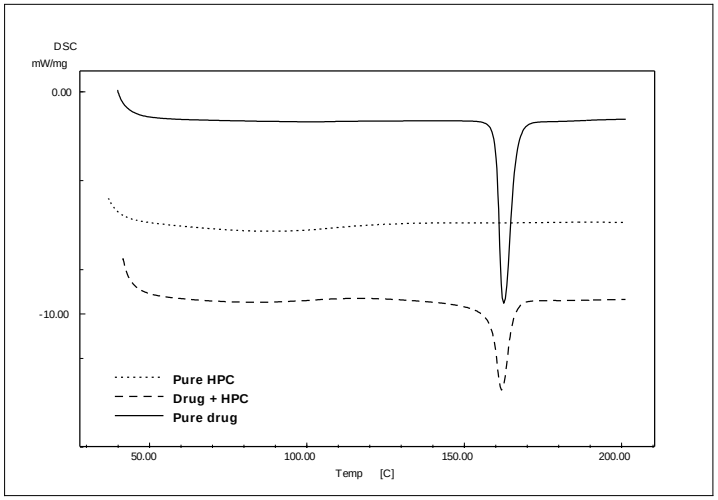
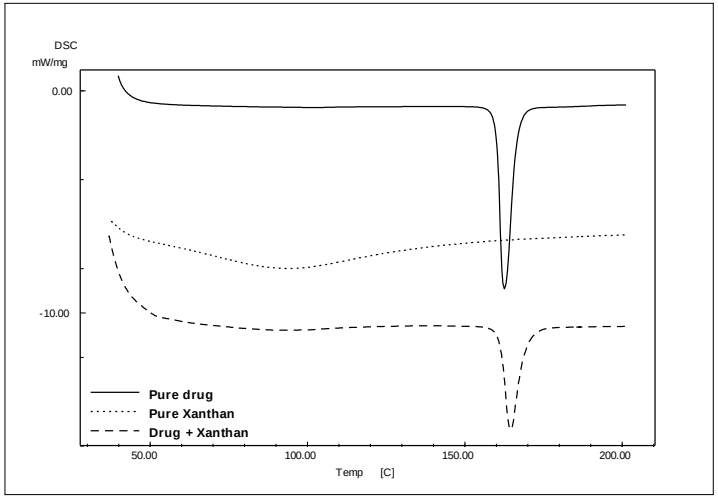


(d)

Fig 5.7: Representative DSC thermograms of physical admixtures of drug with (a) EC (b) EL100 (c) CP (d) ES100 stored at CRT for six months



(a)



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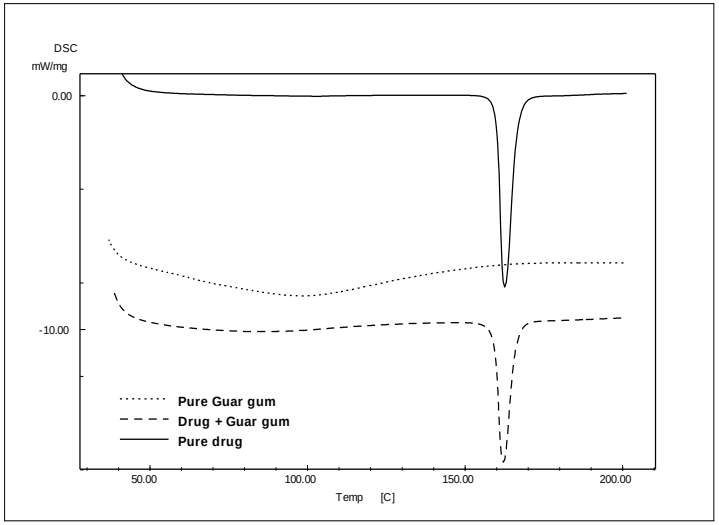

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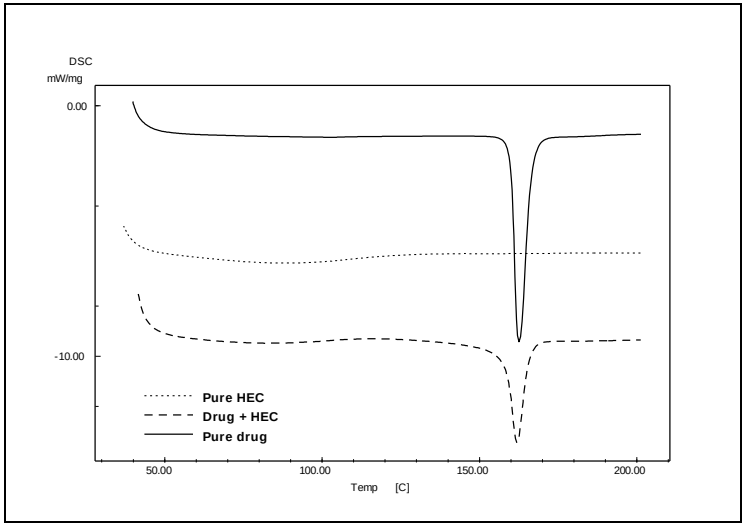
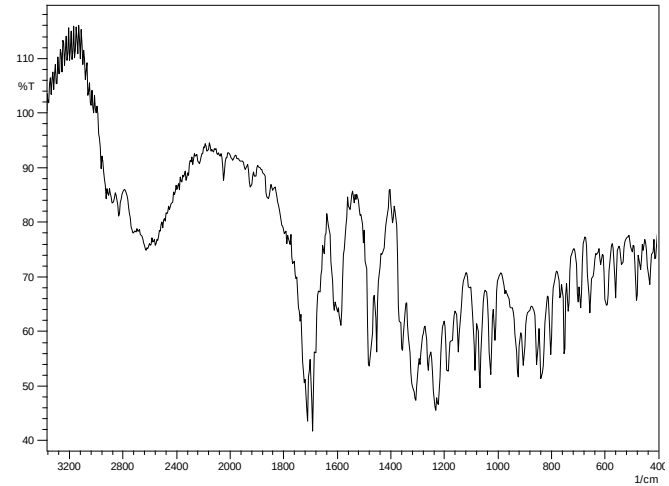
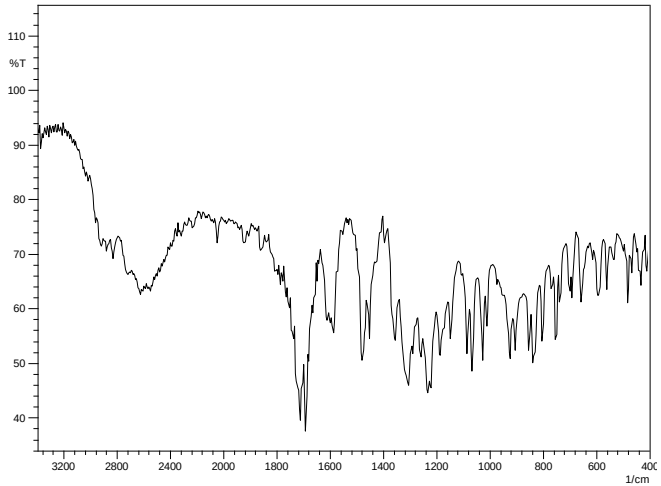


(d)

Fig 5.8: Representative DSC thermograms of physical admixtures of drug with (a) HPC (b) XG (c) GG (d) HEC stored at CRT for six months



(a)



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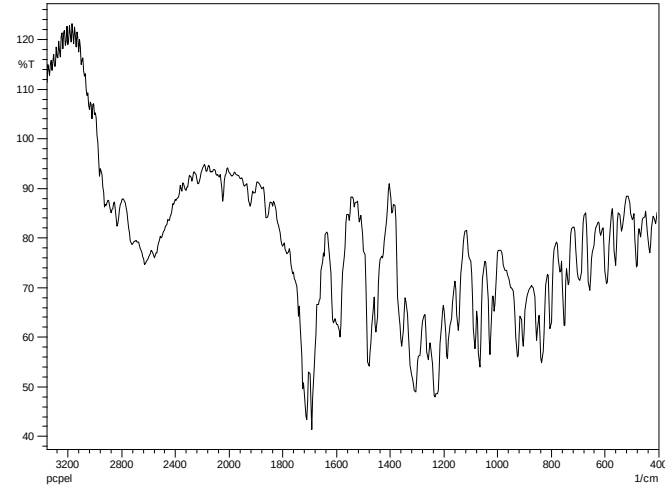

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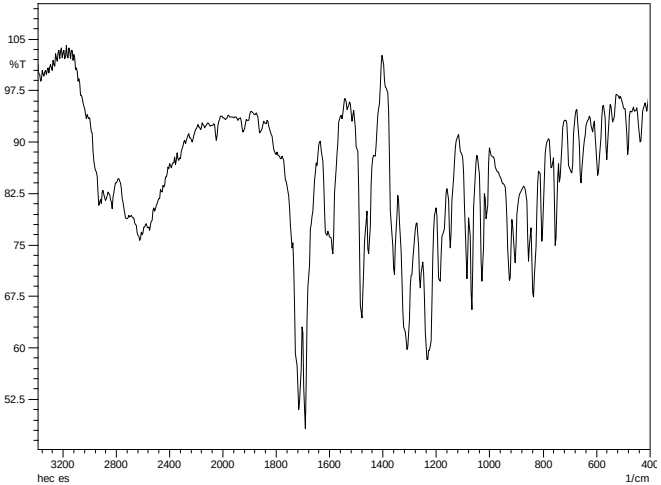


(d)

Fig 5.9: Representative FTIR spectra of physical admixtures of drug with (a) EL100 (b) HEC (c) PCP (d) EC stored at CRT for six months

5.4. Formulation design and development

For an ideal colon targeted drug delivery system, the drug release should be prevented in the stomach and small intestine (Ibekwe et al., 2004). Release of drugs must be completed within the residence time in the colon. Approaches that have been reported for achieving site specific drug release in colon like prodrugs, pH, time and enzyme controlled systems have been reviewed in detail in Chapter One. One of the most commonly employed approach for colon specific delivery is coating the drug delivery system with pH sensitive polymers (Ashford et al. 1993). The pH sensitive polymers for colonic delivery are designed to be solubilized at a pH around 7.0 so as to exploit the increase of pH in the large intestine. However, as the pH of the colon drops from 7.0 in the terminal ileum to 6.5 in the ascending colon (due to the fermentation of undigested food by colonic bacteria leading to the formation of organic acids which lower the pH), it is possible that coatings which dissolve at pH 7.0 would release the active agent in the ileum itself rather than in the colon. On the other hand, if the coating is too thick or non-uniform, there is a possibility that no drug will be released in the colon (Ashford et al. 1993). Studies carried out in the recent past have also shown that tablets coated with Eudragit polymers demonstrated erratic performance in vivo and many tablets failed to disintegrate inside the human body (Ibekwe et al., 2006, 2008). Another drawback of employing polymers that dissolve at higher pH values (> 7.0), is that they may fail to release the drug in patients with inflammatory bowel disease whose luminal pH does not exceed the threshold pH for dissolution of the pH sensitive polymer (Ewe et al.,1999).

A bimodal (sigmoidal) release profile characterized by negligible to slower release in the initial stage (0-6 h) followed by controlled release during later stage (6-16 h) will ensure targeted drug release to the colon. Solid dosage forms with such release characteristics can be designed either by coating the drug core with pH sensitive polymer or incorporating the drug in polymeric matrix. Since the in vivo performance of tablet formulation coated with pH sensitive polymer is highly variable, an alternative means of averting this problem is by the use of a combined pH and transit time controlled matrix embedded system. By suitable modulation of matrix properties, it is possible to confer a bimodal (sigmoidal) release profile to the drug delivery system with negligible to slower release in the initial phase (0-6 h) followed by immediate or controlled release suited for colon targeting purpose. Such a system will help reduce the improbability in drug release from colon specific drug delivery system due to thin pH gradient between small and large intestine, pH changes in diseased states and variable gastric residence times (Friend 1991; Rubinstein 2005). Further, matrix embedded systems are simple to manufacture and also easy to reproduce and scale-up.

Polymers that show pH sensitive swelling and/or erosion property can modulate release in a biphasic pattern corresponding to the pH changes of the GI tract. However, one major issue with such pH sensitive polymers is maintenance of adequate matrix strength during the sojourn of the formulation in the upper GI tract upto colon.

Single unit systems: The rationale for polymer selection for developing a matrix system for single unit systems was that it should form strong and stable matrices with the potential of giving pH and time controlled release. Several anionic and non-ionic polymers that were either hydrophilic or hydrophobic were selected for this purpose. Cationic polymers were not used as these would swell in gastric pH and give high initial release.

Anionic polymers: These polymers have negatively charged groups and are sensitive to pH changes. As the pH of the environment increases from acidic to alkaline side, these polymers ionize to varying degrees and either dissolve or swell or erode and this property can be used to trigger drug release in the relatively alkaline environment of the distal ileum and the colon where pH is usually greater than 7.0. The anionic polymers selected for the present study were Eudragit L100 (EL100), Eudragit S100 (ES100), Polycarbophil (PCP), Carbopol (CP) and Xanthan Gum (XG).

Eudragit L100 (EL100) and Eudragit S100 (ES100) are copolymers of methyl methacrylate and methacrylic acid with varying degrees of carboxylic acid substitution. EL100 dissolves at pH 6.0 while ES100 dissolves at pH 7.0. These pH sensitive polymers are hydrophobic and water - insoluble (Mehta et al., 2001). These two polymers have been extensively employed either individually or in combination with other polymers for coating tablet and other formulations intended for colonic delivery (Leopold, 1999; Kumar and Mishra, 2008). Although there are reports on the use of Eudragit in matrix tablets for various purposes like modulating microenvironmental pH (Al-Taani and Tashtoush, 2003), and achieving pH based erosion controlled systems (Akiyama et al., 1994) yet there were no reports on the use of these polymers in matrix form for colonic delivery. However, a technique based on hot melt extrusion has been reported to design sustained release matrix tablets of 5- amino salicylic acid using EL100 and ES100 for colonic delivery (Bruce et al., 2005).

Polycarbophil (PCP) and Carbopol (CP) are high molecular weight poly acrylic acid polymers, are hydrophilic in nature and show pH dependent swelling behavior above pH range of 6.0 - 7.0. The polymeric chains are cross-linked to form micro gel-like structures. PCP is crosslinked with divinyl glycol (Grabovac et al., 2005) while CP is crosslinked with allyl sucrose. These polymers have been mostly employed for their mucoadhesive potential in

oral and buccal delivery (Luessen et al., 1995). However, the pH dependent swelling property of these polymers has not been investigated for their colon targeting potential.

Another type of anionic hydrophilic polymer is Xanthan gum (XG), a polysaccharide based natural gum produced by *Xanthomonas campestris* bacterium. This polymer has been used as a release retardant polymer in matrix alone and in combination with other polymers to form time dependent swelling controlled systems and drug release is through diffusion from the swollen xanthan gum matrix (Andreopoulos and Tarantili, 2001; Vendruscolo et al., 2005). It has also been used as a copolymer with guar gum to form matrix bases and compression coats for enhanced gel strength in colonic delivery (Sinha et al., 2005).

Nonionic polymers : In addition to anionic polymers, certain non-ionic polymers were employed and investigated for their colon targeting potential. The major feature of non-ionic polymers is they are insensitive to pH changes in the environment and give pH independent drug release. Further, drug release from such matrices can be modulated by suitable manipulation of matrix properties. The polymers that were selected in this category were ethylcellulose (EC), hydroxy ethyl cellulose (HEC), hydroxy propyl cellulose (HPC) and guar gum (GG).

Ethylcellulose (EC) has been used as a hydrophobic retardant matrix base in several sustained and controlled release dosage forms (Saha et al., 2001; Sajeev and Saha, 2001). Micronized EC, both alone and in combination with excipients, has been extensively investigated as a press coating outer shell around a drug core for imparting delayed release properties (Lin et al. 2001, 2004). EC has been used in film coating as a single polymer (Sinha and Kumria 2003) and in combination with amylose (Leong et al. 2002) for delaying drug release in colon targeted systems. A timed release device for theophylline in a matrix composed of EC and hydroxyl ethyl cellulose coated with Eudragit S100 has also been reported as a colon specific drug delivery system (Alvarez-Fuentes et al. 2004).

Hydroxy ethyl cellulose (HEC) and hydroxy propyl cellulose (HPC) are non-ionic derivatives of cellulose ethers used as hydrophilic matrix bases for controlled release (Sinha and Rohera, 2002). Polymer swelling is dependent on hydration of the matrix by the dissolution medium and drug release occurs predominantly by polymer swelling, erosion and diffusion though the hydrated gel. The use of HEC as part of a compression coat for developing delayed release system for colon targeting has been reported (Peerpattana et al., 2004). Except for one report, a detailed survey of literature did not reveal the use of these polymers for colon targeting purpose.

Guar gum (GG) is a non-ionic naturally occurring galactomannan polysaccharide and used as a release retarding polymer for several drugs. It also has the additional property of being selectively degraded by the colonic bacteria, and has been extensively employed as a matrix base or compression coat over tablets for colon specific delivery (Prasad et al., 1998; Krishnaiah et al., 1998). Analysis of available literature indicated that the use of guar gum for colon targeting purpose has several limitations. When used alone, very high percentage of guar gum is required in the matrix base (Momin and Pundarikakshudu, 2004; Al-Saidan et al., 2005) or compression coat (Krishnaiah et al., 2002, 2003) to achieve the desired retardation in the initial phase. Alternately, it has to be used in combination with other polymers (Sinha & Kumria, 2004) or suitably coated with enteric polymers (Mundargi, et al., 2007; Ravi et al., 2008). For some of the other reported guar gum based formulations, the drug release is slow and controlled by microbial degradation in the colon (Sinha et al., 2005). Such systems may be unsuitable for patients whose colon transit time is low (in the range of 10-12 h) such as in case of diarrheal symptoms. Further, when antibiotics and antibacterials are co-administered, the microflora of the colon is disturbed and this may affect the degradation of guar gum matrix (Krishnaiah et al., 2001). Therefore, the formulation design was to evaluate effect of individual polymer type and proportion on drug release from designed matrices and also study the effect of combination of anionic and non-ionic polymers in varying proportions on the sigmoidal release profile.

Multi unit systems: Multi unit dosage forms, when compared with the conventional drug delivery systems, provide more consistent and reproducible transit through GI tract (Bott et al. 2004). In addition, microparticulate dosage forms have longer colonic residence time (Davis et al., 1986; Follonier and Doelker 1992). Several multi unit based microparticulate systems have been reported for colon targeting (Asghar and Chandran, 2006). One of the other objectives of the present investigation was to explore the possibility of employing ethylcellulose in combination with Eudragit L100 or S100 as a single polymeric system to form a pH and transit time controlled multiparticulate formulation of indomethacin for colonic delivery. Most of the multiparticulate systems for colon targeting employ an outer coating of a suitable pH sensitive polymer like Eudragit derivatives to provide protection to drug release in gastric and intestinal environment like crosslinked chitosan microcapsules coated with Eudragit L100 and Eudragit S100 (Chourasia and Jain, 2004), budesonide-loaded PLGA microparticles coated with Eudragit S100 (Krishnamachari et al., 2007), heparin loaded microparticles coated with Eudragit P-4135F (Meissner et al., 2007), and Eudragit-coated pectin microcapsules for colon targeting of 5-fluorouracil (Paharia et al., 2007). However, as discussed previously, coated microparticulate systems like single unit systems

may also show inconsistent and uncertain release behavior in vivo. An alternate strategy to the coating approach is the matrix system in which drug is embedded in an enteric or pH sensitive polymeric matrix based microspheres. These matrix based polymeric microspheres are expected to show pH dependent dissolution and gradually dissolve during transit through GI tract with progressive increase in pH.

For the purpose of designing matrix based multiparticulate systems for colonic delivery, it was decided to develop a controlled release system as it has been shown in previous reports that continuous drug release is advantageous during transit through the large intestine (Lamprecht et al. 2003). Therefore, ethyl cellulose was selected as the rate controlling polymer as the use of EC in microencapsulation of different drugs is well reported (Sajeev et al., 2002; Benita and Donbrow, 2006). In order to confer pH dependent release properties, EL100 or ES100 were proposed to be incorporated as part of the matrix base.

5.5. Physical characterization of designed tablet based formulations

The prepared tablets from all the batches were found to be of good quality with acceptable physical characteristics. The results of tablet characteristics are present in Table 5.10 to 5.14. The crushing strength was found to range between 4.5 – 5.0 kg across all batches of formulations. The percentage friability and weight variation in all the formulations was ≤ 0.5% and ± 5.0% respectively. The average drug content across all batches of formulations was within a variation of ± 5% from the theoretical formula value. The low value of weight variation, optimal crushing strength and friability, and high degree of drug content uniformity suggested that wet granulation is an acceptable method of manufacturing matrix embedded formulation of indomethacin for colon specific delivery.

Table 5.10a: Physical characterization of single polymer based formulations

Batches	Physical Characterization				
	Drug content [a] (mg/ tablet)	Weight variation [b] (%)	Crushing strength[c] (kg)	Friability[d] (NMT %)	Thickness [e] (mm)
(a) EC					
IEC5	75.5 ± 0.4	±4.2	4.5 (±0.1)	0.4	1.98 (±0.02)
IEC10	72.6 ± 0.3	±4.5	4.5 (±0.4)	0.3	2.07 (±0.01)
IEC20	75.3 ± 1.5	±3.6	4.8 (±0.1)	0.1	2.17 (±0.02)
(b) EL100					
IEL25	74.0 ± 1.5	±0.1	4.7 (±0.1)	0.2	2.15 (±0.02)
IEL50	72.5 ± 1.4	±2.0	5.0 (±0.1)	0.3	2.19 (±0.01)
(c) ES100					
IES25	75.7 ± 2.0	±1.6	4.7 (±0.2)	0.5	2.12 (±0.03)
IES50	75.9 ± 1.6	±0.4	4.8 (±0.2)	0.3	2.14 (±0.01)

[a] mean ± SD (n = 10); [b] SD from the mean value (n = 20); [c] mean ± SD (n = 10); [d] mean of 10 tablets; [e] mean ± SD (n = 5)
The diameter of the tablets was 0.70 ± 0.01 cm

Table 5.10b: Physical characterization of single polymer based formulations

Batches	Physical Characterization				
	Drug content [a] (mg/ tablet)	Weight variation [b] (%)	Crushing strength [c] (kg)	Friability [d] (NMT %)	Thickness [e] (mm)
(a) PCP					
IPCP5	74.5 ± 0.1	± 2.8	5.0 (±0.1)	0.5	1.99 (±0.01)
IPCP10	73.4 ± 0.3	± 0.3	4.9 (±0.2)	0.2	2.02 (±0.02)
IPCP20	75.4 ± 0.4	± 3.7	4.6 (±0.2)	0.4	2.12 (±0.01)
(b) CP					
ICP5	75.5 ± 0.4	± 4.2	4.5 (±0.1)	0.4	1.98 (±0.02)
ICP10	76.8 ± 1.2	± 3.0	4.6 (±0.1)	0.2	2.01 (±0.01)
ICP20	74.1 ± 0.1	± 1.5	4.5 (±0.2)	0.3	2.05 (±0.02)
(c) XG					
IXG5	73.5 ± 0.2	± 4.2	4.7 (±0.2)	0.3	1.88 (±0.02)
IXG10	74.7 ± 0.1	± 1.0	4.6 (±0.3)	0.2	2.04 (±0.01)
IXG20	72.6 ± 0.3	± 4.5	4.5 (±0.4)	0.3	2.06 (±0.01)
(d) HEC					
IHEC5	72.5 ± 2.6	± 5.0	4.5 (±0.3)	0.2	1.89 (±0.01)
IHEC10	75.1 ± 2.1	± 2.6	4.8 (±0.2)	0.2	2.03 (±0.02)
IHEC20	76.7 ± 1.1	± 5.0	5.0 (±0.1)	0.3	2.06 (±0.02)
(e) HPC					
IHPC5	73.5 ± 1.2	± 3.6	4.6 (±0.3)	0.1	1.87 (±0.02)
IHPC10	73.4 ± 2.3	± 1.3	4.7 (±0.3)	0.1	2.05 (±0.01)
IHPC20	75.6 ± 1.4	± 4.0	4.7 (±0.2)	0.3	2.18 (±0.01)
(f) GG					
IGG5	74.5 ± 0.3	± 4.1	4.5 (±0.1)	0.4	1.99 (± 0.01)
IGG10	74.2 ± 0.2	± 1.2	4.5 (±0.2)	0.1	2.02 (± 0.02)
IGG20	73.2 ± 0.4	± 2.5	4.7(±0.3)	0.2	2.04 (± 0.01)

[a] mean ± SD (n = 10); [b] SD from the mean value (n = 20); [c] mean ± SD (n = 10); [d] mean of 10 tablets; [e] mean ± SD (n = 5)

The diameter of the tablets was 0.70 ± 0.01 cm

Table 5.11: Physical characterization of formulations prepared using combination of EL100 and ES100

Batches	Physical Characterization				
	Drug content [a] (mg/ tablet)	Weight variation [b] (%)	Crushing strength [c] (kg)	Friability [d] (NMT %)	Thickness [e] (mm)
IEL10ES15	73.9 ± 1.2	± 1.8	4.8 (±0.2)	0.2	1.97 (±0.02)
IEL12.5ES12.5	76.6 ± 1.6	± 3.3	4.8 (±0.1)	0.2	1.96 (±0.04)
IEL15ES10	73.4 ± 1.8	± 1.9	4.8 (±0.2)	0.5	1.99 (±0.02)
IEL20ES30	72.2 ± 1.0	± 4.0	4.9 (±0.1)	0.2	2.09 (±0.02)
IEL25ES25	75.3 ± 1.5	± 3.6	4.8 (±0.1)	0.1	2.07 (±0.01)
IEL30ES20	75.3 ± 1.6	± 2.8	4.6 (±0.3)	0.2	2.06 (±0.01)

[a] mean ± SD (n = 10); [b] SD from the mean value (n = 20); [c] mean ± SD (n = 10); [d] mean of 10 tablets; [e] mean ± SD (n = 5)

The diameter of the tablets was 0.70 ± 0.01 cm

Table 5.12: Physical characterization of tablet formulations prepared using combination of EC with other polymers

Batches	Physical Characterization				
	Drug content (mg/ tablet) [a]	Weight variation [b] (%)	Crushing strength [c] (kg)	Friability [d] (NMT %)	Thickness [e] (mm)
(a) EC + EL100					
IEL15EC10	75.3 ± 1.2	±1.2	4.6 (±0.2)	0.1	1.95 (±0.03)
IEL20EC5	73.8 ± 1.3	±4.4	4.9 (±0.1)	0.3	1.96 (±0.02)
IEL30EC20	74.1 ± 1.5	±1.7	4.5 (±0.1)	0.3	2.12 (±0.01)
IEL40EC10	72.8 ± 1.3	±1.5	4.8 (±0.1)	0.2	2.11 (±0.04)
(b) EC +ES100					
IES15EC10	74.4 ± 1.5	±3.7	4.9 (±0.1)	0.4	2.13 (±0.02)
IES20EC5	76.4 ± 1.4	±4.6	4.7 (±0.2)	0.2	2.12 (±0.01)
IES30EC20	72.5 ± 1.4	±1.3	4.9 (±0.1)	0.3	1.99 (±0.02)
IES40EC10	72.7 ± 2.1	±4.7	4.8 (±0.2)	0.2	2.00 (±0.01)
(c) EC+ PCP					
IPCP10EC5	73.2 ± 0.1	±4.6	4.6 (±0.8)	0.4	1.81 (±0.01)
IPCP10EC10	74.1 ± 3.2	±1.0	4.7 (±0.3)	0.2	1.82 (±0.02)
IPCP10EC20	76.3 ± 0.3	±3.0	4.7 (±0.2)	0.1	2.06 (±0.01)
IPCP10EC40	74.2 ± 0.2	±5.1	5.0 (±0.1)	0.5	2.08 (±0.01)
IPCP20EC5	72.8 ± 0.1	±2.3	4.4 (±0.3)	0.1	2.06 (±0.02)
IPCP20EC10	72.3 ± 0.4	±5.6	4.1 (±0.1)	0.4	2.06 (±0.01)
IPCP20EC20	74.2 ± 0.1	±3.8	5.0 (±0.1)	0.2	2.08 (±0.02)
IPCP20EC40	73.4 ± 0.1	±4.6	4.7 (±0.1)	0.5	2.09 (±0.02)
(d) EC+ CP					
ICP10EC5	75.3 ± 0.1	±2.0	4.1 (±0.1)	0.3	1.85 (±0.01)
ICP10EC10	75.6 ± 0.1	±3.2	4.2 (±0.4)	0.4	1.89 (±0.02)
ICP10EC20	73.6 ± 0.2	±2.5	4.6 (±0.2)	0.5	2.07 (±0.01)
ICP10EC40	75.1 ± 0.2	±4.6	4.9 (±0.3)	0.4	2.09 (±0.01)
ICP20EC5	73.8 ± 0.1	±5.0	5.0 (±0.1)	0.5	2.05 (±0.02)
ICP20EC10	74.9 ± 0.2	±2.5	4.8 (±0.2)	0.5	2.07 (±0.01)
ICP20EC20	74.5 ± 0.1	±5.0	4.8 (±0.5)	0.4	2.08 (±0.02)
ICP20EC40	73.1 ± 0.2	±4.6	4.9 (±0.2)	0.3	2.11 (±0.01)

[a] mean ± SD (n = 10); [b] SD from the mean value (n = 20); [c] mean ± SD (n = 10); [d] mean of 10 tablets;
[e] mean ± SD (n = 5)
The diameter of the tablets was 0.70 ± 0.01 cm

Table 5.13: Physical characterization of tablet formulations prepared using combination of EL100 with other polymers

Batches	Physical Characterization				
	Drug content [a] (mg/ tablet)	Weight variation [b] (%)	Crushing strength[c] (kg)	Friability[d] (NMT %)	Thickness [e] (mm)
(a) EL100 + PCP					
IPCP5EL10	76.6 ± 2.2	±2.7	4.6 (±0.3)	0.2	2.04 (±0.01)
IPCP5EL20	76.5 ± 1.2	±1.8	4.7 (±0.3)	0.2	2.05 (±0.01)
IPCP10EL20	76.8 ± 2.4	±2.2	5.0 (±0.1)	0.1	2.14 (±0.02)
IPCP10EL40	73.1 ± 2.1	±1.9	4.9 (±0.2)	0.4	2.15 (±0.03)
IPCP20EL40	74.6 ± 0.2	±0.3	4.8 (±0.2)	0.1	2.16 (±0.01)
(b) EL100 + CP					
ICP5EL10	73.9 ± 0.2	±3.3	4.7 (±0.3)	0.4	1.95 (±0.01)
ICP5EL20	74.7 ± 2.1	±2.6	4.7 (±0.4)	0.1	2.03 (±0.02)
ICP10EL20	74.4 ± 0.4	±4.7	4.6 (±0.3)	0.2	2.05 (±0.03)
ICP10EL40	75.5 ± 2.3	±3.1	4.5 (±0.2)	0.2	2.05 (±0.02)
ICP20EL40	74.0 ± 0.4	±1.0	4.9 (±0.2)	0.4	2.06 (±0.02)
(c) EL100 + XG					
IXG5EL5	76.3 ± 0.4	±3.5	4.5 (±0.2)	0.4	1.85 (±0.01)
IXG5EL10	73.8 ± 0.3	±4.0	4.6 (±0.3)	0.2	1.89 (±0.01)
IXG5EL20	75.0 ± 0.1	±1.8	4.8 (±0.3)	0.3	2.01 (±0.02)
IXG5EL40	75.4 ± 0.3	±2.8	5.0 (±0.0)	0.5	2.05 (±0.02)
IXG10EL10	74.1 ± 0.2	±0.8	4.6 (±0.2)	0.2	2.05 (±0.01)
IXG10EL20	74.2 ± 0.1	±0.2	4.5 (±0.1)	0.3	2.09 (±0.01)
(d) EL100 + HEC					
IHEC5EL10	73.8 ± 1.5	±2.6	4.6 (±0.1)	0.2	1.85 (±0.01)
IHEC5EL20	74.9 ± 2.1	±3.1	4.5 (±0.4)	0.4	1.89 (±0.02)
IHEC10EL20	74.6 ± 1.3	±2.5	4.6 (±0.2)	0.4	2.01 (±0.01)
IHEC10EL40	75.4 ± 1.4	±4.6	4.9 (±0.1)	0.3	2.04 (±0.01)
IHEC20EL40	74.2 ± 2.0	±5.0	5.0 (±0.1)	0.4	2.09 (±0.02)
(e) EL100 + HPC					
IHPC5EL10	73.6 ± 2.5	±4.6	4.6 (±0.4)	0.5	1.89 (±0.01)
IHPC5EL20	73.4 ± 1.9	±1.0	4.7 (±0.3)	0.2	1.95 (±0.01)
IHPC10EL20	75.5 ± 1.7	±3.0	4.7 (±0.2)	0.1	2.00 (±0.02)
IHPC10EL40	73.6 ± 2.2	±5.0	5.0 (±0.1)	0.3	2.06 (±0.02)
IHPC20EL40	74.3 ± 1.2	±2.3	4.6 (±0.3)	0.1	2.08 (±0.01)
(f) EL100 + GG					
IGG5EL5	76.2 ± 0.2	±3.6	4.6(±0.1)	0.3	1.88 (±0.02)
IGG5EL10	73.8 ± 0.2	±2.3	4.5 (±0.1)	0.1	1.90 (±0.02)
IGG5EL20	75.0 ± 0.2	±1.8	4.7 (±0.3)	0.2	2.01 (±0.01)
IGG5EL40	75.4 ± 0.3	±2.3	4.9 (±0.1)	0.4	2.02 (±0.02)
IGG10EL10	74.2 ± 0.2	±2.8	4.5 (±0.2)	0.5	2.05 (±0.02)
IGG10EL20	73.2 ± 0.2	±0.5	4.7 (±0.2)	0.4	2.07 (±0.01)

[a] mean ± SD (n = 10); [b] SD from the mean value (n = 20); [c] mean ± SD (n = 10); [d] mean of 10 tablets;
[e] mean ± SD (n = 5)
The diameter of the tablets was 0.70 ± 0.01 cm

Table 5.14: Physical characterization of tablet formulations prepared using combination of ES100 with other polymers

Batches	Physical Characterization				
	Drug content [a] (mg/ tablet)	Weight variation [b] (%)	Crushing strength[c] (kg)	Friability[d] (NMT %)	Thickness [e] (mm)
(a) ES100 + PCP					
IPCP5ES10	74.6 ± 2.1	±2.4	4.2 (±0.1)	0.3	2.02 (±0.01)
IPCP5ES20	72.5 ± 1.4	±1.9	4.3 (±0.1)	0.3	2.04 (±0.01)
IPCP10ES20	75.8 ± 2.4	±2.4	4.5 (±0.3)	0.2	2.12 (±0.02)
IPCP10ES40	72.6 ± 2.1	±1.8	4.3 (±0.2)	0.1	2.12 (±0.03)
IPCP20ES40	73.4 ± 0.2	±1.3	4.6 (±0.2)	0.1	2.13 (±0.02)
(b) ES100 + CP					
ICP5ES10	74.9 ± 0.2	±3.5	4.7 (±0.2)	0.5	1.95 (±0.01)
ICP5ES20	72.7 ± 2.4	±2.5	4.2 (±0.3)	0.4	2.03 (±0.02)
ICP10ES20	73.4 ± 0.4	±4.7	4.6 (±0.1)	0.3	2.05 (±0.03)
ICP10ES40	74.5 ± 2.3	±3.2	4.4 (±0.2)	0.2	2.12 (±0.02)
ICP20ES40	76.8 ± 1.4	±4.1	4.8 (±0.2)	0.4	2.12 (±0.01)
(c) ES100 + XG					
IXG5ES5	74.7 ± 0.2	±0.8	5.0 (±0.1)	0.4	1.92 (±0.02)
IXG5ES10	74.6 ± 0.2	±2.5	4.9 (±0.1)	0.3	1.93 (±0.02)
IXG5ES20	72.6 ± 0.3	±2.7	4.5 (±0.2)	0.3	2.02 (±0.02)
IXG5ES40	76.9 ± 0.1	±1.2	4.5 (±0.3)	0.4	2.04 (±0.01)
IXG10ES10	76.3 ± 0.4	±1.7	4.7 (±0.3)	0.5	2.07 (±0.02)
IXG10ES20	73.5 ± 0.1	±4.8	4.8 (±0.1)	0.2	2.08 (±0.02)
(d) ES100 + HEC					
IHEC5ES10	72.6 ± 2.2	±2.6	4.5 (±0.3)	0.1	1.75 (±0.02)
IHEC5ES20	73.5 ± 2.5	±4.8	4.5 (±0.1)	0.3	1.80 (±0.01)
IHEC10ES20	74.7 ± 1.3	±2.5	4.7 (±0.1)	0.3	1.99 (±0.01)
IHEC10ES40	72.8 ± 1.5	±2.3	4.8 (±0.3)	0.1	2.02 (±0.02)
IHEC20ES40	72.6 ± 1.5	±4.9	4.6 (±0.1)	0.4	2.03 (±0.01)
(e) ES100 + HPC					
IHPC5ES10	73.6 ± 1.4	±5.0	4.5 (±0.1)	0.4	2.09 (±0.01)
IHPC5ES20	73.8 ± 1.2	±3.8	5.0 (±0.1)	0.2	2.13 (±0.02)
IHPC10ES20	75.1 ± 1.7	±4.6	5.0 (±0.1)	0.3	2.15 (±0.02)
IHPC10ES40	73.7 ± 1.6	±4.6	4.6 (±0.1)	0.4	2.16 (±0.02)
IHPC20ES40	75.5 ± 0.2	±1.0	4.7 (±0.2)	0.2	2.16 (±0.01)
(f) ES100 + GG					
IGG5ES10	74.2 ± 0.1	±2.3	4.8 (±0.2)	0.4	1.92 (±0.03)
IGG5ES20	74.2 ± 0.2	±1.2	4.8 (±0.2)	0.4	1.98 (±0.03)
IGG5ES40	75.3 ± 0.3	±1.3	4.7 (±0.2)	0.3	2.01 (±0.01)
IGG10ES10	73.4 ± 0.2	±1.9	4.6 (±0.2)	0.5	2.03 (±0.01)
IGG10ES20	74.2 ± 0.2	±4.9	4.6 (±0.1)	0.3	2.08 (±0.03)

[a] mean ± SD (n = 10); [b] SD from the mean value (n = 20); [c] mean ± SD (n = 10); [d] mean of 10 tablets; [e] mean ± SD (n = 5)

The diameter of the tablets was 0.70 ± 0.01 cm

5.6. In vitro release studies

5.6.1. Theoretical target release

For an ideal colon targeted drug delivery system, the drug release should be prevented in the stomach and small intestine. Release of drugs must be completed within the residence time of the dosage form in the colon. The mean gastric residence time for single solid dosage forms in fasting state is 0.25–2 h and the transit time through the small intestine is approximately 3 h (Wilson and Washington, 2000). Colonic residence is highly variable and is reported to vary from 10 h (Follonier and Doelker, 1992) to 40 h (Hinton et al., 1969). In another report, the transit times through the colon vary from 7 to 24 h (Marvola et al., 2008). Therefore, it was assumed that for colon targeting purpose, a 14 to 16 h controlled release formulation with negligible to no release in the first 4-6 h would be suitable. An initial release of less than 7-10% of the administered dose in first 4-6 h was considered acceptable. Further, this initial lag time of 4-6 h would ensure the passage of the formulation intact to the distal ileum or proximal colon without appreciable drug loss. Moreover, such a design would also ensure that maximum drug release would occur even in cases when colonic transit time is on the lower side as is the case in various pathologies of the bowel. Based on these assumptions, a theoretical target release profile was defined as shown in the figures corresponding to pH gradient dissolution conditions. The predicted release profile for each formulation beyond 14 h up to 24 h is shown in the figures as a dotted line.

5.6.2. Optimization of dissolution medium

The in vitro release profiles of drugs carried by Eudragit based systems are normally investigated in buffers like phosphate buffers with pH ranging from 6.5 to 7.5 after a pretest in acid medium (Li et al., 1991). Indomethacin, an indole acetic acid derivative with a pKa of 4.5, has been reported to have solubility of 3.66 µg/ml in pH 1.2 and 1975 µg/ml in pH 7.2 at 37°C (Valizadeh et al., 2004). Our studies revealed that drug (present in a micronized form) had a solubility of ~ 53 µg/ml at 25°C and ~ 80 µg/ml at 37°C in distilled water which could be due to its ionization at the pH of distilled water (6.8-7.0). This solubility provides for release upto 25 mg of administered dose at 37°C without attaining saturation solubility in distilled water. Therefore, dissolution was carried out in 500 ml distilled water for the first 2 h as saturation solubility was not achieved in distilled water even when 35-40% of the complete dose is released. Also, Eudragits are insoluble in water just as in pH 1.2 (Kislalioglu et al., 1991; Ammar et al., 1997), inspite of the fact that

pH of distilled water (6.8-7.0) is near the threshold dissolution pH of Eudragit. Eudragits do not dissolve in distilled water due to the negligible ionic strength of distilled water as the dissolution of Eudragit depends on ionic strength and buffer capacity of the medium (Fadda and Basit, 2005). In addition, previous reports have shown that as the patient consumes a tablet with a good quantity of water, dissolution of poorly water soluble drugs can be done in distilled water for the initial period (Sheu et al., 1992; Saha et al., 2001; Sajeev and Saha, 2001). During preliminary studies it was observed that distilled water could discriminate well between the various formulations with and without EL100 or ES100.

The release studies were further (post 2h) investigated in phosphate buffer of pH 7.4 (ionic strength of 0.129 and osmolality of 228 mOsm/ kg) (Ibekwe et al., 2006). It was reported that the carboxylic acid group present in Eudragits reacts with the phosphate bases (HPO_4^{2-}) in the buffer resulting in increase in Eudragit dissolution rate (Chan et al., 2001). Also, the drug was freely soluble at this pH and this medium simulates the alkaline environment of distal small intestine and colon.

The primary objective of the formulation design was to prevent initial release in the 4-6 h and then obtain controlled release and also ensure complete release within 14-16 h (during the residence time of formulation in colon). The preliminary release study condition would therefore indicate the controlled release kinetics of designed formulations in phosphate buffer pH 7.4. Hence, for further, pH gradient studies (0-2 h pH 1.2; 2-4 h pH 4.5; and 4 h to end of study pH 7.4) only those matrices were selected which showed good controlled release and near complete release in 14-16 h in phosphate buffer of previous dissolution conditions. The performance of selected formulations (based on preliminary dissolution profile as mentioned above) was evaluated in a pH gradient system in order to investigate the performance of formulations in situations mimicking actual GI tract pH change. The dissolution profiles of all the formulations were compared with respect to release rate constant (K), time taken for 10% release ($t_{10\%}$), time taken for 90% release ($t_{90\%}$) and n values.

5.6.3. Effect of polymer type on indomethacin release from single polymer matrix bases

To study the effect of polymer type on drug release from single polymeric matrix, various polymer types studied were namely, hydrophobic non-ionic polymer (Ethyl cellulose (EC); ionic pH sensitive Eudragit L100 (EL100) and S100 (ES100); hydrophilic ionic polymer polycarbophil (PCP), carbopol (CP), xanthan gum (XG) and hydrophilic

non-ionic polymer hydroxy ethyl cellulose (HEC), hydroxy propyl cellulose (HEC), and guar gum (GG). The effect of polymer type and proportion on indomethacin release from above category of polymers is discussed in the following sections.

5.6.3.1. Effect of hydrophobic nonionic polymer (ethyl cellulose) on indomethacin release

The release profiles from matrix tablets containing EC at varying proportion of 5, 10 and 20% w/w of drug, i.e., IEC5, IEC10 and IEC20 respectively, are shown in Fig 5.10. The K values for the three formulations were obtained as 2.576 $h^{-1.02}$ and 2.566 $h^{-0.92}$ and 3.401 $h^{-0.73}$. It was observed that initial drug release (first 2 h) from the matrix formulations was negligible with only 3-4% drug release. However, the overall drug release rate from all the matrices was extremely slow with only 30% release in 14 h of study for IEC5. This could be because of the hydrophobic nature of both the polymer (EC) and the drug (log P 3.8), that slowed down the penetration of dissolution medium into the matrix, thereby resulting in low release rates.

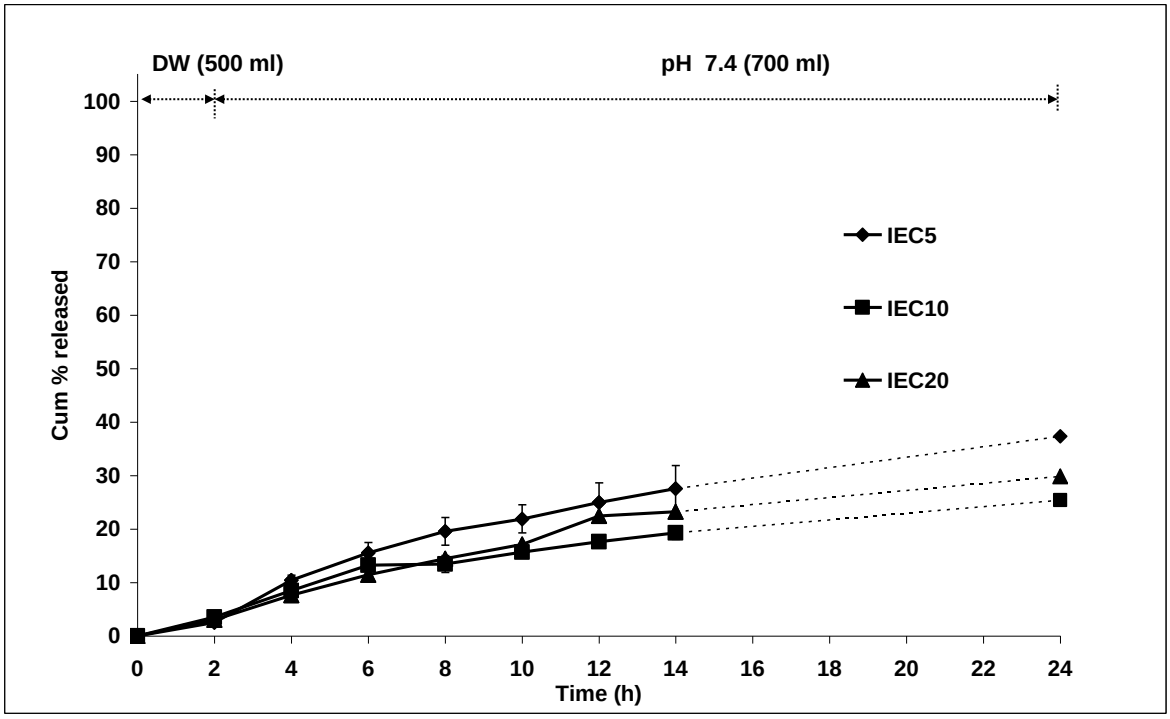


Fig 5.10: Release profile of indomethacin from EC based matrix tablets at varying polymer proportion. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The 'n' values from the power equation (Table 5.15) indicated that increase in the relative proportion of EC shifted the drug release mechanism from super case-II (erosion based

release) for IEC5 to anomalous non-Fickian type for IEC20. Such a matrix design would be unsuitable for colonic delivery.

