Design and Evaluation of Mucoadhesive Buccal Delivery Systems of Lercanidipine Hydrochloride

THESIS

Submitted in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

By
SHRIKANT YASHWANT CHARDE

Under the supervision of
PROF. RANENDRA N. SAHA

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
PILANI (RAJASTHAN) INDIA
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CERTIFICATE

This is to certify that the thesis entitled **"Design and Evaluation of Mucoadhesive Buccal Delivery Systems of Lercanidipine Hydrochloride"** submitted by **Shrikant Yashwant Charde**, ID No. 2001PHXF414 for award of Ph.D. Degree of the Institute, embodies original work done by him under my supervision.

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Shrikant Y. Charde

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List of Abbreviations and Symbols

#	Sieve size
%	Percentage
% Bias	Measure of accuracy of analytical method
% CDR	Percentage cumulative drug released
%RSD	Percentage relative standard deviation
%RTD	Percentage remaining to be degraded
µg.h/l	Micro gram hour per liter
µg/ml	Microgram per milliliter
µm	Micro meter
λ_{max}	Wavelength of maximum absorbance
(R)	Rectus
(S)	Sinistrus
<	Less than
=	Equal to
µg/l	Microgram per liter
µg/ml	Microgram per milli liter
µl	Micro liter
ACN	Acetonitrile
AT	Accelerated temperature (40 ± 2°C/ 75 ± 5 % RH)
ATP	Adenosine tri phosphate
$AUC_{(0-\infty)}$	Area under serum concentration-time curve
AUMC	Area under first moments curve of serum concentration-time profile
BP	Blood pressure
CCB	Calcium channel blocker
CH	Chitosan
cm^2	Centimeter square
C_{MAX}	Maximum plasma concentration
C_{max}	Maximum serum concentration
CMC	Carboxy methyl cellulose
Conc.	Concentration
CP	Carbopol 934P
CRT	Controlled room temperature (25 ± 2°C/60 ± 5 % RH)
CYP	Cytochrome
DHP	Di hydro pyridine
DSC	Differential scanning calorimetry
EC	Ethyl cellulose
EDTA	Ethylene di amine tetra acetic acid
et al.	Co-workers
F	Calculated or tabulated value of statistical test analysis of variance
F_r	Relative bioavailability
FT	Refrigerated temperature (5 ± 2°C)
FTIR	Fourier transform infrared

g	Gram
GI	Gastro-intestinal
H	Hour
HCl	Hydrochloric acid
HEC	Hydroxy ethyl cellulose
HPC	Hydroxy propyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxy propyl methyl cellulose
HPMC K100M	Hydroxy propyl methyl cellulose 100000 cPs
HPMC K15M	Hydroxy propyl methyl cellulose 15000 cPs
HPMC K4M	Hydroxy propyl methyl cellulose 4000 cPs
HQC	Higher quality control sample
ICH	International conference on harmonization
IR	Infrared
J/g	Joules per gram
K	Release rate constant
K^+	Potassium
K_{deg}	Degradation rate constant
l	Liter
l/day	Liter per day
l/kg	Liter per kilo gram
LCMS	Liquid chromatography coupled with mass spectrophotometer
LER	Lercanidipine hydrochloride
LHRH	Luteinizing hormone-releasing hormone
Log % RTD	Log percentage remaining to be degraded
Log P	Log of oil water partition coefficient
LQC	Lower quality control sample
M	Molar
MCG	Membrane coating granule
mg	Milligram
mg/day	Milligram per day
mg/ml	Milligram per milli liter
min	Minutes
ml	Milli liter
ml/min	Milli liter per minute
mM	Millimolar
mm	Millimeter
mm/sec	Millimeter per second
MQC	Medium quality control sample
MRT	Mean residence time
MSSR	Mean sum of square residuals
N	Newton
NaCMC	Sodium carboxy methyl cellulose
ng/ml	Nanogram per milli liter