Table 5.15: Release kinetics characterization of drug release from EC matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IEC5	0.9629	3.06×10^{-2}	2.576	1.02	3.7	38.8
IEC10	0.9934	2.14×10^{-3}	2.566	0.92	4.4	45.6
IEC20	0.9716	4.01×10^{-3}	3.401	0.73	4.3	48.8

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

5.6.3.2. Effect of hydrophobic ionic pH sensitive polymers on indomethacin release

(i) Effect of Eudragit L100

The release profiles from matrix tablets containing EL100 at 25% and 50% w/w of drug, i.e., IEL25 and IEL50 respectively, are shown in Fig 5.11. The K values for the two formulations were obtained as 2.659 $h^{-1.48}$ and 2.486 $h^{-1.20}$ respectively. The drug release in the initial phase was negligible (less than 5% drug release) followed by increase in release rate post 2 h in pH 7.4 medium. Increasing the polymer proportion from 25% to 50% considerably retarded the overall rate of drug release from the matrix with $t_{90\%}$ increasing from 10.8 h for IEL25 to 19.9 h for IEL50 (Table 5.16, Fig 5.11). The $t_{10\%}$ ranged from 2.1 h for IEL25 to 2.8 h for IEL50 (Table 5.16). The release mechanism was found to be super case II indicating increasing in rate of release with time due to matrix erosion and increase in overall porosity of the matrix due to pH dependent ionization of carboxylic group of the polymer by HPO_4^{2-} groups of the buffering agent.

(ii) Effect of Eudragit S100

When ES100 was used in place of EL100 at same polymer proportion (25% and 50% w/w of drug), i.e., IES25 and IES50 respectively, comparatively higher retardation was obtained in the case of ES100 as shown in Fig 5.12. This was shown by the K values for the two formulations that were obtained as 1.137 $h^{-1.40}$ and 1.782 $h^{-1.16}$ respectively. This can be attributed to the fact that EL100 dissolves at a pH less than 7.0 resulting in higher swelling and erosion in phosphate buffer pH 7.4 (Khan et al. 1999). Increasing the polymer proportion from 25% to 50% did not considerably retard the overall rate of drug release from the matrix. The $t_{10\%}$ and $t_{90\%}$ at both polymer proportion of 25% and 50% w/w of drug were not found to be significantly different (Table 5.16).

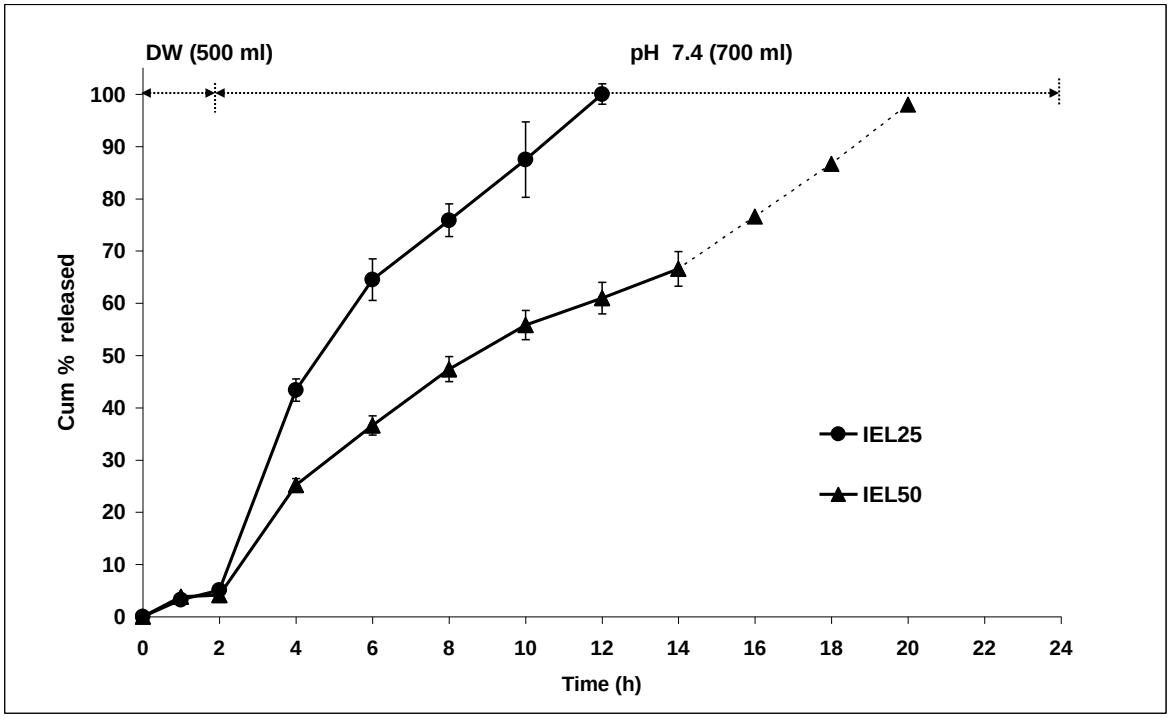


Fig 5.11: Release profile of indomethacin from EL100 based matrix tablets at total polymer proportion of 25% w/w and 50% w/w of drug. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

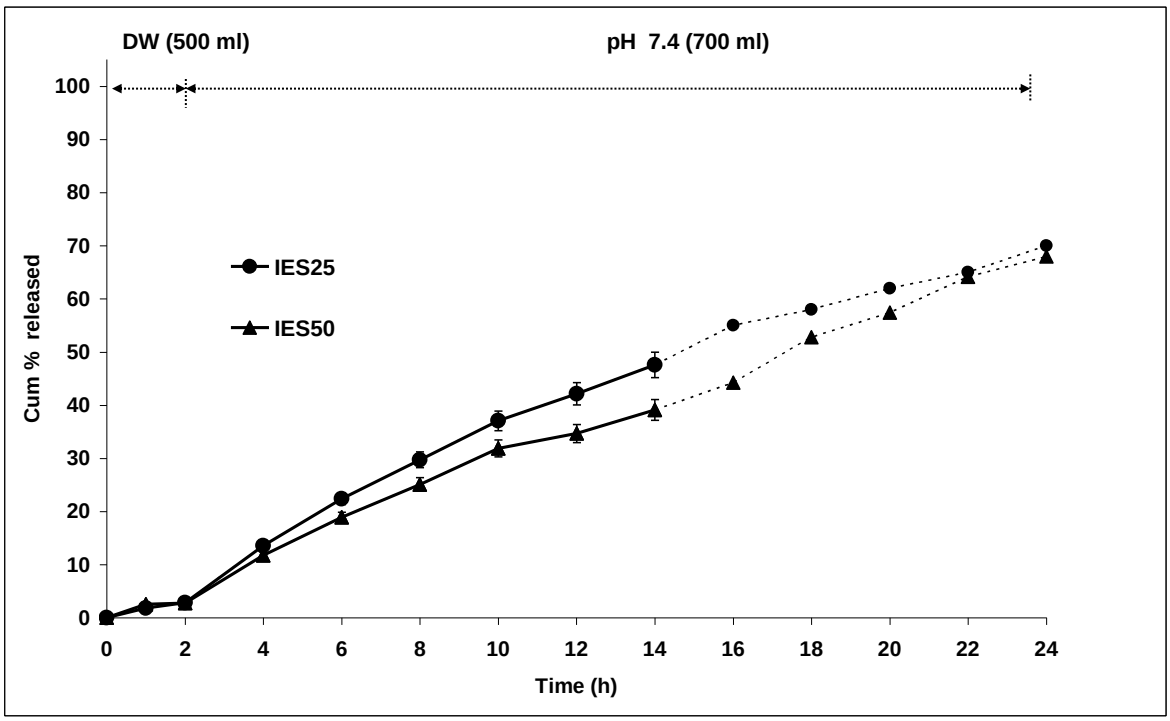


Fig 5.12: Release profile of indomethacin from ES100 based matrix tablets at total polymer proportion of 25% w/w and 50% w/w of drug. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.16: Release kinetics characterization of drug release from EL100 or ES100 matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IEL25	0.9130	3.75×10^{-2}	2.659	1.48	2.1	10.8
IEL50	0.9480	4.34×10^{-3}	2.486	1.20	2.8	19.9
IES25	0.9750	1.16×10^{-3}	1.137	1.40	3.5	26.7
IES50	0.9760	7.19×10^{-3}	1.782	1.16	3.7	29.4

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

5.6.3.3. Effect of hydrophilic ionic polymers on indomethacin release

(i) Effect of Polycarbophil

Polycarbophil has been known to exhibit a great degree of swelling, at pH above 6.8 due to ionization of acidic groups on the polymer (Hosny, 1993; Taylan et al., 1996). This results in formation of a gel layer which impart controlled release characteristics. Drug release has been reported to occur by a combination of diffusion controlled and swelling (chain relaxation) mechanism.

The drug release profiles for matrix tablets containing PCP alone at 5, 10 and 20% w/w of drug are shown in Fig 5.13. The K values for the three formulations IPCP5, IPCP10 and IPCP20 were obtained as 4.556 $h^{-1.29}$, 4.169 $h^{-1.46}$ and 11.442 $h^{-1.03}$ respectively. In the present case, it was found that increasing the relative proportion of PCP resulted in faster rate of drug release from the matrix with tablets containing PCP at 20% w/w of drug showing minimum retardation in initial as well as overall drug release. The drug release in the initial phase (first 2 h) varied from 5% for IPCP5 to 20% for IPCP20 in 2 h and complete release was obtained within 8-12 h for all the formulations (Fig 5.13). The 'n' values for these formulations varied from 1.03 to 1.46 indicating the drug release mechanism to be super case II type (Table 5.17).

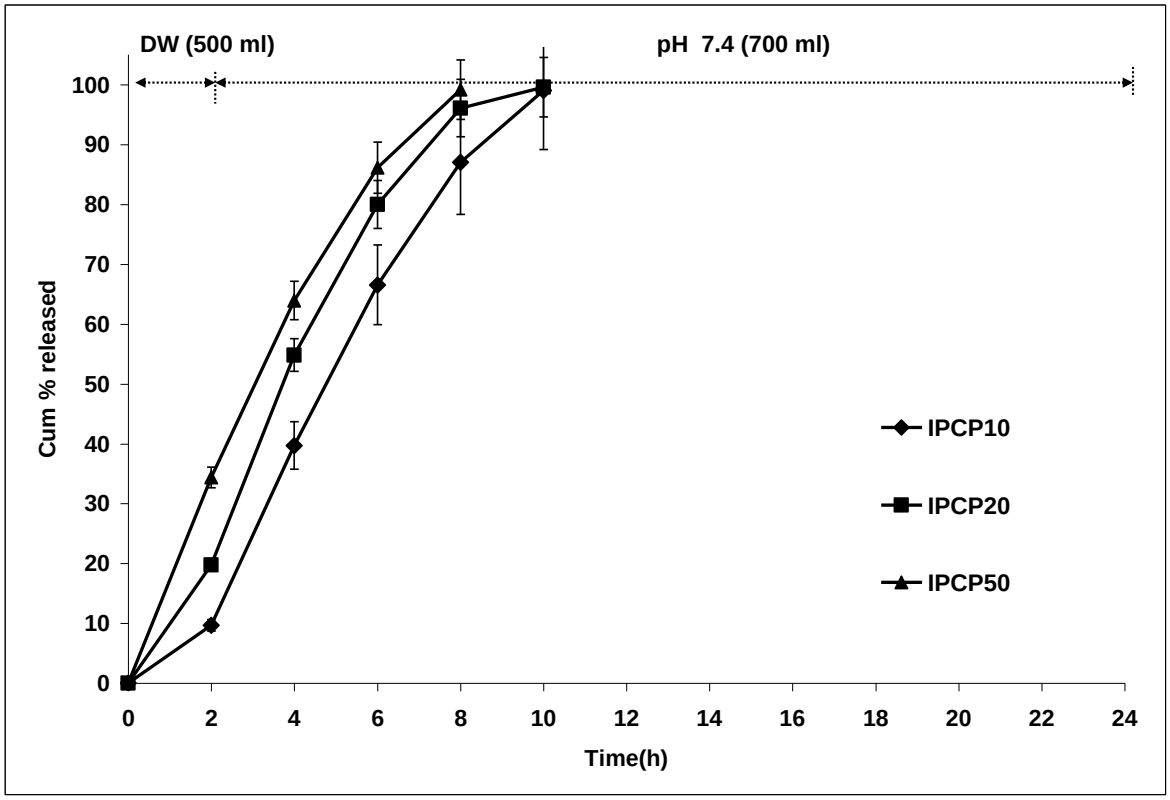


Fig 5.13: Release profile of indomethacin from matrix tablets with varying proportion of PCP. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

(ii) Effect of carbopol

On the other hand, the matrix bases composed of carbopol alone in 5, 10 and 20% w/w of drug were found to swell quickly initially and then provide controlled release of the drug proportional to the amount of polymer in formulation (Fig 5.14). The values for the release rate constant K for the three formulations ICP5, ICP10 and ICP20 were obtained as 12.995 $h^{-0.49}$, 5.411 $h^{-0.74}$ and 6.382 $h^{-0.62}$ respectively. All formulations showed an initial release of 15-20% in 2 h with release of only 45% in 14 h for ICP5. In case of ICP10 and ICP20 the release was still slower with only 28% and 20% drug release respectively in 14 h. In this case, values of 'n' varied from 0.49 to 0.74 indicating anomalous (non- Fickian) type mechanism of drug release (Table 5.17). The initial drug release from carbopol matrix is attributed to the quick penetration of water into pores of the polymer matrix and diffusion of the drug though these pores prior to complete gel formation as reported previously (Cooper et al., 1995; Huang and Schwartz, 1995). With increase in pH, the ionization of the carboxylic acid groups increases leading to ionic repulsion and swelling of the polymer. This explains the non-Fickian (anomalous) nature of the drug release mechanism.

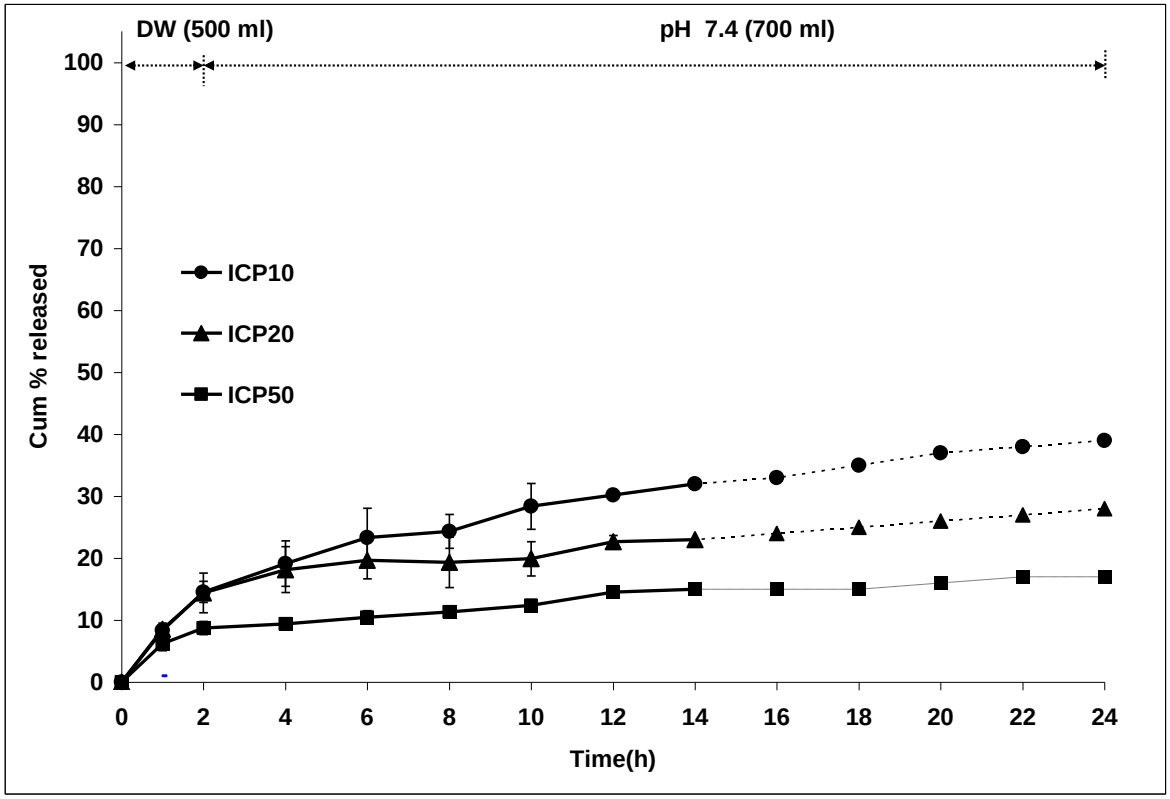


Fig 5.14: Release profile of indomethacin from matrix tablets with varying proportion of CP. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

(iii) Effect of Xanthan Gum

A plot of cumulative percentage released versus time for matrix tablets of indomethacin prepared using varying proportions of xanthan gum alone (5, 10 and 20% w/w of drug) is shown in Fig 5.15. It was observed that the initial percentage released from all the formulations was quite high (35% for IXG5 and 15-20% for the others) in the first 2 h followed by a slower and more controlled release during the later stages depending on the proportion of the polymer in the matrix. The K values for the three formulations IXG5, IXG10 and IXG20 were obtained as 11.679 $h^{-1.10}$, 5.220 $h^{-1.09}$ and 15.707 $h^{-0.55}$ respectively. When used as a matrix base, xanthan gum forms a time dependent swelling controlled system and drug release is through diffusion from the swollen xanthan gum matrix (Phaechamud and Ritthidej, 2007). According to another report, the high initial swelling of xanthan gum based matrices resulted in the release of a significant drug load from the formulation during the early drug release phase (Yeole et al., 2006). This property can make xanthan gum alone unsuitable as a polymeric matrix base for colonic delivery because there will be high drug loss in the upper GI tract upon oral administration.

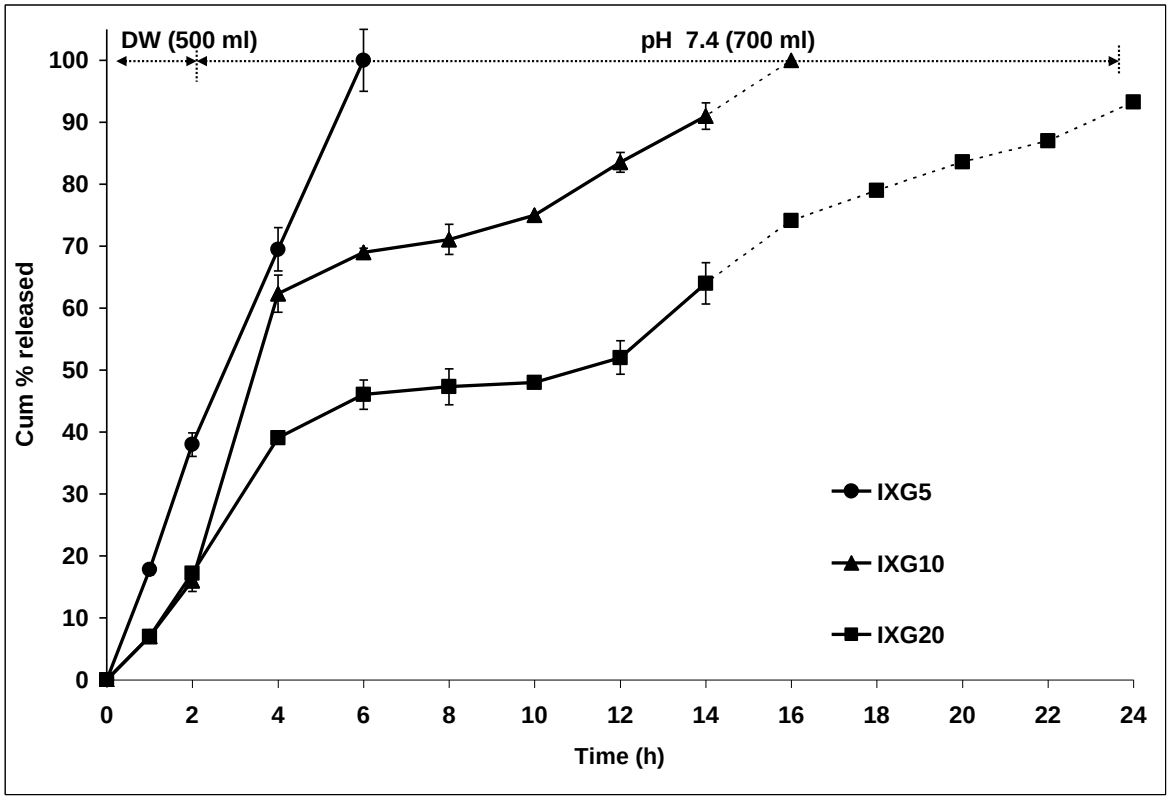


Fig 5.15: Release profile of indomethacin from matrix tablets with varying proportion of xanthan gum. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The release data when fitted to the power equation (Table 5.17) to get the calculated values of $t_{90\%}$ indicated 5.4 h for IXG5 which was extended to 23.9 h for IXG20 when the proportion of xanthan gum was increased from 5% to 20% w/w of drug respectively. The use of higher proportions of xanthan gum resulted in the formation of a thick polymeric gel layer which acted as a barrier to drug diffusion. The values of 1.13 (in case of IXG5) and 0.55 (in case of IXG20) for the diffusional exponent 'n' indicated a shift in the release mechanism from super case II ($n > 1.0$) to anomalous non- Fickian type ($0.45 < n < 0.89$). In case of IXG5 and IXG10 with a relatively higher proportion of indomethacin, drug release took place due to erosion of tablet surface, due to limited swelling of xanthan gum in the presence of a hydrophobic drug. On the other hand, due to the relatively higher proportion of xanthan gum in IXG20, the drug release mechanism was obtained as anomalous due to swelling of xanthan gum and diffusion of drug through the swollen layer.

Table 5.17: Release kinetics characterization of drug release from PCP / CP / XG based matrix tablets

Batches	Release kinetics					
	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]
IPCP5	0.9696	1.48 x10^{-3}	4.556	1.29	2.3	10.1
IPCP10	0.9791	4.60 x10^{-3}	4.169	1.46	2.0	8.2
IPCP20	0.9808	7.12 x10^{-3}	11.442	1.03	1.1	7.6
ICP5	0.9981	4.81 x10^{-2}	12.995	0.49	1.2	34.5
ICP10	0.9721	4.00 x10^{-2}	5.411	0.74	1.0	39.8
ICP20	0.9736	4.50 x10^{-2}	6.382	0.62	1.0	41.2
IXG5	0.9504	1.27 x 10^{-3}	11.679	1.10	0.5	5.4
IXG10	0.9204	1.83 x 10^{-2}	5.220	1.09	1.2	13.7
IXG20	0.9167	1.23 x 10^{-2}	15.707	0.55	1.1	23.9

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

5.6.3.4. Effect of hydrophilic nonionic polymer on indomethacin release

(i) Effect of hydroxy ethyl cellulose

In the case of non-ionic hydrophilic polymeric matrix systems that are comprised of HEC (or even in case of HPC), the surface of the matrix initially hydrates during dissolution to generate an outer viscous gel layer. This phase is then sequentially followed by matrix bulk hydration, swelling and erosion. The overall dissolution rate is controlled by the rate of matrix swelling and drug diffusion through the gel layer (Siegel et al., 1984; Bain et al., 1991).

A plot of cumulative percentage drug released against time for the matrix bases composed of varying proportions (5, 10 and 20% w/w of drug) of HEC is shown in Fig 5.16. It was observed that drug release was rapid with an initial burst (around 30 - 40% in first 2 h) with complete release within 4 h ($t_{90\%}$ of 3.5 h) suggesting that HEC could not sustain drug release from the matrix in the proportions employed (Table 5.18). The corresponding K values for the three formulations IHEC5, IHEC10 and IHEC20 were obtained as 14.428 $h^{-1.45}$, 18.550 $h^{-1.26}$ and 12.663 $h^{-1.55}$ respectively.

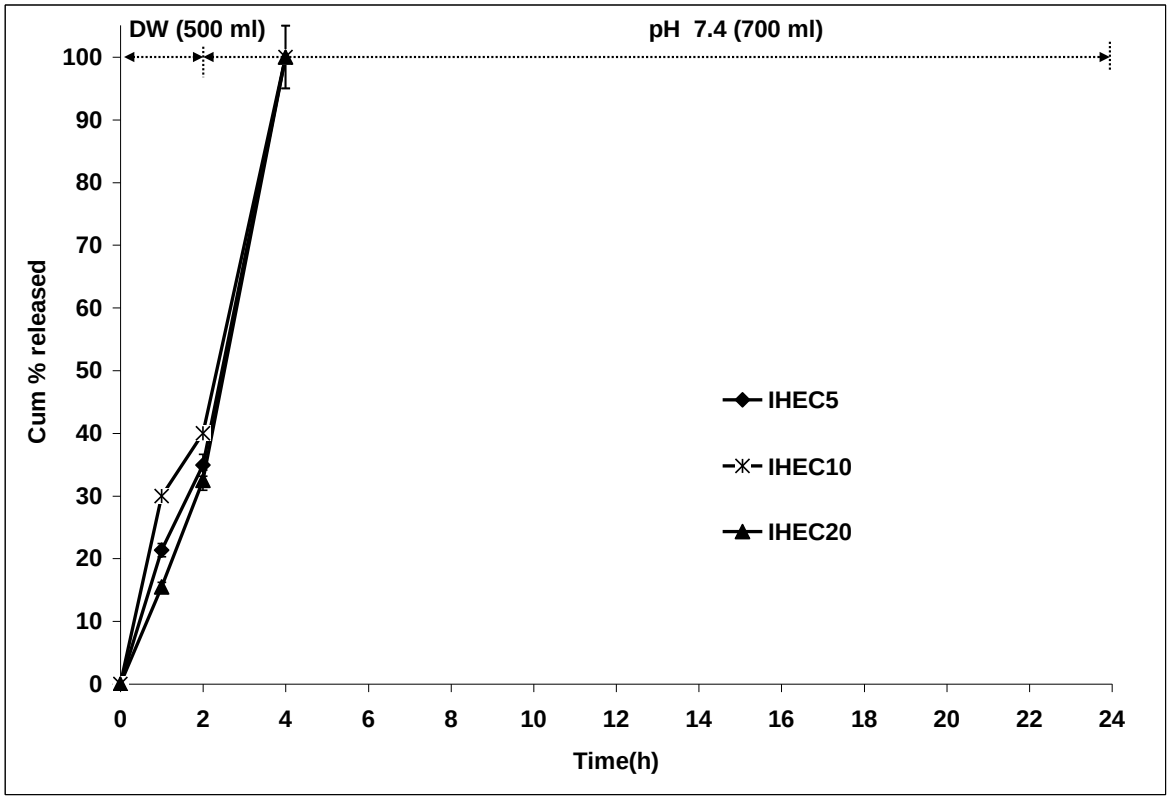


Fig 5.16: Release profile of indomethacin from matrix tablets with varying proportion of HEC. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

(ii) Effect of hydroxy propyl cellulose

In the case of matrix bases composed of drug with HPC in varying proportion (5, 10 and 20% w/w of drug), the formulations showed low initial release of less than 2% in 2 h and 10-12% in 5-6.5 h (Fig 5.17). The K values for the three formulations IHPC5, IHPC10 and IHPC20 were obtained as 2.533 $h^{-1.08}$, 0.796 $h^{-1.44}$ and 0.528 $h^{-1.54}$ respectively. The expected duration of drug release (in terms of calculated $t_{90\%}$) was found to be more than 26.5 h for IHPC5 and 28.0 h for IHPC20, indicating considerable deviation from the design requirement of 80-90% release in 14 -16 h (Table 5.18). Release mechanism in case of all the levels of HPC was found to be super case II indicating erosion along with swelling as the primary mechanism of drug release from HPC matrix.

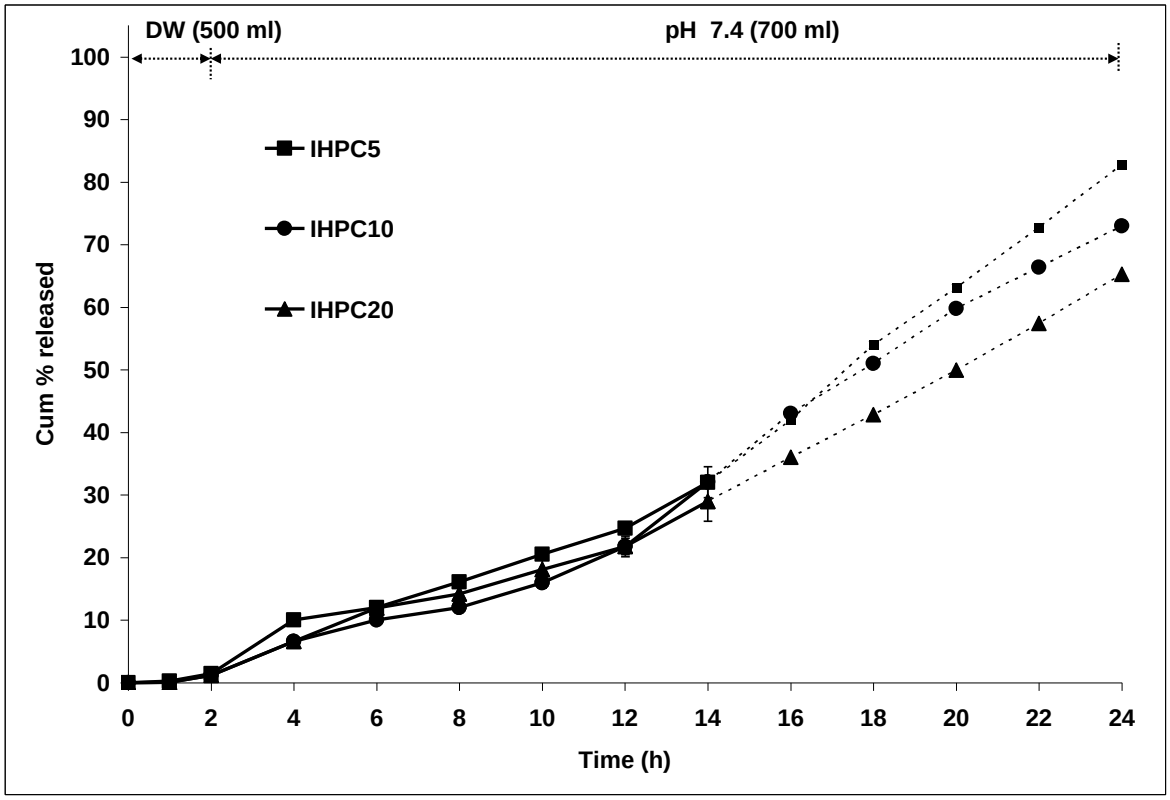


Fig 5.17: Release profile of indomethacin from matrix tablets with varying proportion of HPC. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

(iii) Effect of guar gum

In case of the matrix bases comprising of guar gum in similar proportions (5, 10 and 20% w/w of drug), the in vitro drug release profiles are shown in Fig 5.18. The K values for the three formulations IGG5, IGG10 and IGG20 were obtained as 15.041 $h^{-1.34}$, 17.104 $h^{-1.22}$ and 22.218 $h^{-0.93}$ respectively. In the present study, it was observed that guar gum proportions upto 20% w/w of drug in matrix base (IGG20) could not retard the drug release from the matrix. Formulations with 5% guar gum (IGG5) were found to give very rapid drug release (nearly 45% in 2 h) followed by complete release in 4 h (Table 5.18). Formulations with 10% (IGG10) and 20% (IGG20) of the polymer were also found to swell rapidly with 30% drug release in the first 2 h followed by disintegration of the matrix resulting in complete release in 4-5 h. It has been shown that when present in lower proportions in a matrix base, guar gum tends to swell and dissolve rapidly owing to its high hydrophilicity (Sinha and Kumria, 2003), which explains the rapid release of drug from the matrix in the present case also. In higher concentrations however, upon exposure to dissolution and biological fluids, the gum hydrates slowly and forms a viscous gel layer that slows down penetration of the seeping fluid and diffusion of drug from the core

(Krishnaiah et al., 2002; Jain et al., 2007). From the present study, it could be concluded that guar gum alone was not useful for colonic delivery in the proportions employed.

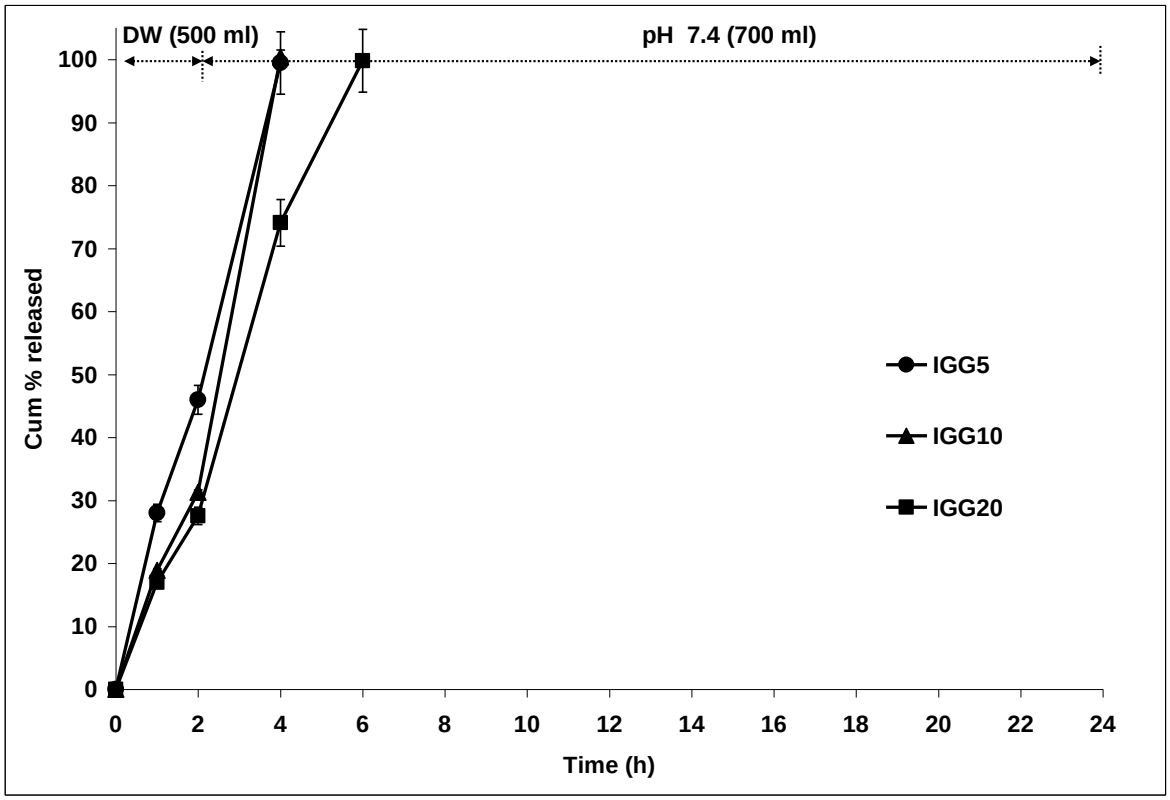


Fig 5.18: Release profile of indomethacin from matrices with varying proportions of guar gum. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.18: Release kinetics characterization of drug release from HEC/ HPC/GG based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IHEC5	1.000	0.00	14.428	1.45	0.7	3.5
IHEC10	1.000	0.00	18.550	1.26	0.8	3.5
IHEC20	1.000	0.00	12.663	1.55	0.8	3.5
IHPC5	0.9956	1.07×10^{-3}	2.533	1.08	4.0	26.5
IHPC10	0.9603	1.85×10^{-2}	0.796	1.44	4.3	26.7
IHPC20	0.9783	1.13×10^{-2}	0.528	1.54	4.8	28.0
IGG5	0.9923	1.23×10^{-3}	15.041	1.34	0.4	3.8
IGG10	0.9748	1.13×10^{-3}	17.104	1.22	0.6	3.9
IGG20	0.9886	1.24×10^{-3}	22.218	0.93	0.3	4.5

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

5.6.4. Effect of combination of pH sensitive polymers (EL100 and ES100) on indomethacin release

In order to investigate the effect of polymer combination, the first combination of polymers that was tried was Eudragit L100 and Eudragit S100. When used as coating polymers, both EL100 and ES100 are recommended to be used in combination as neither polymer alone is suitable for colonic delivery (Khan et al., 1999). EL100 dissolves above pH 6.0 and ES100 above pH 7.0. The combination of these two polymers in various ratios makes it possible to manipulate drug release within 6.0-7.0 pH range. This can ensure that the release of drug from formulation will occur even when the pH value of the GI tract does not reach more than 6.8. Therefore, indomethacin matrix tablets comprising of varying proportion of EL100 and ES100 were developed.

The release profiles from the matrix tablets containing both EL100 and ES100 in relative ratios of 3:2, 1:1, and 2:3 respectively, i.e., IEL15ES10, IEL12.5ES12.5, IEL10ES15, at total polymer proportion of 25% w/w of drug, are shown in Fig 5.19. The K values for the three formulations were obtained as 3.174 $h^{-1.66}$, 0.489 $h^{-1.96}$ and 1.895 $h^{-1.44}$ respectively. An increase in the proportion of ES100 (relative to EL100) indicated no significant difference in the initial drug release parameters but affected the overall release kinetics (Table 5.19).

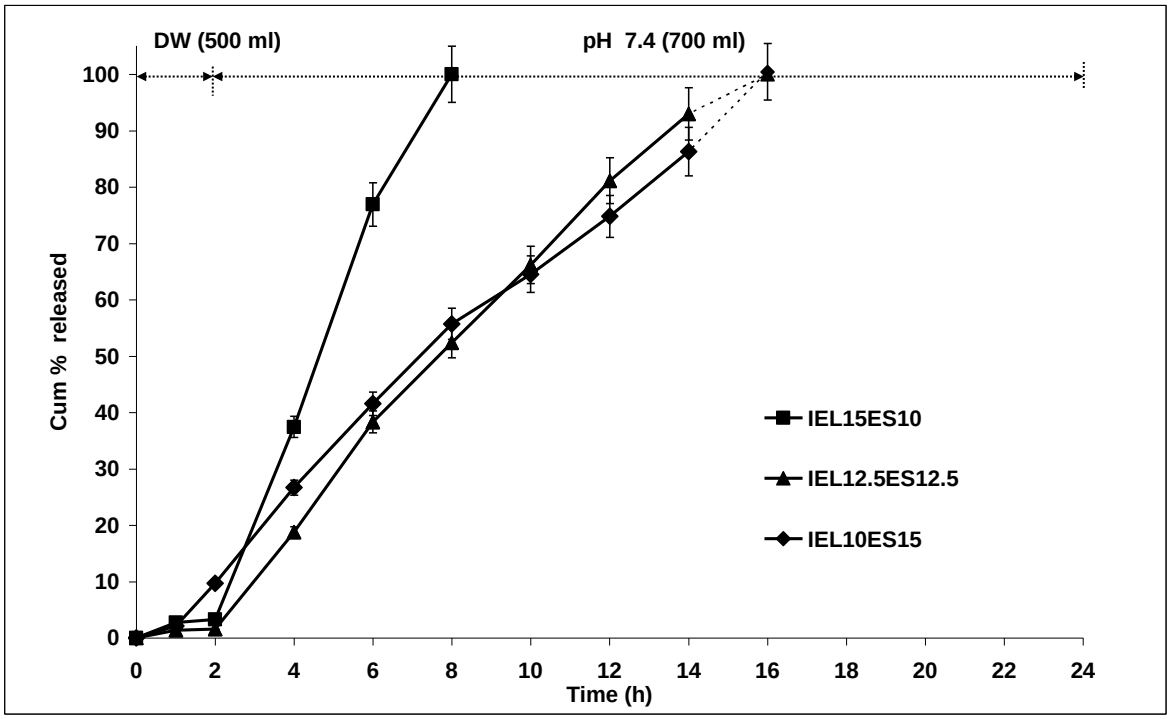


Fig 5.19: Release profile of matrix tablets of indomethacin containing combination of EL100 and ES100 in varying ratios at 25%w/w of drug. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The $t_{10\%}$ value was found to be 2.8, 2.9 and 2.5 h for IEL15ES10, IEL12.5ES12.5, and IEL10ES15 respectively. The $t_{90\%}$ value was extended from 7.5 h for IEL15ES10 to 14.3 h for IEL12.5ES12.5 with increase in relative proportion of ES100 and further to 14.6 h in case of IEL10ES15. Thus, retardation in indomethacin release from these matrices in alkaline pH was found to depend on the relative proportion of EL100 and ES100 in the polymer matrix. The decrease in drug release rate post 2 h in pH 7.4 with increase in relative proportion of ES100 is attributed to the presence of lower percentage of carboxylic acid groups on ES100 resulting in slower rate of matrix erosion (Mehta et al., 2001).

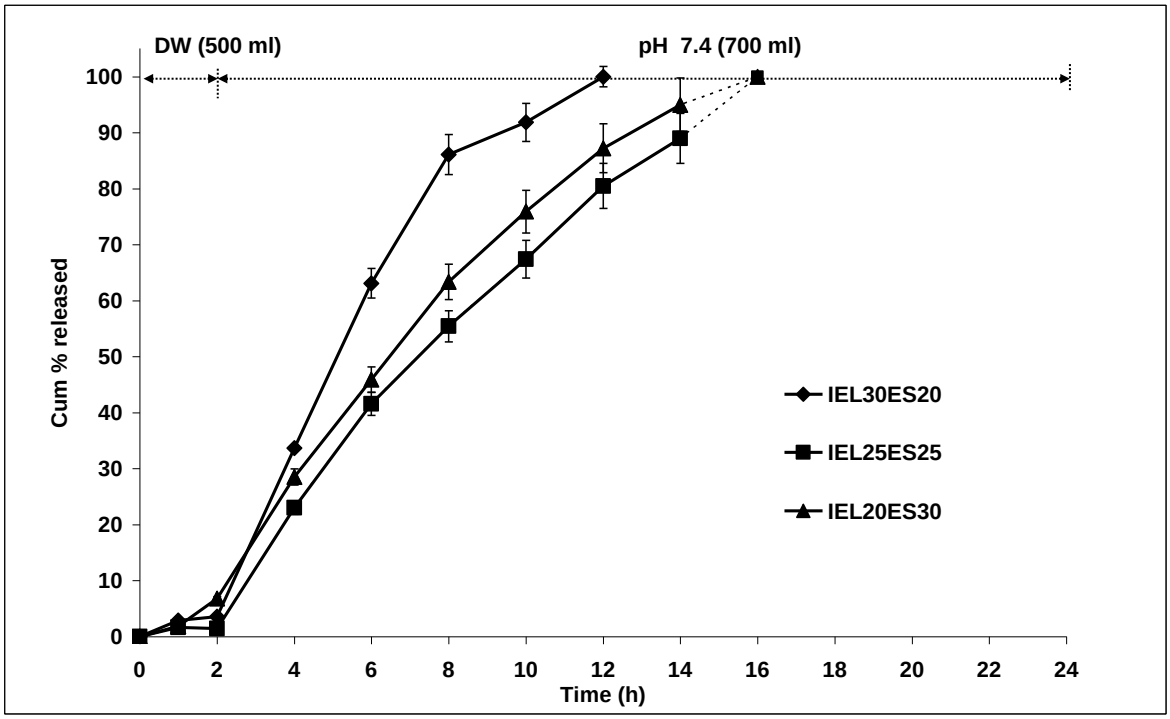


Fig 5.20: Release profile of matrix tablets of indomethacin containing combination of EL100 and ES100 in varying ratios at 50%w/w of drug. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

When these matrices were compared at similar relative polymer ratios at 50% w/w of drug, i.e., IEL30ES20, IEL25ES25, and IEL20ES30 release profiles quite similar to those obtained in case of 25% w/w of the drug were observed (Fig 5.20). The K values for the three formulations were obtained as 1.827 $h^{-1.70}$, 0.459 $h^{-1.93}$ and 4.498 $h^{-1.19}$ respectively. The results indicated no advantage upon increasing the total polymer content from 25% to 50% in the matrix as the $t_{10\%}$ and the $t_{90\%}$ values were not improved significantly (Table 5.19). Since the 'n' values (Table 5.19) for these series of formulations was in the range of 1.19 to 1.96 ($n > 1.0$; super case II type), it can be concluded that erosion of the Eudragit matrix in alkaline pH was the primary mechanism of drug release (Carelli et al., 2000).

Table 5.19: Release kinetics characterization of drug release from EL100 and ES100 based matrix tablets

Formula	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IEL15ES10	0.9531	1.25×10^{-2}	3.174	1.66	2.8	7.5
IEL12.5ES12.5	0.9050	6.82×10^{-2}	0.489	1.96	2.9	14.3
IEL10ES15	0.9800	3.22×10^{-3}	1.895	1.44	2.5	14.6
IEL30ES20	0.9360	5.77×10^{-2}	1.827	1.70	2.3	9.9
IEL25ES25	0.9921	7.08×10^{-4}	0.459	1.93	2.6	15.4
IEL20ES30	0.9836	6.23×10^{-3}	4.498	1.19	2.2	12.4

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

5.6.5. Effect of ethyl cellulose in combination with other polymers on indomethacin release

The effect of varying proportion of ethyl cellulose was investigated on drug release from ionic and pH sensitive polymer based indomethacin matrix tablets. It was envisaged that presence of EC would inhibit initial matrix swelling in distilled water and therefore impart suitable lag time in initial drug release. Further, EC would retard the overall drug release from the matrix and therefore provide controlled release.

5.6.5.1. Effect of ethyl cellulose in combination with EL100 or ES100 on indomethacin release

(i) Ethyl cellulose + Eudragit L100

Effect of EC in EL100 was studied at both 25% and 50% w/w level of the polymer. In these formulations the EL100 to EC ratio was varied as 4:1 or 3:2. The release profiles from the matrix tablets containing EL100 and EC in the ratio 3:2 or 4:1 at 25% w/w of drug, i.e., IEL20EC5 and IEL15EC10 are shown in Fig 5.21 and corresponding to 50% w/w of the drug (IEL30EC20 and IEL40EC1) are shown in Fig 5.22. The K values for the formulations IEL20EC5 and IEL15EC10 were obtained as 3.727 $h^{-1.57}$and 0.355 $h^{-1.89}$ respectively. Incorporation of EC in the matrix retarded the release rate when compared to EL100 alone (Fig 5.11) at both 25% and 50% w/w levels of total polymer proportion (Fig 5.21 and Fig 5.22) except in case of IEL20EC5. It was observed that increasing the relative proportion of EC from 5% to 10% as in the case of (IEL20EC5 or IEL40EC10) and from 10% to 20% (IEL15EC10 or IEL30EC20) retarded the release rate and also extended the total duration of release (Table 5.20).