NIR	Near infra red
nm	Nanometer
^{o}C	Degree centigrade
p	Probability
PC	Polycarbophil
PEG	Polyethylene glycol
PEO	Polyethylene oxide (Polyox WSR 1105)
pH	Negative log to the base 10 of hydrogen ion concentration
$P_{o/w}$	Apparent partition coefficient
PVA	Polyvinyl alcohol
PVP	Polyvinyl pyrollidine
QC	Quality control
R	Correlation coefficient
R^2	Regression coefficient
R_f	Retention factor
RH	Relative humidity
RPM	Revolutions per minute
RT	Retention time
S	Slope of the least square regression line
SD	Standard deviation
Sec	Seconds
SSF	Simulated salivary fluid
$t_{1/2}$	Half life
$t_{50\%}$	Time taken for 50% of drug release from formulations
$t_{90\%}$	Time taken for drug concentration to come down to 90% of original concentration
TDW	Triple distilled water
TLC	Thin layer chromatography
T_{max}	Time to reach maximum concentration
UK	United Kingdom
USP	United States Pharmacopoeia
UV	Ultraviolet
v/v	Volume by volume
VIS	Visible
σ	Standard deviation of *y* intercept of regression equation
cPs	Centi poise
cm^{-1}	Centimeter inverse
h^{-1}	Hour inverse

Abstract

The objective of this work was to design and evaluate mucoadhesive buccal tablets of lercanidipine hydrochloride. To achieve this broad objective analytical methods for accurate and precise estimation of drug in variety of samples were developed. Extensive preformulation studies were carried out for establishing pharmaceutically relevant physicochemical properties of the drug. Buccal mucoadhesive controlled release tablets of the drug were prepared and evaluated for in vitro and in vivo performance.

Formulations were prepared using various mucoadhesive and rate controlling polymers either alone or in combination using direct compression technique. Effect of ionic nature, hydrophilicity/ hydrophobicity and swelling behavior of the polymer on in vitro drug release and mucoadhesion was assessed. Designed formulations were characterized for drug content, weight variation, friability, thickness and hardness. Further, in vivo studies of selected formulations were carried out in rabbits to establish the bioavailability relative to oral dose. In vivo human acceptability studies were carried out for assessing compatibility and acceptability of designed formulations in humans.

Results indicated that all the developed and validated methods were accurate and precise and facilitated estimation of drug in variety of samples. Preformulation studies indicated poor solubility of drug with high partition coefficient. Drug was found to be photosensitive in solution and solid state stability studies. Drug was compatible with all the selected excipients.

The designed tablets possessed good physical characteristics with acceptable weight variation and good content uniformity. The designed formulations were stable for at least two years when stored at controlled room temperature. Drug release and mucoadhesive behavior of designed tablets was affected by polymer proportion, polymer combination, polymer hydrophilicity/ hydrophobicity and swelling behavior of the polymer. The release mechanism in almost all the designed formulations was anomalous non Fickian transport. The deigned tablets possessed good mucoadhesive characteristics.

The designed buccal mucoadhesive tablets were non-irritating and acceptable in human subjects. In vivo studies of selected formulations in rabbits demonstrated significant increase in bioavailability of drug relative to orally administered drug.

It can be concluded that the designed formulations have potential to overcome the disadvantage of poor and erratic bioavailability associated with presently marketed preparations. The method used for manufacturing was relatively simple and can easily be adopted in conventional formulation manufacturing units on a commercial scale.

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Chapter 1
Literature Survey and Objectives

1.1 Introduction

The oral route of drug delivery is traditionally the most preferred route of systemic drug administration by physicians and patients. Oral route offers distinct advantages over other conventional drug delivery routes like topical and parenteral. However, peroral administration of certain drugs has disadvantages such as unpredictable and erratic absorption, GI intolerance, incomplete absorption, degradation of drug in GI contents and presystemic metabolism resulting in reduced bioavailability. Parenteral route of drug administration is the only established route that overcomes the drawbacks associated with orally less efficient or inefficient drugs. However, parenteral formulations are costly, have least patient compliance, and self administration is difficult. Consequently, over the last decade, there has been a particular interest in investigating other absorptive mucosae as potential sites for systemic delivery of drugs. Transmucosal routes of drug delivery (i.e., the mucosal linings of the nasal, rectal, vaginal, ocular and oral cavity) offer distinct advantages over peroral administration for systemic and local drug delivery (Shojaei, 1998). Major advantages of transmucosal routes include possible bypass of first pass effect and avoidance of metabolism/degradation of drug within the GI tract (Rathbone and Hasgraft, 1991).