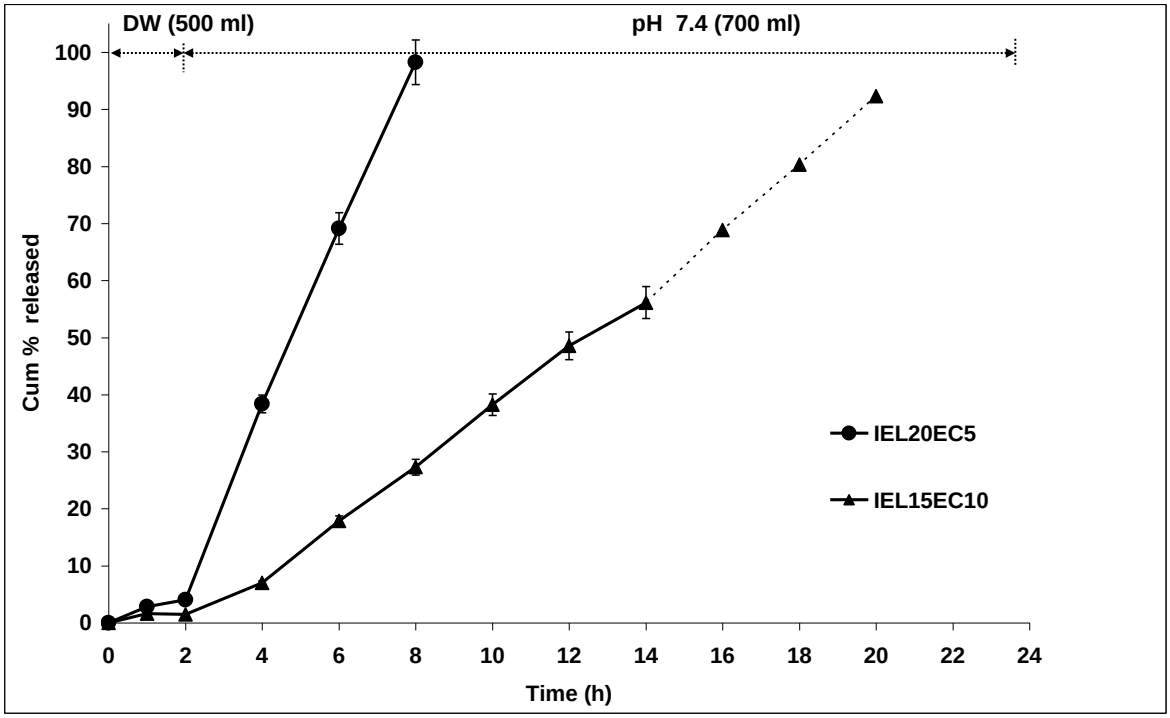


Fig 5.21: Release profile of matrix tablets of indomethacin containing combination of EL100 and EC in varying ratios at 25% w/w of drug. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

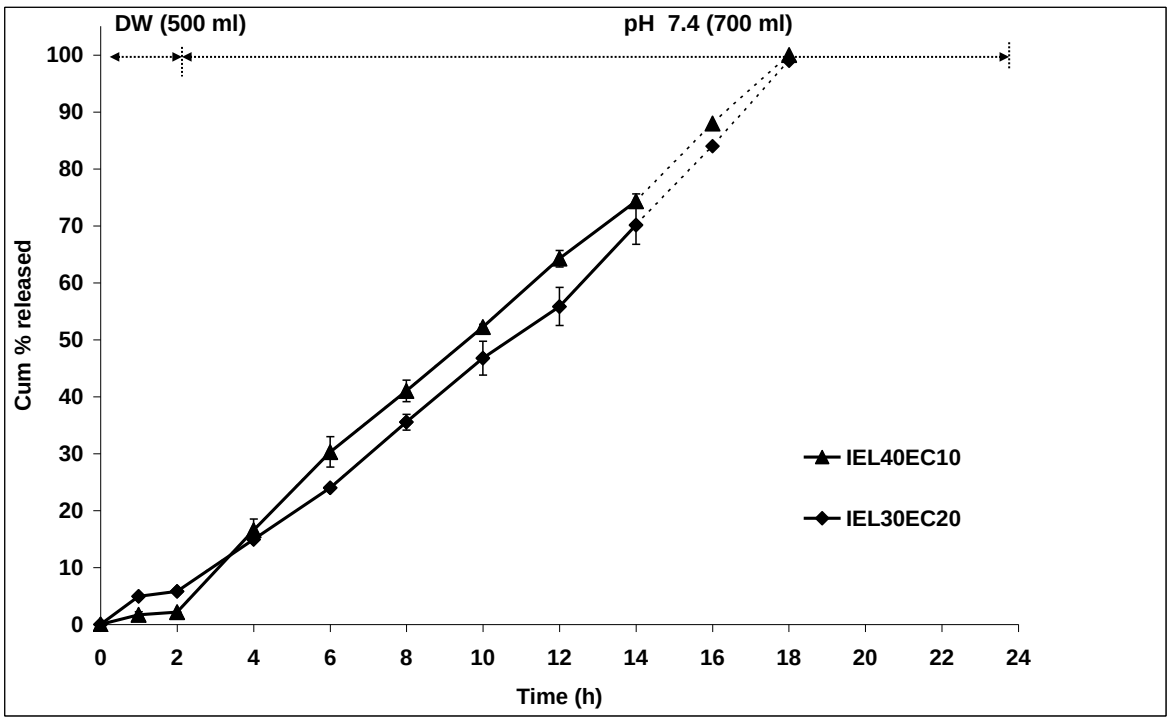


Fig 5.22: Release profile of matrix tablets of indomethacin containing combination of EL100 and EC in varying ratios at 50%w/w of drug. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

In case of total polymer proportion at 25% w/w of the drug, $t_{10\%}$ increased from 2.3 h for IEL20EC5 to 2.9 h for IEL15EC10 while $t_{90\%}$ increased significantly from 7.6 h for IEL20EC5 to 18.5 h for IEL15EC10. At 25% w/w of the drug, the increase in EC

percentage from 4:1 to 3:2, resulted in the formation of a tight non-porous matrix allowing for very slow penetration of external media and slower release rates hence significantly extending the duration of release and retarding the release rate (Fig 5.21). But in case of IEL40EC10 and IEL30EC20, with total polymer proportion of 50% w/w of drug, the $t_{10\%}$ increased from 3.0 h for IEL40EC10 to 4.1 h for IEL30EC20 but the $t_{90\%}$ value only marginally increased from 16.0 h for IEL40EC10 to 16.8 h for IEL30EC20 (Fig 5.22). It has been shown in earlier studies that high levels of EC reduce drug release rates on account of formation of a strong matrix with reduced porosity (Khan and Zhu, 1999; Sajeev and Saha, 2001; Saha et al., 2002). This increases diffusional path length leading to reduced dissolution media penetration through the micropores resulting in slower drug release. But increasing the total polymer proportion to 50% w/w of drug did not offer any additional advantage in terms of $t_{10\%}$ and $t_{90\%}$. The reason for such phenomenon is attributed to the presence of relatively higher proportion of EL100 in such matrix. As EL100 dissoluted above pH 6.0, the matrix with higher EL100 proportion cannot maintain matrix integrity and tortousity at pH 7.4, increasing the relative proportion of EC in this case was not found to have any impact.

Table 5.20: Release kinetics characterization of drug release from EL100 and EC matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IEL20EC5	0.9140	4.95×10^{-2}	3.727	1.57	2.3	7.6
IEL15EC10	0.9921	5.47×10^{-4}	0.355	1.89	2.9	18.5
IEL40EC10	0.9690	6.38×10^{-3}	1.131	1.58	3.0	16.0
IEL30EC20	0.9995	7.49×10^{-4}	2.348	1.29	4.1	16.8

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

The drug release mechanism for EC + EL100 based matrix tablets was super case II (n > 1), due to matrix erosion implying that the presence of ethyl cellulose did not change the primary mechanism of release that was observed for matrix bases with EL100 alone. It was also observed that sigmoidal release profiles were obtained in all the cases and except for IEL20EC5 that showed slightly higher release rates, all other formulations nearly completed release within 16-18 h, and thereby only slightly deviating from the requirement for complete release in 14- 16 h.

(ii) Ethyl cellulose + Eudragit S100

The release profiles from the matrix tablets comprising of ES100 and EC (4:1 and 3:2 ratios) at 25% and 50% w/w of drug, are shown in Fig 5.23(a & b) respectively.

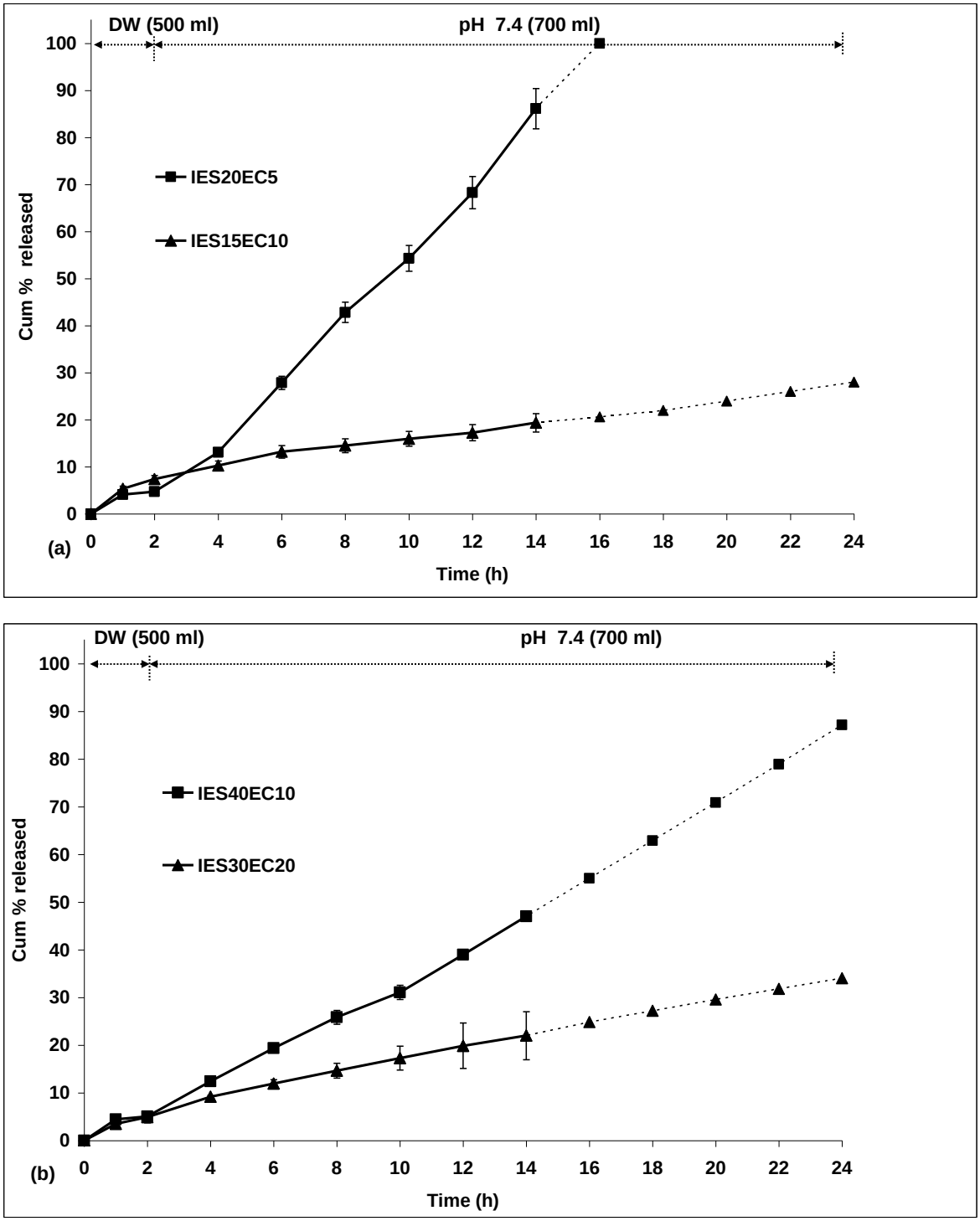


Fig 5.23. Release profile of matrix tablets of indomethacin containing combination of ES100 and EC in varying ratios at total polymer proportion of (a) 25%w/w of drug and (b) 50%w/w of drug. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The formulations IES20EC5 and IES15EC10 were marginally different in terms of initial release as is evident from the $t_{10\%}$ values (Table 5.21) for the two formulations (3.4 h and 3.8 h respectively). As the proportion of EC was increased from 5% (4:1) to 10%, the $t_{90\%}$ values were drastically increased from 14.8 h for IES20EC5 to 38.5 h for IES15EC10.

From the release profiles of matrices at 50% w/w of drug in similar relative ratios, i.e., IES40EC10 and IES30EC20, the $t_{10\%}$ values (Table 5.21) of 3.4 h for IES40EC10 and 4.2 h for IES30EC20 indicated good retardation in the initial release (Fig 5.23b). However, the $t_{90\%}$ values for the two formulations (IES40EC10: 39.6 h, IES30EC20: 28.5 h) were significantly higher than the expected duration of release.

The release mechanism from ES100 matrices in combination with EC was also found to be similar to that observed for EC + EL100 matrices and was super case II release (n > 1.0) indicating erosion of the polymer matrix at higher pH as the primary mechanism of drug release. Except for IES20EC5 with a sigmoidal profile that was within acceptable limits, all other formulations showed significant deviation from the desired target release of 80-90% in 14 -16 h. In comparison to EC + EL100 matrices, the designed matrices of EC + ES100 showed lower rate of drug release at all polymer ratios and proportion due to the nature of ES100 that erodes at pH > 7.0 when compared to the optimal dissolution pH of 6.0 for EL100.

Table 5.21: Release kinetics characterization of drug release from ES100 and EC based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IES20EC5	0.9980	5.30×10^{-4}	1.581	1.5	3.4	14.8
IES15EC10	0.9976	3.08×10^{-4}	1.947	1.05	3.8	38.5
IES40EC10	0.9850	4.54×10^{-4}	1.563	1.3	3.4	39.6
IES30EC20	0.9976	2.97×10^{-4}	2.671	1.05	4.2	28.5

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

5.6.5.2. Effect of ethyl cellulose in combination with PCP or CP on indomethacin release

(i) Ethyl cellulose + Polycarbophil

The release profiles from matrices containing PCP at 10% w/w of drug and varying levels of EC (from 5% to 40% w/w of drug) revealed that increasing the relative proportion of EC in the matrix retarded drug release during the initial as well as the later stages as shown in Fig 5.24. The $t_{10\%}$ values ranged from 3.8 h to 5.9 h when EC content was increased from 5 to 40% w/w of drug in 10% PCP matrix (Table 5.22). Similarly, the $t_{90\%}$ values varied from 14.5 h to 19.9 h when EC content was increased from 5 to 40% w/w of drug in 10% PCP matrix.

Compared to indomethacin matrix bases with PCP alone, the drug release profile from EC + PCP matrix were significantly different. There was retardation in initial release and extension in duration of release (Fig 5.24). The inclusion of ethylcellulose in a hydrophilic polymer (HPMC) matrix has been previously shown to control drug release due to the decreased penetration of the solvent molecules in the presence of hydrophobic polymer leading to decreased diffusion of the drug from the matrix (Tiwari et al., 2003). In the present case too, the presence of EC would have slowed down the penetration of dissolution medium and thereby prevented initial matrix swelling. With subsequent rise in pH to 7.4, ionization of acrylate groups on polycarbopihil resulted in repulsion between polymer chains leading to polymer relaxation manifested as swelling of matrix (Hosny, 1993). This swelling allowed for relatively easier penetration of dissolution medium into matrix and enhanced drug release.

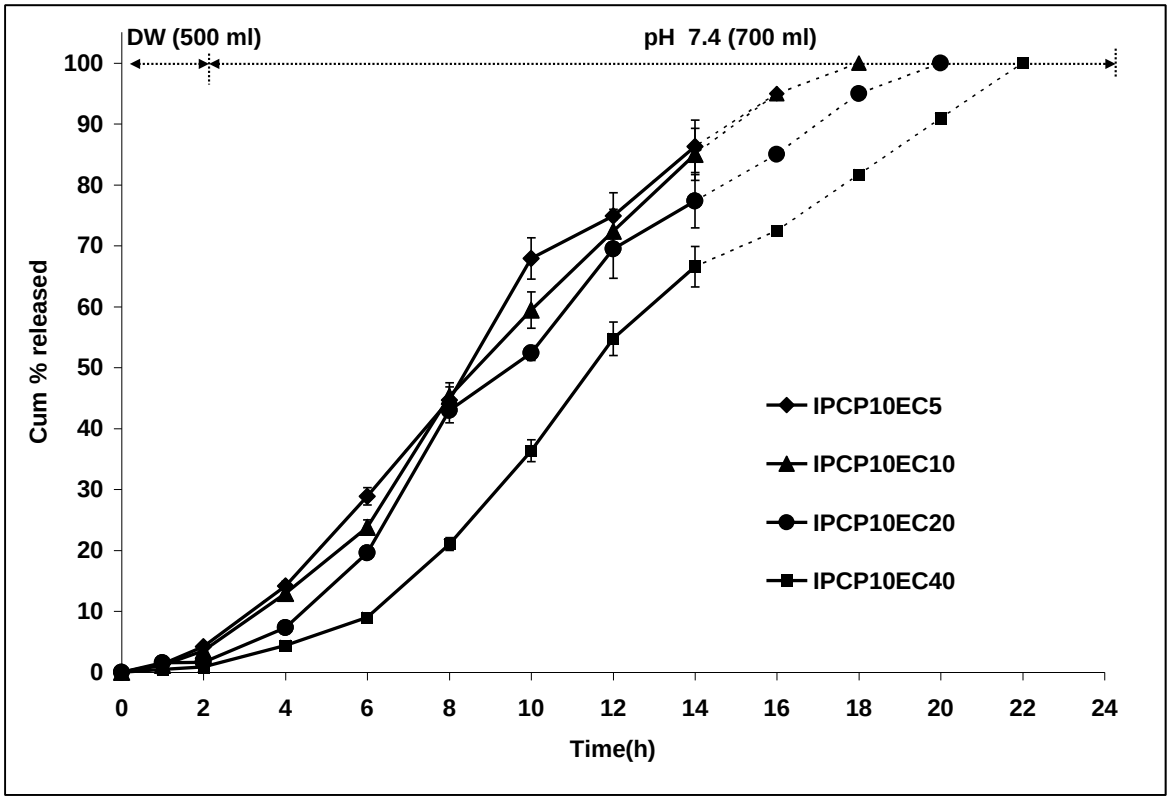


Fig 5.24: Release profile of indomethacin from matrix tablets showing effect of varying proportion of EC on 10% PCP matrix tablets. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

When the relative proportion of PCP in the matrix was increased from 10% to 20% w/w of drug (with EC proportion as earlier), higher release rates were observed when compared to corresponding 10% PCP matrix with EC (Table 5.22). This could be due to enhanced swelling of the matrix with increase in percentage of hydrophilic polymer (Fig. 5.25).

However, the $t_{10\%}$ and $t_{90\%}$ depended on the proportion of EC resulting in highest release rates for IPCP20EC5 and slowest in case of for IPCP20EC40.

In a previous study, the effect of incorporating a hydrophobic polymer Eudragit RL100 on the mucoadhesion and other properties of matrices composed of polycarbophil has been reported (Tirosh et al., 2001). Eudragit RL100 was able to significantly decrease the swelling of polycarbophil films in a concentration-dependent manner. The swelling of the mixture was less in pH 5.0 than in pH 7.4. The amount of Eudragit RL100 correlated well with the rate of erosion of the matrix. A similar effect was observed in our case with incorporation of EC in polycarbophil matrix.

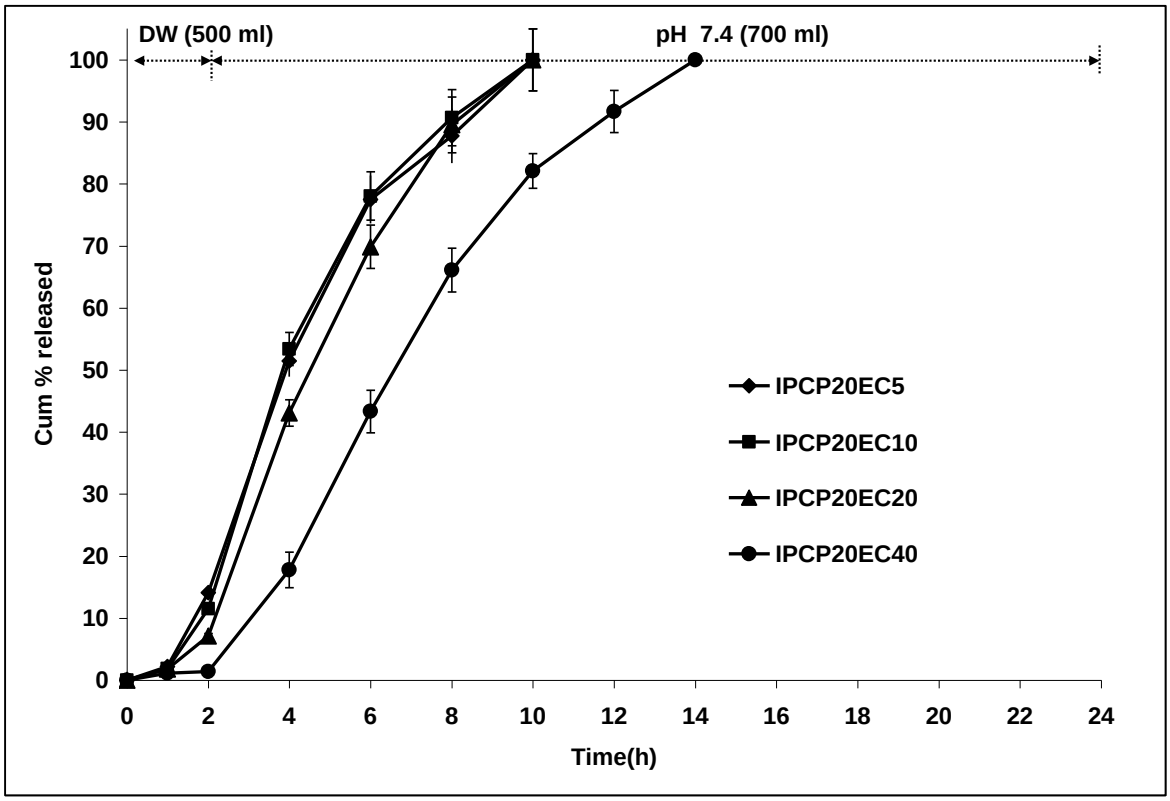


Fig 5.25: Release profile of indomethacin from matrix tablets showing effect of varying proportion of EC on 20% PCP matrix tablets. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Further, 'n' values (Table 5.22) indicate super case II mechanism which can be attributed to matrix swelling due to the hydrophilic component accompanied with erosion. EC (at 5 to 40% w/w of drug) thus could impart a sigmoidal pattern to the drug release profiles from the prepared PCP matrices (Fig 5.24 and 5.25).Therefore, it was concluded that inclusion of ethyl cellulose in a polycarbophil matrix base resulted in the formation of a delayed release system with 10% PCP matrix showing more desirable release profile for colon targeting than the corresponding 20% PCP matrix.

Table 5.22: Release kinetics characterization of drug release from PCP and EC based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
10% PCP with varying EC proportion						
IPCP10EC5	0.9968	5.74×10^{-3}	1.281	1.59	3.8	14.5
IPCP10EC10	0.9978	4.94×10^{-3}	1.067	1.65	3.9	14.7
IPCP10EC20	0.9834	2.45×10^{-3}	1.145	1.55	4.6	16.7
IPCP10EC40	0.9775	7.50×10^{-3}	4.260	1.02	5.9	19.9
20% PCP with varying EC proportion						
IPCP20EC5	0.9474	3.72×10^{-3}	7.495	1.21	1.6	7.8
IPCP20EC10	0.9518	3.39×10^{-2}	5.959	1.33	1.8	7.7
IPCP20EC20	0.9674	6.68×10^{-2}	3.035	1.64	2.2	7.9
IPCP20EC40	0.9782	2.87×10^{-3}	0.484	2.11	3.8	11.9

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

(ii) Ethyl cellulose + Carbopol

The effect of EC on drug release from 10% CP matrices is shown in Fig 5.26. Increase in relative proportion of EC (from 5% to 40% w/w of drug) resulted in decrease in the release rate of the drug from 2.714 $h^{-1.26}$ for ICP10EC5 to 1.497 $h^{-1.25}$ for ICP10EC40. This effect was similar to that observed for PCP + EC matrices. The $t_{10\%}$ values ranged from 4.2 h to 6.8 h and the $t_{90\%}$ values extended from 16.1 h to 26.5 h respectively when the relative proportion of EC increased from 5% to 40% w/w of drug in 10% CP matrix. Thus, in case of CP and EC based matrices, both the hydrophilic and hydrophobic polymer were observed to contribute together in retarding drug release from the matrix as opposed to PCP and EC matrix bases wherein increase in PCP proportion enhanced drug release rates. Therefore, when the relative proportion of CP in the matrix was increased from 10% w/w to 20% w/w of drug and EC levels varied from 5% to 40% w/w of drug, it resulted in a still greater retardation in the release rates as shown in Fig 5.27 and Table 5.23.

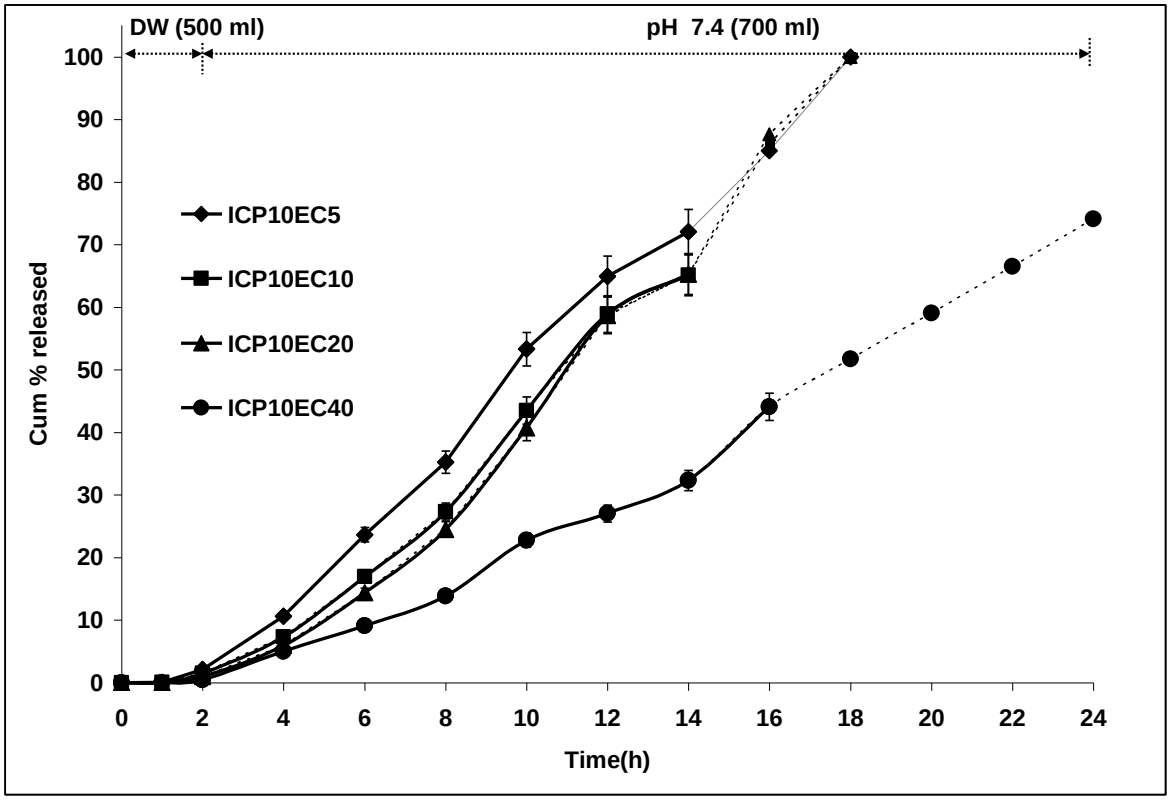


Fig 5.26: Release profile of indomethacin from matrix tablets showing effect of varying proportion of EC on 10% CP matrix tablets. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.23: Release kinetics characterization of drug release from CP and EC based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
10% CP with varying EC proportion						
ICP10EC5	0.9823	2.97×10^{-3}	2.714	1.26	4.2	16.1
ICP10EC10	0.9931	1.32×10^{-3}	2.155	1.34	5.0	16.2
ICP10EC20	0.9918	4.94×10^{-3}	1.473	1.48	5.5	16.1
ICP10EC40	0.9892	3.46×10^{-3}	1.497	1.25	6.8	26.5
20% CP with varying EC proportion						
ICP20EC5	0.9860	4.58×10^{-3}	0.396	1.87	4.7	18.2
ICP20EC10	0.9835	2.85×10^{-3}	0.267	1.94	5.5	20.1
ICP20EC20	0.9993	6.09×10^{-3}	1.043	1.44	6.1	22.1
ICP20EC40	0.9772	2.81×10^{-2}	1.355	1.23	7.2	30.3

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

The $t_{10\%}$ values ranged from 4.7 h to 7.2 h and the $t_{90\%}$ values extended from 18.2 h to 30.3 h respectively when the relative proportion of EC increased from 5% to 40% w/w of

drug in 20% CP matrix (Table 5.23, Fig 5.27). The combination of EC and CP resulted in the formation of matrix of high gel strength with very low porosity and high tortousity that resulted in very slow drug release rates from these matrices.

Therefore, it was concluded that 10% CP matrices with optimized proportion of EC can achieve suitable time delay in initial drug release. The values of the diffusional exponent 'n' in case of CP and EC based formulations indicate a super case II type of release implying that drug release occurred by matrix swelling and erosion. Also, polymer relaxation in alkaline medium contributed to enhanced drug release.

The use of 10% and 20% CP matrix with different proportions of EC resulted in a deviation in some cases (ICP10EC40, ICP20EC20, ICP20EC40) from the theoretical target release beyond 12 h ($t_{90\%}$ > 14-16 h), but may still be valuable for colonic delivery in cases where patient colonic transit times are severely prolonged.

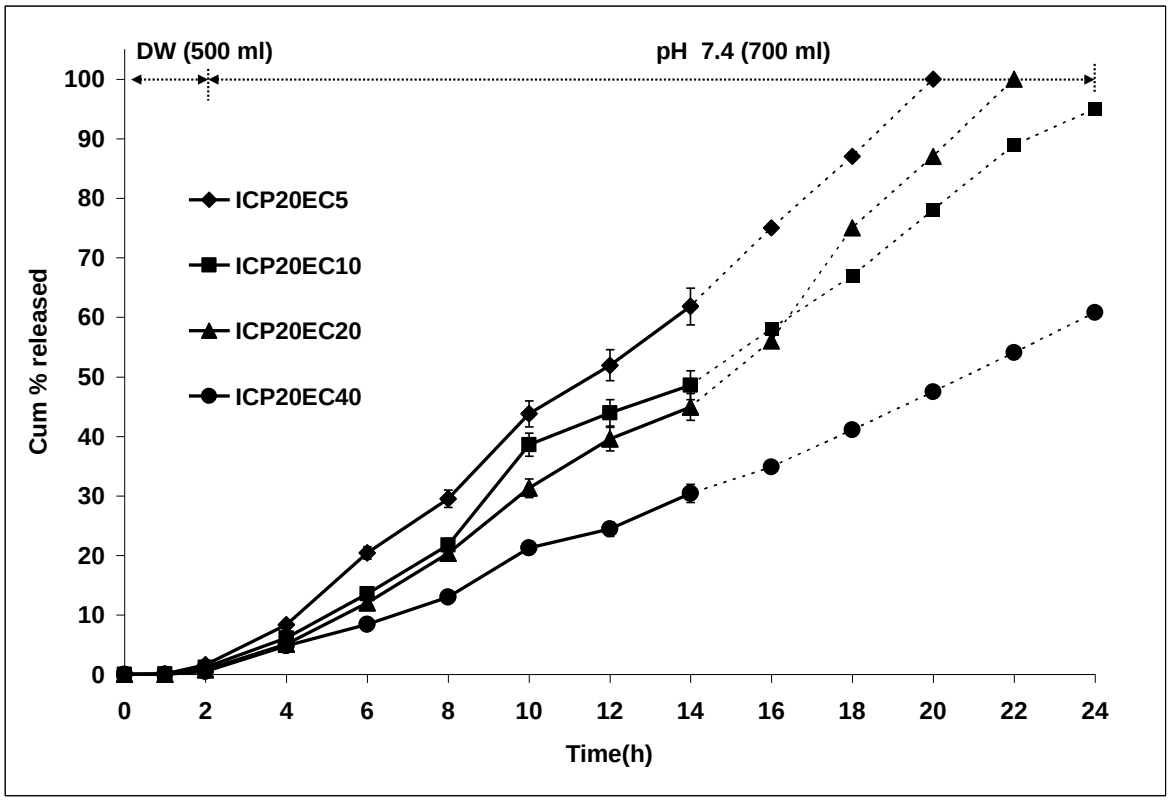


Fig 5.27: Release profile of indomethacin from matrix tablets showing effect of varying proportion of EC on 20% CP matrix tablets. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

5.6.6. Effect of Eudragit L100 in combination with other polymers on indomethacin release

The effect of Eudragit L100 in combination with different ionic and non ionic polymers on drug release was investigated. The ionic polymers selected were polycarbophil, carbopol

and xanthan gum while non ionic polymers chosen for the study were hydroxy ethyl cellulose, hydroxy propyl cellulose and guar gum. The ionic polymers selected (PCP, CP and XG) possess inherent swelling properties in alkaline pH due to the presence of acidic and anionic functional groups and nonionic polymers selected (HEC, HPC and GG) swell independent of pH changes. Therefore, it was considered worthwhile to investigate if the two sets of polymers in combination with EL100 confer similar type of pH and transit time dependent release to indomethacin for colonic delivery.

5.6.6.1. Effect of Eudragit L100 on indomethacin release from hydrophilic ionic polymer matrix bases

(i) Eudragit L100 + Polycarbophil

The in vitro release profiles from matrix bases composed using varying relative proportions of EL100 and PCP are shown in Fig 5.28. It was observed that presence of EL100 resulted in negligible release in the period of 2 h from all the formulations (Fig 5.28). Between IPCP5EL10 and IPCP5EL20, comparatively better retardation in the initial release of the drug was observed for IPCP5EL20 as compared to IPCP5EL10. The $t_{10\%}$ values for the two formulations were obtained as 2.9 h for IPCP5EL10 and 3.2 h for IPCP5EL20 (Table 5.24). The corresponding $t_{90\%}$ values were obtained as 14.7 h for IPCP5EL10 and 15.7 h for IPCP5EL20. However, when the relative proportion of EL100 was increased to 40% w/w of drug in a matrix containing 10% w/w of PCP (IPCP10EL40), $t_{10\%}$ value of 3.9 h was obtained which was comparable to 3.8 h for IPCP10EL20. The extent of release, however, was more prolonged for IPCP10EL40 ($t_{90\%}$ of 17.4 h) as compared to IPCP10EL20 ($t_{90\%}$ of 16.7 h) (Table 5.24). This could be on account of increase in relative proportion of hydrophobic polymer content. However, when the PCP level was doubled from 10% (IPCP10EL40) to 20% (IPCP20EL40) keeping proportion of EL100 constant at 40% w/w in the matrix, the release rate was found to increase slightly as shown by the lower $t_{10\%}$ and $t_{90\%}$ values (Table 5.24). This can be attributed to the increase in relative proportion of hydrophilic component of the matrix.

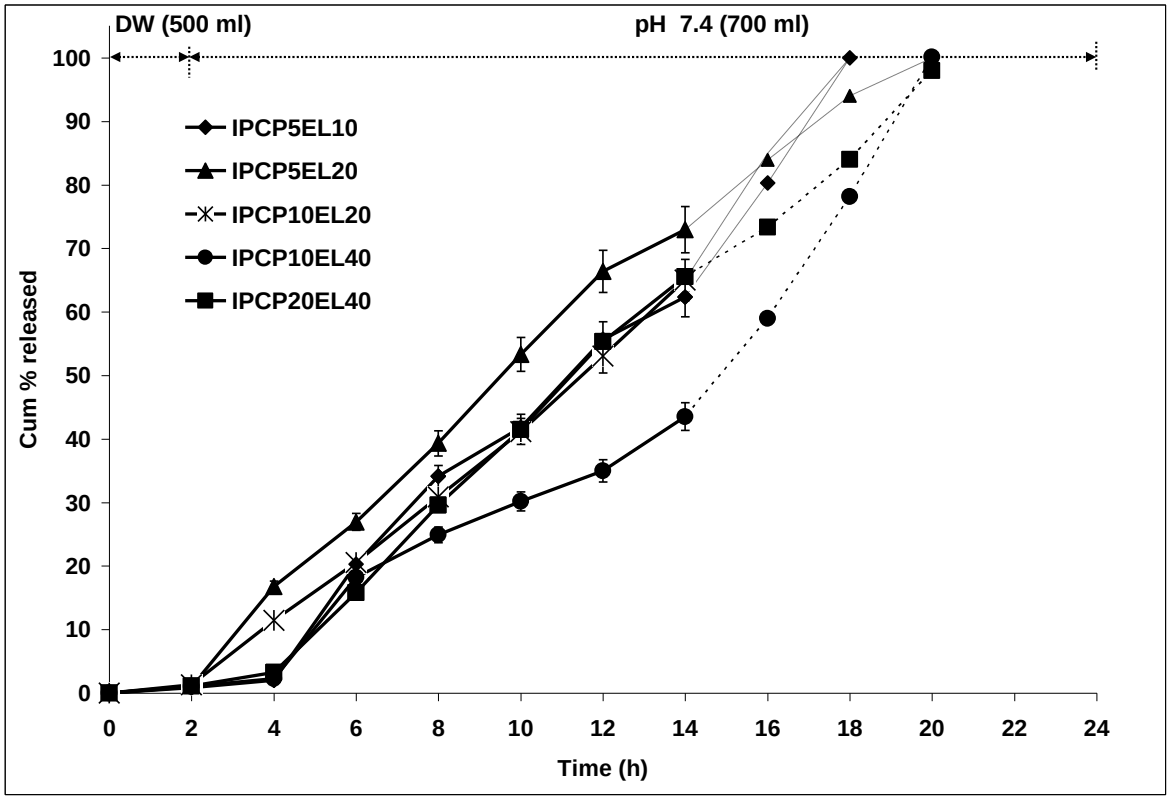


Fig 5.28: Release profile of indomethacin from PCP based matrix tablets showing effect of varying proportion of EL100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.24: Release kinetics characterization of drug release from PCP and EL100 based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IPCP5EL10	0.9448	5.96×10^{-2}	2.885	1.31	2.9	14.7
IPCP5EL20	0.9500	6.14×10^{-2}	0.559	1.90	3.2	15.7
IPCP10EL20	0.9763	1.49×10^{-2}	0.567	1.81	3.8	16.7
IPCP10EL40	0.9554	6.13×10^{-2}	0.700	1.70	3.9	17.4
IPCP20EL40	0.9826	6.26×10^{-3}	1.264	1.56	3.1	15.4

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

The presence of EL100 in matrix could have inhibited initial matrix swelling that was previously observed for indomethacin matrix bases prepared using PCP alone and this explains the retardation in initial release while gradual dissolution of EL100 in alkaline pH accompanied with swelling of PCP polymer enhanced drug release post 2 h in pH 7.4. The high 'n' values for all these formulations indicate super case II release and further support this theory suggesting polymer relaxation due to PCP and erosion due to EL100 as the mechanism of release (Durig et al, 1999). It was concluded that all

formulations based on combination of PCP and EL100 with low initial drug release and complete release within the desired time- frame of 14-16 h have the potential for site specific delivery to colon.

(ii) Eudragit L100 + Carbopol

A plot of cumulative percentage drug released versus time for matrix tablets of indomethacin prepared using carbopol with varying proportion of EL100 is shown in Fig 5.29. It was found that on increasing the relative proportion of EL100 from 10% to 20% w/w of drug, there was proportionate retardation in the initial release rate as indicated by the $t_{10\%}$ values (ICP5EL10: 2.8 h and ICP5EL20: 4.8 h). The duration of release (in terms of 90% drug release) was similarly extended from 13.9 h for ICP5EL10 to 20.8 h for ICP5EL20. Similarly, when EL100 was varied from 20% (ICP10EL20) to 40% (ICP10EL40) in 10% CP matrix, a relatively greater retardation in drug release was observed which resulted in corresponding lower drug release rates giving higher $t_{10\%}$ (3.1 h for ICP10EL20 and 6.5 h for ICP10EL40) and $t_{90\%}$ values (14.1 h for ICP10EL20 and 22.4 h for ICP10EL40) (Table 5.25). Thus, presence of EL100 had a pronounced effect on CP matrix with greater retardation in initial release and overall release when compared to corresponding CP matrix alone or EL100 + PCP matrices. When the CP level was doubled from 10% (ICP10EL40) to 20% (ICP20EL40) in 40% EL100 matrix, there was slight increase in drug release rate as is evident from the $t_{10\%}$ (5.2 h) and $t_{90\%}$ (18.8 h) for ICP20EL40. This could be due to increase in relative proportion of hydrophilic polymer. Between ICP10EL20 and ICP10EL40, the increase in relative proportion of EL100 decreased the release rates resulting in higher values for $t_{10\%}$ and $t_{90\%}$ (Table 5.25). The combination of EL100 and CP probably resulted in the formation of matrix of high gel strength in a similar way to that observed for EC + CP matrices with very low porosity and high tortousity that resulted in very slow drug release rates from these matrices. Amongst the various formulations studied, ICP5EL10, ICP10EL20 and ICP20EL40 can serve as potential colon specific drug delivery systems.

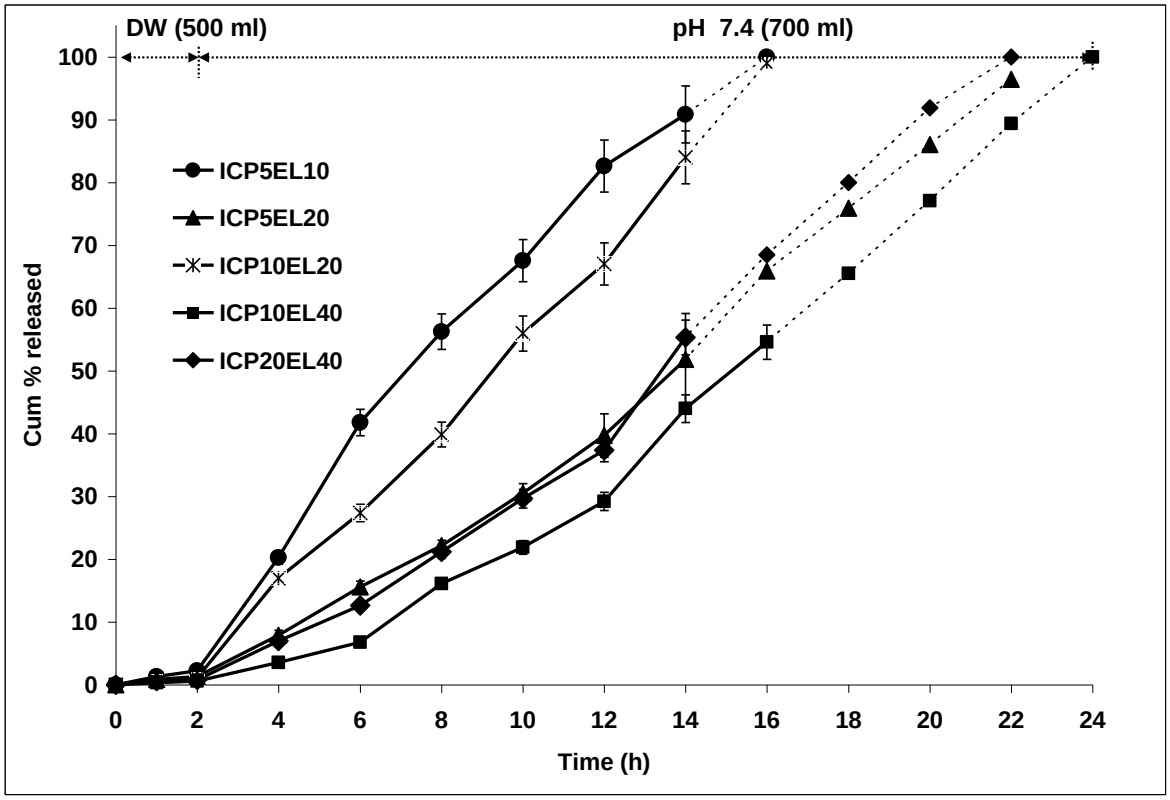


Fig 5.29: Release profile of indomethacin from CP based matrix tablets showing effect of varying proportion of EL100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.25: Release kinetics characterization of drug release from CP and EL100 based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
ICP5EL10	0.9596	5.56×10^{-2}	3.825	1.20	2.8	13.9
ICP5EL20	0.9889	3.50×10^{-3}	0.382	1.80	4.8	20.8
ICP10EL20	0.9528	2.19×10^{-2}	3.760	1.20	3.1	14.1
ICP10EL40	0.9919	8.09×10^{-3}	0.277	1.86	6.5	22.4
ICP20EL40	0.9848	5.88×10^{-3}	0.341	1.90	5.2	18.8

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

(iii) Eudragit L100 + Xanthan Gum

For the matrix tablets prepared using xanthan gum at 5% w/w of drug with varying proportions of Eudragit L100 (5, 10, 20 and 40% w/w of drug), the in vitro drug release profiles are shown in Fig 5.30. The K values for the formulations showed a corresponding decrease from 7.327 $h^{-1.08}$ for IXG5EL5 to 1.402 $h^{-1.27}$ for IXG5EL40. The initial percentage of drug release from all the formulations in the first 2 h was almost negligible (less than 5%) in distilled water followed by an linear increase in release rate post 2 h in pH

7.4 that depended on the proportion of EL100. The release kinetics data for the various formulations reveal $t_{10\%}$ ranging from 2.9 h for IXG5EL5 to 5.2 h for IXG5EL40 implying significant inhibition in the initial drug release (Table 5.26). It was observed that post 2 h, the release of drug from the formulations was extended from more than 10.2 h for IXG5EL5 to more than 26.5 h for IXG5EL40 indicating extension in duration of release with corresponding increase in relative proportion of EL100. The drug release from these formulations was observed to depend on the relative proportion of EL100 and not on xanthan gum. Thus, regulating the amount of EL100 in 5% xanthan gum matrix base could confer desired retardation in initial phase followed by controlled release ranging from 12 to 28 h.

With increase in the relative proportion of EL100 from 10% (IXG5EL10) to 20% (IXG5EL20), a proportionate retardation was observed in the corresponding initial release rates resulting in enhanced $t_{10\%}$ values from 3.6 h for IXG5EL10 to 4.2 h for IXG5EL20. The drug release duration was similarly extended from beyond 13.6 h for IXG5EL10 to 17.7 h for IXG5EL20. This was attributed to increase in total polymer content that resulted in the formation of a relatively strong matrix with decreased porosity and increased tortousity.

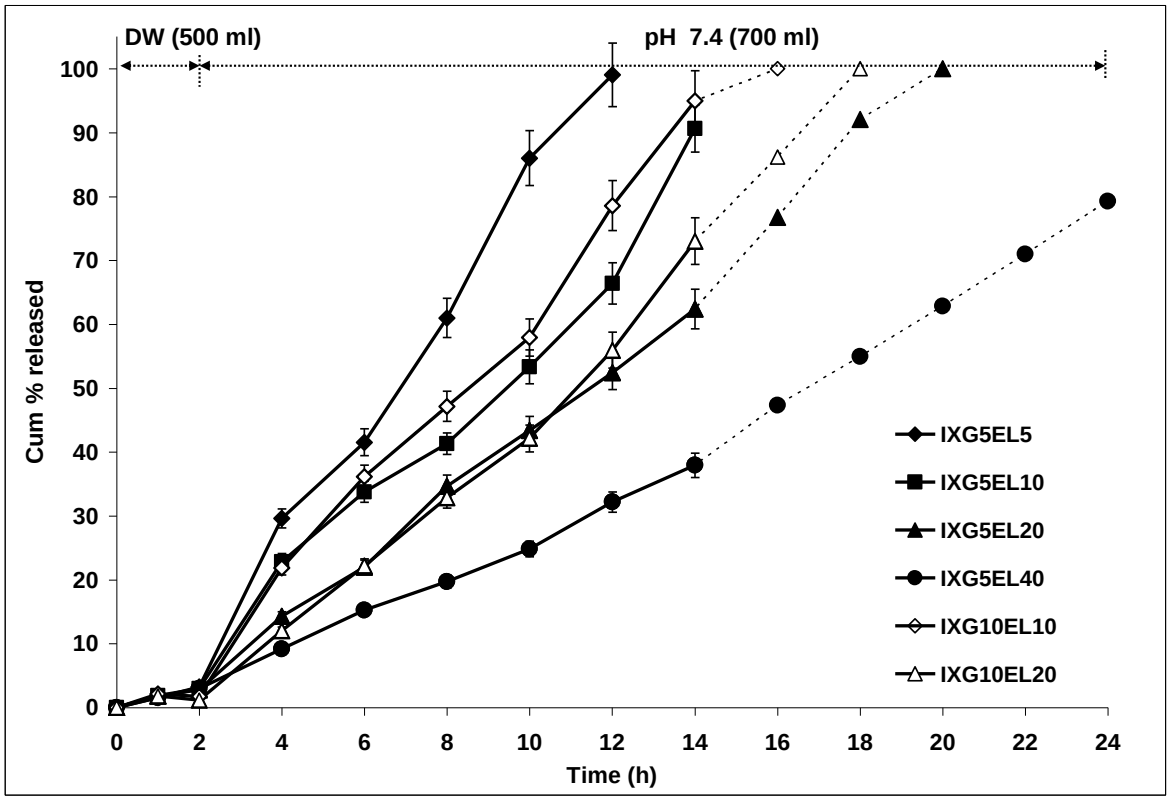


Fig 5.30: Release profile of indomethacin from XG based matrix tablets showing effect of varying proportion of EL100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Further, when the relative proportion of EL100 was increased from 10% (IXG10EL10) to 20% (IXG10EL20), a similar effect on the initial drug release rate ($t_{10\%}$ varied from 3.7 h for IXG10EL10 to 4.6 h for IXG10EL20) was observed. The drug release duration was extended from beyond 13.3 h for IXG10EL10 to 16.2 h for IXG10EL20. On the other hand, when the proportion of EL100 was kept constant at 1% w/w of drug and relative proportion of XG varied from 5% (IXG5EL10) to 10% (IXG10EL10) the release kinetics were insignificantly altered. Similar results were observed when XG proportion was increased from 5% to 10% in 20% EL100 matrices.

The presence of pH based polymer EL100 was able to control the initial rapid swelling of xanthan gum based matrices and thereby prevent the high percentage of drug release which was previously observed for formulations prepared with xanthan gum alone (45% release for IXG5 in 2 h).

Table 5.26: Release kinetics characterization of drug release from XG and EL100 based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IXG5EL5	0.9942	3.24×10^{-4}	7.327	1.08	2.9	10.2
IXG5EL10	0.9827	3.42×10^{-3}	1.346	1.61	3.6	13.6
IXG5EL20	0.9926	2.67×10^{-4}	1.047	1.55	4.2	17.7
IXG5EL40	0.9852	1.05×10^{-3}	1.402	1.27	5.2	26.5
IXG10EL10	0.9876	2.13×10^{-3}	5.945	1.05	3.7	13.3
IXG10EL20	0.9967	7.22×10^{-4}	2.409	1.30	4.6	16.2

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

Further, as discussed in previous sections, EL100 in combination with XG in a polymeric base could impart a pH responsive drug release character. With increase in the pH of dissolution medium to 7.4, an increase in the drug release rate was observed on account of matrix erosion due to dissolution of EL100 at alkaline pH > 7.0. The formation of a porous matrix then facilitated enhanced diffusion of the drug through the pores. This was further confirmed by the values of 'n' for XG + EL100 matrices that ranged from 1.05 to 1.61 indicating release mechanism to be super case II type due to increase in matrix erosion along with swelling of xanthan gum post 2 h in pH 7.4 medium. With the exception of IXG5EL40, all other formulations demonstrated pH and transit time dependent sigmoidal drug release characteristics suitable for colonic delivery (Fig 5.30).

5.6.4.3.2. Effect of Eudragit L100 on indomethacin release from hydrophilic non ionic polymer matrix bases

(i) Eudragit L100 + Hydroxy ethyl cellulose

The in vitro release profiles from matrix bases composed of HEC with varying proportions of EL100 are shown in Fig 5.31. It was observed that presence of EL100 could significantly decrease the initial drug release from all the formulations with negligible release in the first 2 h. The release kinetics were slightly altered as observed from $t_{10\%}$ of 2.4 h for IHEC5EL10 and 2.5 h for IHEC5EL20, followed with controlled release extending upto 9-11 h ($t_{90\%}$ of 9.6 h) for IHEC5EL10 and 10-12 h ($t_{90\%}$ of 10.8 h) for IHEC5EL20 (Table 5.27). Although HEC is a non-ionic polymer, yet a significant pH dependent effect was observed in the release which depended on the relative proportion of EL100 in the matrix. In a 20% EL100 matrix, increase in the proportion of HEC from 5% (IHEC5EL20) to 10% w/w of drug (IHEC10EL20) resulted in minor change in dissolution profiles, implying little influence of change of HEC proportion. With K values of 1.213 $h^{-1.81}$ for IHEC5EL20 and 1.246 $h^{-1.91}$ for IHEC10EL20, the $t_{90\%}$ was 10.8 h and 9.4 h respectively.

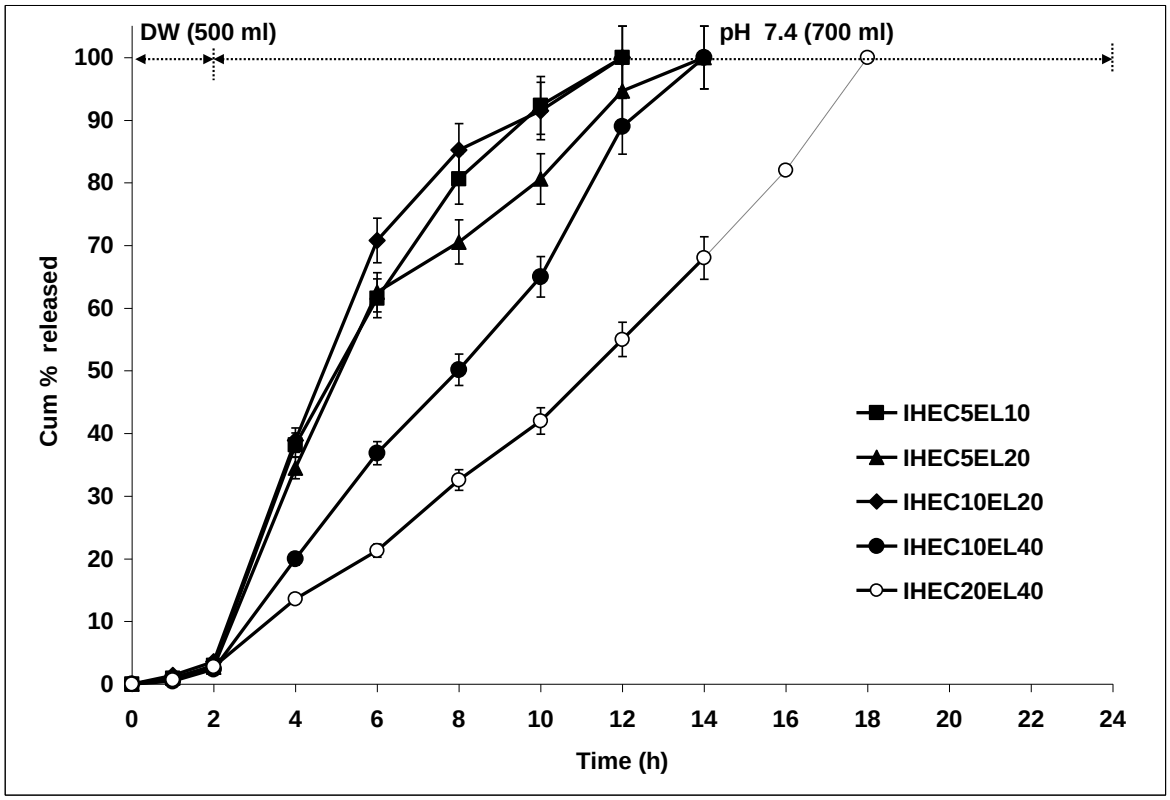


Fig 5.31: Release profile of indomethacin from HEC based matrix tablets showing effect of varying proportion of EL100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

When the relative proportion of EL100 was increased from 20% to 40% in 10% HEC matrix, there was proportionate decrease in the initial release rate shown by the increase in $t_{10\%}$ value of 2.6 h for IHEC10EL20 to 3.2 h for IHEC10EL40 (Table 5.27). The drug release duration in terms of $t_{90\%}$ was similarly extended from 9.4 h (IHEC10EL20) to 11.7 h (IHEC10EL40). When the HEC level was doubled from 10% (IHEC10EL40) to 20% (IHEC20EL40) in 40% EL100 matrix, the release rate was further retarded as indicated by $t_{10\%}$ of 3.7 h and $t_{90\%}$ of 17.1 h for the latter formulation. This could be on account of increase in total polymer content that resulted in decrease in matrix porosity, thus impeding penetration of dissolution medium into the pores of the matrix.

Therefore, it can be concluded that regulating amount of EL100 in matrix can confer the desired delay in the initial drug release period from HEC matrix based formulation. Except for IHEC20EL40 with $t_{90\%}$ of 17.1 h, other formulations had $t_{90\%}$ ranging from 9.4 h to 11.7 h, implying relatively rapid dissolution of Eudragit L100 in pH 7.4 from 5% and 10% HEC matrix that enhanced the drug release rates in alkaline pH.

Table 5.27: Release kinetics characterization of drug release from HEC and EL100 based matrix tablets

Batches	Release kinetics					
	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]
IHEC5EL10	0.9454	5.37×10^{-2}	0.999	1.99	2.4	9.6
IHEC5EL20	0.9297	5.76×10^{-2}	1.213	1.81	2.5	10.8
IHEC10EL20	0.9441	5.50×10^{-2}	1.246	1.91	2.6	9.4
IHEC10EL40	0.9974	4.32×10^{-4}	7.692	1.00	3.2	11.7
IHEC20EL40	0.9989	1.88×10^{-4}	4.566	1.05	3.7	17.1

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

In case of IHEC20EL40, however, relatively higher percentage of HEC (20% w/w of drug) was responsible for lower rate of matrix erosion and therefore, slower drug release rate in alkaline medium. In this series, all the formulations showed a sigmoidal release profile with $t_{10\%}$ ranging from 2.4 to 3.7 h and $t_{90\%}$ ranging from 9.4 to 17.1 h, nearly approaching the theoretical target drug release profile in most cases.

For non-ionic hydrophilic polymeric matrix systems like HEC and HPC, the drug release is controlled by the rate of matrix swelling and drug diffusion through the gel layer (Bain et al., 1991). Although the release mechanism was expected to be more of a Fickian or diffusion based nature, yet values of diffusional exponent ‘n’ for this series of formulations range from 1.0 to 1.9 indicating the release mechanism to be of case II type

(zero-order, n = 1) or super case II type (n >1.0) which may be attributed to several mechanisms operating simultaneously like diffusion, matrix swelling and erosion process due to the dissolution of Eudragit L100 in matrix (Bettini et al., 1995).

(ii) Eudragit L100 + Hydroxy propyl cellulose

As reported in previous sections, a pH and transit time dependent sigmoidal release profile was observed in case of indomethacin formulations prepared using HPC in combination with EL100 when compared to drug release from HPC based matrices only. A plot of cumulative percentage released versus time for matrix tablets of indomethacin prepared using HPC with varying proportion of EL100 is shown in Fig 5.32. Increase in the relative proportion of EL100 from 10% (IHPC5EL10) to 20% (IHPC5EL20) in 5% HPC matrix, resulted in retardation in the initial release with $t_{10\%}$ values obtained as 2.5 h for IHPC5EL10 and 2.8 h for IHPC5EL20 (Table 5.28). But the duration of release (in terms of $t_{90\%}$) was decreased from 16.2 h for IHPC5EL10 to 14.5 h for IHPC5EL20 which could be due to more rapid erosion of matrix due to dissolution of EL100. When HPC was increased from 5% (IHPC5EL20) to 10% (IHPC10EL20) in 20% EL100 matrix, the release rate decreased further to give higher $t_{10\%}$ (3.2 h) and $t_{90\%}$ (14.5 h) for IHPC10EL20. Similarly, when EL100 was varied from 20% (IHPC10EL20) to 40% (IHPC10EL40) in 10% HPC matrix, slightly greater retardation in drug release was observed which resulted in corresponding lower initial release resulting in higher $t_{10\%}$ (3.2 h for IHPC10EL20 and 3.4 h for IHPC10EL40). The $t_{90\%}$ values did not vary much for the two formulations (14.5 h for IHPC10EL20 and 14.2 h for IHPC10EL40) (Table 5.28). Further, when the HPC level was doubled from 10% (IHPC10EL40) to 20% HPC (IHPC20EL40) in 40% EL100 matrix, there was a slight change in the release profile as is evident from the $t_{10\%}$ and $t_{90\%}$ value of 3.5 h and 14.9 h for IHPC20EL40 (Fig 5.32). As previously stated, this could be due to increase in total polymer content and decrease in matrix porosity.

Hence, it can be concluded that desired $t_{10\%}$ can be achieved by employing suitable proportion of EL100 in HPC matrix base. Both HPC and EL100 in combination were found to retard drug release rate in the initial phase ($t_{10\%}$ ranging from 2.5 h to 3.5 h), while release in the later stages depended largely on Eudragit L100 that dissolves at pH 6.0 and enhanced drug release in alkaline medium due to rapid matrix erosion. Effect of EL100 proportion in HPC matrix was more pronounced at low HPC proportions. Drug release was complete within 14-16 h for most of the formulations, thereby nearly approaching the theoretical target release profile.

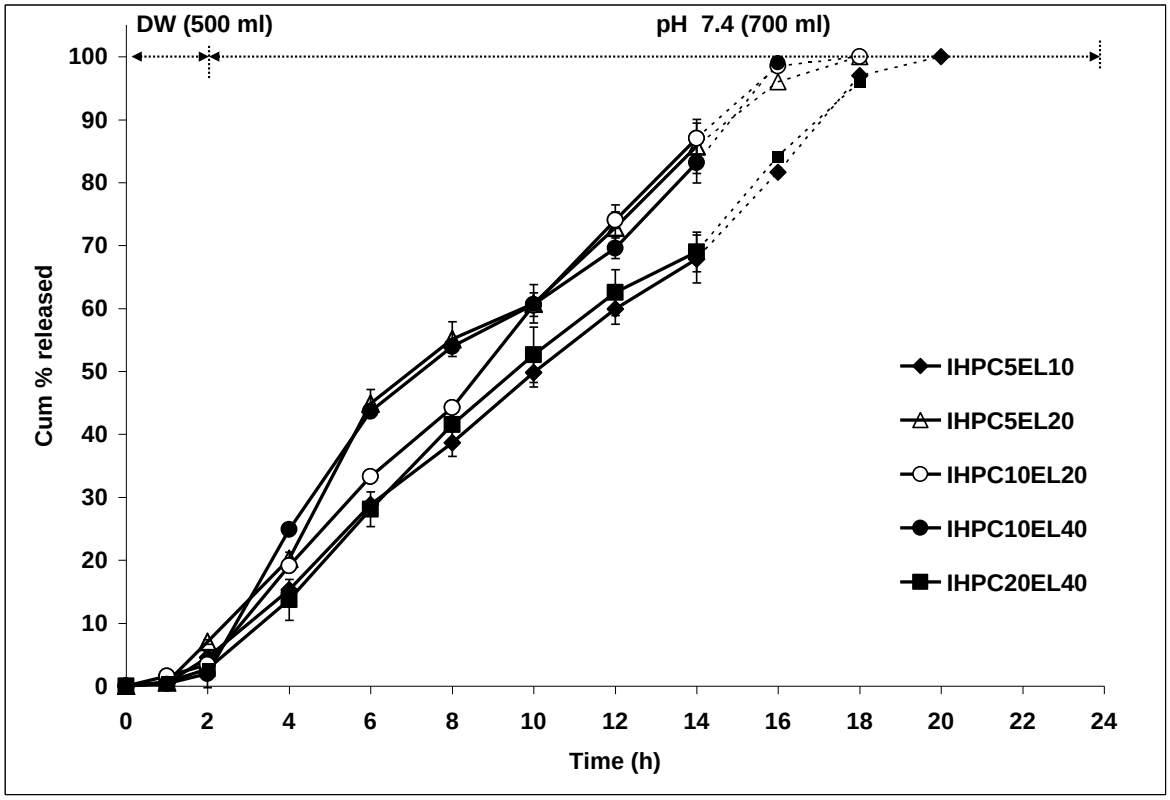


Fig 5.32: Release profile of indomethacin from HPC based matrix tablets showing effect of varying proportion of EL100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.28: Release kinetics characterization of drug release from HPC and EL100 based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IHPC5EL10	0.9942	8.24×10^{-4}	6.385	0.95	2.5	16.2
IHPC5EL20	0.9827	6.47×10^{-3}	3.015	1.27	2.8	14.5
IHPC10EL20	0.9926	1.67×10^{-4}	8.109	0.90	3.2	14.5
IHPC10EL40	0.9852	1.25×10^{-3}	12.973	0.73	3.4	14.2
IHPC20EL40	0.9876	2.14×10^{-3}	5.277	1.05	3.5	14.9

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

(iii) Eudragit L100 + Guar gum

For guar gum matrices, the formulations containing 5% guar gum with EL100 in varying proportions, (5, 10, 20 and 40% w/w of drug), the in vitro drug release profiles are shown in Fig 5.33. It was found that on increasing the relative proportion of EL100 from 5% to 40% w/w of drug, there was significant retardation in the initial release rate (2-7% release in 2 h for all formulations) [as compared to 5% guar gum matrix (IGG5)] followed by increase in release rate post 2 h in phosphate buffer media. The $t_{10\%}$ extended from 2.2 h for

IGG5EL5 to 3.2 h for IGG5EL40 (Table 5.29). The duration of drug release post 2 h was extended from beyond 13.5 h for IGG5EL5 to beyond 14.1 h for IGG5EL40. The release kinetics calculated for these formulations were significantly different when compared to the matrix bases containing guar gum alone (Fig 5.33).

Increasing the relative proportion of EL100 from 10 to 20% in formulations prepared using 5% guar gum (IGG5EL10 and IGG5EL20) did not result in a significant change in drug release kinetics. There was no appreciable difference between the two formulations (IGG5EL10 and IGG5EL20) with respect to the drug release rates and dissolution profiles were almost similar for the two.

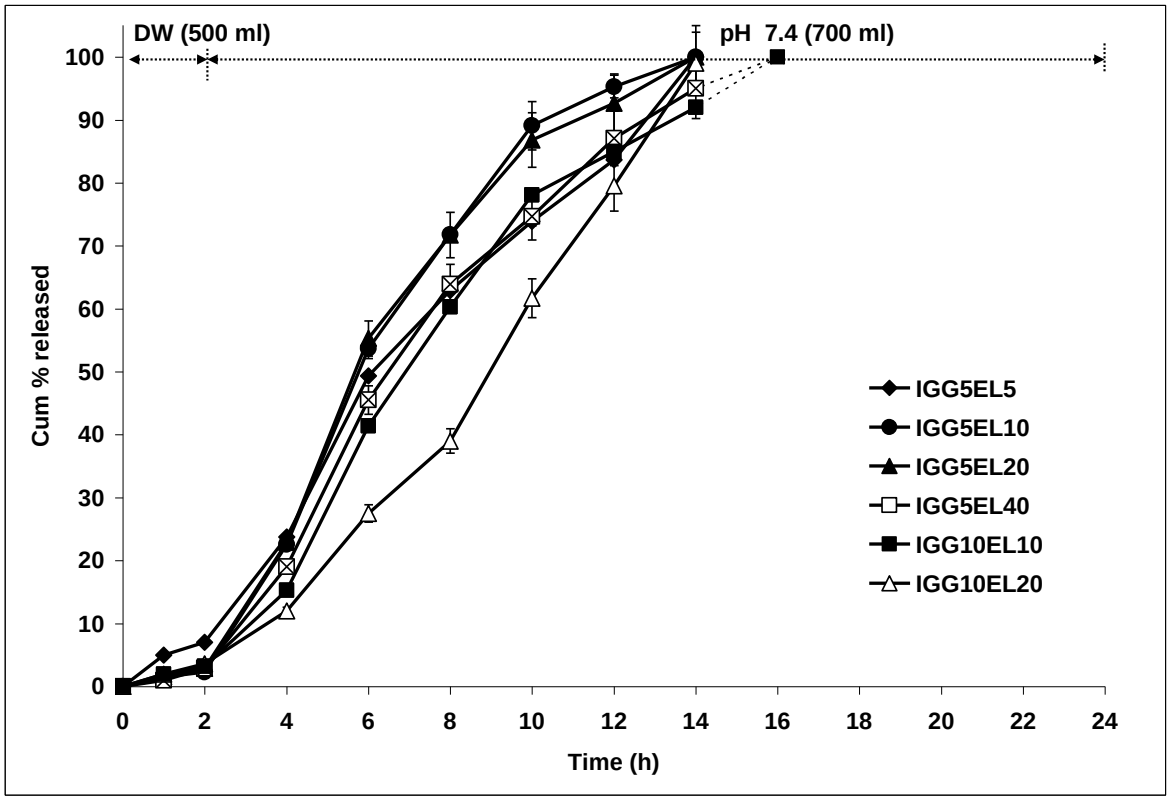


Fig 5.33: Release profile of indomethacin from GG based matrix tablets showing effect of varying proportion of EL100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

However, a statistically significant difference was obtained between the dissolution profiles of IGG10EL10 and IGG10EL20 and drug release was extended from beyond 11.4 h for IGG10EL10 to beyond 13.9 h for IGG10EL20. This may be due to the presence of relatively higher proportion of EL100 in case of IGG10EL20. With increase in relative proportion of guar gum from 5% (IGG5EL10) to 10% (IGG10EL10) at 10% EL100 in the matrix, drug release was extended from beyond 10.3 h to beyond 11.4 h (Table 5.29). Similarly, in the case of IGG5EL20 and IGG10EL20, the release kinetics were almost similar with respect to the initial release for the two formulations while the duration release

was slightly more extended in case of IGG10EL20 ($t_{90\%}$ of 13.9 h). Thus, increase in percentage of guar gum did not affect the release rates of the formulations significantly. This may be due to decreased swelling of guar gum in the presence of EL100.

In case of all these formulations, the drug release mechanism was inferred as being predominantly erosion based (n>1.0; super case II) due to the presence of EL100 (Table 5.29). It has been reported earlier that hydration of guar gum is independent of the pH of the medium (Krishnaiah et al., 1998). From the present study, it could be inferred that presence of EL100 in the guar gum matrix base could effectively control the initial swelling and impart pH dependent drug release kinetics. Further, the dissolution of Eudragit L100 in alkaline medium was responsible for matrix erosion which enhanced the drug release rate during the later phase of drug release.

Table 5.29: Release kinetics characterization of drug release from GG and EL100 based matrix tablets

Batches	Release kinetics					
	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]
IGG5EL5	0.9927	2.04×10^{-4}	3.760	1.22	2.2	13.5
IGG5EL10	0.9759	2.12×10^{-3}	1.149	1.87	3.2	10.3
IGG5EL20	0.9838	2.87×10^{-4}	1.494	1.75	3.0	10.4
IGG5EL40	0.9885	1.25×10^{-4}	0.901	1.74	3.2	14.1
IGG10EL10	0.9863	2.13×10^{-3}	1.336	1.73	3.5	11.4
IGG10EL20	0.9947	3.54×10^{-4}	1.370	1.59	3.2	13.9

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

5.6.7. Effect of Eudragit S100 in combination with other polymers on indomethacin release

The effect of Eudragit S100 in combination with different ionic and non-ionic polymers on drug release was also investigated. The polymers selected were same as described in previous section for studying the effect of EL100. The purpose of the investigation was to find out if higher pH threshold solubility for Eudragit S100 is capable of conferring better retardation in initial release as compared to corresponding EL100 matrices and at the same time completing drug release in the stipulated time frame of 14-16 h.

5.6.7.1. Effect of Eudragit S100 on indomethacin release from hydrophilic ionic polymer

(i) Eudragit S100 + Polycarbophil

The effect of incorporating ES100 in varying proportions in a PCP matrix is shown in Fig 5.34. Although there was significant decrease in the initial release rate when compared to pure PCP based formulations, the overall release rates were relatively higher than the corresponding PCP + EL100 matrices. For the matrix containing 5% PCP with the relative proportion of ES100 increased from 10% (IPCP5ES10), to 20% (IPCP5ES20), the duration of release (in terms of $t_{90\%}$) was reduced from 12.0 h for IPCP5ES10 to 8.8 h for IPCP5ES20 (Table 5.30). However, in the case of IPCP10ES20, there was excellent retardation in the initial release phase ($t_{10\%}$ of 6.1 h) and complete release was observed to occur within 14 h ($t_{90\%}$ of 13.2 h) indicating good potential of the formulation to be used as colon specific drug delivery system (Fig 5.34).

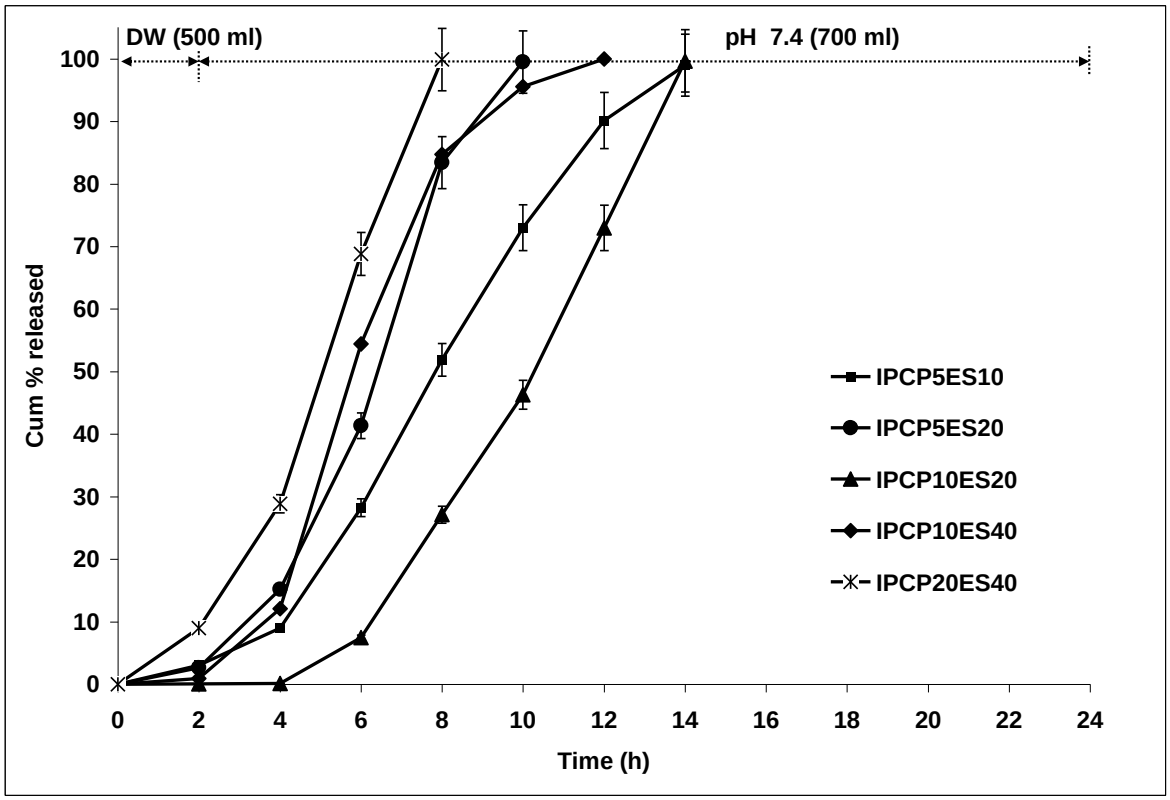


Fig 5.34: Release profile of indomethacin from PCP based matrix tablets showing effect of varying proportion of ES100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

In matrix containing 10% PCP, when the relative proportion of ES100 was increased from 20% (IPCP10ES20) to 40% (IPCP10ES40), the release rates increased to give relatively lower $t_{10\%}$ (3.8 h) and $t_{90\%}$ (8.9 h) values for IPCP10ES40. Similarly,

increase in PCP proportion from 10% (IPCP10ES40) to 20% (IPCP20ES40) in 40% ES100 matrix, the release rates were found to be significantly increased [$t_{10\%}$ (2.1 h) and $t_{90\%}$ (7.5 h) for IPCP20ES40].

In this series of formulations (PCP + ES100 matrices), the drug release rate was found to increase when the polymer proportion was increased due to increase in matrix porosity. The increase in total polymer content with respect to a hydrophobic drug could have caused decreased hydrophobicity of the matrix, which explains for enhanced swelling of the matrix. At alkaline pH (7.4), with increase in the proportion of ES100, matrix swelling and erosion also increased, facilitating rapid drug release (Table 5.30). Amongst the different formulations investigated, IPCP5ES10 and IPCP10ES20 showed a significantly sigmoidal release profile suitable for colonic delivery.

Table 5.30: Release kinetics characterization of drug release from PCP and ES100 based matrix tablets

Batches	Release kinetics					
	r[a]	**MSSR**	**K[b]**	**n[c]**	**$t_{10\%}$[d]**	**$t_{90\%}$[e]**
IPCP5ES10	0.9916	2.25×10^{-3}	0.782	1.91	4.1	12.0
IPCP5ES20	0.9710	7.32×10^{-3}	1.324	1.94	3.3	8.8
IPCP10ES20	0.9638	6.68×10^{-2}	1.210	1.67	6.1	13.2
IPCP10ES40	0.9069	5.90×10^{-2}	9.47	1.03	3.8	8.9
IPCP20ES40	0.9517	3.57×10^{-2}	6.825	1.28	2.1	7.5

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

(ii) Eudragit S100 + Carbopol

In the formulations containing 5% CP with ES100 varied from 10% to 20% w/w of drug, it was found that on increasing the relative proportion of ES100, the rate of drug release was increased with the $t_{10\%}$ values decreasing from 2.6 h for ICP5ES10 to 2.2 h for ICP5ES20 followed by controlled release for 7-8 h (Fig 5.35). Similarly, when the relative proportion of ES100 varied from 20% (ICP10ES20) to 40% (ICP10ES40) in 10% CP matrix, it resulted in higher release rates giving lower $t_{10\%}$ (1.2 h for ICP10ES20 and 1.1 h for ICP10ES40) and $t_{90\%}$ values (6.0 h for ICP10ES20 and 4.1 h for ICP10ES40) (Table 5.31). This observation was similar to the one in which ES100 was incorporated in PCP matrices and can be attributed to the nature of matrix formed between ES100 and CP. The enhanced matrix porosity of ES100 based matrices resulted in rapid erosion of the matrix post 2 h.

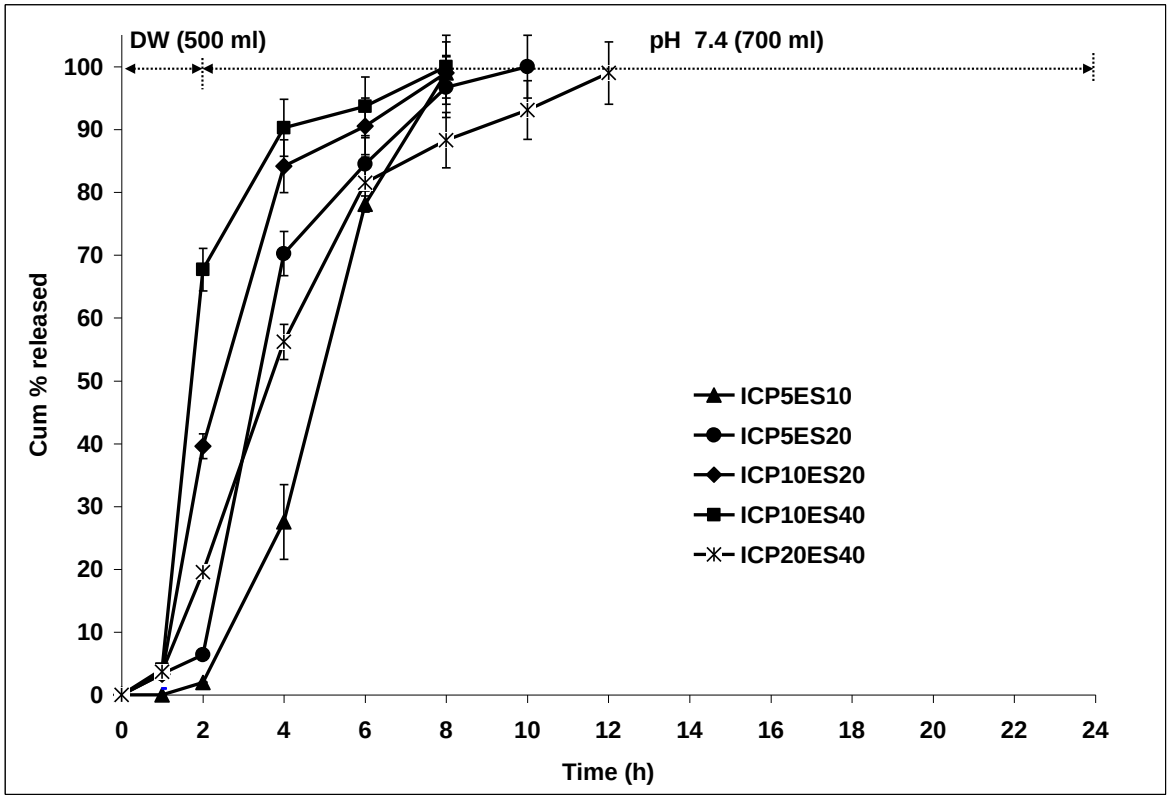


Fig 5.35: Release profile of indomethacin from CP based matrix tablets showing effect of varying proportion of ES100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.31: Release kinetics characterization of drug release from CP and ES100 based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
ICP5ES10	0.9721	7.64×10^{-3}	2.303	1.87	2.6	7.1
ICP5ES20	0.9277	5.28×10^{-2}	1.909	1.98	2.2	7.0
ICP10ES20	0.9873	5.88×10^{-3}	5.028	1.61	1.2	6.0
ICP10ES40	0.9372	5.85×10^{-2}	11.798	1.44	1.1	4.1
ICP20ES40	0.9483	6.18×10^{-2}	2.680	1.67	1.5	8.2

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

Alternately, when the CP level was doubled from 10% (ICP10ES40) to 20% (ICP20ES40) in matrix containing 40% ES100, there was corresponding decrease in the rate of drug release from 11.798 $h^{-1.44}$ to 2.680 $h^{-1.67}$ with the $t_{10\%}$ and $t_{90\%}$ values in case of ICP20ES40 increasing to 1.5 h and 8.2 h respectively (Table 5.31). The probable reason for this could be the ability of carbopol to swell in alkaline pH resulting in a gel barrier to diffusion of drug. An overall decrease in $t_{10\%}$ and $t_{90\%}$ was observed with increase in relative proportion of ES100 in matrix (Fig 5.35). All the ES100 + CP based formulations

showed rapid release in alkaline pH and would be suitable only for patients with lower GI and colonic transit times.

(iii) Eudragit S100 + Xanthan Gum

A plot of cumulative percentage released versus time for matrix tablets of indomethacin prepared using xanthan gum at 5% w/w of drug with varying proportion of ES100 (5, 10, 20 and 40% w/w of drug) is shown in Fig 5.36. It was observed that on increasing the relative proportion of ES100 from 5% to 40% w/w of drug, there was proportionately greater retardation in the initial release rate as indicated by the $t_{10\%}$ (ranging from 2.1 h for IXG5ES5 to 6.0 h for IXG5ES40). The values for the release rate constant K for the formulations showed a decrease from 12.159 $h^{-0.79}$ for IXG5ES5 to 0.938 $h^{-1.26}$ for IXG5ES40. Similarly, drug release was extended from beyond 12.6 h for IXG5ES5 to beyond 37.4 h for IXG5ES40 (Table 5.32). The release kinetics calculated for these formulations were significantly different when compared to the matrix base IXG5 (containing xanthan gum alone). The $t_{10\%}$ and $t_{90\%}$ values were found to be higher in the case of ES100 based xanthan gum matrices when compared to EL100 probably due to the difference in threshold pH solubility (pH 6.0 for EL100, 7.0 for ES100) of the two polymers.

For xanthan gum - EL100 formulations, the drug release rate was found to depend on the relative proportion of ES100 as shown by the increase in $t_{10\%}$ and $t_{90\%}$ values with increase in proportion of ES100 (Table 5.32). Although good retardation in the initial release phase was observed, there was considerable deviation from the theoretical target of 80-90% release in 14-16 h at higher ES100 proportion. When the relative proportion of xanthan gum in the matrix was increased from 5% (IXG5ES10) to 10% of drug (IXG10ES10), a relatively greater swelling of the matrix might have occurred that resulted in significantly high release rates (Fig 5.36). The significantly lowered $t_{10\%}$ (1.0 h) and $t_{90\%}$ (9.2 h) values for IXG10ES10 are indicative of this. Similar release kinetics were observed for IXG10ES20 ($t_{10\%}$ of 2.0 h and $t_{90\%}$ of 9.1 h) which were significantly different from IXG5ES20.

The values of n for XG + ES100 series ranged from 0.40 to 1.26 indicating that with increase in total polymer content of the matrix release mechanism shifted from Fickian diffusion to anomalous (matrix swelling and diffusion) to super case II (erosion type), implying that drug release occurred by a combination of several processes like diffusion, swelling of hydrophilic component (polymer relaxation) and erosion of matrix (due to dissolution of Eudragit S100) in alkaline media.

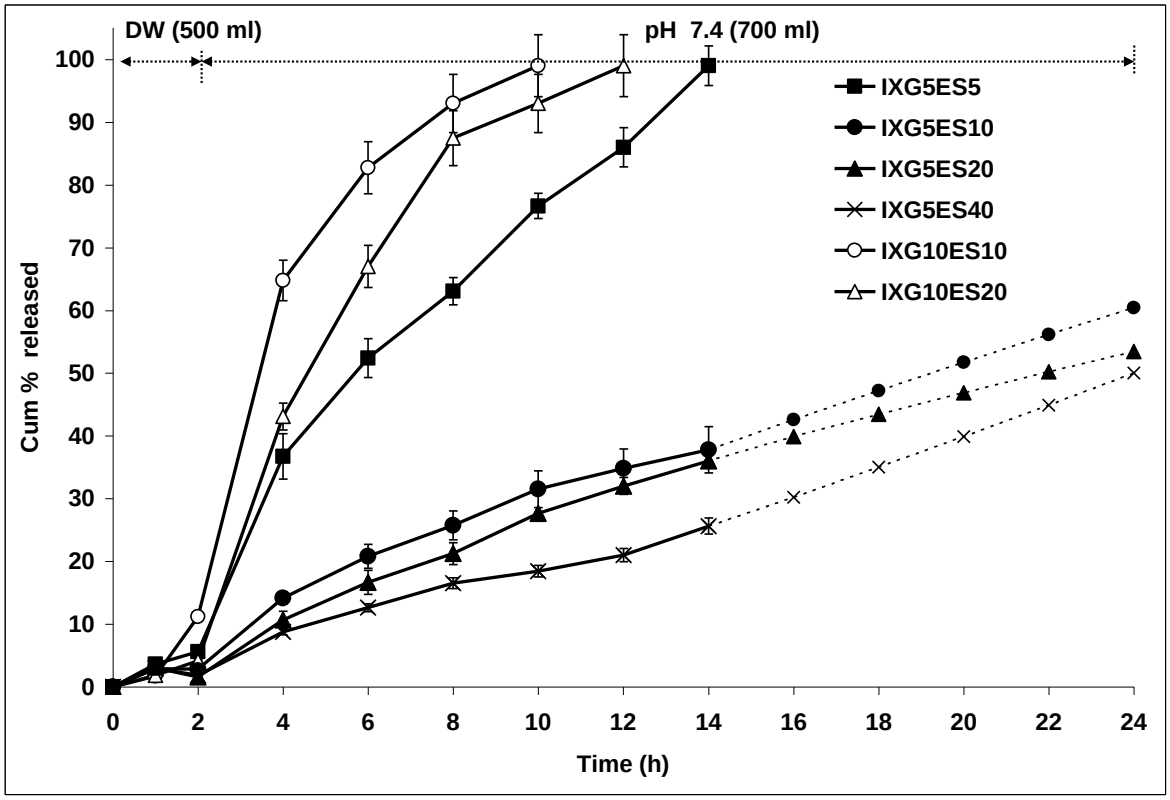


Fig 5.36: Release profile of indomethacin from XG based matrix tablets showing effect of varying proportion of ES100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Table 5.32: Release kinetics characterization of drug release from XG and ES100 based matrix tablets

Batches	Release kinetics					
	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]
IXG5ES5	0.9950	9.45×10^{-4}	12.159	0.79	2.1	12.6
IXG5ES10	0.9979	3.57×10^{-4}	5.417	0.78	4.1	36.7
IXG5ES20	0.9822	1.30×10^{-2}	4.950	0.79	5.2	39.3
IXG5ES40	0.9758	3.53×10^{-3}	0.938	1.26	6.0	37.4
IXG10ES10	0.9234	1.73×10^{-2}	6.276	0.40	1.0	9.2
IXG10ES20	0.9425	1.63×10^{-2}	6.358	0.86	2.0	9.1

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

Therefore, it was concluded that IXG5ES5 and matrices with 10% xanthan gum (IXG10ES10 and IXG10ES20) with varying proportions of ES100 demonstrated desirable release kinetics in vitro and indicate good potential for site specific controlled drug delivery to the colon.

5.6.7.2. Effect of Eudragit S100 on indomethacin release from hydrophilic non ionic polymer

(i) Eudragit S100 + Hydroxy ethyl cellulose

The effect of incorporating ES100 in varying proportions in a HEC matrix is shown in Fig 5.37. Drug release was found to depend on the relative proportion of both HEC and ES100. For the matrix containing 5% HEC, with the relative proportion of ES100 increased from 10% (IHEC5ES10) to 20% (IHEC5ES20), the initial drug release rates were quite high with $t_{10\%}$ of 0.8 h and 1.7 h respectively.

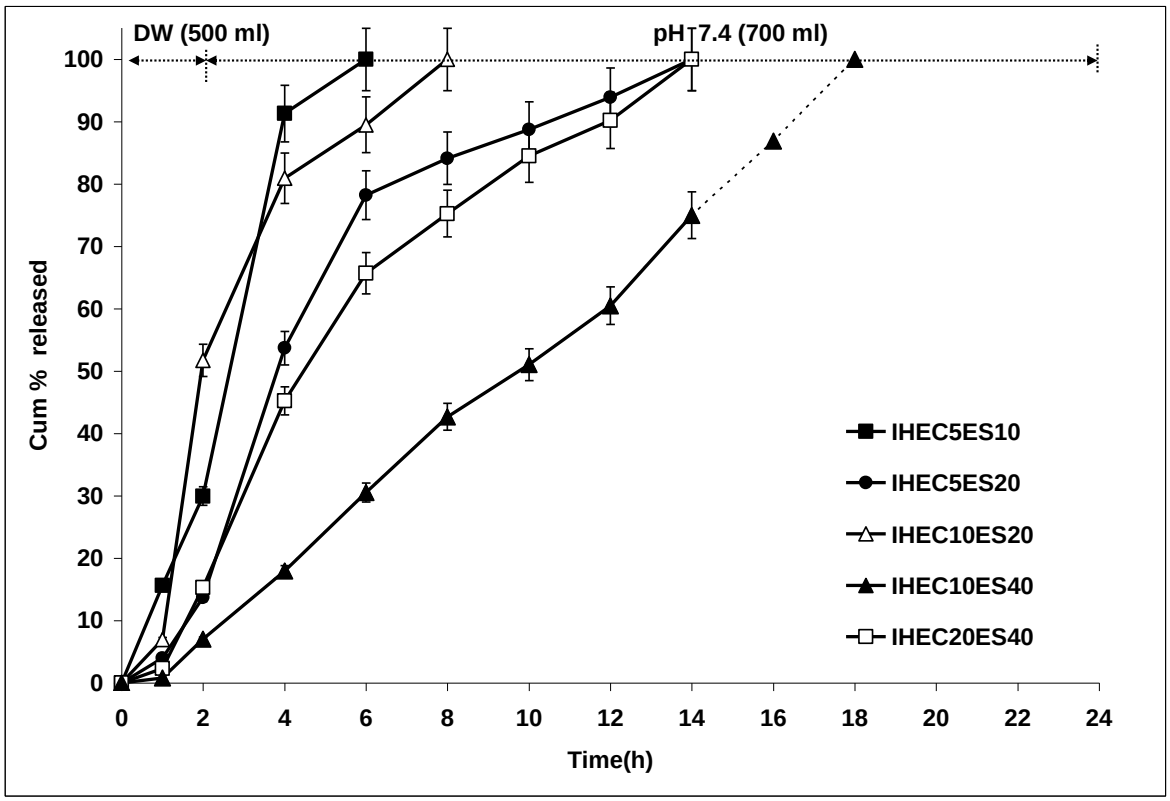


Fig 5.37: Release profile of indomethacin from HEC based matrix tablets showing effect of varying proportion of ES100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The corresponding duration of release was extended from 4.8 h (IHEC5ES10) to 10.4 h (IHEC5ES20) (Table 5.33). Conversely, at 20% ES100, increase in the proportion of HEC from 5% (IHEC5ES20) to 10% (IHEC10ES20) resulted in significantly enhanced release rates ($t_{10\%}$ of 1.1 h and $t_{90\%}$ of 6.3 h for IHEC10ES20). This could be due to the increase in relative hydrophilicity of the matrix. Further, in matrix containing 10% w/w of HEC, when the relative proportion of ES100 was increased from 20% (IHEC10ES20) to 40% w/w of drug (IHEC10ES40), the release rates decreased significantly with $t_{10\%}$ increasing from 1.1 h to 3.0 h and $t_{90\%}$ from 6.3 h to 15.8 h for IHEC10ES40.

Whereas, when relative proportion of HEC was increased from 10% (IHEC10ES40) to 20% (IHEC20ES40) in 40% ES100 matrix, the release rate increased resulting in relatively lowered values of $t_{10\%}$ (2.1 h) and $t_{90\%}$ (11.6 h) for IHEC20ES40. However, it was observed in case of HEC + ES100 matrices, drug release in the initial phase was not controlled to the extent as in case of corresponding HEC + EL100 matrices. This implied that higher percentage of ES100 was required to confer the desired bimodal drug release characteristics to an HEC matrix.