Further, recent developments in the field of molecular biology and gene technology is resulting in generation of many macromolecular drugs including peptides, proteins, polysaccharides and nucleic acids possessing superior pharmacological efficacy with site specificity. However, the main constraint for the oral delivery of these drugs as potential therapeutic agents is their extensive presystemic metabolism, instability in acidic environment resulting in inadequate and erratic oral absorption. This recent development has further led to investigation of alternative drug delivery routes (Soyani and Chien, 1996; Veuillez et al., 2001).

The nasal route has become increasingly popular for systemic delivery of some peptide and protein drugs. Drug absorption from nasal mucosa is very rapid because of rich vasculature and high permeability (Brahmankar and Jaiswal, 1995). Nasal mucosa for systemic drug delivery has been extensively investigated by many research groups (Tengamnuay and Mitra 1990; Shao and Mitra, 1992) and the route has almost reached commercial status with several drugs including LHRH (Adjei et al., 1992) and calcitonin (Dal Negra et al., 1991). The potential irritation and the irreversible damage to the ciliary action of the nasal cavity from chronic application of nasal dosage forms, as well as the large intra- and inter-subject variability in mucus secretion in the nasal mucosa, significantly affect drug absorption from this site (Beckett and Triggs, 1967). Even though the rectal, vaginal, and ocular mucosae all offer certain advantages, the poor patient

acceptability associated with these sites makes them appropriate for local applications rather than systemic drug administration (Shojaei, 1998).

Within the oral mucosal cavity, systemic delivery of drugs can be achieved by either sublingual delivery, which is systemic delivery of drugs through the mucosal membranes lining the floor of the mouth or buccal delivery, which is drug administration through the mucosal membranes lining the cheeks (Shojaei, 1998).

The sublingual route is the most widely studied of the oral transmucosal routes. The sublingual mucosa has good permeability resulting in quick onset of action and higher bioavailability for many drugs. Moreover, this route is convenient, accessible, and generally well accepted (Deneer et al., 2002). Sublingual dosage forms create a very high drug concentration in the sublingual region before the drug is systemically absorbed across the mucosa. Even though the sublingual mucosa is relatively more permeable than the buccal mucosa, it is not suitable for oral transmucosal controlled delivery of drugs. The sublingual region lacks an expanse of smooth muscle or immobile mucosa and is constantly washed by a considerable amount of saliva. This makes retention of delivery system a difficult proposition. Due to its high permeability and rich blood supply, the sublingual route is capable of producing a quick onset of action making it appropriate for drugs with short delivery period requirements with infrequent dosing regimen as in case of nitroglycerine tablets (Shojaei**,** 1998).

The buccal mucosa is comparatively less permeable than the sublingual area, and is generally not able to provide the rapid absorption and good bioavailability seen after sublingual drug administration (Pimlott and Addy, 1985). Due to two important differences between the sublingual mucosa and buccal mucosa, the latter is a preferred route for systemic transmucosal drug delivery (Harris and Robinson, 1992; Gandhi and Robinson, 1994). First difference is the permeability characteristics of the region. The buccal mucosa is less permeable and is thus not able to give a rapid onset of absorption (i.e. more suitable for a sustained release formulation). Secondly, the buccal mucosa has an expanse of smooth muscle and relatively immobile mucosa which makes it more suitable for retentive drug delivery systems used for oral transmucosal drug delivery. Moreover, as the buccal mucosa is routinely exposed to a variety of different foreign compounds, it is rather robust and less prone to irreversible irritation or damage by dosage forms, or additives such as absorption promoters (Squier, 1991). The virtual lack of Langerhans cells makes the oral mucosa tolerant to potential allergens (Bodde et al., 1990). The venous drainage of the buccal region is not subjected to hepatic first pass metabolism. Drug administration through the buccal mucosa avoids gastrointestinal metabolism/degradation of drug, as the drug bypasses the

destructive acidic/enzymatic environment of the GI tract. Other important advantages of buccal mucosa include low enzymatic activity, painless administration, easy drug withdrawal, facility to include permeation enhancer/enzyme inhibitor or pH modifier in the formulation and flexibility in designing delivery system as multidirectional or unidirectional release systems for local as well as systemic actions (Alur et al., 2001).