Table 5.33: Release kinetics characterization of drug release from HEC and ES100 based matrix tablets

Batches	Release kinetics					
	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]
IHEC5ES10	0.9536	1.54×10^{-2}	14.817	1.15	0.8	4.8
IHEC5ES20	0.9296	1.74×10^{-2}	8.653	1.00	1.7	10.4
IHEC10ES20	0.9140	1.27×10^{-3}	9.016	1.25	1.1	6.3
IHEC10ES40	0.9970	9.22×10^{-4}	3.280	1.20	3.0	15.8
IHEC20ES40	0.9651	7.22×10^{-3}	8.987	0.94	2.1	11.6

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

In case of HEC + ES100 matrices, the mechanism governing release was either case II or super case-II type in which polymer relaxation and erosion were primary mechanism of drug release. Inclusion of ES100 in a hydrophilic polymer matrix resulted in increase in matrix porosity and matrix erosion due to dissolution of Eudragit S100 in alkaline medium, thereby shifting the mechanism towards super case II type. It was concluded that IHEC10ES40 and IHEC20ES40 with nearly acceptable values for $t_{10\%}$ and $t_{90\%}$ release could be useful for colon specific delivery.

(ii) Eudragit S100 + Hydroxy propyl cellulose

In the matrix system containing 5% HPC with ES100 varied from 10% to 20% w/w of drug, it was found that on increasing the relative proportion of ES100, there was significant retardation in the initial release as indicated by the $t_{10\%}$ value of 4.1 h for IHPC5ES10 and 5.5 h for IHPC5ES20, thereby approaching close to the target value for initial release. However, drug release in the later stages was severely prolonged (beyond 20 h) and deviated from the theoretical target of 80-90% release in 14 h (Table 5.34, Fig 5.38). Such a release pattern may only be useful for colonic delivery when colonic transit times are

prolonged. At 20% ES100, when HPC was increased from 5% (IHPC5ES20) to 10% (IHPC10ES20), there was slight increase in release rate indicated by lowered $t_{10\%}$ and $t_{90\%}$ values of 4.2 h and 18.0 h respectively (Table 5.34). When the relative proportion of ES100 was varied from 20% (IHPC10ES20) to 40% (IHPC10ES40) in 10% HPC matrix, there was insignificant change in release in the initial period, while a more rapid rate of release was observed for IHPC10ES40 resulting in lower $t_{90\%}$ values of 14.6 h for IHPC10ES40. Further, increasing the proportion of HPC from 10% (IHPC10ES40) to 20% (IHPC20ES40) in 40% ES100 matrix prolonged $t_{90\%}$ from 14.6 h for IHPC10ES40 to 16.2 h for IHPC20ES40 without much difference in $t_{10\%}$ values (Table 5.34).

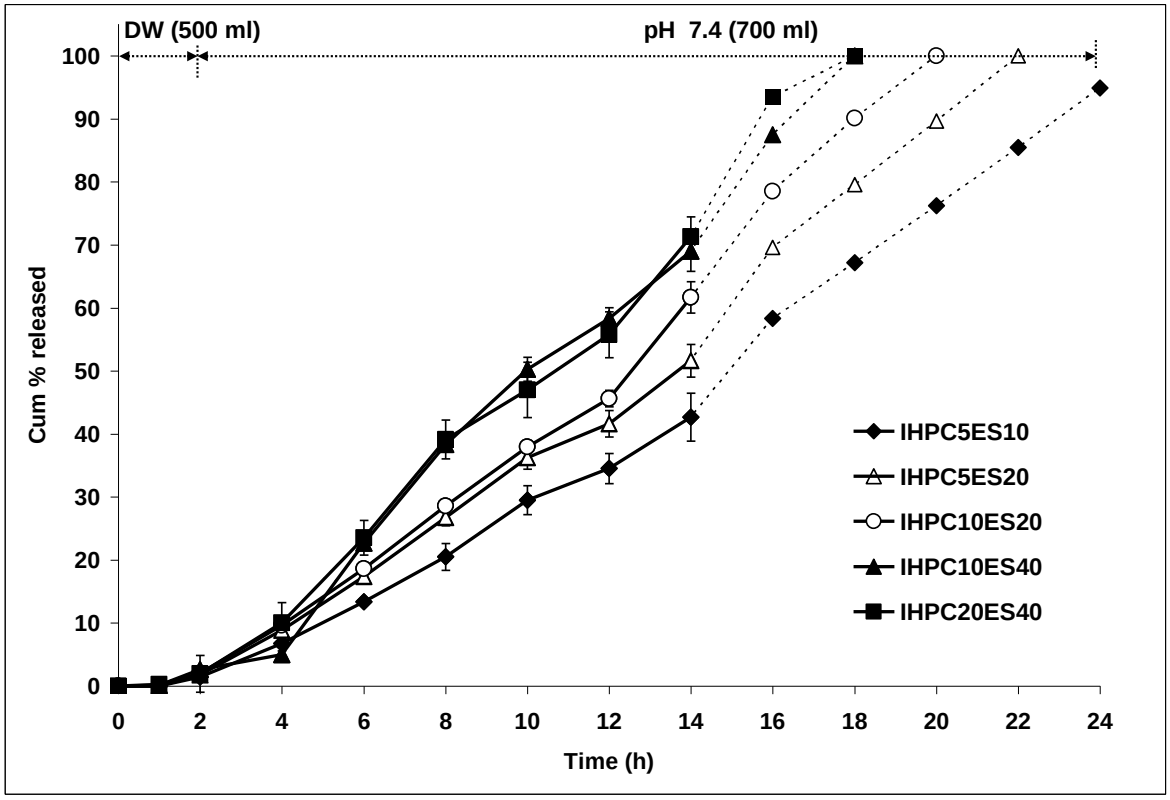


Fig 5.38: Release profile of indomethacin from HPC based matrix tablets showing effect of varying proportion of ES100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

It could be inferred that addition of pH responsive polymers in matrices with suitable proportions of HPC can suitably modify drug release to get sigmoidal release profiles. The values of 'n' in all the formulations of HPC in combination with ES100 indicate super case II type of release (n>1) in all cases implying release by combination of two or more mechanisms, erosion, being the predominant one. It was concluded that except for formulations with 5% HPC in matrix, other formulations showed acceptable release kinetics for potential colonic delivery.

Table 5.34: Release kinetics characterization of drug release from HPC and ES100 based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IHPC5ES10	0.9967	7.26×10^{-4}	2.090	1.20	4.1	23.0
IHPC5ES20	0.9950	9.94×10^{-4}	2.941	1.14	5.5	20.1
IHPC10ES20	0.9979	4.57×10^{-4}	3.059	1.17	4.2	18.0
IHPC10ES40	0.9822	1.90×10^{-2}	3.070	1.26	4.3	14.6
IHPC20ES40	0.9758	3.43×10^{-3}	0.535	1.84	4.2	16.2

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

(iii) Eudragit S100 + Guar gum

The in vitro release profiles of matrix tablets containing guar gum with varying proportion of ES100 are shown in Fig 5.39. In the matrix containing 5% guar gum and proportion of ES100 varying from 10% to 40%, there was proportionate retardation in the release rate of the drug (Table 5.35, Fig 5.39). The reason for this was attributed to the nature of matrix formed between guar gum and ES100. Matrix bases of guar gum with ES100 are possibly more porous in nature and this explains the relatively higher release rates observed for these matrices when compared to matrix bases containing guar gum with EL100. Hence, a higher percentage of ES100 was required to get the desired retardation in drug release.

Table 5.35: Release kinetics characterization of drug release from GG and ES100 based matrix tablets

Batches	Release kinetics					
	r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
IGG5ES10	0.9433	4.67×10^{-3}	17.527	1.14	1.2	4.2
IGG5ES20	0.9851	1.50×10^{-3}	2.687	1.59	2.6	9.1
IGG5ES40	0.9859	2.53×10^{-3}	6.734	1.04	3.9	11.9
IGG10ES10	0.9889	1.63×10^{-3}	5.352	1.59	1.6	5.9
IGG10ES20	0.9937	1.89×10^{-3}	0.493	1.87	3.8	16.2

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

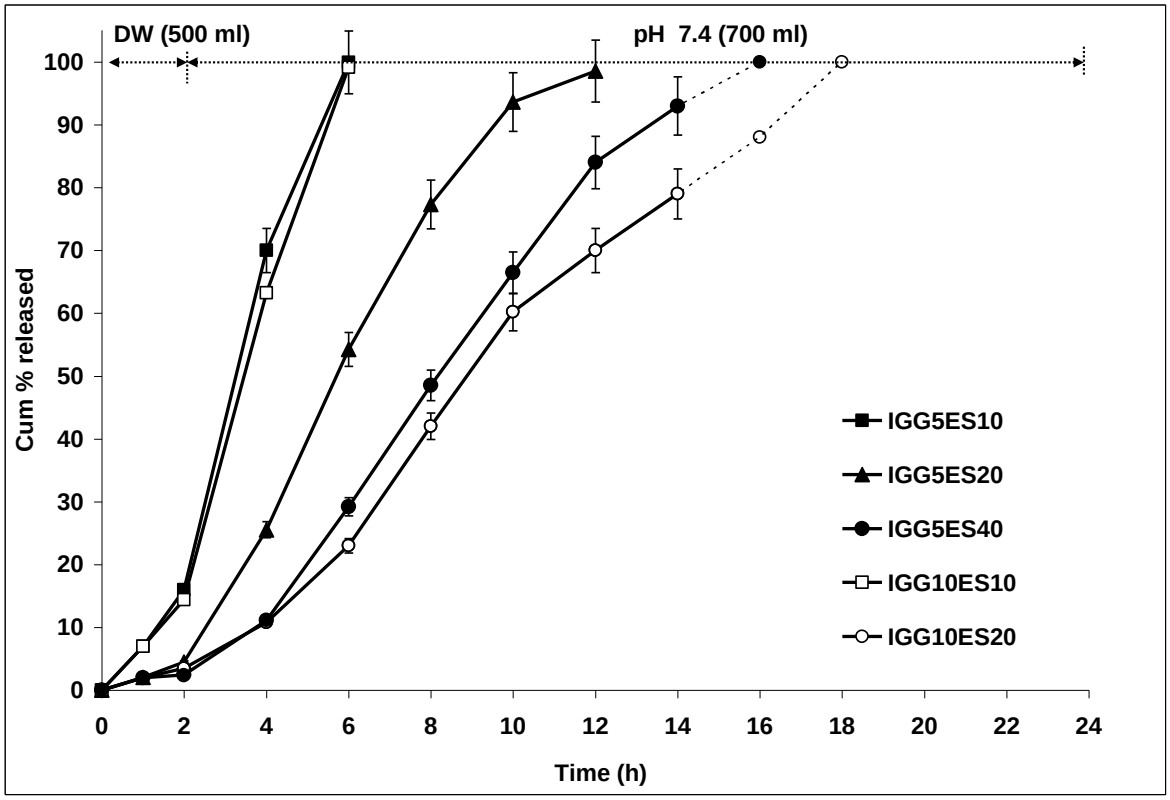


Fig 5.39: Release profile of indomethacin from GG based matrix tablets showing effect of varying proportion of ES100. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

With increase in the level of ES100 from 10% (IGG5ES10) to 20% (IGG5ES20), a corresponding retardation in the drug release rate was observed. The $t_{10\%}$ increased from 1.2 h for IGG5ES10 to 2.6 h for IGG5ES20 (Table 5.35). Similarly, the duration of drug release extended from beyond 4.2 h (IGG5ES10) to beyond 9.1 h (IGG5ES20). When the % of ES100 was further increased to 40% in 5% GG matrix, the rate of release was further retarded with $t_{10\%}$ and $t_{90\%}$ values obtained as 3.9 h and 11.9 h respectively. When the proportion of guar gum was increased from 5% (IGG5ES10) in 10% (IGG10ES10) on 10% ES100 matrix, the change was marginal.

Subsequently, with increase in ES100 from 10% (IGG10ES10) to 20% (IGG10ES20), a significant decrease in the initial and overall release rate was observed which resulted in relatively higher $t_{10\%}$ (3.8 h) and $t_{90\%}$ (16.2 h) values, implying a more suitable polymer proportion of ES100 (Fig 5.39). Amongst the various formulations, IGG5ES20, IGG5ES40 and IGG10ES20 showed good potential for colon specific release.

From the present study, it can be concluded that the use of pH based polymers in combination with hydrophilic polymer(s) to form a polymeric matrix base controls the initial swelling of these polymers to a good extent which could prevent early drug loss from their matrices during upper GI transit. It also confers matrix strength and rigidity to

the formulations, thereby enabling lower proportions of these polymers to be used in matrix bases.

5.7. Effect of simulated GI fluid pH (without enzymes) on release

The in vitro release studies conducted in the initial dissolution conditions were intended to characterize and understand the effect of various polymers alone or in combination to control the initial drug release and also their potential to impart controlled release characteristics in later period of drug release. The ability of the polymeric system employed to ensure complete drug release in the stipulated time - frame of 14-16 hours in the alkaline environment of colon (pH 7.4) was also evaluated. During the course of gastrointestinal transit, an orally ingested formulation may be exposed to various pH conditions ranging from 1.2 in the stomach to 7.4 in the intestine. Therefore, selected formulations from each series were taken for release studies in simulated GI fluid pH (without enzymes). The choice of pH conditions was pH 1.2 for a duration of 2 h (simulated gastric fluid, 350 ml), pH 4.5 for 2 h (simulated duodenal fluid, 600 ml) followed by pH 7.4 (simulated distal ileum and colon, 900 ml) for the remaining period of study. Enzymes or microorganisms were not employed in this study as the choice of polymer and mechanism of drug release envisaged was pH or transit time control. The drug release from the various formulations was compared with the theoretical target values using f_1 (dissimilarity) and f_2 (similarity) factors (Tables 5.36 a & b).

From the series of formulations prepared using combination of EL100 and ES100, formulations IEL15ES10 and IEL10ES15, were selected for in vitro release study in simulated GI fluid pH (without enzymes). The in vitro release profiles for these formulations are shown in Fig 5.40. A slightly greater retardation in release rate was observed when the relative proportion of ES100 in the matrix was increased from 10% to 15% w/w of drug as observed from the $t_{10\%}$ values (IEL15ES10: 3.6 h and IEL10ES15: 3.8 h) and $t_{90\%}$ (IEL15ES10: 14.5 h and IEL10ES15: 15.2 h) (Table 5.36a). The K values for these two formulations were found to be 1.719 $h^{-1.48}$ and 2.105 $h^{-1.38}$. This observation was similar to that observed during preliminary studies of formulations carried out in distilled water (500 ml) followed by pH 7.4 medium wherein retardation in indomethacin release from matrices prepared using EL100 and ES100 in combination was found to depend more on the relative proportion of ES100 in the polymer matrix. The decrease in release rate post 2 h is attributed to the presence of lower percentage of carboxylic acid groups on ES100 that get ionized gradually above pH 7.0 leading to slower matrix erosion

in the latter case (Mehta et al., 2001). The drug release mechanism for these two formulations was found to be super case II type implying release by erosion due to dissolution of both Eudragit polymers in matrix. When the in vitro release profiles of these two formulations were compared with the theoretical target release profile, acceptable values of similarity factor f_2 (54.8 for IEL15ES10 and 51.8 for IEL10ES15) and dissimilarity factor f_1 (7.1 for IEL15ES10 and 5.1 for IEL10ES15) were obtained (Table 5.36a). This implied suitability of the matrix for sigmoidal release profile required for colonic delivery.

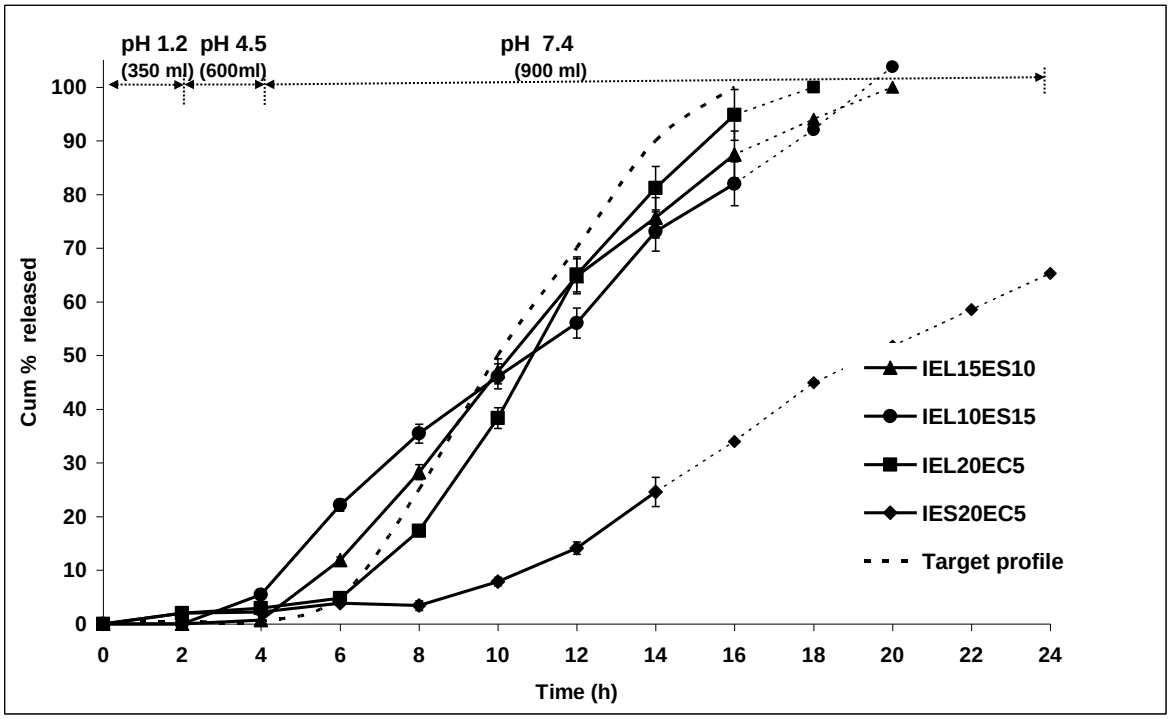


Fig 5.40: Release profile of indomethacin from EL100 or ES100 based matrix tablets in combination with each other or with EC in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

For formulation matrix prepared using combination of EC with EL100 or ES100, the in vitro release profiles are shown in Fig 5.40. The K values for the two formulations were obtained as 0.522 $h^{-1.99}$ and 4.657 $h^{-0.91}$ and the $t_{10\%}$ values obtained as 4.9 h for IEL20EC5 and 8.2 h for IES20EC5 respectively, showing a significant difference in the initial release behavior of the drug from the two matrices (Table 5.36a). The combination of EC with ES100 probably resulted in the formation of a highly hydrophobic matrix wherein the pH sensitive polymer was less susceptible to –COOH group ionization which results in erosion of such polymeric matrix in alkaline environment, resulting in very long lag time for drug release. Such a lag time of 8.2 h as observed in the case of IES20EC5 may be beneficial in certain cases when GI transit times are very high or targeting to the remotely terminal part

of the colon is desired. However, the calculated $t_{90\%}$ values for the two formulations (IEL20EC5: 13.3 h, IES20EC5: 25.9 h) indicated an unacceptable slow release rate for IES20EC5. The n values for the two formulations namely, 1.99 for IEL20EC5 and 0.91 for IES20EC5, indicated differing release mechanisms, signifying an erosion based drug release mechanism for the former and anomalous, non-Fickian (diffusion and polymer relaxation) based mechanism approaching case II (zero order) for the latter. An f_2 value of 59.1 and f_1 value of 3.9 for the release profile of IEL20EC5 indicated good similarity with the target profile. On the other hand, in case of IES20EC5 a very low f_2 value of 1.5 and high f_1 value of 69.9 indicated that IES20EC5 was not suitable for colon specific release under present considerations.

In case of matrices comprising of EC with PCP, the in vitro release profiles of selected formulations, viz., IPCP10EC5, IPCP10EC10, IPCP20EC20 in simulated GI fluid pH (without enzymes), are shown in Fig 5.41. The values for the release rate constants K for these formulations were 0.250 $h^{-2.1}$ for IPCP10EC5, 0.152 $h^{-2.2}$ for IPCP10EC10, 0.399 $h^{-1.95}$ for IPCP20EC20. The $t_{10\%}$ values of 4.5 h for IPCP10EC5 and 9.2 h for IPCP10EC10 and corresponding $t_{90\%}$ of 16.5 h and 18.2 h indicated that increase in the relative proportion of EC retarded the release rate in 10% PCP matrix. However, when the relative proportion of PCP was increased from 10% to 20% in 20% EC matrix, the release rate increased slightly as shown by $t_{10\%}$ of 8.3 h and $t_{90\%}$ of 16.1 h for IPCP20EC20. This was due to increase in relative percentage of hydrophilic polymer in matrix. These findings were similar to those obtained previously for these matrices in preliminary dissolution studies. High values of n that ranged from 1.95 to 2.20 imply that drug release occurred by complex mechanism involving combination of two or more processes. Values of n higher than 1 have been attributed to super case II or polymer relaxation (Korsemeyer et al., 1983; Ranga Rao et al., 1988; Alur et al., 1999). However, other studies have attributed this phenomenon to matrix erosion (Wei et al., 2006). In the present case, it was attributed to combination of matrix erosion, altered permeability of the matrix and matrix swelling. The release profiles in the present case showed a significant bimodal pattern and except for IPCP10EC10, both IPCP10EC5 and IPCP20EC20 showed good similarity with the target release profile with f_2 value of 52.39 for IPCP10EC5 and 54.23 for IPCP20EC20 (Table 5.36a). These two formulations showed less than 10% release in first 6 h with expected complete drug release in 16 h respectively indicating good potential of these matrix bases as pH and transit time controlled systems for colon specific delivery.

In case of matrices prepared using combination of EC with CP, the two formulations selected for study in simulated GI fluid pH were ICP10EC5 and ICP20EC5 as these two formulations showed desirable release kinetics during preliminary studies in the first set up of dissolution conditions. The corresponding in vitro release profiles are shown in Fig 5.41. The values for the release rate constant K for these formulations were 0.032 $h^{-2.84}$ for ICP10EC5 and 0.134 $h^{-2.10}$ for ICP20EC5. Increase in the relative proportion of CP decreased the initial release of drug from the matrix as shown by the $t_{10\%}$ of 5.4 h for ICP10EC5 and 4.3 h for ICP20EC5 but retarded the overall release rate significantly as shown by the $t_{90\%}$ of 16.3 h for ICP10EC5 and 22.2 h for ICP20EC5 values are indicative of this (Table 5.36a).

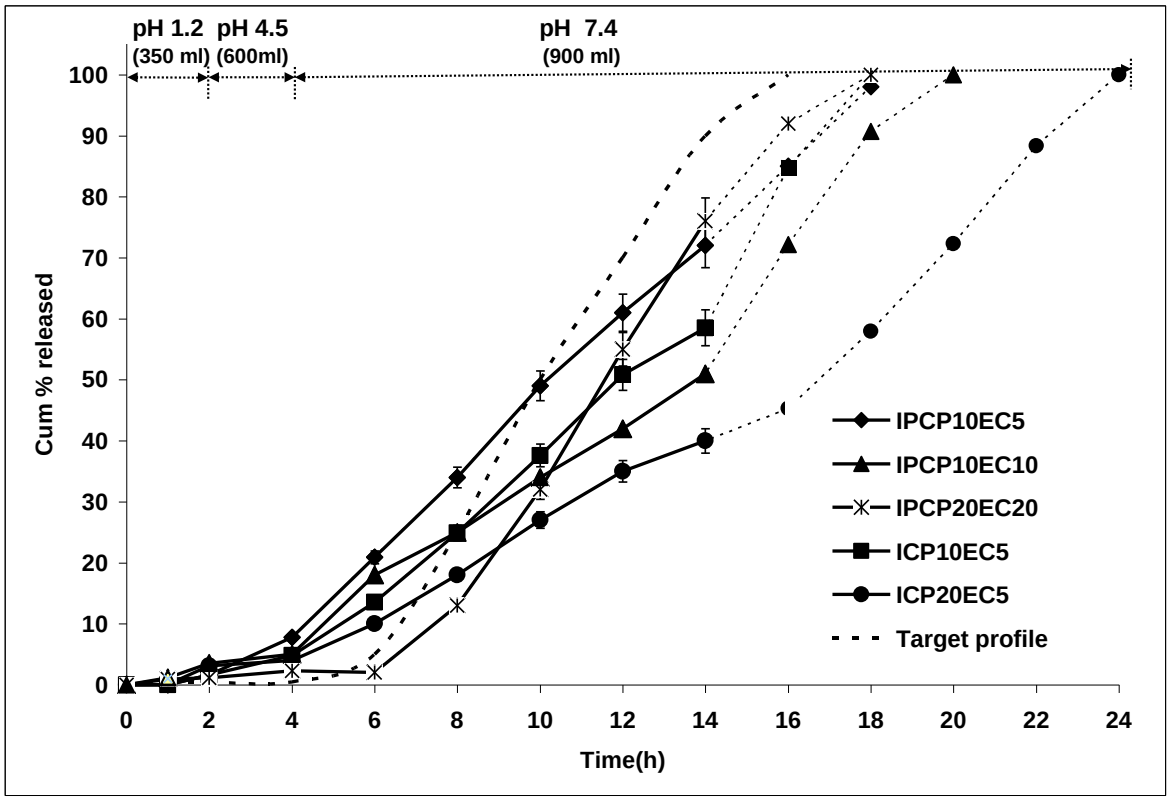


Fig 5.41: Release profile of indomethacin from matrix tablets based on combination of EC with PCP or CP in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The combination of EC with CP resulted in the formation of a tight matrix base which gave highly retarded release. High values of n (2.84 and 2.10) obtained for both the formulations again indicated that drug release occurred by a complex process. None of these formulations approached the target release profile with $f_2 < 50$ and $f_1 > 15$ (Table 5.36a). For matrices prepared with PCP and EL100, the formulations investigated were IPCP5EL10, IPCP5EL20 and IPCP10EL20 (Fig 5.42). The K values for these formulations were found to be 1.014 $h^{-1.85}$ for IPCP5EL10, 0.164 $h^{-2.08}$ for IPCP5EL20, and 1.185 $h^{-1.23}$

for IPCP10EL20. Increase in the relative proportion of EL100 from 10% to 20% in 5% PCP matrix retarded drug release significantly. The $t_{10\%}$ for IPCP5EL10 was 4.7 h and for IPCP5EL20 and IPCP10EL40, was obtained as 7.1 hand 7.2 h respectively. Similarly, $t_{90\%}$ values for these series indicated 11.3 h, 20.5 h and 33.8 h for IPCP5EL10, IPCP5EL20 and IPCP10EL20 respectively, indicating a significant deviation from the target profile for the latter two formulations. Only IPCP5EL10 with an f_2 value of 53.24 and f_1 value of 13.03 showed nearness to the target values amongst these three formulations.

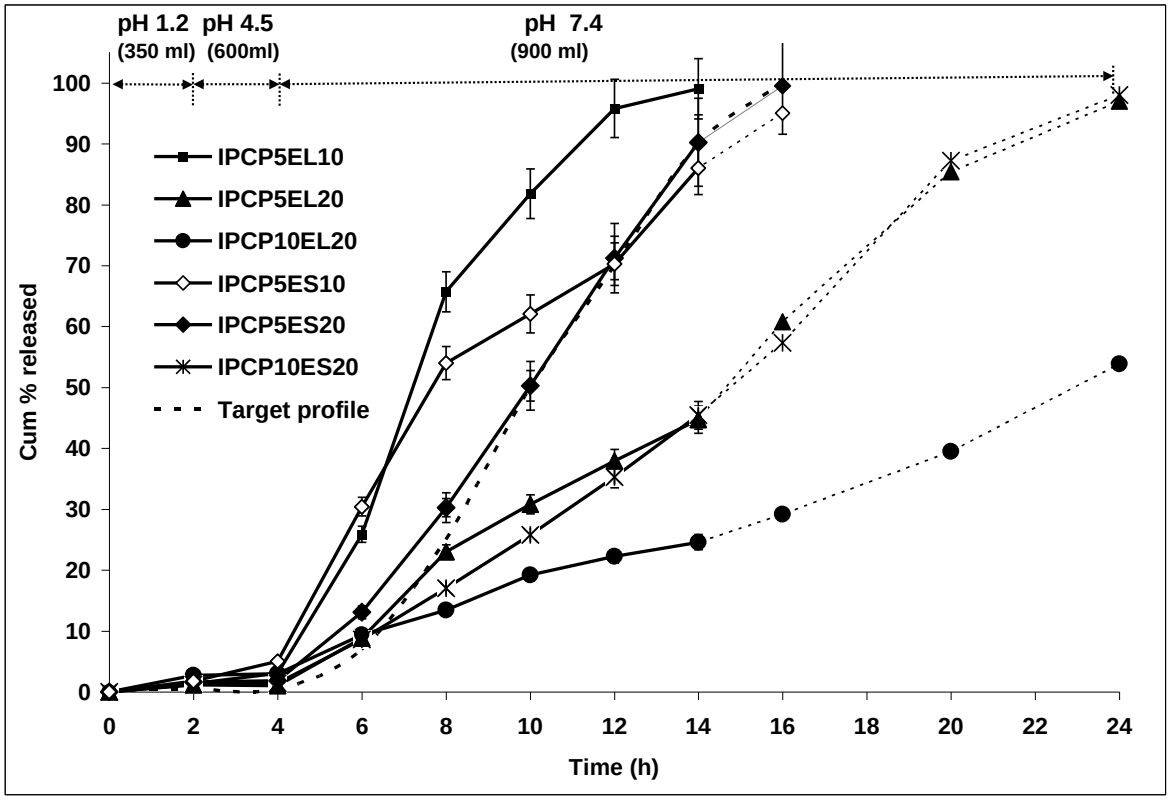


Fig 5.42: Release profile of indomethacin from PCP based matrix tablets in combination with EL100/ES100 in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

In case of matrix bases composed of PCP in combination with ES100, the release profiles for selected formulations IPCP5ES10, IPCP5ES10 and IPCP10ES20 are shown in Fig 5.42. The K values for these formulations were found to be $1.046h^{-1.67}$ for IPCP5ES10, $0.647\ h^{-1.87}$ for IPCP5ES20, and $0.259\ h^{-1.98}$ for IPCP10ES20. The $t_{10\%}$ values ranged from 4.7 h for IPCP5ES10 to 6.7 h for IPCP10ES20 while the $t_{90\%}$ values ranged from 14.4 h for IPCP5ES10 to 19.2 h for IPCP10ES20. Therefore, compared to PCP + EL100 matrices, these formulations showed better release kinetics. Except for IPCP10ES20, other two formulations demonstrated good similarity with the target release (f_2 >50; f_1 < 15) as shown in Table 5.36a. The n values (> 1) for PCP + EL100 and PCP + ES100 indicated drug

release mechanism was erosion based (super case II) due to the presence of Eudragit polymer.

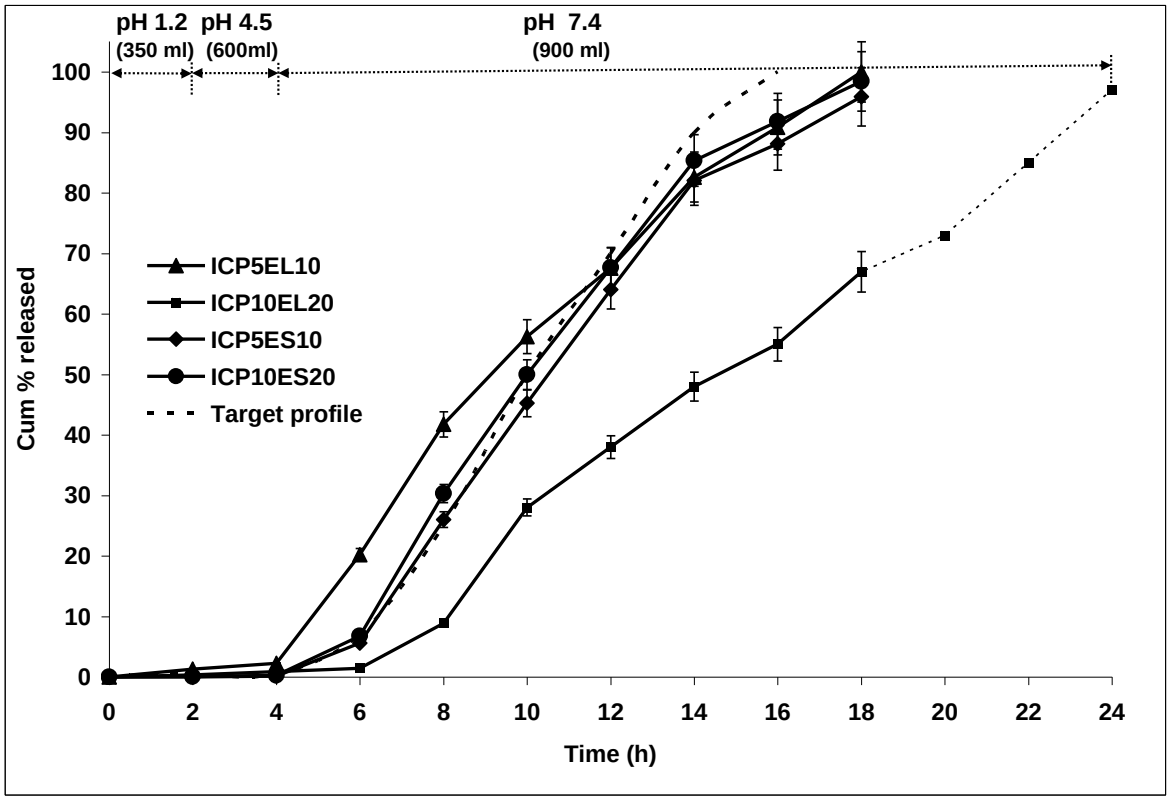


Fig 5.43: Release profile of indomethacin from CP based matrix tablets in combination with EL100/ES100 in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

For matrices prepared using combination of CP with EL100, the in vitro release profiles of two selected formulations (ICP5EL10 and ICP10EL20) in simulated GI fluid pH are shown in Fig 5.43. The values for the release rate constant K for the two formulations were 1.960 $h^{-1.45}$ and 0.106 $h^{-1.99}$. In these two formulations, $t_{10\%}$ value increased significantly from 4.8 h to 7.1 h and $t_{90\%}$ values from 14.0 to 29.6 h when the polymer proportions were doubled from 5% to 10% for CP and from 10% to 20% for EL100, indicating that use of these polymers in a lower percentage is advantageous. The n values for the two formulations (1.45 for ICP5EL10 and 1.99 for ICP10EL20) confirmed the release mechanism as super case II (erosion based). Formulation ICP5EL10 showed near ideal release with f_2 value of 52.41 and f_1 value of 14.91 when compared with the theoretical target release.

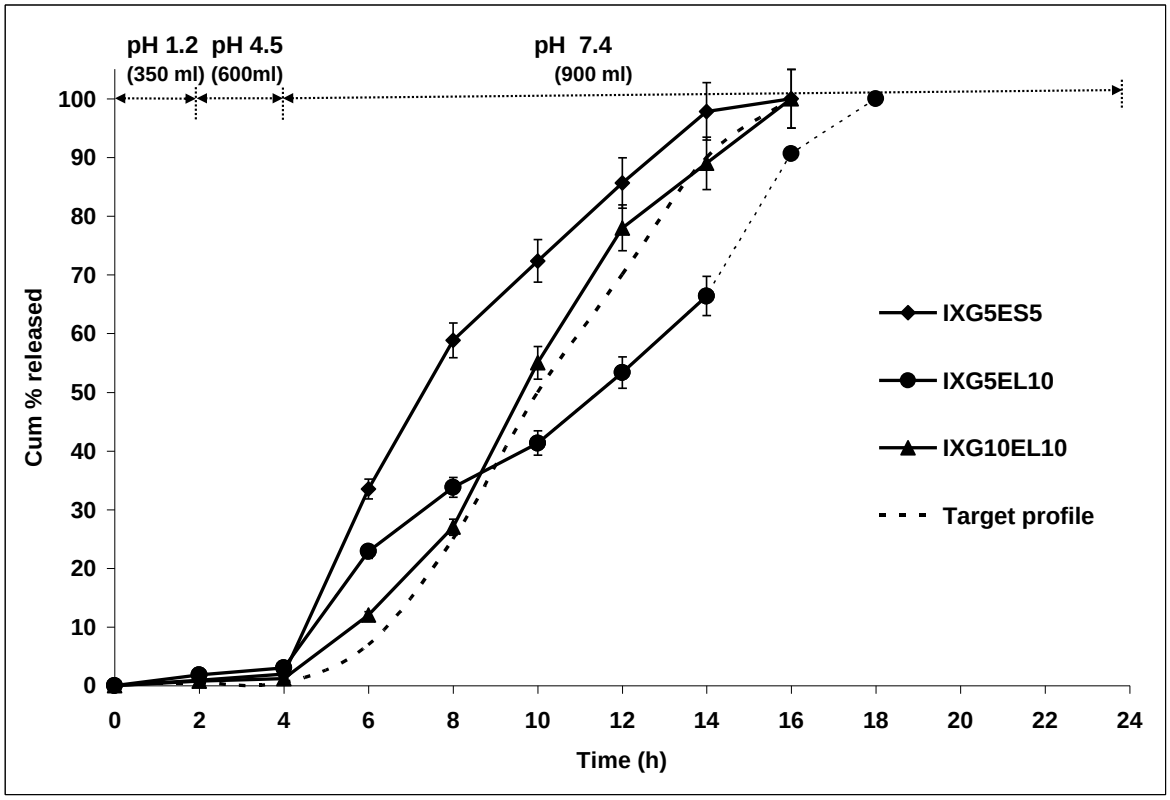


Fig 5.44: Release profile of indomethacin from XG based matrix tablets in combination with EL100/ES100 in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The release profiles of selected formulations ICP5ES10 and ICP10ES20 prepared using CP with ES100 are shown in Fig 5.43. The K values for these formulations were obtained as 1.143 $h^{-1.65}$ and 0.485 $h^{-1.99}$. The release profile in this case showed a different trend when compared with the corresponding CP + EL100matrices discussed previously. When the total polymer proportion was doubled from 5% to 10% for CP and from to 10% to 20% for ES100, there was slightly greater retardation in initial release as shown by $t_{10\%}$ value of 5.9 h for ICP5ES10 that increased to 6.9 h for ICP10ES20. However, there was slight decrease in $t_{90\%}$ from 14.1 h for ICP5ES10 to 13.8 h for ICP10ES20. This observation was again consistent with previous findings reported for these formulations in the first set up of dissolution conditions and was attributed to increase in matrix porosity with increase in relative proportion of ES100 in matrix from 10% to 20%. The high porosity of these matrix bases renders them more susceptible to erosion and this theory is supported by high values of n (1.65 for ICP5ES10 and 1.99 for ICP10ES20) which indicates super case II mechanism. Both the formulations showed good similarity with the theoretical target release profile with f_2 value of 53.89 for ICP5ES10 and 51.67 for ICP10ES20 and corresponding f_1 value as 14.33 and 12.81 respectively indicating their potential for site specific release to colon.

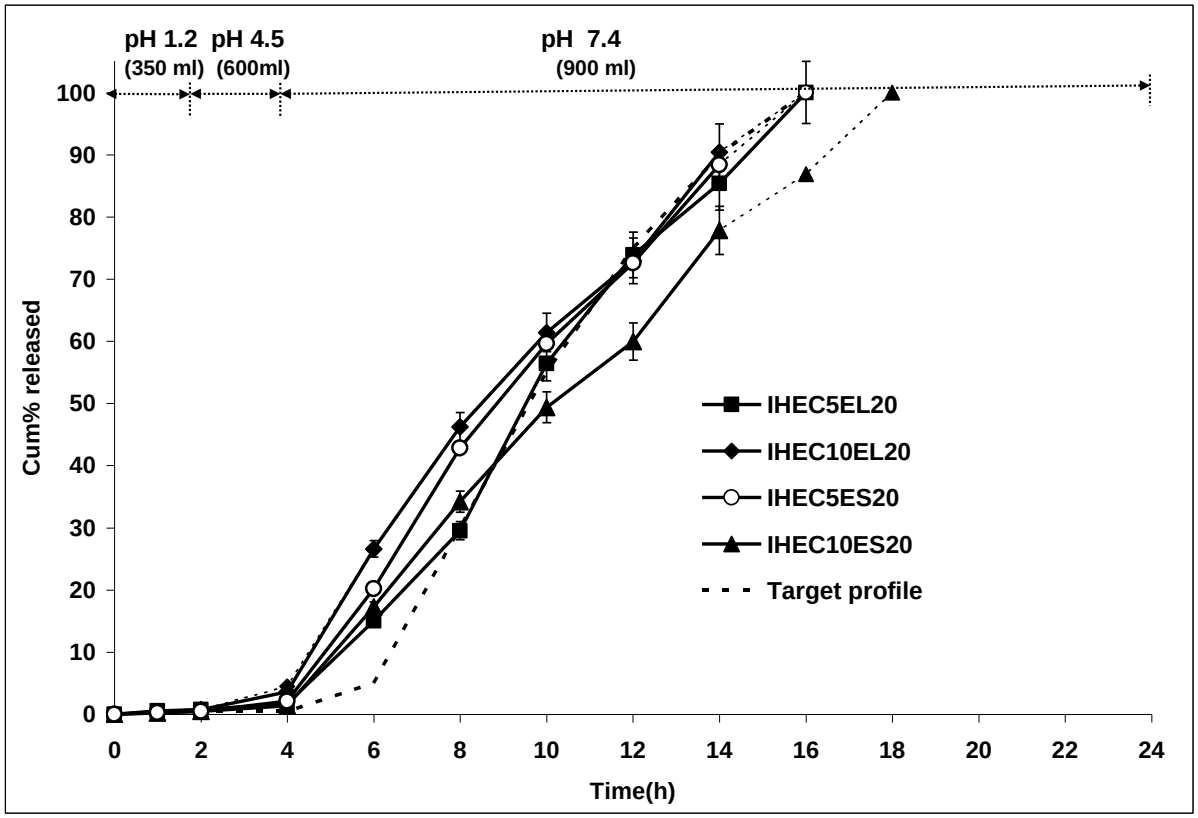


Fig 5.45: Release profile of indomethacin from HEC based matrix tablets in combination with EL100/ES100 in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

For XG based formulations with EL100, the formulations studied were IXG5EL10 and IXG10EL10 while from XG + ES100 series, IXG5ES5 was evaluated for release in simulated GI fluid pH. The corresponding release profiles are presented in Fig 5.44. The values for release rate constant K were 4.016 $h^{-1.12}$ for IXG5EL10 and 10.972 $h^{-0.91}$ for IXG10EL10. Increase in relative proportion of xanthan gum from 5% to 10% in 10% EL100 matrix slightly increased the $t_{10\%}$ from 4.5 h for IXG5EL10 to 4.9 h for IXG10EL10 and decreased the $t_{90\%}$ from 15.9 h for IXG5EL10 to 14.1 h for IXG10EL10 (Table 5.36b). The release mechanism also changed from erosion type release (n value of 1.12 for IXG5EL10) to non-Fickian anomalous approaching case II release (n = 0.91). Higher percentage of XG in EL100 matrix resulted in imparting a swelling and diffusion controlled release mechanism. Formulation IXG10EL10 showed very good similarity with target profile with f_2 value of 71.04 and f_1 value of 2.03.

For formulation prepared using combination of XG with ES100 (IXG5ES5), the drug release profile was characterized by a sigmoidal pattern with $t_{10\%}$ of 4.2 h and $t_{90\%}$ of 12.7 h. The n value of 0.86 indicated the drug release mechanism to be non- Fickian anomalous type due to polymer swelling and diffusion in the presence of XG. The release rate K for

the formulation was 10.114 $h^{-0.86}$ and the release profile showed only a minor deviation from the target profile (Fig 5.44, Table 5.36b) with f_2 value of 41.84 and f_1 value of 13.95. In case of matrix bases composed of HEC in combination with EL100, the release profiles for selected formulations IHEC5EL20 and IHEC10EL20 are shown in Fig 5.45. The K values for these formulations were found to be 18.578 $h^{-0.59}$ for IHEC5EL20 and 17.861 $h^{-0.59}$ for IHEC10EL20. The n values of 0.59 suggest non-Fickian anomalous type of release mechanism based on polymer swelling and diffusion. The $t_{10\%}$ values decreased from 5.1 h for IHEC5EL20 to 4.4 h for IHEC10EL20 while the $t_{90\%}$ values increased from 14.5 h for IHEC5EL20 to 15.5 h for IHEC10EL20. The slightly higher initial release observed in case of IHEC10EL20 was probably because of quick matrix swelling due to presence of relative higher percentage of HEC that resulted in faster release of the drug. But in later stages, gradually swollen HEC polymer matrix resulted in formation of a highly coiled and dense matrix. This structure significantly retarded drug diffusion through the swollen gel layer (Sinha and Rohera, 2002). This explains the higher $t_{90\%}$ value observed for IHEC10EL20. Both formulations (IHEC5EL20 and IHEC10EL20) demonstrated good similarity with the target release with f_2 values of 53.56 and 51.20 and f_1 values of 12.52 and 3.95 respectively as shown in Table 5.36b.

For the matrix bases prepared using HEC with ES100, the release profiles for selected formulations, IHEC5ES20 and IHEC10ES20 are shown in Fig 5.45. The K values for these formulations were found to be 12.745 $h^{-0.72}$ and 10.529 $h^{-0.73}$. When the relative polymer proportion of HEC was increased from 5% to 10% in 20% ES100 matrix, there was retardation in release rate as shown by $t_{10\%}$ value of 4.3 h for IHEC5ES20 that increased to 5.7 h for IHEC10ES20 and $t_{90\%}$ increased from 15.1 h to 18.9 h.

This observation was not consistent with previous findings reported for these formulations when they were evaluated in the first set up of dissolution conditions where increase in relative proportion of HEC enhanced the release rate. In the present case, the retardation in release rate in case of IHEC10ES20 is attributed to formation of a thicker gel matrix that showed gradual swelling in acidic (pH 1.2) and mildy acidic (pH 4.5) conditions followed by slow erosion in alkaline medium. The release mechanism in both cases (n is 0.72 for IHEC5ES20 and 0.73 for IHEC10ES20) is suggestive of swelling and diffusion controlled mechanism (non-Fickian anomalous type). Amongst these two formulations, IHEC5ES20 was found to approach theoretical target values with a similarity factor f_2 of 51.04 and dissimilarity factor f_1 of 11.25.