Similar to any other delivery route, some disadvantages are associated with buccal route. The low permeability of the buccal membrane sometimes results in a low flux for buccal absorption of drugs (Pimlott and Addy, 1985). The total surface area of the membranes of the oral cavity available for drug absorption is only 170 cm^2 (Collins and Dawes, 1987), of which approximately 50 cm^2 represents non-keratinized tissues, including the buccal membrane (Lee et al., 2000). The low surface area seriously limits the size of delivery system and hence the amount of drug that can be loaded on to the delivery system (Shojaei**,** 1998). The continuous secretion of saliva (0.5–2 l/day) leads to substantial dilution of the drug (Gandhi and Robinson, 1994). Swallowing of saliva can also potentially lead to the loss of dissolved or suspended drug. This dissolved or suspended drug might then again be exposed to GI contents and first pass metabolism. These dosage forms are inconvenient when the patient is eating or drinking and sometimes there is a possibility of involuntary removal of the dosage form.

Nevertheless, the advantages and recent progress in delivering a variety of orally less efficient or inefficient drugs, specifically peptides and proteins, render the disadvantages of this route less significant.

1.2 Structure and Environment of Buccal Mucosa

The primary role of the buccal mucosa, like skin, is to protect underlying structures from foreign agents. The surface of buccal mucosa consists of a stratified squamous epithelium which is separated from the underlying connective tissue (lamina propria and submucosa) by a continuous layer of extracellular material called basement membrane (Rathbone and Hasgraft, 1991). This stratified squamous epithelium consists of differentiating layers of cells (keratinocytes) which change in size, shape, and content as they travel from the basal region to the superficial region**.** The epithelial cells increase in size and become flatter as they travel from the basal layers to the superficial layers. There are approximately 40–50 cell layers in epithelium, resulting in a buccal mucosa of 500 - 800 μm thickness (Hayward, 1976) as shown in Figure 1.1(c).

The permeability of the buccal mucosa is greater than that of the skin, but less than that of the intestine (Rojanasakul et al., 1992; Gore et al., 1998; Shojaei, 1998). This is

because of greater surface area provided by the small intestine and the structural differences between each of the tissues, as shown in Figure 1.1 (Miller et al., 2005; Nicolazzo et al., 2005a). The simple columnar epithelium covering the small intestine provides less resistance to drug transfer than the stratified squamous epithelium covering the skin and buccal mucosa accounting for higher permeability of intestine. Buccal mucosa although structurally similar to skin, is either less or not keratinized and hence more permeable.

The composition of oral epithelium also varies depending on the site in the oral cavity. The mucosae of areas subject to mechanical stress (the gingivae and hard palate) are keratinized similar to the skin. The mucosae of the soft palate, sublingual and buccal regions are non-keratinized (Harris and Robinson, 1992). The keratinized epithelia are relatively impermeable to water because of high proportion of lipids like ceramides and acylceramides in them. Non-keratinized epithelia, such as the sublingual and the buccal mucosa do not contain acylceramides and only have small amounts of ceramide (Squier and Wertz, 1996). They also contain small amounts of other polar lipids, mainly cholesterol sulfate and glucosyl ceramides. These epithelia have been found to be considerably more permeable to water than keratinized epithelia (Squier et al., 1991a). Hence it can be said that presence of lipids like ceramides and acylceramides in epithelium is associated with barrier function of mucosa.

A gel like secretion known as mucus, which contains mostly water insoluble glycoproteins covers the entire buccal cavity. Mucus is bound to the apical cell surface and acts as a protective layer to the cells below (Allen et al., 1984). It is composed of several components such as proteins, enzymes, electrolytes and nucleic acids. The composition of mucus varies based on the origin of the mucus secretion in the body (Haas and Lehr, 2002).

1.2.1 Barriers to penetration across buccal mucosa

The upper one-third to one-quarter of the buccal epithelium has been attributed for its barrier function. Experimental evidence for this was first demonstrated with the topical application of horseradish peroxidase to the oral mucosa of monkeys, rabbits and rats, where the protein was unable to penetrate deeper than the top 1–3 cell layers (Squier, 1973). When injected subepithelially, horseradish peroxidase was found within connective tissue and the intercellular spaces of the epithelium, till the region where the membrane-coating granules (MCG's) first appear (Squier, 1973). This suggested that the permeability barrier of buccal mucosa may be attributed to the materials extruded from membrane-coating granules. To ensure that this region was also the barrier to permeation of smaller molecules,

the experiments were repeated using smaller molecules (lanthanum salts), and the results obtained were identical (Squier and Rooney, 1976).

The role of MCG's in the barrier properties of buccal mucosa was further confirmed by good permeability of tissues (junctional epithelium) lacking these granules to tracers like horseradish peroxidase and lanthanum (Tanaka, 1984; Romanowski et al., 1989). Similar results were obtained when these tracers were applied topically to keratinized oral epithelium in tissue culture (Squier et al., 1978). Since these cultured tissues lacked MCG's (Lillie et al., 1980), it became evident that the permeability barrier of oral mucosa could be attributed to contents extruded from the MCG's into the epithelial intercellular spaces.

MCG's have been reported to extrude lipids (mainly ceramides and acylceramides) into the intercellular spaces mainly responsible for barrier function of buccal mucosa (Elias et al., 1979; Elias, 1981). It is believed that the barrier of non-keratinized oral epithelium is also composed of lipid material, since treatment of oral mucosa with chloroform/methanol mixtures resulted in a reduced barrier function (Squier et al., 1991a). To verify the chemical nature of these lipids, various regions of porcine oral cavity were separated, and the lipids present in each region were extracted and identified by thin-layer chromatography (Squier et al., 1986b, 1991a,b; Wertz et al., 1986). The keratinized palatal and gingival mucosae contained high quantities of ceramides and cholesterol, and a low proportion of cholesterol esters and glycosylceramides. Whereas, the non-keratinized buccal and sublingual mucosae showed higher proportion of polar phospholipids, cholesterol esters and minimal amounts of ceramides. Histochemical staining suggested that polar lipids were localized in intercellular spaces of the non-keratinized oral epithelium (Squier et al., 1986b). Therefore, the intercellular lipids of non-keratinized regions of the oral cavity are more polar in nature than the intercellular lipids of keratinized regions. This difference in chemical nature of the lipids has been reported to contribute to the differences in permeability observed between these mucosae (Squier et al., 1991a).

Enzymatic degradation is another barrier to drug permeability across buccal mucosa. Saliva contains moderate levels of esterases, carbohydrases, and phosphatases (Robinson and Yang, 2001) and several other proteolytic enzymes like aminopeptidase (Veuillez et al., 2001). To reach systemic circulation, drugs must overcome this enzymatic barrier (Rathbone and Tucker, 1993; Nielsen and Rassing, 2000; Senel and Hincal, 2001). However, this enzymatic barrier is less effective than the GI enzymatic barrier (Walker et al., 2002).