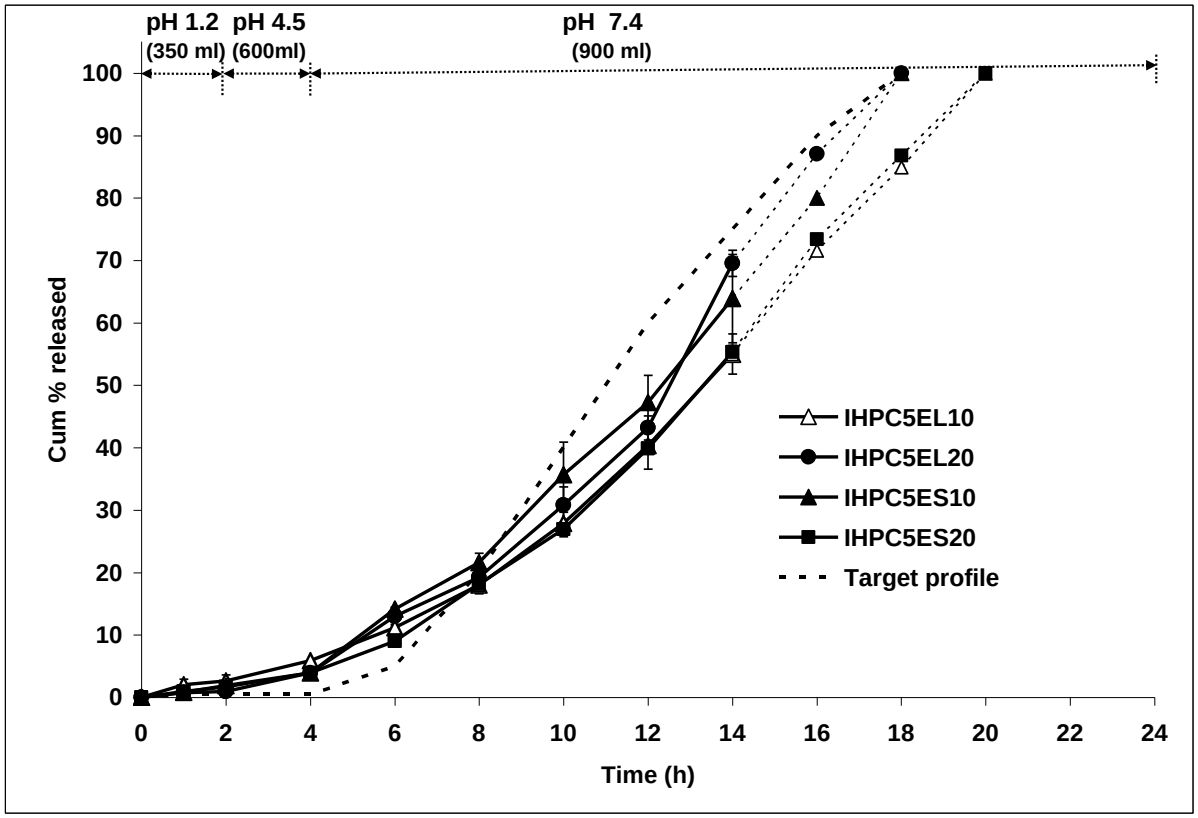


Fig 5.46: Release profile of indomethacin from HPC based matrix tablets in combination with EL100/ES100 in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The in vitro release profiles of the formulations IHPC5EL10 and IHPC5EL20 (prepared with HPC and EL100) studied in simulated GI fluid pH (without enzymes) are shown in Fig 5.46. The values for release rate constant K were obtained as 1.288 $h^{-1.45}$ for IHPC5EL10 and 0.740 $h^{-1.76}$ for IHPC5EL20. Increase in relative proportion of EL100 in this case slightly increased the $t_{10\%}$ from 5.1 h for IHPC5EL10 to 5.3 h for IHPC5EL20 and decreased the $t_{90\%}$ from 18.7 h for IHPC5EL10 to 15.3 h for IHPC5EL20. The release mechanism in both cases was indicative of super case II type (Table 5.36b) implying that drug release from the matrix depended on erosion that in turn depended on dissolution of EL100 in alkaline medium. Between these two formulations, IHPC5EL20 was found to approach theoretical target values with f_2 of 51.25 and f_1 of 12.77. Therefore, this formulation has good potential for site specific drug release to colon (Fig 5.46). In case of selected matrix bases (IHPC5ES10 and IHPC5ES20) comprising of HPC in combination with ES100, the in vitro release profiles are shown in Fig 5.46. The values for release rate constant K were 0.796 $h^{-1.75}$ for IHPC5ES10 and 0.380 $h^{-1.85}$ for IHPC5ES20. In this case, increase in the relative proportion of ES100 from 10% to 20% in 5% HPC matrix showed higher initial release but prolonged the extent of release. This is shown by $t_{10\%}$ (IHPC5ES10: 6.2 h and IHPC5ES20: 5.8 h) and $t_{90\%}$ values (IHPC5ES10: 14.9 h and

IHPC5ES20: 19.2 h). The slow hydration of matrix in the initial phase may be responsible for faster initial release. An f_2 value of 52.4 and f_1 value of 13.26 for the release profile of IHPC5ES10 indicated good similarity with the target profile. However, IHPC5ES20 was found to slightly deviate from the target profile (Table 5.36b).

In case of matrix bases composed of GG in combination with EL100, for selected formulations IGG5EL20 and IGG10EL20 the K values were found to be 1.023 $h^{-1.86}$ and 2.601 $h^{-1.39}$ respectively. The corresponding release profiles are shown in Fig 5.47. The $t_{10\%}$ values were 4.5 h for IGG5EL20 and 4.1 h for IGG10EL20 while the $t_{90\%}$ values were 11.1 h for IGG5EL20 and 12.8 h for IGG10EL20. Therefore, increase in the relative proportion of GG did not affect the drug release significantly in the initial period but extended the duration of release, due to slower matrix erosion. IGG10EL20 was the better formulation amongst the two as it demonstrated good similarity with the target release (f_2 of 51.0 and f_1 of 2.03) as shown in Table 5.36b.

For GG based formulations with ES100, the formulations selected for study were IGG5ES20 and IGG10ES20. The K values were obtained as 1.007 $h^{-1.67}$ for IGG5ES20 and 1.107 $h^{-1.47}$ for IGG10ES20 and the corresponding release profiles are shown in Fig 5.47. In this case, increase in the percentage of guar gum retarded the release rate as shown by the $t_{10\%}$ values obtained as 5.7 h for IGG5ES20 and 5.9 h for IGG10ES20 while the corresponding $t_{90\%}$ values were 14.6 h and 19.7 h respectively. It is possible that higher percentage of swellable hydrophilic polymer resulted in increase in gelling that retarded drug release from the matrix base. The n values for GG + EL100 and GG + ES100 based matrices indicated that drug release mechanism was erosion based (super case II). Formulation IGG5ES20 was found to approach theoretical target values with a similarity factor f_2 of 53.80 and dissimilarity factor f_1 of 3.95. Therefore, this study shows evidence that incorporating pH sensitive polymers in guar gum matrix base can be a useful approach for designing formulations with sigmoidal release profile. Further, guar gum was employed in very low proportions, unlike previous reports where only high quantities of guar gum could attain colon specific delivery (Prasad et al., 1998; Momin and Pundarikakshudu 2004; Al-Saidan et al., 2005).

In summary, it may be observed from Table 5.36 (a & b), that several formulations, viz., IEL15ES10, IEL10ES15, IEL20EC5, IPCP10EC5, IPCP20EC20, IPCP5EL10, IPCP5ES10, IPCP5ES20, ICP5EL10, ICP5ES10, ICP10ES20, IXG10EL10, IHEC5EL20, IHEC10EL20, IHEC5ES20, IHPC5EL20, IHPC5ES10, IGG5EL20 and IGG5ES20 had

shown good similarity with the theoretical target release profile in vitro as shown by their similarity factor f_2 that was greater than 50 and dissimilarity factor f_1 that was less than 15.

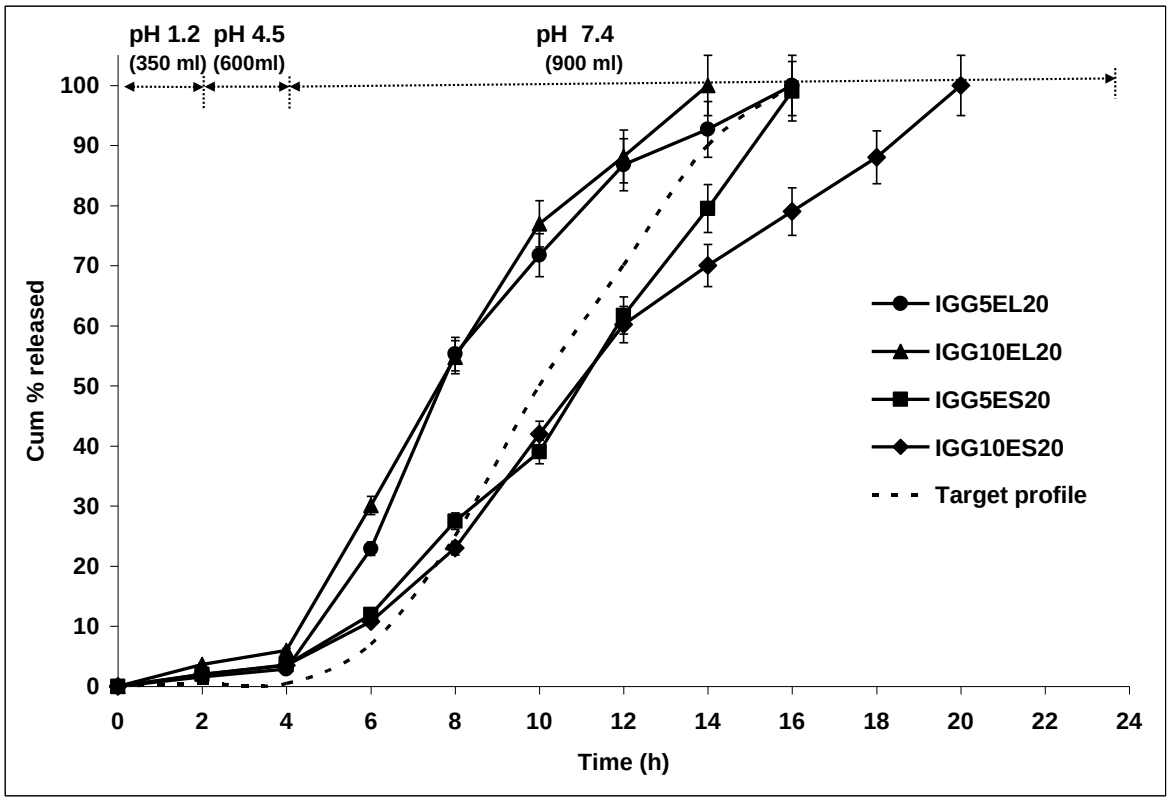


Fig 5.47: Release profile of indomethacin from GG based matrix tablets in combination with EL100/ES100 in simulated GI fluid pH (without enzymes). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Therefore, it could be concluded from the present investigation that presence of either Eudragit L100 or S100 could successfully impart a pH and time dependent sigmoidal release pattern to all hydrophilic and hydrophobic polymer matrix based formulations. The drug release profile from most of the selected formulations in simulated GI fluid (without enzymes) was characterized by an initial lag time period of 4-6 h with low drug release followed by controlled release phase in phosphate buffer media for about 14-16 h. Therefore, these formulations have the potential for pH and time dependent delivery to the colon. Some of these formulations IPCP5ES20, ICP5EL10, IXG10EL10, IHEC5EL20, IHPC5ES10 and IGG5ES20 were selected for further in vivo studies in Wistar rat model and human subjects.

Table 5.36a: Release kinetics of selected formulations in simulated GI fluid pH (without enzymes)

Formulation series	Batches	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]	f_1[#]	f_2[#]
EL100 + ES100	IEL15ES10	0.9799	2.21×10^{-3}	1.719	1.48	3.6	14.5	7.10	54.80
	IEL10ES15	0.9898	1.04×10^{-3}	2.105	1.38	3.8	15.2	5.10	51.80
EL100 + EC	IEL20EC5	0.9632	1.83×10^{-3}	0.522	1.99	4.9	13.3	3.90	59.10
ES100 + EC	IES20EC5	0.9988	2.18×10^{-3}	4.657	0.91	8.2	25.9	69.90	1.50
PCP + EC	IPCP10EC5	0.9836	2.37×10^{-2}	0.250	2.10	4.5	16.5	10.98	52.39
	IPCP10EC10	0.9982	5.29×10^{-3}	0.152	2.20	9.2	18.2	36.32	12.87
	IPCP20EC20	0.9870	5.87×10^{-3}	0.399	1.95	8.3	16.1	13.98	54.23
CP + EC	ICP10EC5	0.9884	3.68×10^{-2}	0.032	2.84	5.4	16.3	15.31	49.87
	ICP20EC5	0.9882	5.55×10^{-3}	0.134	2.10	4.3	22.2	34.80	18.97
PCP + EL100	IPCP5EL10	0.9648	3.27×10^{-3}	1.014	1.85	4.7	11.3	13.03	53.24
	IPCP5EL20	0.9415	1.23×10^{-3}	0.164	2.08	7.1	20.5	52.70	11.80
	IPCP10EL20	0.9018	1.03×10^{-3}	1.185	1.23	7.2	33.8	12.44	7.89
PCP + ES100	IPCP5ES10	0.9648	3.27×10^{-3}	1.046	1.67	4.7	14.4	12.67	61.76
	IPCP5ES20	0.9876	1.62×10^{-3}	0.647	1.87	4.2	14.0	10.63	52.30
	IPCP10ES20	0.9623	1.23×10^{-3}	0.259	1.98	6.7	19.2	23.26	42.81
CP + EL100	ICP5EL10	0.9785	3.67×10^{-2}	1.960	1.45	4.8	14.0	14.91	52.41
	ICP10EL20	0.9913	5.28×10^{-3}	0.106	1.99	7.1	29.6	33.08	12.90
CP + ES100	ICP5ES10	0.9885	5.17×10^{-3}	1.143	1.65	5.9	14.1	14.33	53.89
	ICP10ES20	0.9913	6.03×10^{-3}	0.485	1.99	6.9	13.8	12.81	51.67

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h).

[#] Release data are compared to the theoretical target release profile. For similarity, f_1 should be < 15 and f_2 > 50.

Table 5.36b: Release kinetics of selected formulations in simulated GI fluid pH (without enzymes)

Formulation series	Batches	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]	f_1[#]	f_2[#]
XG + EL100	IXG5EL10	0.9812	2.21×10^{-3}	4.016	1.12	4.5	15.9	12.95	48.35
	IXG10EL10	0.9012	4.86×10^{-3}	10.972	0.91	4.9	14.1	2.03	71.04
XG + ES100	IXG5ES5	0.9613	3.27×10^{-3}	10.114	0.86	4.2	12.7	13.95	41.84
HEC + EL100	IHEC5EL20	0.9752	2.82×10^{-3}	18.578	0.59	5.1	14.5	12.52	53.56
	IHEC10EL20	0.9910	3.18×10^{-3}	17.861	0.59	4.4	15.5	3.95	51.20
HEC + ES100	IHEC5ES20	0.9809	1.01×10^{-3}	12.745	0.72	4.3	15.1	11.25	51.04
	IHEC10ES20	0.9898	1.74×10^{-3}	10.529	0.73	5.7	18.9	25.48	33.85
HPC + EL100	IHPC5EL10	0.9982	5.86×10^{-4}	1.288	1.45	5.1	18.7	54.96	6.20
	IHPC5EL20	0.9932	4.81×10^{-3}	0.740	1.76	5.3	15.3	12.77	51.25
HPC + ES100	IHPC5ES10	0.9900	3.29×10^{-3}	0.796	1.75	6.2	14.9	13.26	52.40
	IHPC5ES20	0.9930	5.11×10^{-3}	0.380	1.85	5.8	19.2	12.09	49.80
GG + EL100	IGG5EL20	0.9637	4.26×10^{-3}	1.023	1.86	4.5	11.1	2.03	51.00
	IGG10EL20	0.9395	1.03×10^{-3}	2.601	1.39	4.1	12.8	19.40	21.30
GG + ES100	IGG5ES20	0.9988	4.07×10^{-3}	1.007	1.67	5.7	14.6	3.95	53.80
	IGG10ES20	0.9989	1.23×10^{-3}	1.107	1.47	5.9	19.7	14.9	34.50

[a] Correlation coefficient [b] Release rate constant [c] Diffusional exponent indicative of the release mechanism [d] Time for 10% of the drug release (h) [e] Time for 90% of the drug release (h).

[#] Release data are compared to the theoretical target release profile. For similarity, f_1 should be < 15 and f_2 > 50.

5.8. Preparation and characterization of microspheres

Matrix microsphere based oral controlled release formulations were designed for indomethacin by varying the proportion of EC as the retardant material by solvent evaporation technique. Ethyl cellulose (EC) a hydrophobic water insoluble polymer has been widely used for microencapsulation of number of drugs to retard their release or improve the stability (Sajeev et al., 2002; Benita and Donbrow, 2006). In order to confer pH dependent properties for colonic delivery, EL100 and ES100 as pH sensitive polymers were added to the internal phase to be incorporated as part of the coat. The technique employed in the present study was coacervation- phase separation induced by solvent evaporation. Physical characteristics, micromeritics, and in vitro release studies were carried out to evaluate the release characteristics of drug from these microspheres.

5.8.1. Physical characterization of microspheres

The characteristics of the microspheres such as drug entrapment efficiency and release of drug to a large extent depend on several factors like the aqueous solubility of the drug, the type of organic solvent or solvent mixture used, the phase ratio of the emulsion system, the temperature, and the type and concentration of emulsion stabilizers (Bodmeier et al., 1987).

The drug content per 50 mg of microspheres, entrapment efficiency, particle size distribution and yield for each formulation is given in Table 5.37. The microspheres prepared with differing internal: external phase ratios resulted in microspheres with varied physical characteristics (Fig 5.48). Only microspheres prepared with both 1:1 and 1:9 internal: external phase ratio revealed acceptable physical characteristics in terms of sphericity and free flow (absence of agglomeration) for microspheres (Fig 5.48). The probable reason could be that more uniform dispersion was obtained in case of 1:1 and 1:9 internal to external phase ratios that resulted in better and more uniform encapsulation. The microsphere surface was smooth, but exhibited tiny pores in some cases. Further, microspheres prepared with internal to external phase ratio of 1:1 and 1:9 resulted in higher drug loading and entrapment efficiency. The particle size was found to be dependent on internal to external phase ratio (Table 5.37) as these ratios impacted uniformity of emulsion formed. The particle size distribution was found to be lowest in case of M1 (1:1) and M5 (1:9). When the relative proportion of EC was varied at two different internal to external phase ratio within each series, particle size marginally increased with increase in EC proportion (Fig 5.49). However, lower particle size was

observed in case of higher liquid paraffin content (1:9 internal to external phase ratio) in the emulsion system (Table 5.37). This may be due to relatively higher shearing rate imparted to the system (at 1:9 phase ratio) due to low volume of internal phase in case of M5EC1 resulting in the formation of smaller emulsion droplets and thereby creating relatively smaller microspheres.

In case of microspheres of EC prepared in combination with EL100 or ES100, the average particle size was found to be distributed over a narrow range of about 70-80 µm. With increase in proportion of EL100 or ES100, the particle diameters did not change significantly (Table 5.37). This was considered advantageous as particle size smaller than 200µ show predictable gastric emptying and reproducible GI transit (Hardy et al., 1988; Watts et al., 1992). Further, optical microscopy revealed spherical shape and smooth surface of microspheres implying formation of homogenous polymer mixture and uniform distribution of coat around the core as shown in Fig 5.50(a) and (b) for M1EC1EL1 and M1EC1ES1 respectively. The product yield was good (about 90%) in all the cases, drug content was about 70-80% with high entrapment efficiency of 70-85% indicating feasibility of the method as opposed to previous studies that have shown that an oil/oil emulsification method might not be a suitable choice for microencapsulating lipophilic drugs (Lamprecht et al., 2004). It could be concluded that the internal to external phase ratio and the relative proportion of polymers employed affect the nature and yield of microspheres formed.

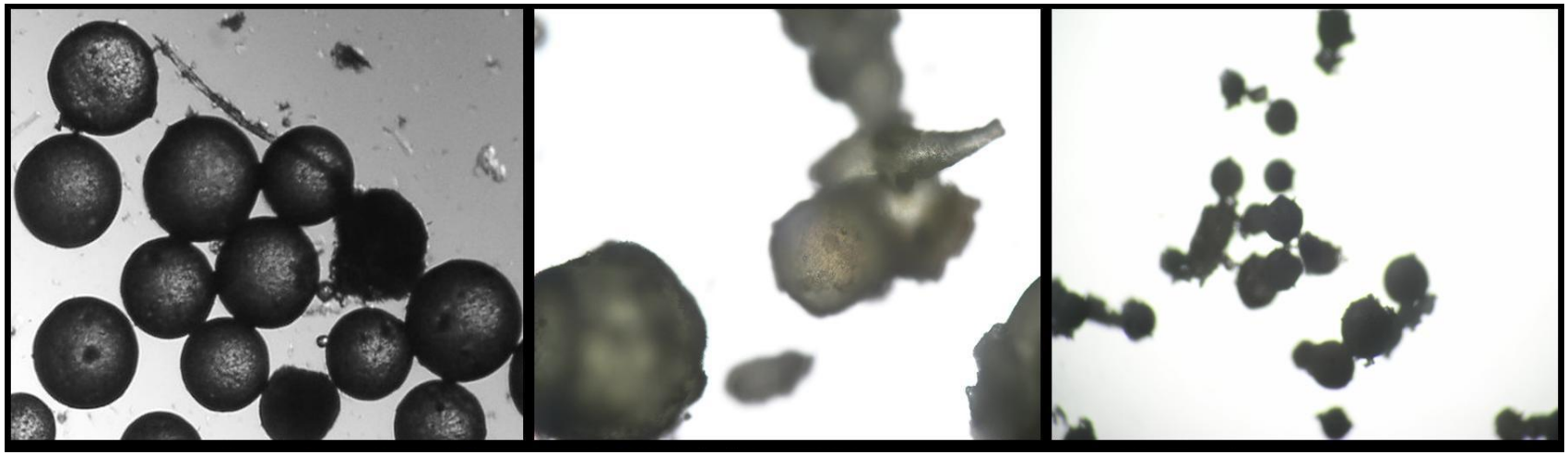
5.8.2. In vitro release studies of designed microspheres

The in vitro release profiles were studied in terms of n, $t_{10\%}$, and $t_{90\%}$ release. In vitro release studies of the EC microparticles in differing ratios of internal to external phase showed that the time for 10% ($t_{10\%}$) indomethacin release was extended to 2.4 h for M1 (internal to external phase ratio 1: 1) to 7.0 h for M5 (internal to external phase ratio 1: 9). Similarly, time for 90% ($t_{90\%}$) indomethacin release was extended to 22.3 h for M1 (internal to external phase ratio 1: 1) to 33.5 h for M5 (internal to external phase ratio 1: 9). The in vitro release profiles are shown in Fig 5.51 and corresponding data is presented in Table 5.38. The difference in release rates between these formulations (M1 to M5) with varying internal to external phase ratio is attributed to the nature of microparticles formed.

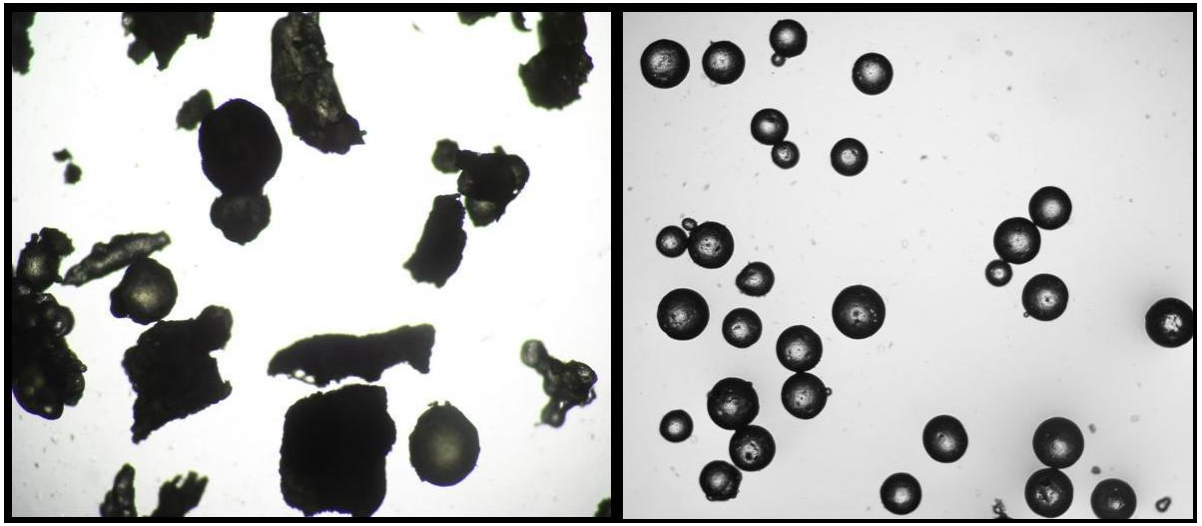
Table 5.37: Physical characterization of microspheres

Batches	Yield (%)	Drug Content [a] (mg)	Entrapment efficiency (%)	Particle size [b] (µm)	Physical appearance
M1	95.3	39.98 ± 2.8	79.95	132.6 ± 23.8	Spherical, discrete
M2	91.3	38.22 ± 5.7	76.44	353.86 ± 140	Spherical ,agglomerated
M3	87.4	39.02 ± 6.7	78.03	152.1 ± 55.7	Irregular in shape
M4	90.0	38.48 ± 4.8	76.97	161.2 ± 60.7	Slightly irregular in shape
M5	97.4	35.45 ± 3.8	70.91	62.2 ± 24.8	Spherical, discrete
M1EC1	95.2	36.88 ± 6.5	73.76	131.9 ± 66.9	Spherical, discrete
M1EC2	82.7	41.52 ± 4.7	83.03	133.2 ± 54.9	Spherical, discrete
M1EC3	88.5	39.40 ± 2.3	78.80	159.1 ± 16.8	Spherical, discrete
M5EC1	92.2	40.99 ± 3.8	81.99	87.4 ± 29.7	Spherical, discrete
M5EC2	90.6	37.32 ± 2.4	74.65	114.5 ± 47.2	Spherical, discrete
M5EC3	89.7	35.77 ± 1.2	71.53	120.4 ± 17.4	Spherical, discrete
M1EC1EL1	92.3	42.08 ± 1.9	84.16	72.7 ± 35.7	Spherical, discrete
M1EC1EL2	83.8	40.12 ± 2.6	80.23	74.2 ± 28.8	Spherical, discrete
M1EC1EL3	89.2	38.08 ± 3.4	76.15	80.4 ± 31.5	Spherical, discrete
M1EC1ES1	89.5	40.20 ± 4.9	80.41	79.5 ± 45.1	Spherical, discrete
M1EC1ES2	86.9	35.47 ± 5.7	70.94	78.1 ± 46.8	Spherical, discrete
M1EC1ES3	89.9	41.95 ± 6.3	83.89	81.2 ± 38.7	Spherical, discrete
M5EC1EL1	94.4	35.21 ± 4.8	70.43	76.5 ± 43.8	Spherical, discrete
M5EC1ES1	90.6	37.52 ± 2.5	75.03	79.9 ± 42.9	Spherical, discrete

[a] Expressed as mg /per 50 mg of microparticles from 3 batches; [b] mean ± SD of 100 particles from 3 batches



(a) (b) (c)



(d) (e)

Fig 5.48: Images of microspheres prepared using varying internal : external phase ratio (a) M1 (1:1), (b) M2 (1:3), (c) M3 (1:5), (d) M4 (1:7), (e) M5 (1:9)

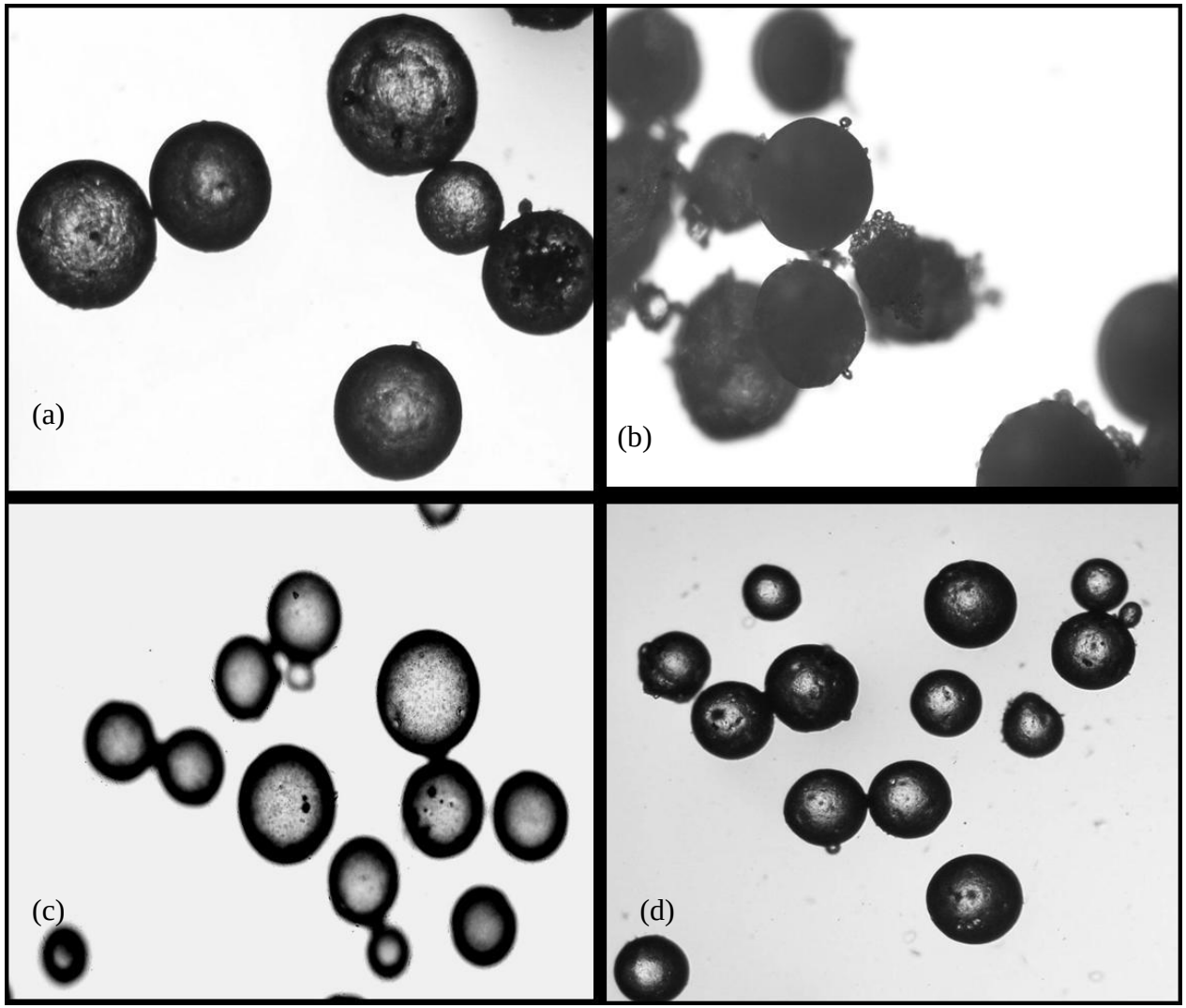
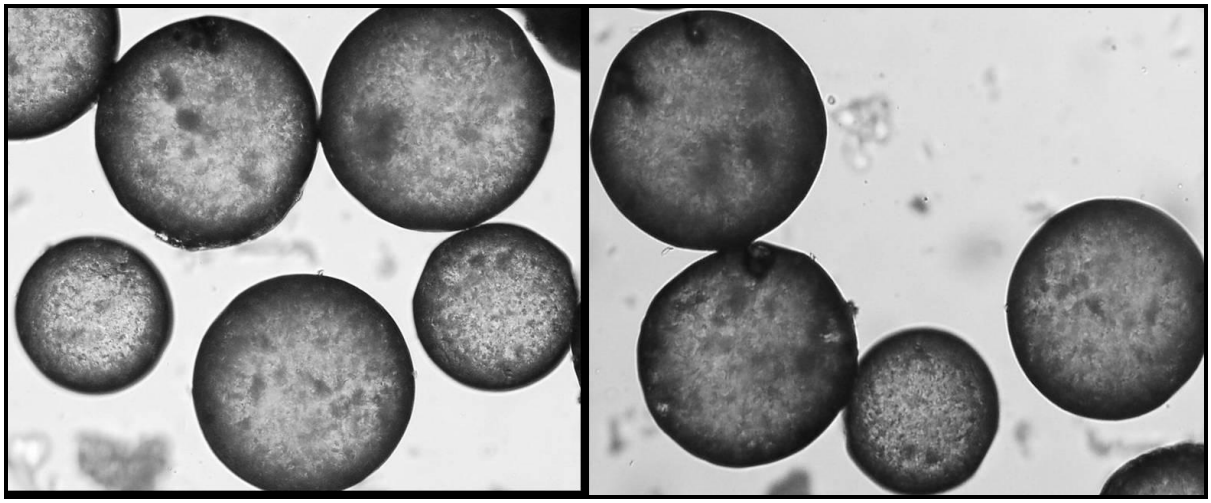


Fig 5.49: Image of microspheres from batches showing effect of varying proportion of EC (a) M1EC1 (1.25% w/w) and (b) M1EC1 (5% w/w) at 1:1 internal to external phase ratio and (c) M5EC1 (1.25% w/w) and (d) M5EC1 (5% w/w) at 1:9 internal to external phase ratio (at 100X magnification).



(a) **(b)**

Fig. 5.50: Photograph of microspheres prepared from batches showing EC in combination with (a) EL100 (M1EC1EL1) (b) ES100 (M1EC1ES1) at 400X magnification.

The formulations M2, M3 and M4 were either agglomerated or irregular in shape, thus affecting surface area and matrix properties. Only internal to external phase ratio of 1:1 and 1:9 resulted in discrete, free flowing and spherical particles.

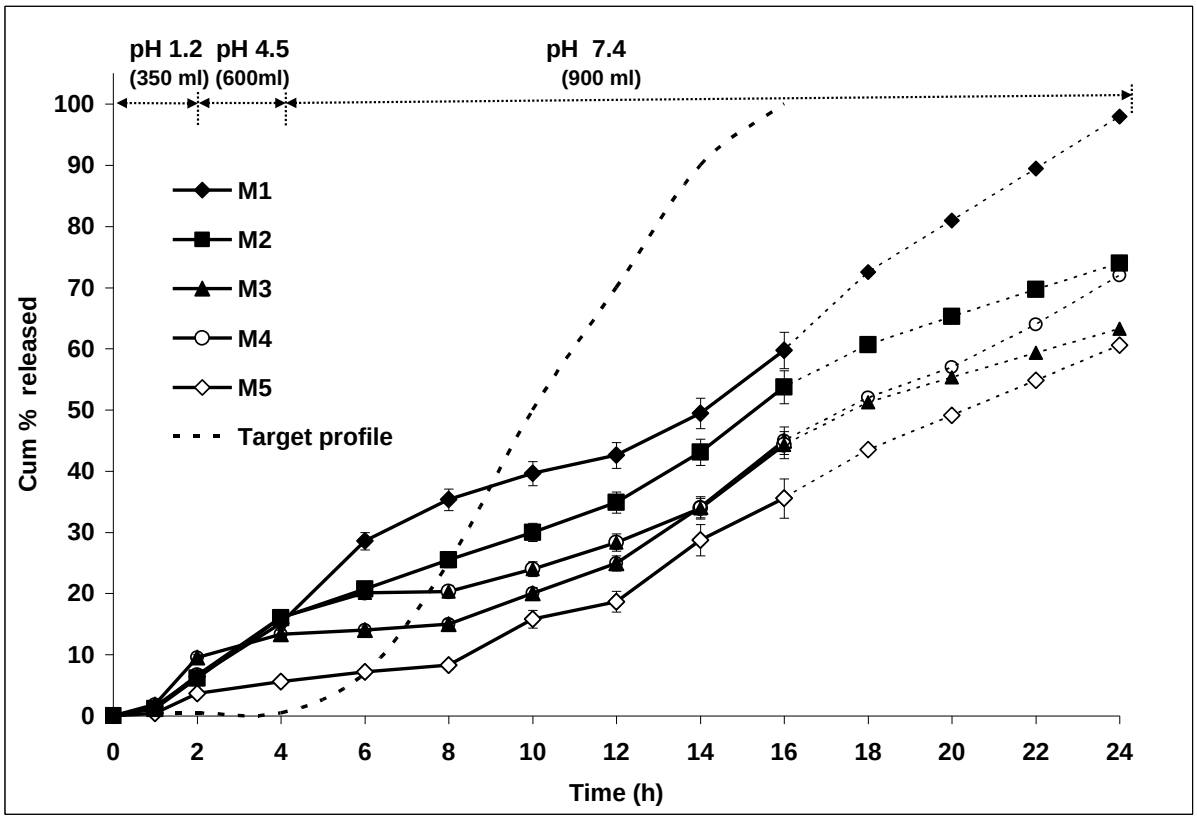


Fig 5.51: Release profile of indomethacin from EC microspheres with varying internal: external phase ratio. Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Between M1 and M5, the drastic change in external phase ratio from 1 part to 9 parts might have affected the degree of agitation and the evaporation of internal phase (acetone)

that in turn affected the size and thickness of the coat of the microparticles, thereby affecting drug release rates. Further, higher retardation in release in case of M3, M4 and M5 could be attributed to the presence of lipophilic surfactant (Span 80) that formed a protective outer layer that could not be removed after washing with petroleum ether (Lamprecht et al., 2004). The microspheres prepared with internal to external phase ratio of 1:1 and 1:9 showed desirable physical attributes, and release profiles and therefore, further studies were carried out at internal to external phase ratio of both 1:1 and 1:9

In case of formulations prepared using EC with internal to external phase ratio of 1:1, varying proportion of EC in the coating solution extended the release of 90% drug from 14.2 h (M1EC1) to 24.3 h (M1EC3) corresponding to increase in concentration of EC from 1.25% to 5% w/w of drug (Table 5.38, Fig 5.52). The results indicate that increasing the proportion of EC decreased the release rate which may be attributed to the slower rate of diffusion of dissolution medium into the microspheres due to increased thickness of the coat. The extent of surface pores decreased with decreasing core: coat ratio (Sajeev et al., 2002).

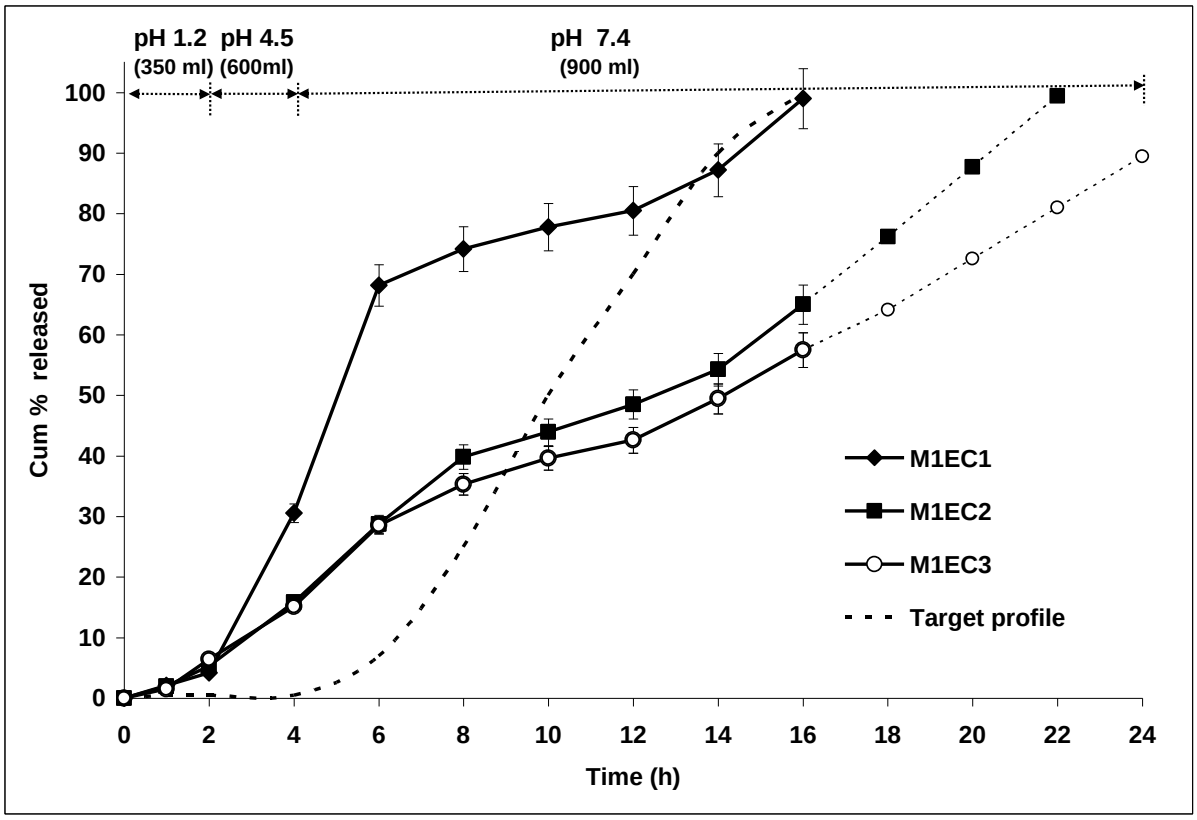


Fig 5.52: Release profile of indomethacin from microspheres with varying proportion of EC (phase ratio 1:1). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

This indicates absence of burst effect as the drug is present in a dispersed form inside the microsphere matrix making negligible to low drug availability on matrix microsphere surface.

When internal to external phase ratio was changed to 1:9 from 1:1 and the proportion of EC was varied, similar type of effect was observed. The drug release was more retarded with increase in EC concentration in the microsphere (Fig 5.53, Table 5.38). The $t_{10\%}$ of indomethacin release was extended from 1.1 h for M5EC1 (1.25% EC) to 3.1 h for M5EC3 (5% EC) while the $t_{90\%}$ of indomethacin release was extended from 5.8 h for M5EC1 to 31.3 h for M5EC3. More extended release in case of microspheres with 1:9 phase ratio as compared to 1:1 can be attributed to better uniformity in emulsion formation at higher proportion of external phase. The diffusion exponent from the power equation indicated super case II release from all these formulations (Table 5.38).

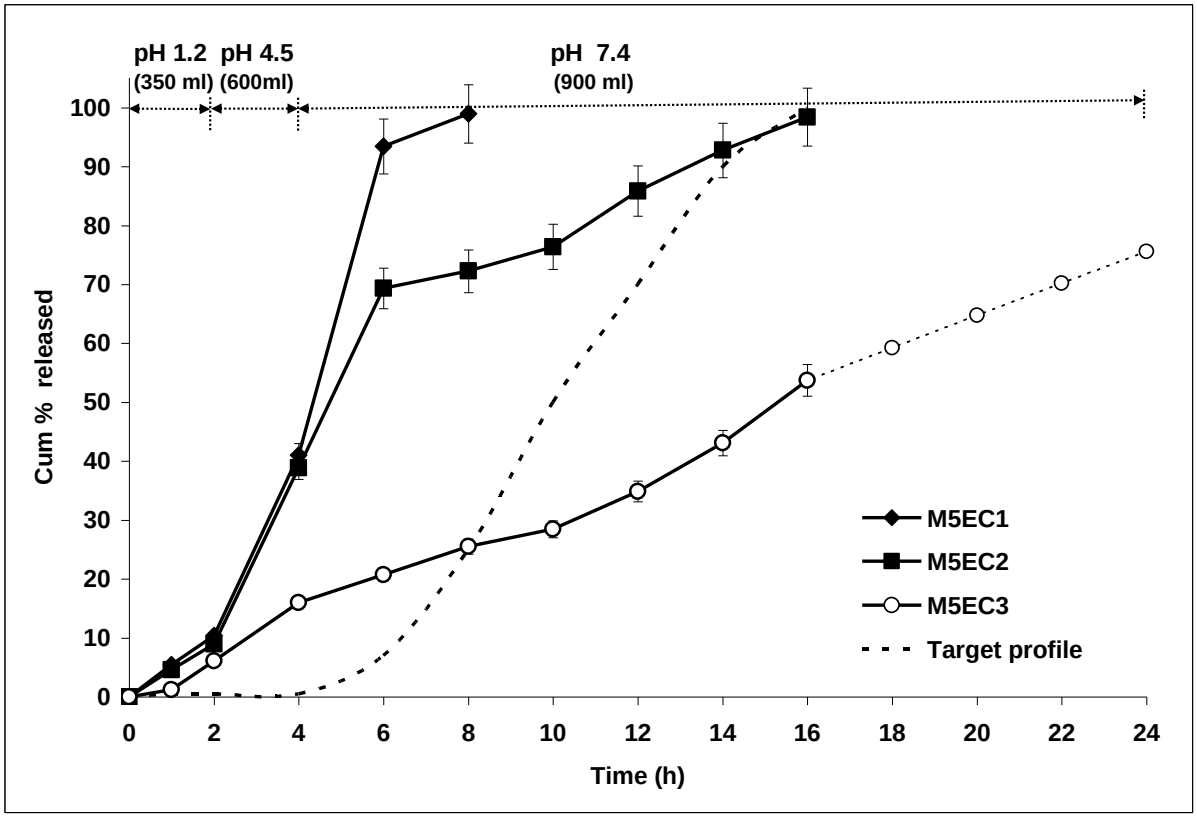


Fig 5.53: Release profile of indomethacin from microspheres with varying proportion of EC (phase ratio 1:9). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

In case of formulations prepared with EC at 1.25% w/v of internal phase and varying proportion of EL100 (0.5 to 1.25% w/v of internal phase) with internal to external phase ratio of 1:1, in vitro release studies indicate significant increase in $t_{10\%}$ from 0.7 h for microparticle prepared with EC alone (M1EC1) to 4.0 h for M1EC1EL1. This was found to increase further to 5.9 h for M1EC1EL3 with increase in proportion of EL100 (Fig 5.54). Similarly, the $t_{90\%}$ increased from 14.2 h for M1EC1 to 14.7 h for M1EC1EL1 which further extended to 15.9 h for M1EC1EL3. All microspheres with EC and EL100 combinations were found to

approach the ideal theoretical target release profile and were considered suitable for site specific release to colon. Eudragit L100 underwent gradual erosion at pH values exceeding the threshold of polymer ionization (pH 6.0) which enhanced matrix erosion and resulted in enhanced rate of release (Mehta et al., 2001). The diffusional exponent 'n' from the power law equation indicated super case II mechanism which is again indicative of erosion process. The drug release was found to depend on both EC and EL100 concentration in the matrix base. An increase in proportion of EL100 resulted in more modulated release as observed in case of M1EC1EL2 and M1EC1EL3. Increase in total polymer content could have decreased penetration of dissolution medium into the microspheres, and thereby resulting in slower release (Fig 5.54).

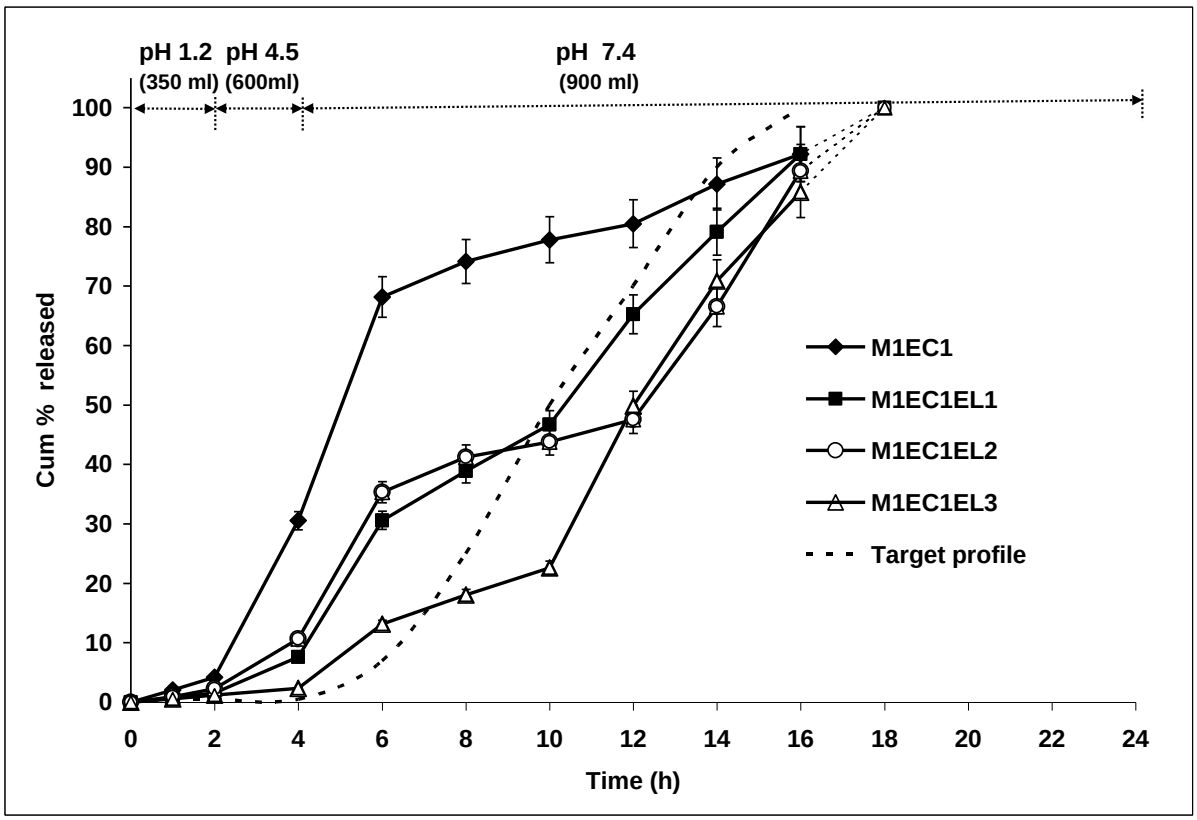


Fig 5.54: Release profile of indomethacin from EC microspheres with varying proportion of EL100 (phase ratio 1:1). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

Similarly, in case of formulations prepared with EC at 1.25% and varying proportion of ES100 (0.5 to 1.25% w/v of internal phase) with internal to external phase ratio of 1:1, in vitro release studies indicate significant increase in $t_{10\%}$ from 0.7 h for microparticles prepared with EC alone (M1EC1) to 4.9 h for M1EC1ES1. Increase in proportion of ES100 extended the $t_{10\%}$ significantly to 5.5 h observed for M1EC1ES3 (Fig 5.55). The $t_{90\%}$ increased from 14.2 h M1EC1 to 17.5 h for M1EC1ES1 with further extension to 19.2 h for

M1EC1ES3. Here, both M1EC1ES1 and M1EC1ES2 were found to follow desirable release pattern with release profile manifesting super case II release indicating matrix erosion and polymer relaxation as mechanism of release.

Table 5.38: Release parameters for microparticulate based formulations

Internal : external phase ratio	Formulation	Release kinetics					
		r^a	MSSR	K^b	n^c	$t_{10\%}{}^d$	$t_{90\%}{}^e$
a) Release parameters of indomethacin-EC microspheres with different internal to external phase ratio.							
1:1	**M1**	0.9805	3.46×10^{-3}	1.857	1.25	2.4	22.3
1:3	**M2**	0.9412	2.74×10^{-2}	4.134	0.94	3.1	26.5
1:5	**M3**	0.9522	2.89×10^{-2}	2.143	1.10	4.1	29.9
1:7	**M4**	0.9639	3.97×10^{-2}	0.712	1.40	3.5	31.7
1:9	**M5**	0.9638	1.29×10^{-2}	0.637	1.41	7.0	33.5
b) Release parameters of indomethacin microspheres with varying EC proportion							
	M1EC1	0.9560	3.62×10^{-2}	1.870	1.46	0.7	14.2
1:1	**M1EC2**	0.9882	3.14×10^{-3}	2.039	1.26	2.3	20.2
	M1EC3	0.9797	2.27×10^{-3}	1.668	1.25	2.4	24.3
	M5EC1	0.9807	3.63×10^{-3}	12.788	1.11	1.1	5.8
1:9	**M5EC2**	0.9781	4.63×10^{-3}	5.747	1.06	2.0	13.4
	M5EC3	0.9671	1.56×10^{-2}	2.420	1.05	3.1	31.3
c) Release parameters of indomethacin-EC microspheres with varying EL100 proportion							
1:1	**M1EC1EL1**	0.9850	2.11×10^{-3}	1.396	1.55	4.0	14.7
	M1EC1EL2	0.9860	3.47×10^{-3}	1.572	1.45	4.7	16.3
	M1EC1EL3	0.9698	2.84×10^{-2}	2.091	1.36	5.9	15.9
1:9	**M5EC1EL1**	0.9819	4.19×10^{-3}	5.896	0.96	3.9	17.1
d) Release parameters of indomethacin-EC microspheres with varying ES100 proportion							
	M1EC1ES1	0.9782	3.40×10^{-3}	1.733	1.38	4.9	17.5
1:1	**M1EC1ES2**	0.9659	3.01×10^{-2}	1.208	1.50	5.3	17.7
	M1EC1ES3	0.9617	1.13×10^{-2}	1.102	1.49	5.5	19.2
1:9	**M5EC1ES1**	0.9959	1.61×10^{-4}	4.829	0.97	3.7	20.4

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

Formulation M1EC1ES3, although extended the release beyond 20 h, yet was found to be unsuitable for colonic delivery as more than 30% of drug was left remaining to be released at the end of 16 h. Between EL100 and ES100 based formulations, slightly higher retardation in release rate was observed for EC+ES100 matrices, which could be because of the higher pH range (> 7.0) required for dissolution of ES100 due to the lower percentage of methacrylic acid units in ES100 than EL100 (Ashford et al., 1993).

With internal to external phase ratio of 1:9, three formulations were studied; microparticles prepared with 1.25% EC (M5EC1) alone and those prepared with 1.25% EC and 1.25% of either EL100 (M5EC1EL1) or ES100 (M5EC1ES1) respectively (Fig 5.56). It was observed that release kinetics was significantly altered in presence of pH sensitive polymers. The $t_{10\%}$ was significantly extended in case of EC microparticles with EL100 (M5EC1EL1) and ES100 (M5EC1ES1) with $t_{10\%}$ of 3.9 h and 3.7 h respectively which differed significantly from M5EC1 ($t_{10\%}$ of 1.1 h). The extent of release in terms of $t_{90\%}$ was similarly prolonged from 5.8 h for M5EC1 to 17.1 h for M5EC1EL1 and 20.4 h for M5EC1ES1 respectively.

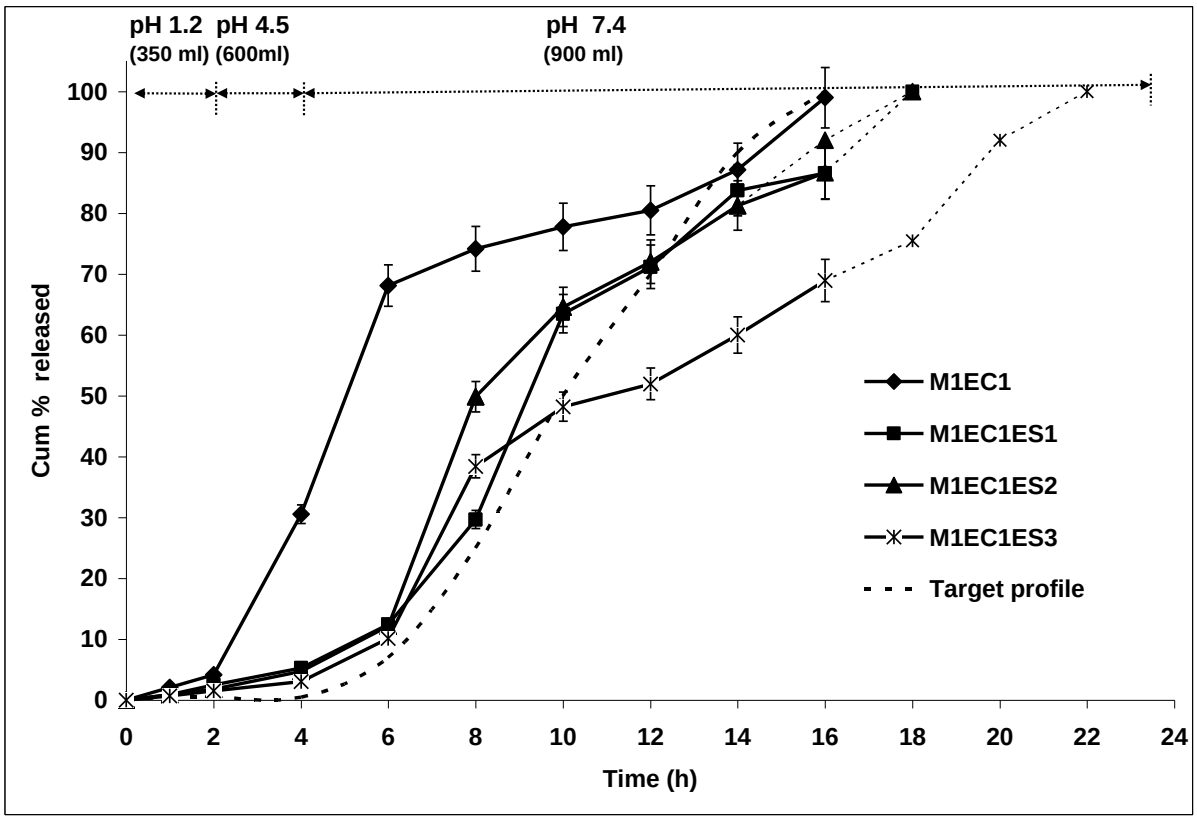


Fig 5.55: Release profile of indomethacin from EC microspheres with varying proportion of ES100 (phase ratio 1:1). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

The present study has shown that pH sensitive polymers EL100 and ES100 can be employed to form matrix base microspheres with ethyl cellulose and can modulate pH dependent and transit time based indomethacin release from EC microspheres with controlled release characteristics. EL100 and ES100 in combination with EC in acetone and liquid paraffin emulsion resulted in homogenous polymer dispersion giving good microspheres with pH dependent release profile. Previously, oil/oil emulsion based microencapsulation of drug loaded hydrophobic core with EL100 and ES100 were reported using acetone/2-propanol mixture with liquid paraffin for ondensetron and budesonide (Rodriguez et al., 1998). These microparticles were reported to possess pH dependent release profile. However, incorporating

EL100 or ES100 along with a hydrophobic polymer (EC) to form microspheres possessing pH and transit time dependent release has not been reported earlier.

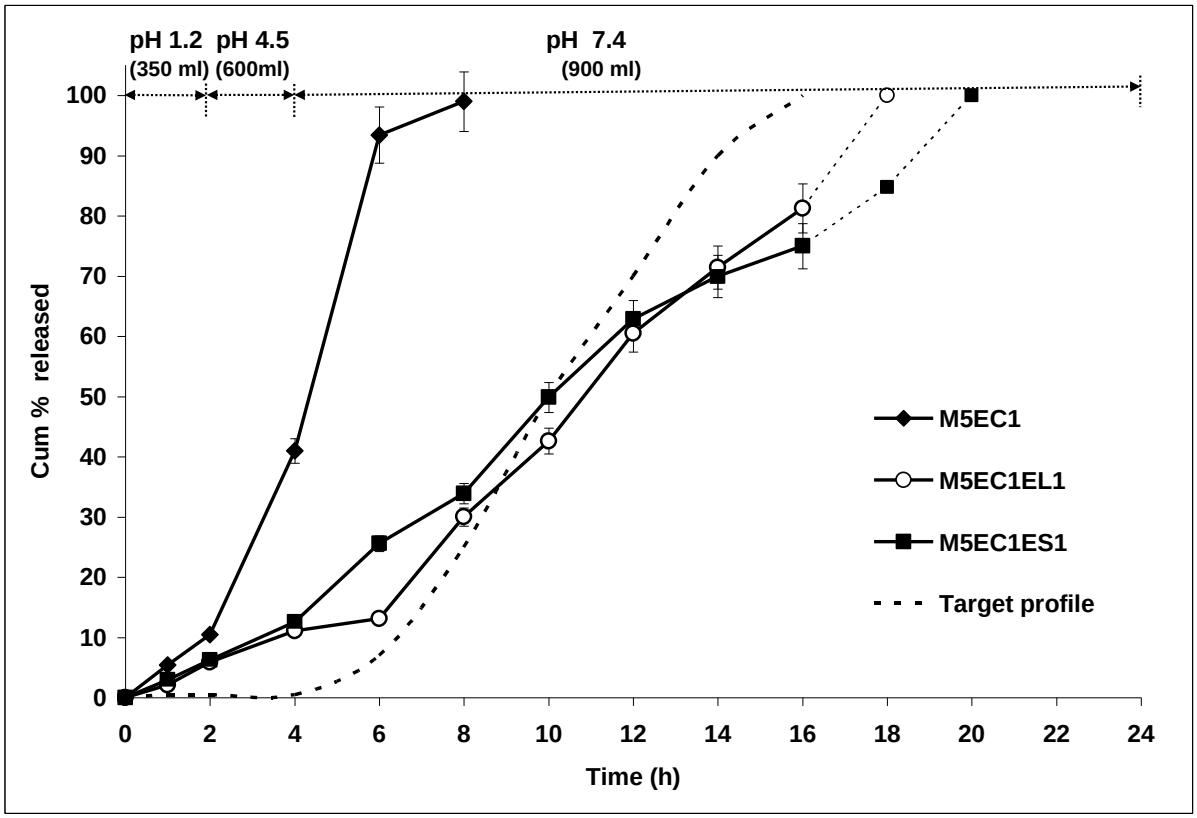


Fig 5.56: Release profile of indomethacin from EC microspheres with EL100 / ES100 (phase ratio 1:9). Each data point represents the average of two batches done in triplicate with standard deviation. Each dotted line represents the predicted release profile beyond 14 h upto 24 h based on power law equation.

For designed microspheres, the values of 'n' ranged from 0.9 to 1.46, across all the series of formulations indicating a release mechanism to be case II to super case II indicating polymer relaxation and erosion as the primary factors governing drug release. At lower pH, either hydrophobicity of EC or inertness of EL100 and ES100 in acidic environment contribute to low drug release. Whereas at weakly acidic to neutral pH, ionization of methacrylic acid moiety in EL100 and ES100, cause electrostatic repulsion of polymer chains that disrupt the matrix and result in enhanced drug release. Further, erosion of EL100 and ES100 at higher pH results in the formation of pores in the matrix which contribute to enhanced drug release (Akiyama et al., 1994).

5.9. Batch reproducibility and stability on storage

No significant difference was observed in the release profile of different batches of each matrix formulation, indicating that the manufacturing process employed was reliable and reproducible. Further, there was no change in the physical appearance of the different formulations at the end of the six month storage period at accelerated conditions 40 ± 2°C/75 ± 5% RH. The formulations were also subjected to estimation of drug content, in vitro drug

release and DSC and FTIR studies upon storage (Fig 5.57 & 5.58). There was no significant change in drug content (Table 5.39). The predicted $T_{90\%}$ values from batches stored at controlled as well as accelerated conditions indicate a stable shelf life for span of 2-3 years.

Table 5.39: Stability data of prepared formulations

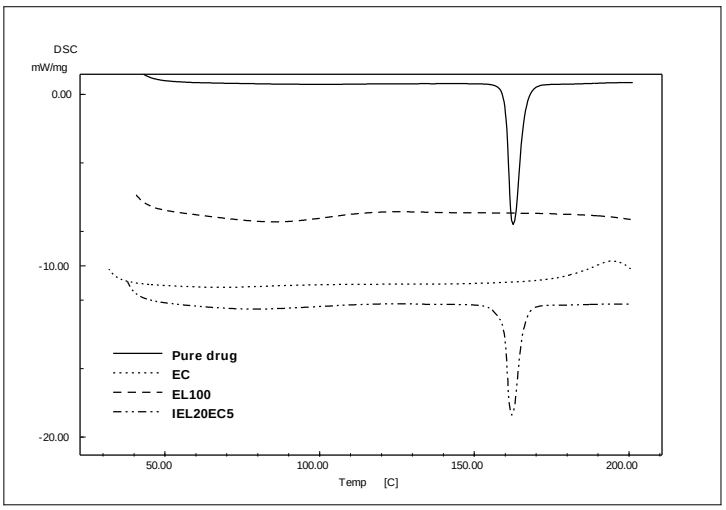
Batches		CRT			ATC	
Formulation code	**Physical appearance ***	**K_{deg} x 10^3 ($month^{-1}$)**	**$T_{90\%}$ (months)**	**Physical appearance***	**K_{deg} x 10^3 ($month^{-1}$)**	**$T_{90\%}$ (months)**
IEL20EC5	Unchanged	2.686	39.2	Unchanged	3.280	32.1
IEL15ES10	Unchanged	3.250	32.4	Unchanged	4.179	25.2
IPCP5ES20	Unchanged	2.171	48.5	Unchanged	2.304	45.7
ICP5EL10	Unchanged	2.299	45.8	Unchanged	2.909	36.2
IGG5ES20	Unchanged	4.113	25.6	Unchanged	6.017	17.5
IXG10EL10	Unchanged	3.052	34.5	Unchanged	3.669	28.7
IHEC5EL20	Unchanged	2.885	36.5	Unchanged	3.134	33.6
IHPC5ES10	Unchanged	2.816	37.4	Unchanged	3.210	32.8
MIEC1EL3	Unchanged	3.522	29.9	Unchanged	4.406	23.9

K_{deg} - first order degradation rate constant; $T_{90\%}$ - time taken for drug to degrade to 90% of the labeled claim; * at the end of six month storage period

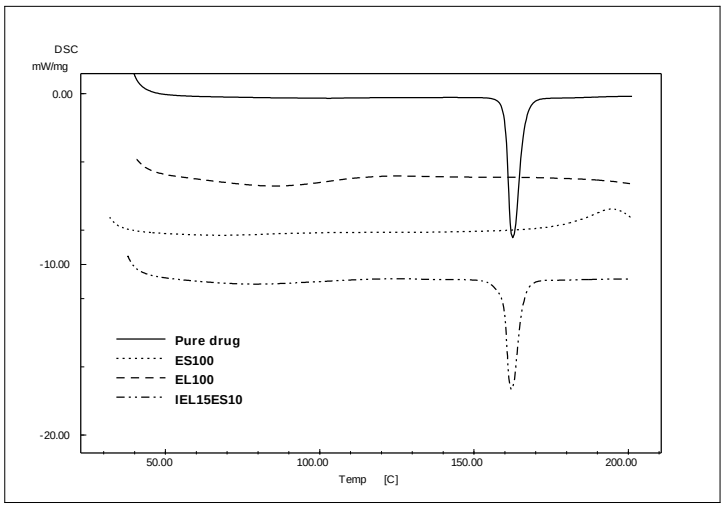
Further, in vitro release studies carried out on the formulations stored at accelerated test conditions for six months indicated no statistically significant change in the drug release profiles when compared to formulations analyzed at zero time. These results imply good stability of the different products on long term storage.

DSC thermogram of indomethacin revealed a sharp melting endothermic peak of the drug at 161°C which corresponds with the melting point of pure drug (158-161°C) with an enthalpy value of -57.2 J/g. There was little or no difference between the endothermic peak obtained for the pure drug and the different formulations before and after storage (Fig 5.57 a, b & c). A slight reduction in enthalpy value to 52.3 J/g with little broadening of the endothermic peak was observed in some cases which might be due to the mixing process that lowers the purity of the different components (Verma and Garg, 2001).

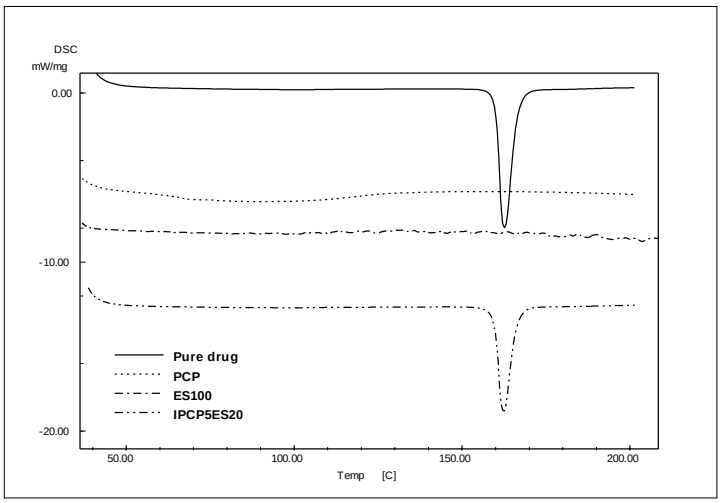
The FTIR spectrum for pure indomethacin revealed peaks at 1730 cm^{-1} for the carbonyl group of –COOH, a broad peak from 2000 to 3000 cm^{-1} for the –OH group and a peak at 1375 cm^{-1} due to stretching of $–CH_3$ group (Fig 5.58 a, b & c). There was no significant change in the IR spectrum of drug in formulations. All peaks due to the different functional groups of pure drug were well preserved even after storage at accelerated conditions for a period of six months implying absence of chemical interaction between the drug and the formulation excipients.



(a)

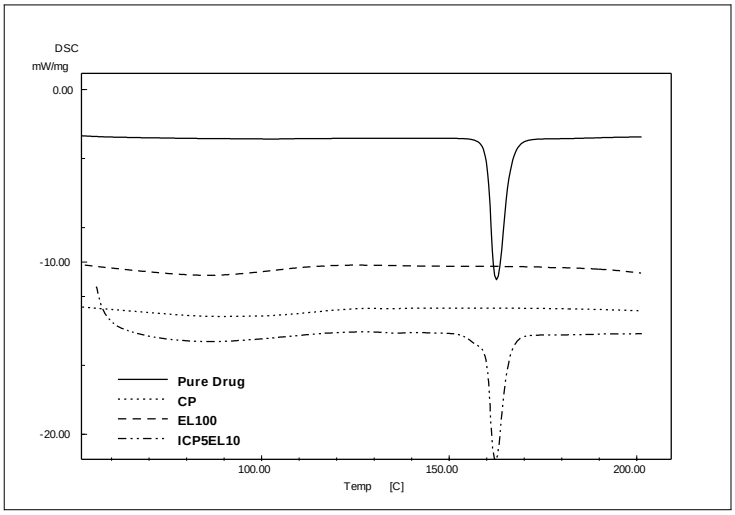


(b)

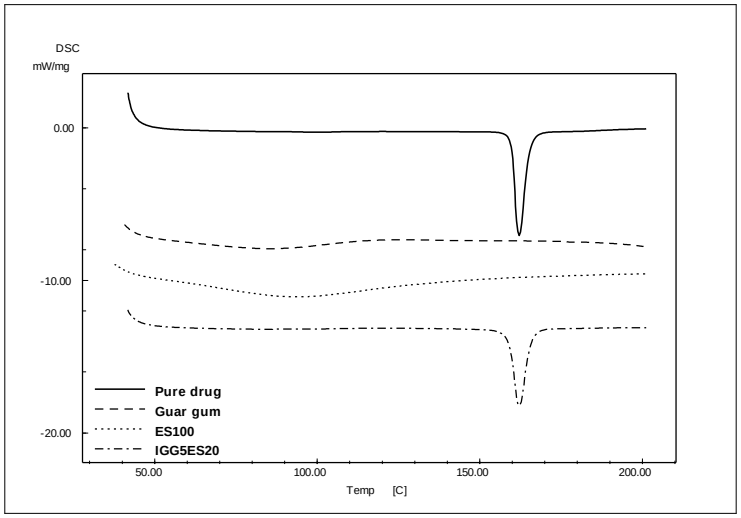


(c)

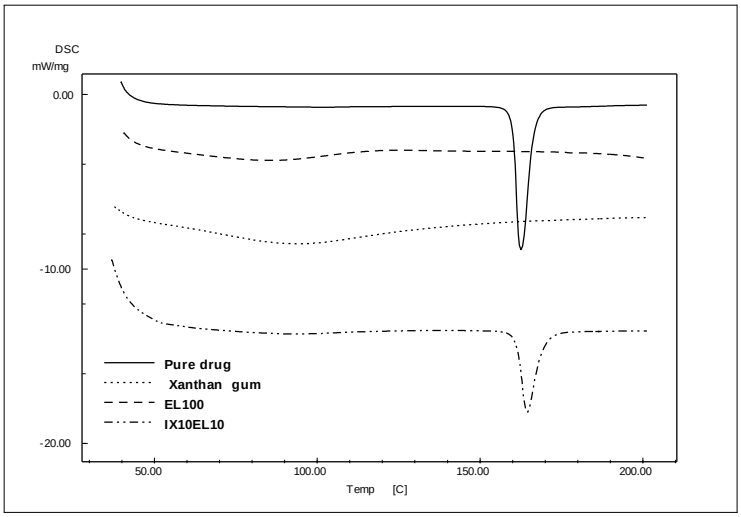
Fig 5.57 a: Representative DSC thermograms of formulations stored at accelerated conditions for six months (a) IEL20EC5 (b) IEL15ES10 (c) IPCP5ES20



(a)

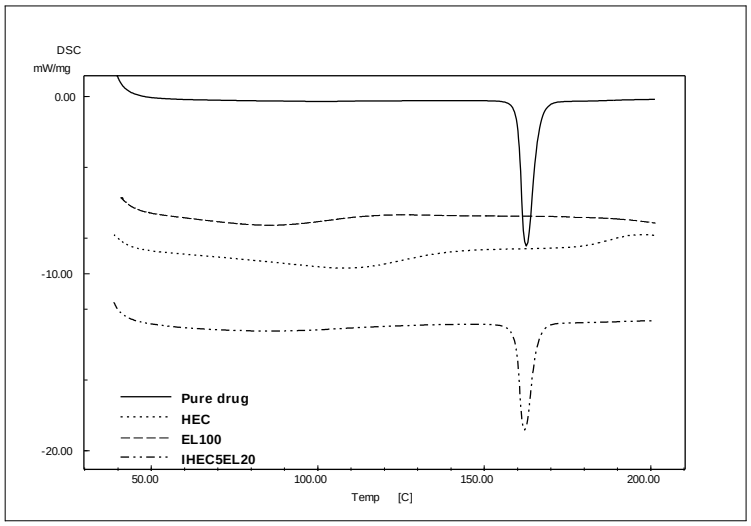


(b)

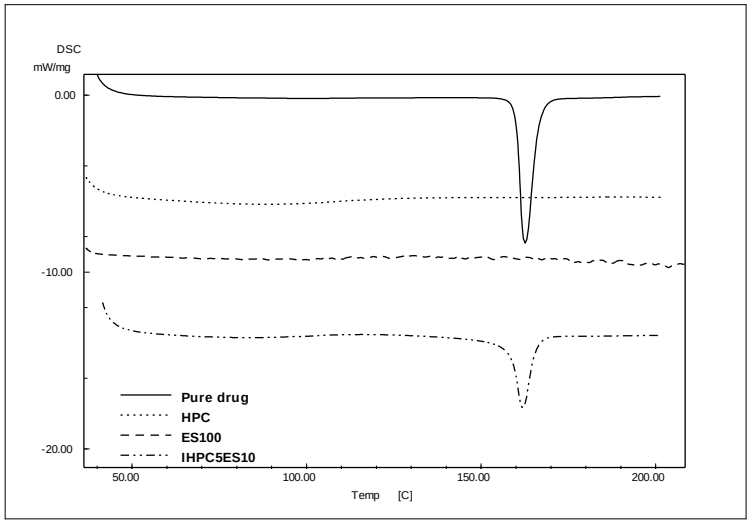


(c)

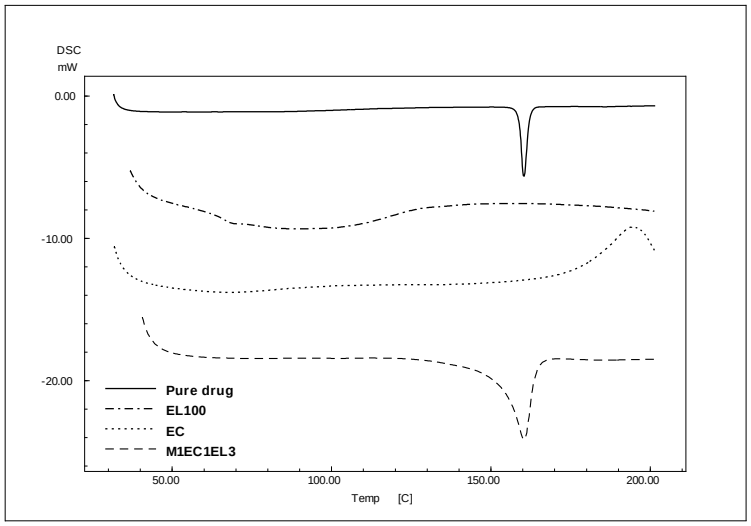
Fig 5.57 b: Representative DSC thermograms of formulations stored at accelerated conditions for six months (a) ICP5EL10 (b) IGG5ES20 (c) IXG10EL10



(a)

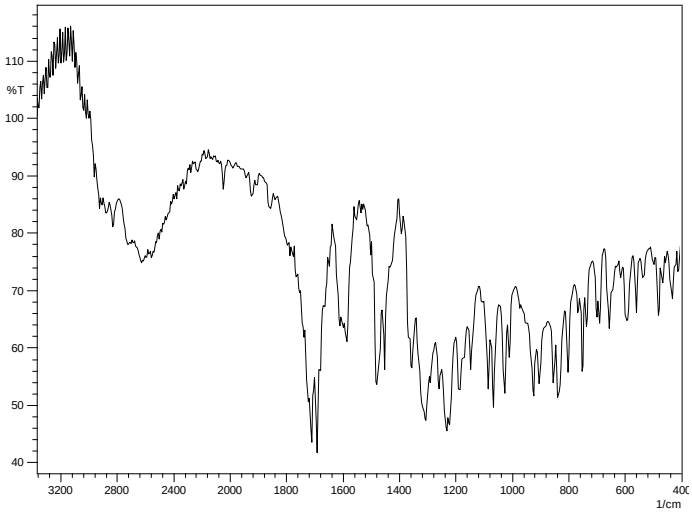


(b)

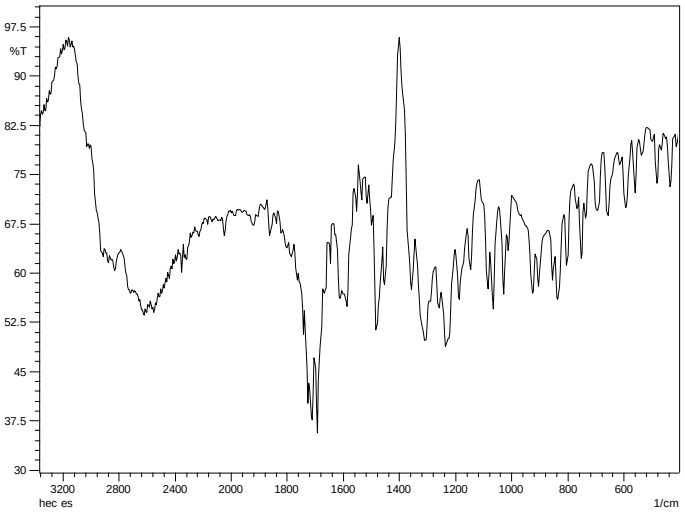


(c)

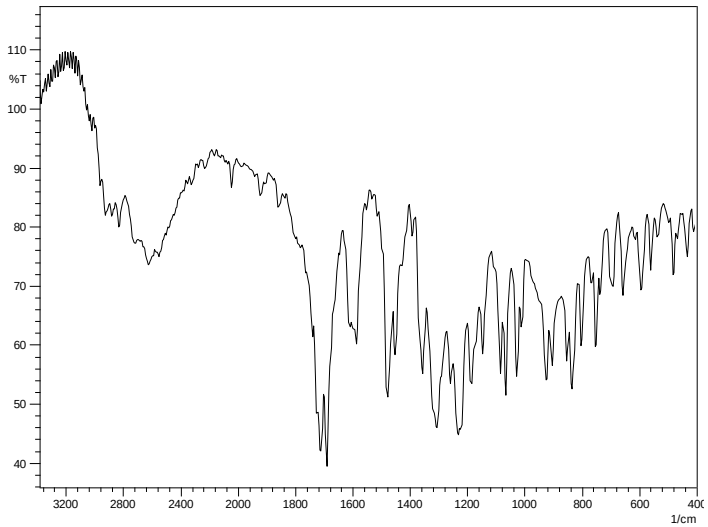
Fig 5.57c: Representative DSC thermograms of formulations stored at accelerated conditions for six months (a) IHEC5EL20 (b) IHPC5ES10 (c) M1EC1EL3



(a)

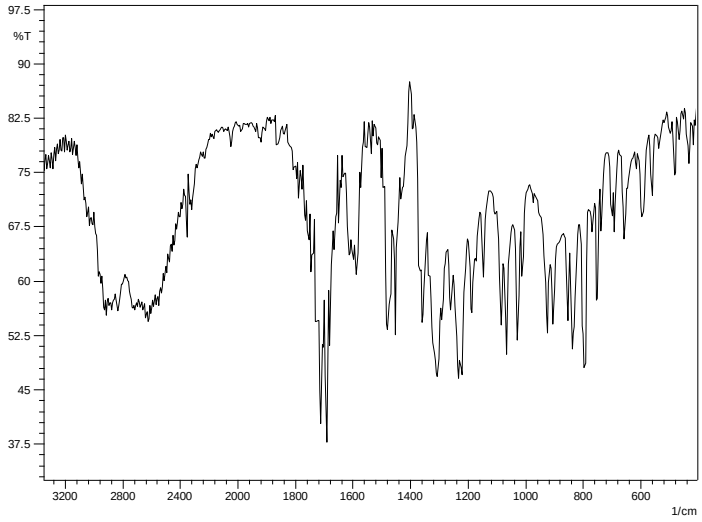


(b)

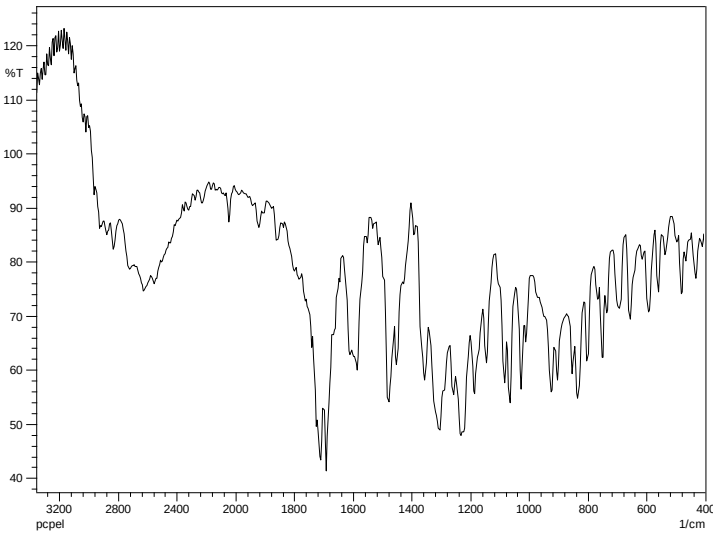


(c)

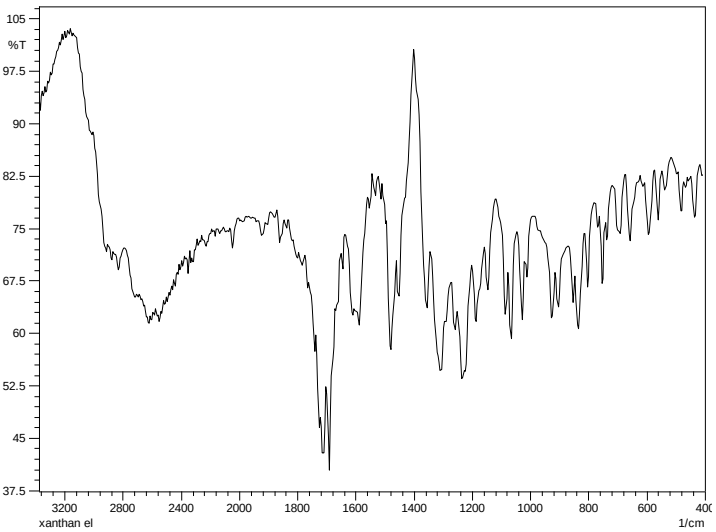
Fig 5.58a: Representative IR spectra of stability samples of formulations (a) IEL20EC5 (b) IEL15ES10 (c) IPCP5ES20



(a)

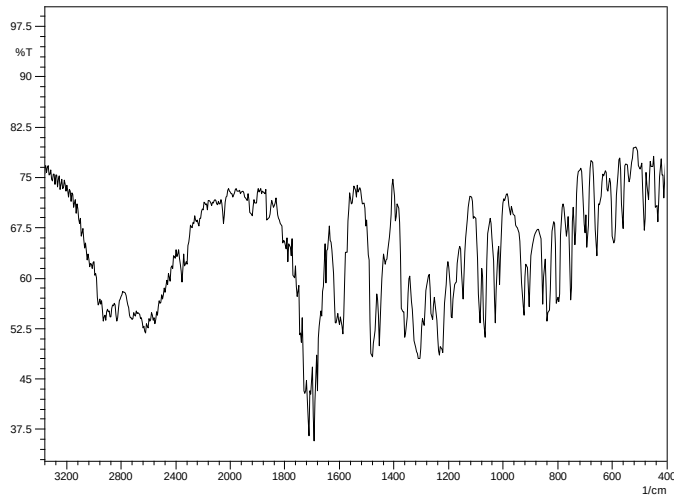


(b)

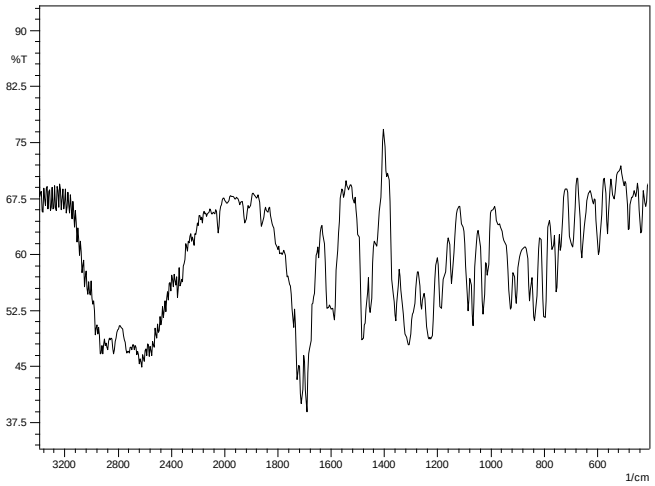


(c)

Fig 5.58b: Representative IR spectra of stability samples of formulations (a) ICP5EL10 (b) IGG5ES20 (c) IXG10EL10



(a)



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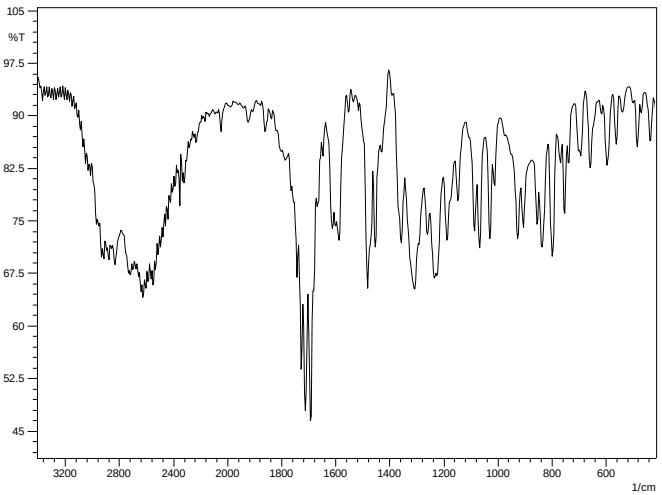


(c)

Fig 5.58c: Representative IR spectra of stability samples of formulations (a) IHEC5EL20 (b) IHPC5ES10 (c) M1EC1EL3

5.10. In vivo evaluation of selected formulations in animal model (Wistar rat)

The preclinical in vivo fate of several drugs and dosage forms has been conventionally studied using suitable rodent models (Kararli, 1995). Studies have shown the suitability of the rat model in generating data pertaining to the absorption and other pharmacokinetic parameters of drugs (Haruta et al., 2002), drug- receptor interactions, permeability studies, as well as in vivo dissolution and/or disintegration of dosage forms like the gelatin capsule shells and mini tablets (Hu et al., 1999; Wong et al., 2006) and colon targeted pellets (Tuleu et al., 2001). The pH changes that occur along the GI tract of female Wistar rats have been recently reported by McConell et al. (2008), the details of which are presented in Appendix - II. Broadly, the pH in a fasted female Wistar rat varies from 3.90 ± 1.0 in stomach to 5.89 ± 0.3 in duodenum and 6.13 ± 0.3 in jejunum. The pH decreases slightly to 5.93 ± 0.4 in ileum and increases to 6.58 ± 0.4 in caecum and becomes 6.23 ± 0.4 in proximal colon. The GI transit time for a suspension formulation in fasted female Sprague-Dawley rats has been reported to vary from 0-2 h in stomach, 2-4 h in small intestine and caecum and between 4-6 h in colon (Ciftci and Groves, 1996). The formulation was found to be eliminated from the body between 6-8 h.

As part of in vivo screening of formulations, GI transit study of selected formulations was carried out in healthy Wistar rats (both male and female) to investigate the influence of physiological environment of GI tract on pH and transit time dependent drug release from the different matrices. It was also envisaged to check if there is similarity between in vitro release of drug in simulated human GI fluid and the in vivo release during transit. The theoretical target release profile in simulated GI fluid pH for formulations that were designed for in vivo studies was redefined to match with the GI transit and pH changes that occur in a rat model. These formulations were expected to show negligible to low drug release from 0-4 h followed by controlled release for 8-10 h after oral administration. For this purpose, selected formulations were designed as mini tablets of approximately 4 mm diameter and formulation parameters were optimized as before to ensure that statistically significant similarity is obtained with the theoretical target in vitro drug release profile in simulated GI fluid pH (without enzymes) (Fig 5.59). The formulations that were selected were representative of each matrix type, viz., IPCP5ES20, ICP5EL10, IXG10EL10, IHEC5EL20, IHPC5ES10 and IGG5ES20. The in vitro release profile(s) of 4 mm diameter formulations showed good similarity with the theoretical target release in simulated GI fluid pH without enzymes ($f_2 > 50$; $f_1 < 15$) (Table 5.40). In order to investigate the GI transit pattern of the microsphere based formulations, formulation

M1EC1EL3 was administered orally in suspension form (50mg/2ml) to the animals described previously in Chapter 4 (Section 4.9).

The animals were sacrificed at fixed time intervals of 2, 4, 6 and 8 h and the respective formulations (tablets or microparticles) were recovered after a surgical intervention that exposed the gastrointestinal tract. The position of each recovered formulation was expressed as distance traveled (in cm) from the stomach (Table 5.41). The corresponding residual drug content analysis is presented in Fig 5.60 (a & b).

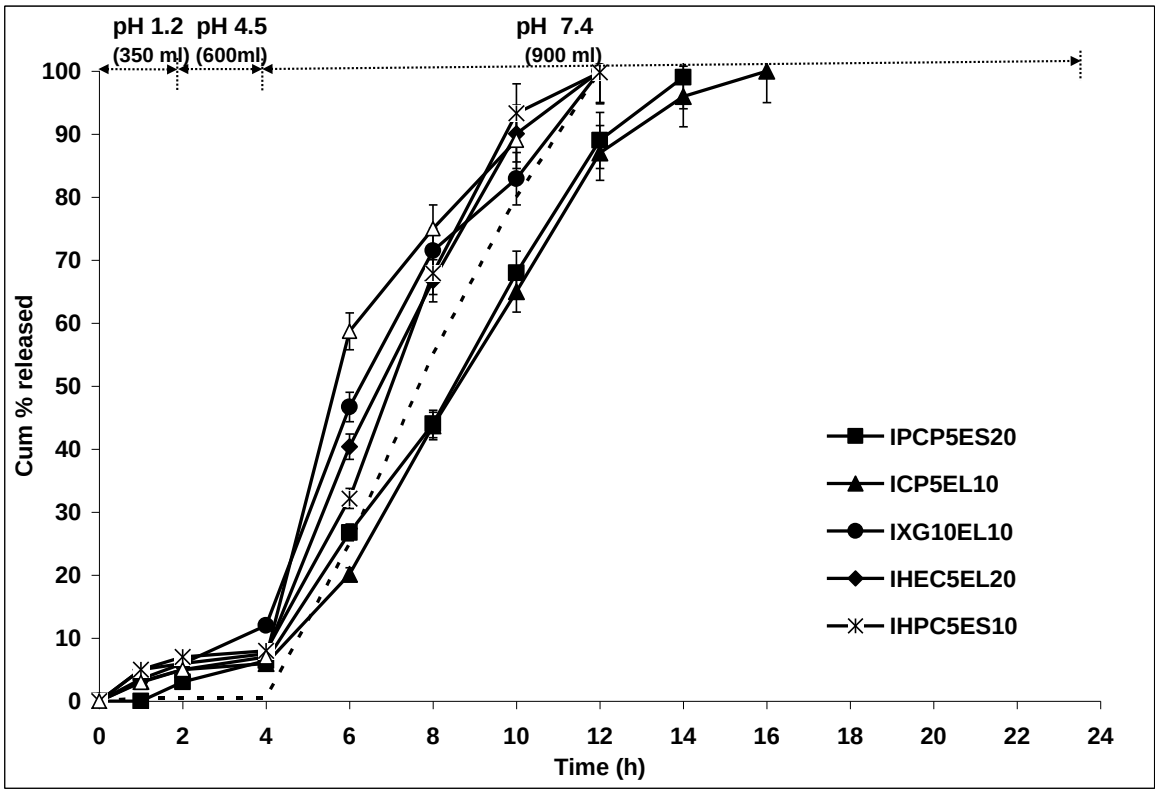


Fig 5.59: Release profile of indomethacin from mini tablets (4mm diameter) in simulated GI fluid. Each data point represents the average of two batches done in triplicate with standard deviation.

Table 5.40: Release kinetics characterization of drug release from mini tablets (4mm diameter) in simulated GI fluid pH (without enzymes)

Batches	r[a]	MSSR	K[b]	n[c]	$t_{10\%}$[d]	$t_{90\%}$[e]	f_1[#]	f_2[#]
IPCP5ES20	0.9853	1.80×10^{-3}	0.793	1.82	4.0	13.5	14.2	51.2
ICP5EL10	0.9700	3.11×10^{-3}	1.130	1.65	3.7	14.1	11.7	50.6
IXG10EL10	0.9517	1.45×10^{-3}	1.164	1.87	3.2	10.2	12.3	53.2
IHEC5EL20	0.9519	5.43×10^{-3}	0.414	2.32	3.9	10.1	11.4	54.8
IHPC5ES10	0.9699	4.45×10^{-3}	0.381	2.35	4.0	10.2	6.7	52.4
IGG5ES20	0.9071	3.56×10^{-3}	0.267	2.55	4.1	9.8	8.9	50.2

[a] Correlation coefficient; [b] Release rate constant; [c] Diffusional exponent indicative of the release mechanism; [d] Time for 10% of the drug release (h); [e] Time for 90% of the drug release (h)

In case of IPCP5ES20, the tablet was recovered at 6.17 ± 1.04 cm at 2 h (duodenal region) 29.70 ± 2.12 cm at 4 h (jejunum region), 63.35 ± 5.87 cm at 6 h (ileum) and at 118.00 ± 12.73 at 8 h (caecum). The percentage drug recovered from the formulations at these time points was 94.3% (at 2 h), 89.0% (at 4 h), 65.3% (at 6 h) and 58.2% (at 8 h) (Fig 5.60a). The reported pH values corresponding to these regions are 5.8 ± 0.3 (duodenum), 6.13 ± 0.3 (jejunum), 5.93 ± 0.4 (ileum) and 6.58 ± 0.4 (caecum) (McConell et al., 2008). It was observed that only 11% of drug was lost from the formulation in the slightly acidic to neutral environment of rat small intestine upto 4 h. Between 4 h and 6 h, the drug released from the formulation was around 25% which could be due to the relatively alkaline pH (6.58 ± 0.4) of caecum. The % drug that was recovered from the colon at 8 h was around 58.2%, implying loss of another 7% drug during transit from caecum to colon and overall release at 8 h was around 42% (Fig 5.60a). From the in vitro release studies carried out in simulated GI fluid pH (without enzymes) (Fig 5.59), it was found that for IPCP5ES20, around 38.8% drug was released in 8 h indicating good correlation between the in vitro release profile of IPCP5ES20 with its in vivo release in rat model.

The formulation ICP5EL10 was found to show a similar transit pattern as IPCP5ES20 (Table 5.41). The drug recovered at various time points was 95.4% (at 2h in the duodenal region), 87.3% (at 4 h in jejunum), 75.6% (at 6 h at distal ileum) and 55.7% (at 8h in proximal colon) (Fig 5.60a). In this case, about 12.7 % drug was released from the formulation upto 4 h and another 11.7% was released in distal small intestine. Therefore, around 25% drug was released from the formulation in the first 6 h. The tablet was located at the transverse colon at a distance of 126.50 ± 7.78 cm from the stomach at 8 h and showed drug recovery of 55.7%, implying that nearly 20% more of drug released occurred between distal small intestine and the transverse colon (Table 5.41). The overall drug loss from the formulation upto 8 h was 44.3%. A look at the in vitro drug release profile of ICP5EL10 in simulated GI fluid pH (without enzymes) indicated that around 20.1% drug was released at 6 h and 43.68% drug released occurred at 8 h implying that inspite of the differences in pH and transit time between human beings and rat model, in vitro release profiles were quite correlated with the in vivo release behavior.

In case of IXG10EL10, the observed GI transit pattern was quite different from the other two formulations discussed above. The tablet was recovered at 19.25 ± 2.47 cm at 2 h (jejunum), 67.50 ± 10.61 cm at 4 h (proximal small intestine), 115.50 ± 3.54 cm at 6 h (caecum) and at 128.50 ± 1.34 cm at 8 h (distal colon) (Table 5.41). The formulation was

found to show rapid transit in the upper position of the GI tract but the colon arrival time was same as the previous two formulations. The relatively rapid GI transit of IXG10EL10 is attributed to the density of the formulation when compared to the other two previously discussed formulations. Compared to polycarbophil and carbopol, xanthan gum probably exhibited lesser matrix swelling and therefore, was less buoyant and therefore, showed a quicker GI transit. The percentage drug recovered from the formulations at these time points was 85.3% (at 2 h), 80.8% (at 4 h), 25.5% (at 6 h) and 15.6% (at 8 h). Therefore, the overall drug loss from the formulation upto 8 h was 84.4% (Fig 5.60a). The drug released in vitro of IXG10EL10 in simulated GI fluid pH (without enzymes) at the end of 8^{th} h was 72%. The higher release rate is attributed to possible rapid matrix erosion due to the presence of microbial enzymes like xanthanase in rat GI tract that can act on xanthan gum which is a polysaccharide.

Table 5.41: GI transit data of selected formulations in Wistar rats

Formulation	**Mean distance from stomach (cm)**			
	2 h	4 h	6 h	8 h
IPCP5ES20	6.17 ± 1.04	29.70 ± 2.12	63.35 ± 5.87	118.00 ± 12.73
ICP5EL10	8.67 ± 4.16	29.75 ± 3.18	80.45 ± 1.77	126.50 ± 7.78
IXG10EL10	19.25 ± 2.47	67.50 ± 10.61	115.50 ± 3.54	128.50 ± 1.34
IHEC5EL20	9.77 ± 5.97	37.60 ± 7.21	52.50 ± 17.68	121.25 ± 3.18
IHPC5ES10	*	75.65 ± 3.04	119.00 ± 2.83	127.50 ± 3.64
IGG5ES20	46.00 ± 4.88	102.00 ± 14.14	#	Not found in body
M1EC1EL3	---------- 48.5 to 92.5cm[a] ----------			123 to 128[b] cm

Total length of intestine from stomach = 130 cm (approx)

Data is expressed as mean ± SD (n = 3)

*Tablet was found in the stomach in all cases

Tablet fragments were observed at a distance of 82.5cm and 122cm from the stomach in one case and tablet pieces spread across small intestine and colon in other cases.

[a] Spread throughout small intestine; [b] Spread throughout colon

For the formulation IHEC5EL20, the transit of tablet was similar to that observed for IPCP5ES20 and ICP5EL10. The tablet was recovered at 9.77 ± 5.97cm at 2 h (jejunum), 37.60 ± 7.21cm at 4 h (small intestine), and 52.50 ± 17.68 cm at 6 h (distal small intestine) and at 121.25 ± 3.18 cm at 8 h (proximal colon). The percentage drug recovered from the formulations at these time points was 98.2% (at 2 h), 92.5% (at 4 h), 67.9% (at 6 h) and 35.6% (at 8 h). For this formulation, only 7.5 % drug was released from the formulation upto 4 h when the tablet was recovered from small intestine. The drug release was

relatively rapid thereafter and another 25% more drug was released between 4 h and 6 h in distal small intestine. This may be due to the relatively higher pH of distal small intestine that crossed the threshold pH range (pH 6.0) for dissolution of Eudragit L100. The tablet was located at the proximal colon at a distance of 121.25 ± 3.18 cm from the stomach at 8 h and showed drug recovery of 35.6%, indicating that 32.3% drug released occurred between distal small intestine and the proximal colon (Fig 5.60b). Therefore, the drug release from the formulation was pH dependent and release rate increased in relatively alkaline environment of distal small intestine, caecum and colon within a pH range of 6.0 - 7.0. The overall release from the formulation at 8^{th} h was 64.4% which correlates with the in vitro release value of 65.8% for IHEC5EL20 in simulated GI fluid pH (without enzymes).

In case of IHPC5ES10 the formulation was recovered from the stomach at 2 h and then apparently had a rapid transit afterwards as it was recovered at a distance of 75.65 ± 3.04 cm from stomach (distal small intestine) at 4 h. The tablet was recovered from caecum at a distance of 119.00 ± 2.83 at 6 h and was found in distal colon (127.50 ± 3.64 cm from stomach) at 8 h. There was minimum drug release from the tablet in the initial phase as evident from high recovery values of 98.4% and 85.2% at 2^{nd} and 4^{th} h respectively from the formulation, implying that the formulation resisted drug release in stomach and small intestine. As observed in case of IHEC5EL20, maximum drug release (around 40%) was observed in caecum and near caecal region and another 15% was released during transit from caecum through colon (Fig 5.60b). The overall drug release from the formulation upto 8 h was around 70% which again showed good correlation with in vitro release value of 68% from the same formulation in simulated GI fluid pH.

A very rapid rate of GI transit occurred for IGG5ES20. The tablet was recovered at a distance of 46.00 ± 4.88 cm from the stomach in small intestine region at as early as 2 h and near caecum at 4 h. Thereafter, only tablet fractions and fragments were obtained at different positions in small intestine and colon. The percentage drug recovered from the formulation was 95% at 2 h and 78% at 4 h, implying that only 22% of drug was released from the matrix upto 4^{th} h. The release rate was significantly enhanced after that as only 20% of residual drug was recovered at the end of 6^{th} h from caecal region. These observations in case of a guar gum matrix were attributed to significant erosion that occurred during transit. Although drug release was minimum in the first 4 h, yet high erosion resulted in breakdown and disintegration of matrix and near complete release in

6th h. Further, as explained in case of xanthan gum, these matrices are prone to degradation by microorganisms that probably enhanced matrix erosion.

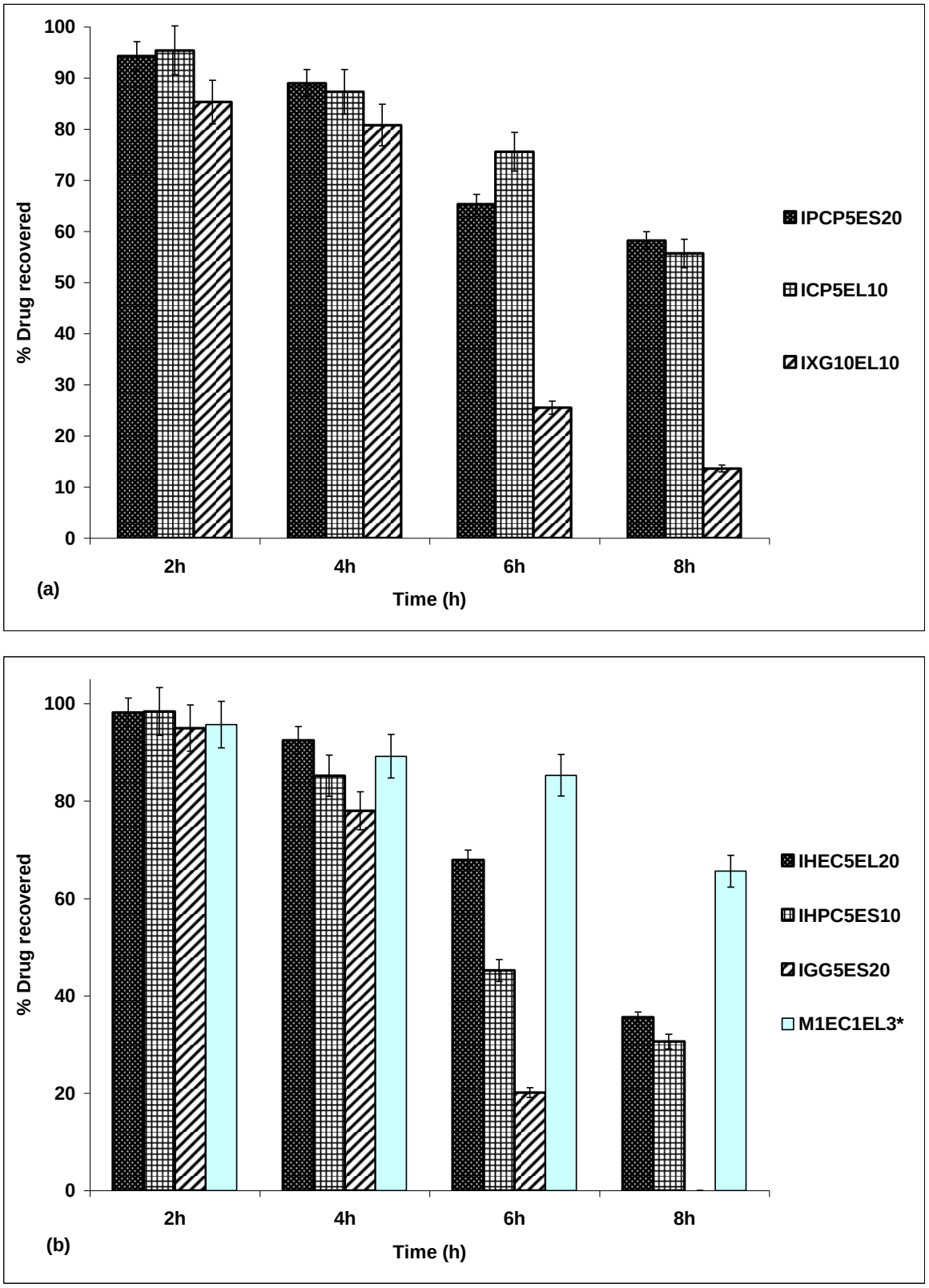


Fig 5.60: Residual drug content obtained for mini tablets recovered at various time points during GI transit study in rats (n = 3) for (a) IPCP5ES20, ICP5EL10, IXG10EL10 (b) IHEC5EL20, IHPC5ES10, IGG5ES20 and M1EC1EL3
*** expressed as drug content recovered per 20 mg of microspheres**

Hence, it can be concluded that drug release in vivo depended on formulation matrix type and surrounding pH conditions. In case of microspheres, the transit was very slow and major portion of microspheres was observed spread across small intestine (at a distance of 48.5 to 92.5cm from the stomach) in the region before caecum till 6 h. It was also found that percentage drug recovered (expressed as drug content per 20 mg of microspheres) at 2^{nd}, 4^{th} and 6^{th} h was 95.7%, 89.2% and 85.3% respectively indicating very small amount of drug release in the precaecal region. This was probably because the microspheres moved as algomerated mass, thereby reducing effective surface area and showing low release. In addition, since the animals were fasted, the luminal fluid content was low and this explains the stagnation of microspheres before caecum. In previous reports also, microparticles have been shown to have very slow transit through GI tract in rat (Ciftci and Groves, 1996) (Table 5.41, Fig 5.60b). A fraction of administered microspheres passed through caecum and were detected in colon at 8^{th} h. Drug content analysis of the recovered microspheres showed that 20% of drug was released between 6^{th} h and 8^{th} h in the region between caecum to colon.

Therefore, studies in animal model gave a preliminary idea about initial drug release from tablets and their pH and transit time dependency in overall release. A good correlation was found between in vitro drug release data and in vivo transit pattern and drug release for all the formulations.

5.11. In vivo evaluation of selected formulations in human subjects

The GI transit and residence time of any dosage form in different parts of human GI tract is affected by factors like size, density, volume that affect its GI transit in terms of stomach emptying, small intestinal transit and residence at ileo-caecal junction (Price et al. 1993; Podzeck et al. 2007). For successful design of colon specific dosage form, it is of paramount importance that studies are done on human subjects to confirm whether the dosage form is able to withstand mechanical stress exerted by the different motility patterns of the GI tract and resist the high internal pressure of colon (Abrahamsson et al. 1993, 1998). This will in turn affect the drug release and colon specificity.

The suitability of gamma scintigraphy for the evaluation of colon specific formulations has been previously established (Wilding et al., 2001). Gamma scintigraphy is a diagnostic tool which helps in the visualization of any dosage form during its transit and can give information about the times at which it (1) leaves the stomach, (2) arrives at the colon, (3)

begins to disintegrate and (4) completely disintegrates. This can be correlated with the absorption or the release profile of the drug from the dosage form.

An essential objective of the present study was to examine the in vivo fate of these formulations with respect to matrix integrity during GI and colonic transit and to determine the residence time of formulations in various parts of GI tract (stomach, small intestine and colon). In addition, it would help clarify whether the formulations adhere to GI mucosa during transit or whether there is stagnation of formulation in any region of the GI tract, especially the ileo caecal junction.

The in vivo transit of selected designed formulations was evaluated in healthy human subjects using the technique of gamma scintigraphy. A preliminary trial was carried out in which the subjects were screened for gastric emptying in fasted condition. It is reported that gastric emptying shows high degree of inter and intra subject variability (Price et al., 1993). Since the study was conducted on purely pilot basis with *n* =1 per formulation, the fasted subjects were initially standardized with respect to their gastric emptying parameters. A clearance of 100 ml of radio labeled water (1MBq) in 60 min was taken to be criteria for the inclusion of subjects in the study. Therefore, 100 ml of radiolabelled water was administered to each fasted subject and the dynamic images were acquired for 1 h in 64 x 64 matrix and 60 frames were acquired of 1 min each (Fig 5.61). The screened 8 subjects with mean gastric emptying ($T_{1/2}$) greater than 30 ± 15 min were removed from further participation in the study (Table 5.42). A sample subject screening data for 8 subjects is presented in Table 5.42.

Table 5.42: Results of human subject standardization with respect to gastric emptying time for GI and colonic transit studies

Subject	$T_{1/2}$ (min)	Clearance at 60 min (%)	Subject inclusion
1	24	100	√
2	29	85	√
3	Φ	37.6	×
4	29.0	100	√
5	24.0	100	√
6	Φ	29.1	×
7	21.0	100	√
8	46	65.2	√

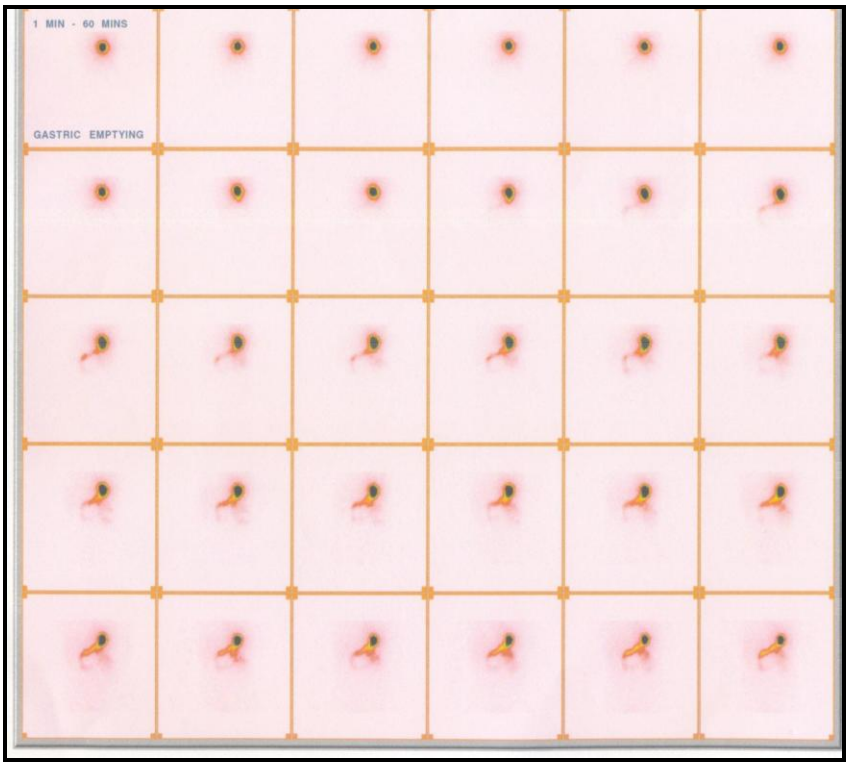
Φ Could not be computed as 60% clearance was greater than 60 min

√ - indicates subject inclusion; × - indicates exclusion from study

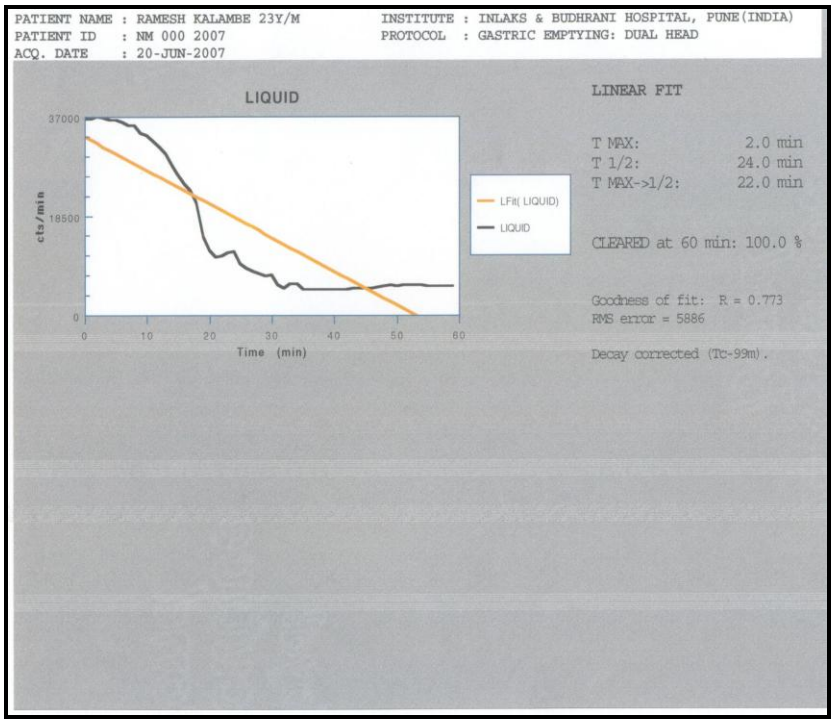
One formulation from each series of matrix type, i.e., IPCP5ES20, ICP5EL10, IXG10EL10, IHEC5EL20, IHPC5ES10 and IGG5ES20 which was previously tested for their transit and release behavior in vivo in Wistar rat model were evaluated for their in vivo GI transit, residence time and matrix integrity in human subjects. The data with respect to GI and colonic transit for the different formulations is shown in Table 5.43.

Formulation IPCP5ES20 showed a gastric emptying of 1.6 h, small intestine transit of 1.65 h, and colon arrival time of 3.25 h (Table 5.43). The residence time of dosage form in the colon was 16.75 h. The formulation did not adhere to gastric, intestinal or colonic mucosa at any time during transit (Fig 5.62). The presence of ES100 in matrix probably decreased the swellability and mucoadhesiveness of polycarbophil. Erosion of matrix was expected to be minimum as the formulation appeared intact with minimum leaching of radioactive tracer during transit. Slight spreading of the radioactive tracer was visible at 2.5 h (Fig 5.62). The subject had consumed light breakfast with juice after 2 h that might have led to mild leaching of tracer from tablet surface. The radioactivity detected in the bladder can be taken as an indication of matrix erosion. Strong radioactivity could be detected at 4.5 h in bladder (Fig 5.62). It is possible that matrix erosion was maximum between 3.4 to 4.5 h when the tablet moved from ileocaecal junction (ICJ) through the ascending colon. Further, the tablet was found to enter the colon without any hindrance. In some previous reports, large size tablets (> 10 mm size) have been shown to have long stagnation times at ICJ (Davis et al., 1989) and then traverse rapidly though ascending colon while smaller sized tablet enter the colon smoothly and have longer residence times in the ascending colon and hepatic flexure (Price et al., 1993; Adkin et al., 1993). In the present case also, small tablet size could be responsible for smooth entry into the colon. Moreover, the tablet appeared intact upto 7.5 h of study and would have eroded during transit as residual radioactivity was observed throughout the colon when the image was obtained at 24 h post administration.

For ICP5EL10, a gastric emptying of 1.5 h was observed followed by small intestinal transit time of 4.25 h and a colon arrival time of 5.75 h (Table 5.43). At 24 h post dosing, the tablet was observed in the rectum prior to defecation (Fig 5.63), so the residence time of the dosage form in colon which was calculated as difference between time of defecation and colon arrival time was about 18.25 h. As observed in case of IPCP5ES20, the formulation was intact during transit, did not show any mucoadhesiveness or prolonged residence anywhere during transit and appeared to have very little erosion.



(a)



(b)

Fig 5.61 : (a) Gastric emptying calculated by acquisition of dynamic images taken in 60 frames of 1 min each and (b) expressed in terms of $T_{1/2}$ and clearance at 60 min.

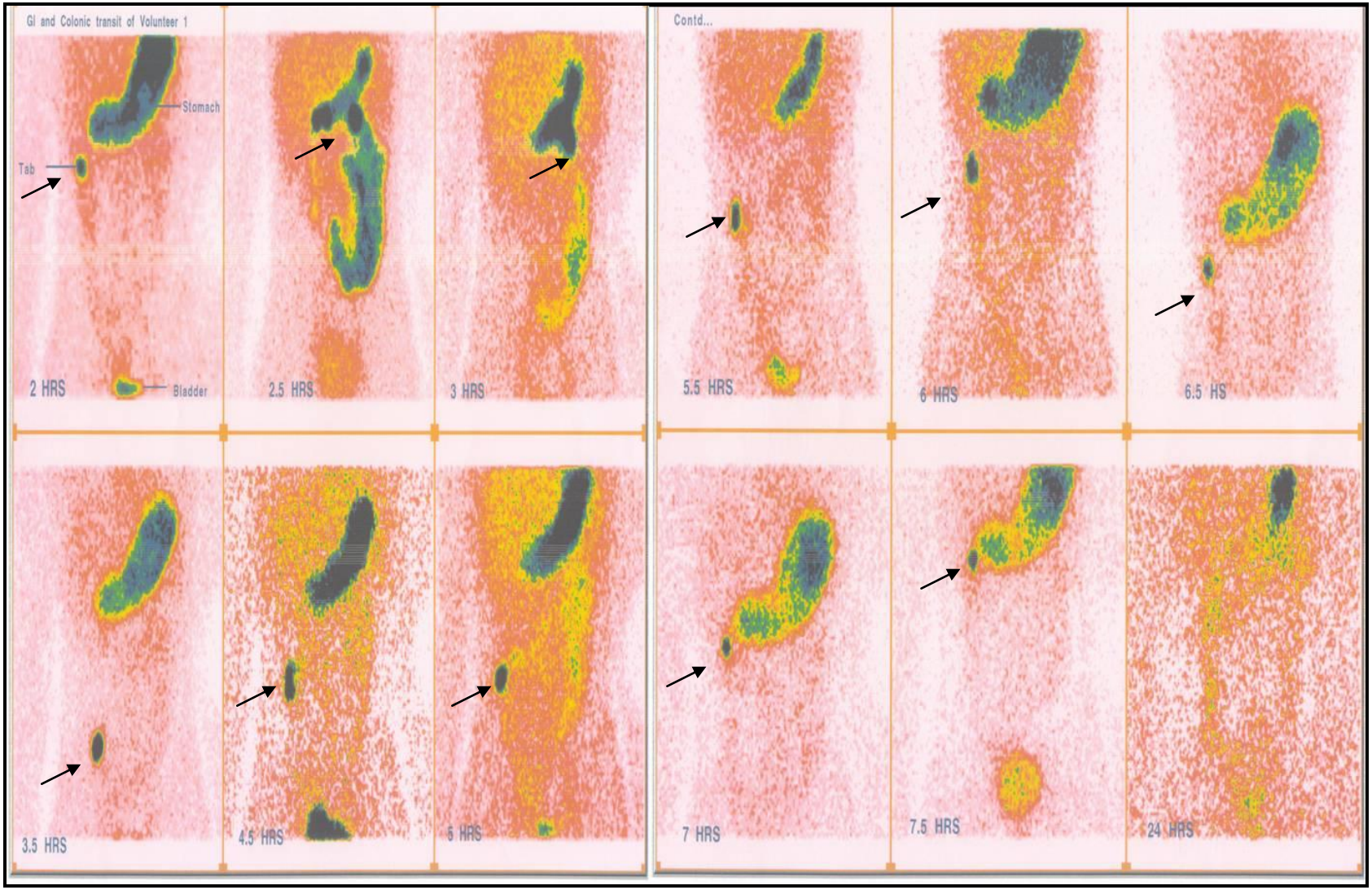


Fig 5.62: Gamma scintigraphic images showing GI and colonic transit of PCP based formulation (IPCP5ES20) in human subject. Arrow indicates tablet position.

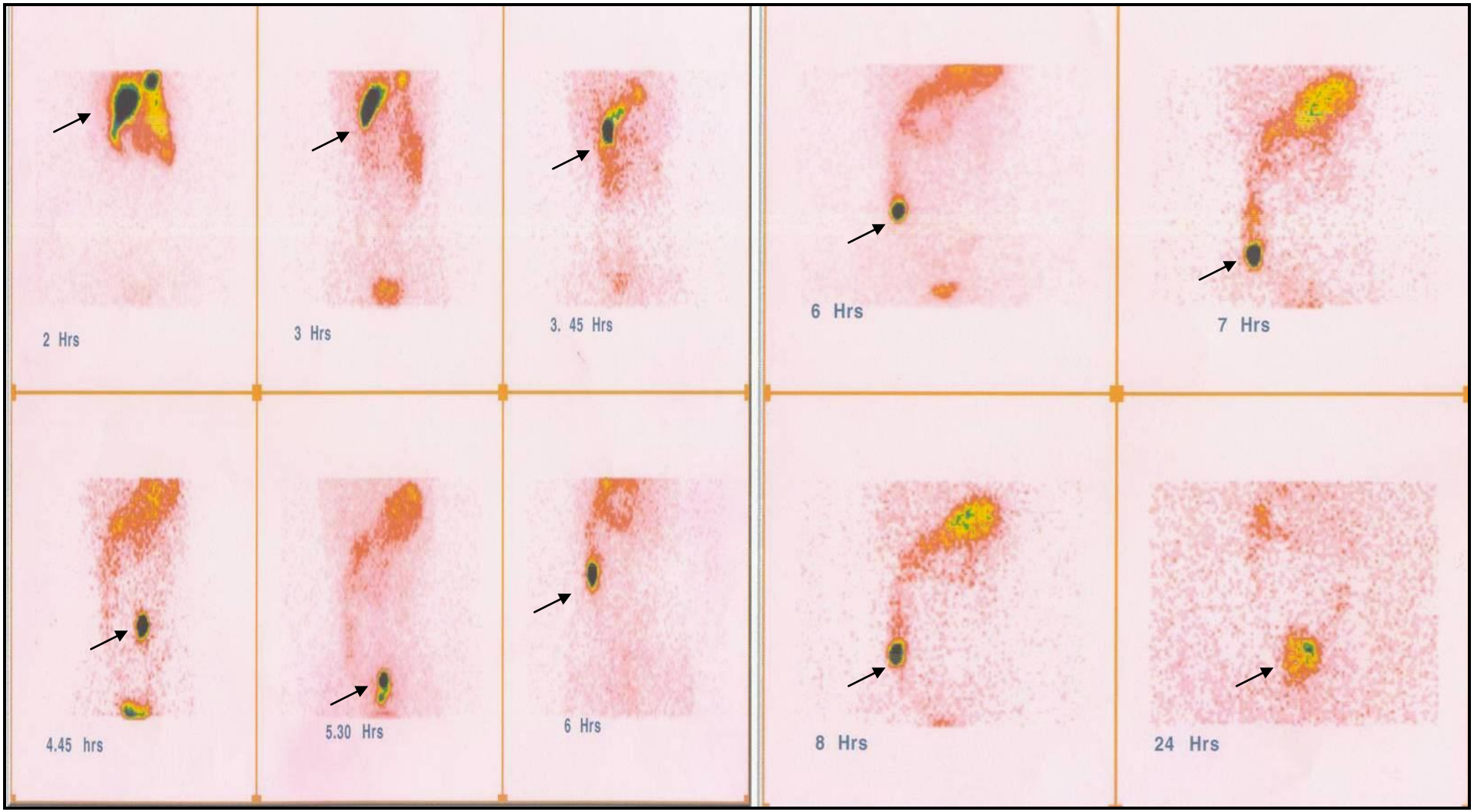


Fig 5.63: Gamma scintigraphic images showing GI and colonic transit of CP based formulation ICP5EL10 in human subject. Arrow indicates tablet position.

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In case of IXG10EL10, gamma scintigraphic analysis showed that the formulations remained intact in stomach upto 1.5 h after which it passed into the duodenum. At 2nd h, the matrix started to disintegrate and in subsequent images at 2.5 and 3 h, showed no traces of tablet in proximal small intestine (Fig 5.64a).The quick disintegration of tablet may be attributed to either loss of matrix strength of this formulation due to adsorption of radioactive tracer in saline or high erosion of matrix by microorganisms of the gut.

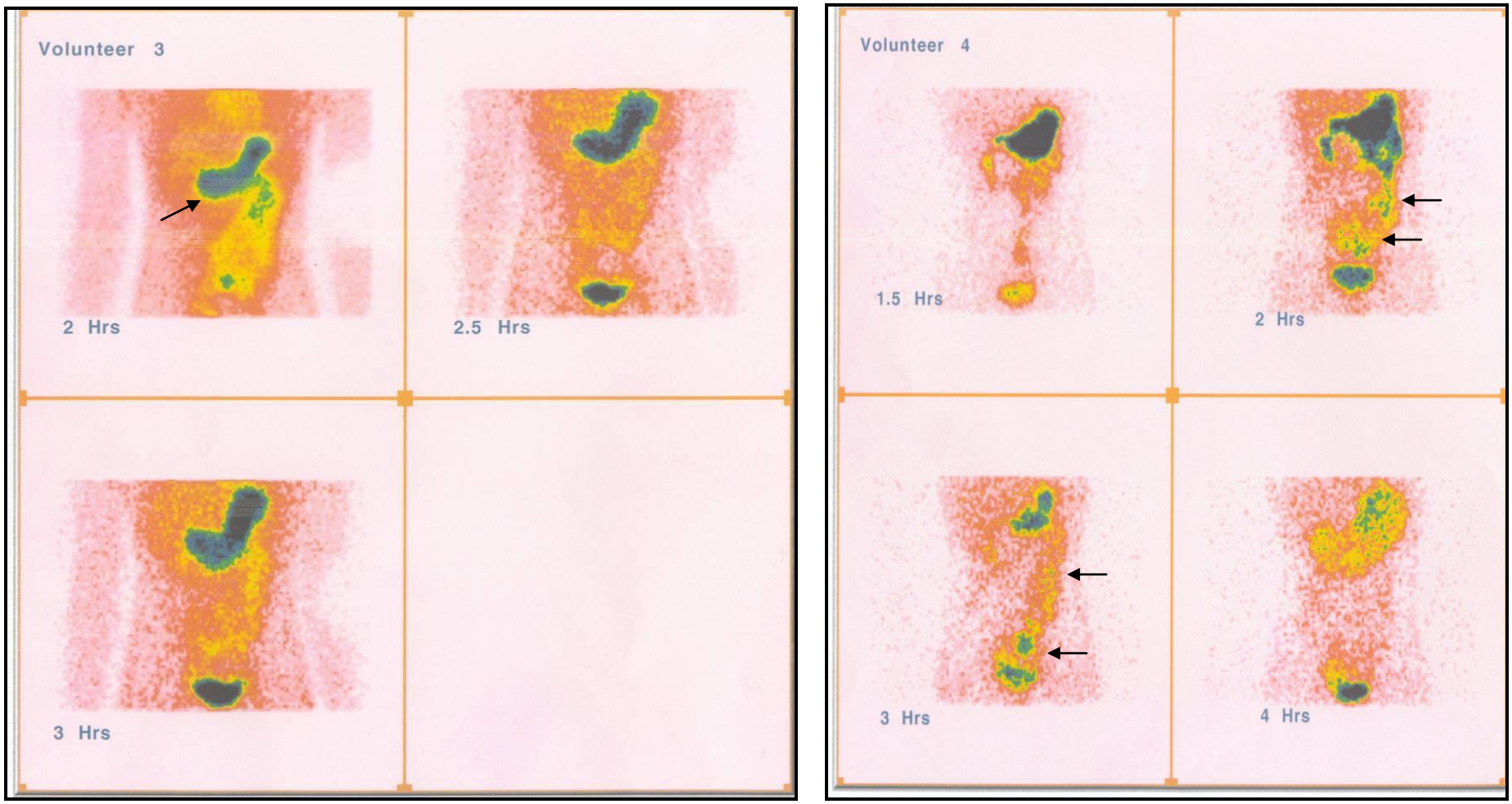
Formulation IHEC5EL20 also had a similar fate in vivo. The tablet was found to be intact in stomach during gastric emptying study uptil 1.5 h and when subsequent images were obtained at 2nd and 3rd h, the matrix was found to be disintegrating quickly and completely broke down at 4th h, during transit through small intestine (Fig 5.64b). The disintegration of matrix in human subject implies poor matrix strength that could not withstand the intense peristaltic pressures of the small intestine.

Table 5.43: Results of GI and colonic transit study for selected formulations in human subjects (n = 1)

Subject No	Formulation code	Gastric emptying time (h)	Colon arrival time (h)	Small intestinal transit time (h)[a]	Time of defecation (h) [b]	Colon residence time (h)	Observed disintegration time and position of dosage form (h), if any
1	IPCP5ES20	1.6	3.25	1.65	20	16.75	-
2	ICP5EL10	1.5	5.75	4.25	24	18.25	-
4	IXG10EL10	1.5	-	-	-	-	2 (duodenum)
5	IHEC5EL20	0.8	-	-	-	-	2 (duodenum)
7	IHPC5ES10	2.5	6	3.5	21	15	-
8	IGG5ES20	0.6	-	-	-	-	1.5 (ileum)

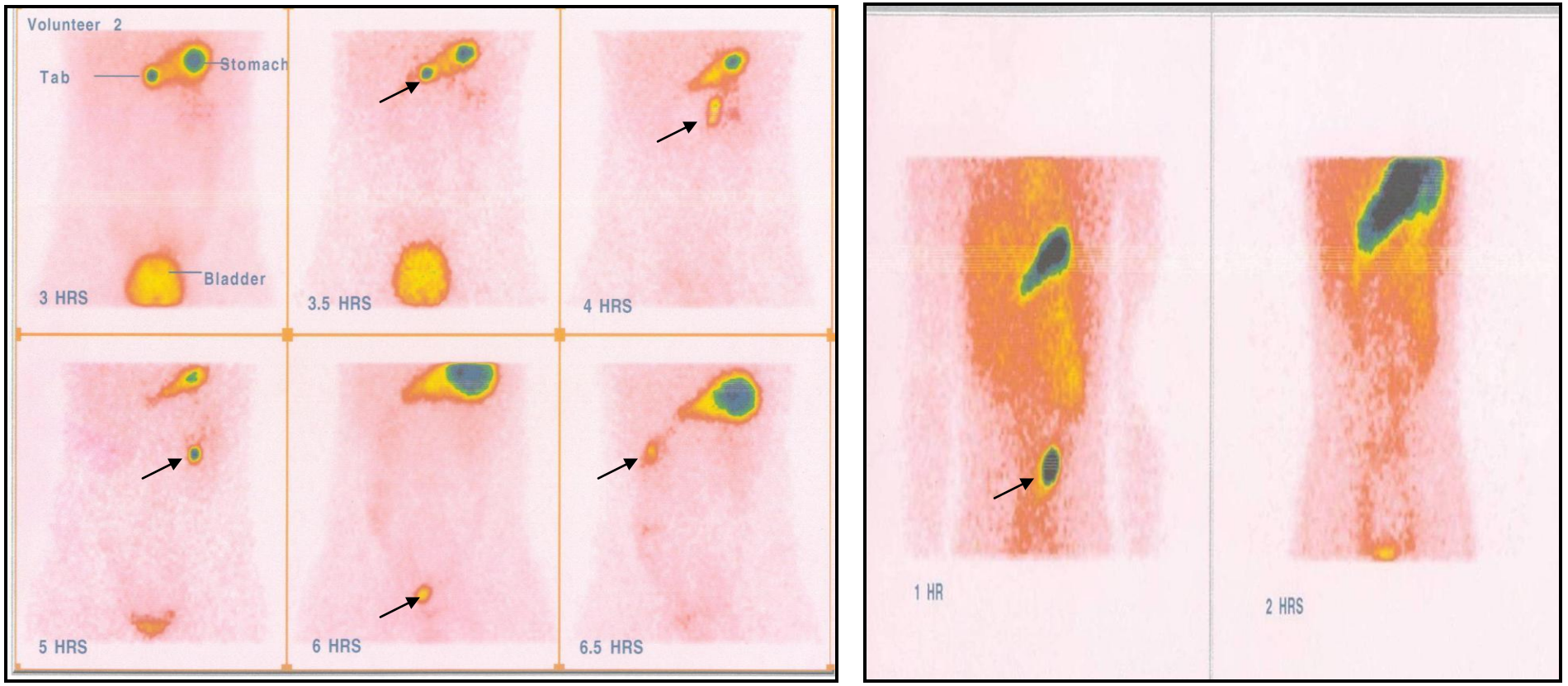
[a]colon arrival time - gastric emptying time = small intestinal transit time

[b] time of defecation - colon arrival time = colon residence time



(a) (b)

Fig 5.64: Gamma scintigraphic images showing GI transit of (a) XG based formulation (IXG10EL10) and (b) HEC based formulation (IHEC5EL20) in human subjects. Arrow indicates tablet position.



(a) **(b)**

Fig 5.65: Gamma scintigraphic images showing GI and colonic transit of (a) HPC based formulation (IHPC5ES10) and (b) GG based formulation (IGG5ES20) in human subjects. Arrow indicates tablet position.

However, formulation based on HPC with ES100 (IHPC5ES10) showed similar transit parameters as IPCP5ES20 and ICP5EL10. The tablet had a gastric emptying time of 2.5 h, small intestine transit of 3.5 h, and colon arrival time of 6.0 h. The residence time of dosage form in the colon was 15 h (Table 5.43). The formulation was intact during transit and showed no signs of fragmentation or disintegration during first 6.5 h of study (Fig 5.65a). Further, it did not show bioadhesion and stasis at ileo-caecal junction. The formulation had shown good retardation in initial release when tested in rat model upto 4 h and then a pH dependent release was observed in caecum and colon.

The formulation based on guar gum with ES100 (IGG5ES20) when administered to human subject showed a rapid rate of transit in vivo and was near distal small intestine at 1h (Fig 5.65b). However, the intact tablet matrix could not be observed in subsequent image obtained at 2h, implying possible disintegration. The GG + ES100 matrix was highly porous and prone to erosion which explains high in vitro and in vivo release with subsequent disintegration in both rat model and human subject.

Thus, it can be concluded that matrix tablets prepared by combination of PCP + ES100 (IPCP5ES20), CP + EL100 (ICP5EL10) and HPC + ES100 (IHPC5ES10) showed good ability to withstand GI and colonic transit with pH and transit time controlled sigmoidal release. It was also observed in these formulations that contained mucoadhesive polymers like polycarbophil, carbopol and HPC did not exhibit any bioadhesive property during GI transit and showed no signs of adhesion or stagnation of tablet in any region of the GI tract. This could be attributed to the presence of the pH sensitive polymer(s) which are hydrophobic in nature. The other formulations IHEC5EL20, IXG10EL10 and IGG5ES20 were found to disintegrate during transit through small intestine which was attributed to relatively lower matrix strength of these formulations. The GI transit of formulations further corroborates our assumptions that formulations with a lag time of 5-6 h followed by complete release in 14-16 h would be potentially colon specific.

Chapter Six *Conclusions*

Colorectal cancer is the second leading cause of death from cancer in industrialized countries. Studies have shown that nonsteroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase-2 (COX-2) inhibitors, hold promise as anticancer agents. Of all the NSAIDs that have been explored for their anti cancer potential, indomethacin has been most extensively investigated and has shown good activity against both in vitro as well as in vivo models of colon cancer. However, oral administration of indomethacin by conventional dosage forms will result in severe local upper gastrointestinal side effects and unwanted systemic effects. Therefore, a formulation of indomethacin with negligible to no release in upper gastrointestinal (GI) tract and controlled release in colonic region would achieve therapeutically effective concentration of drug locally in colon and shall reduce the incidence of GI toxicity and systemic adverse effects associated with the drug.

The pH changes that occur along the GI tract can be used to trigger or initiate drug release from dosage forms intended for site specific delivery to colon. Most of the dosage forms for colonic delivery are coated with pH sensitive polymers that dissolve in alkaline pH of the colon. However, concerns have been raised regarding the use of these coated systems for colonic delivery as they have shown unreliable and inconsistent in vivo performance. Despite their limitations, pH based polymer coated systems are still used because of their commercial viability. Further, literature review revealed only two formulations of indomethacin based on pH polymers and both of them employ coating technique.

Therefore, in order to overcome the drawback of pH polymer coated systems, a novel matrix design that would combine the advantages of pH and transit time controlled systems, as an alternative to the coating approach, was proposed. Hence, the primary objective of the thesis was to study the feasibility of developing matrix based systems comprising of pH sensitive polymers alone or in combination with other suitable polymers in an attempt to develop combined pH and time controlled delivery systems for colon targeted release.

For the purpose of drug estimation in in-house prepared formulations, dissolution and stability samples, an analytical method based on UV-Visible spectrophotometry was developed and validated as per ICH guidelines. The analytical method developed was found to be simple, accurate, precise, selective for the drug and robust.

Some preformulation studies were carried out to assess drug solubility in various buffers and to understand the drug stability in solution form and solid state compatibility for enabling selection of formulation excipients and polymers. Preformulation studies revealed that drug showed pH dependent solubility and was stable in intermediate pH range (pH 4.5) and upto 24 h in alkaline buffer pH 7.4. The drug was found to be compatible with all selected polymeric excipients in physical admixtures and showed acceptable shelf life values in both controlled as well as accelerated conditions.

The formulations were designed as single unit (tablet) and multi unit (microsphere) based systems. Single unit systems were prepared as single polymer based systems using different hydrophilic and hydrophobic polymers and as dual polymer based systems using these polymers in combination with pH sensitive polymers. In all cases, matrix embedding technique using wet granulation was employed. The physical characteristics of all the prepared matrices were found to be satisfactory. In vitro release studies, carried out in distilled water for 2 h followed by pH 7.4, indicated that single polymer based systems employing different hydrophilic or hydrophobic polymers did not show desired release patterns for colonic delivery. However, when blended with Eudragit L100 or S100 to form a polymeric matrix, drug release followed a sigmoidal pattern with minimum drug release in the initial phase, followed by a steady rise to give complete release within 14-16 h corresponding to the residence of a dosage form in colon. The release could be modulated by varying polymer type and proportion. The mechanism of release in most formulations was characterized by polymer swelling and matrix erosion (super case II mechanism). The drug release was also investigated in a pH gradient system simulating GI fluid pH changes without enzymes. The drug release profile from selected formulations in simulated GI fluid was characterized by an initial lag time period of 4-6 h with low drug release followed by controlled release phase in phosphate buffer media for about 14-16 h and several formulations like IPCP5ES20, ICP5EL10, IXG10EL10, IHEC5EL20, IHPC5ES10 and IGG5ES20 showed good similarity with target release profile.

For the preparation of microparticulate system, controlled release matrix based microspheres were prepared utilizing ethyl cellulose as the rate controlling polymer alone and in combination with pH responsive polymers Eudragit L100 or Eudragit S100 for pH and time controlled release using technique of phase separation coacervation induced by solvent evaporation. The effect of polymer proportion and internal: external phase ratio on

micromeritics and drug release kinetics were investigated. Spherical, discrete microspheres could be obtained by this process by varying internal to external phase ratio and proportion of both polymers in the matrix. Presence of EL100 or ES100 in EC microspheres was found to be essential for conferring pH dependent drug release profile. The drug release from formulations was found to be sigmoidal with low initial release followed by complete release in 16-18 h for most formulations.

Stability studies as well as drug excipient compatibility studies using thermal analysis and FTIR did not reveal any instability or presence of physical and chemical interaction in these formulations, implying that drug was stable in designed matrices.

In vivo studies of selected formulations in healthy Wistar rats showed that drug release from the formulations was dependent on pH and transit time and there was minimum release from the formulations in the initial period for formulations prepared with hydrophilic polymers in combination with either EL100 or ES100. Some formulations like IXG10EL10 and IGG5ES20 showed poor control on release in vivo.

Three formulations, IPCP5ES20, ICP5EL10, and IHPC5ES10 out of the six formulations screened for transit time and matrix integrity in healthy human subjects showed good matrix integrity and minimum erosion during gastric and colonic transit. Further, a good correlation was found between in vitro release and in vivo transit times. Therefore, it could be concluded that designed matrices have shown good potential for site specific release of indomethacin to colon. A pH and transit time controlled matrix system can offer a suitable platform for colon targeting purpose with minimum drug loss during upper GI transit and maximum drug release in the colon. These formulations can also serve as a feasible alternative to coating technology as the developed technology is easily scalable with good inter batch reproducibility.

Future Prospects

The work may be continued to explore the use of several other hydrophilic and hydrophobic polymers in matrix base for colonic delivery. Newer polymers like Eudragit FS30D that have high pH threshold ranges may be employed in matrix bases and evaluated for colon specific release. Other NSAIDs with potential use in colon cancer can be tried for their adaptability to this matrix platform. The technology may be assessed for scale-up and industrial or commercial viability. More information is needed to correlate drug release with tablet position in healthy and diseased patients. The work may also be extended to explore matrix based nanoparticulate carriers for colonic delivery.

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