1.2.2 Mechanisms of drug permeation

The cellular organization of buccal epithelia is that of stratified squamous epithelium (Figure 1.1). The epithelial cells are surrounded by a relatively hydrophilic intercellular matrix. The intercellular spaces of the epithelia are also filled with polar lipids because of extrusion of contents from the membrane-coating granules (Hau and Heng, 2003). The lipophilic cell membranes of the epithelial cells are thus surrounded by relatively polar intercellular lipids on the outside and a hydrophilic aqueous cytoplasm on the inside. Consequently, the existence of hydrophilic and lipophilic regions in the oral mucosa has led majority of researchers to postulate existence of two routes of drug transport through the buccal mucosa namely paracellular (between the cells) and transcellular (across the cells) (Hau and Heng, 2003; Miller et al., 2005; Nicolazzo et al., 2005a; Rossi et al., 2005; Sudhakar et al., 2006) as shown in Figure 1.2 (Nicolazzo et al., 2005a). The lipophilic drugs penetrate mainly through the more lipophilic transcellular route, while the less lipophilic paracellular route, characterized by loosely packed polar intercellular lipids, is the principle route for absorption of hydrophilic drugs. The major route of drug penetration is dependent upon the physicochemical properties of the drug molecule. Generally, both the routes co-exist for all the drugs but the predominant route is the one with least resistance (O'Driscoll, 2002; Nicolazzo et al., 2005a).

Sometimes absorption is reported to be by the mechanism of endocytosis where the drug molecules are engulfed by the cells (Hau and Heng, 2003). Active transport mechanism has not been reported in literature but acidic stimulation of the salivary glands, with accompanying vasodilatation, facilitates absorption and uptake into the circulatory system (Chen et al., 2002).

Literature suggests that most compounds actually traverse the buccal mucosa via the paracellular route. Few lipophilic compounds (like glycosylceramides) have been histochemically shown to be located in the intercellular spaces of buccal mucosa (Squier et al., 1986b). The significance of paracellular route in buccal permeation was further substantiated by presence of tracer compounds (horseradish peroxidase and lanthanum salts) in intercellular spaces, when applied to the oral mucosa of rabbits, rats, and monkeys (Squier, 1973; Squier and Rooney, 1976). The paracellular route was also found to be the major route of permeation for water, ethanol, cholesterol, and thyrotropin releasing hormone (Squier and Lesch, 1988; Dowty et al., 1992a). Confocal Laser Scanning Microscopy has also been used to determine the route of transport of fluorescently labeled dextrans, and it was shown that these hydrophilic macromolecules also penetrated the oral mucosa via the paracellular route (Hoogstraate et al., 1994, 1996).

The permeability of ionizable drugs across buccal mucosa is believed to follow the pH-partitioning theory characteristic of passive diffusion (Veuillez et al., 2001). Studies have been carried out on lidocaine and nicotine to find out effect of ionization on permeation through buccal mucosa and the results were found to be in accordance with pH partition hypothesis (Okamoto et al., 2001; Nielsen et al., 2002). It is generally believed that increasing non-ionized fraction of ionizable drugs could favor drug penetration through the transcellular route.

1.3 General Considerations in Formulation Design

The limited area for placement of buccal drug delivery system seriously limits the device size and hence the amount of drug that can be loaded onto the device. Generally, a device with the size of 1–3 cm^2 and a daily dose of 25 mg or less is preferred for buccal delivery (Gandhi and Robinson, 1994; Alur et al., 2001). Various factors that should be considered for designing a formulation for transmucosal delivery of drugs through buccal route are discussed in subsequent sections.

1.3.1 Physiological factors

Constant flow of saliva and mobility of the involved tissues challenge drug delivery to the oral cavity. The residence time of drugs delivered to the oral cavity is very short, in the range of 5–10 min (Lee et al., 2000). Consequently, mucoadhesive formulations are used to overcome this problem. These formulations contain a mucoadhesive polymer which helps in retention of delivery system at site of absorption or action for a longer duration. They also provide a means to confine and maintain high local concentrations of the drug to a defined and relatively small region of the mucosa in order to minimize loss to other regions and limit potential side effects (Miller et al., 2005).

The mucus layer covering the buccal mucosa is necessary for bioadhesive systems. Apart from being a physical barrier to drug permeation, because of its short turnover time mucus prevents long term bioadhesion and sustained drug release. Moreover, the presence of bioadhesive polymers on a mucous membrane might alter the turnover of mucin. The maximum duration of delivery however should not exceed reported mucin turnover rate of 12- 24 h in humans (Forstner, 1978). The maximum duration for buccal drug delivery is usually limited to approximately 6-8 h, since eating and drinking may require dosage form removal (Alur et al., 2001). Faster turnover rate of buccal mucosa (3-8 days) also continuously affects its permeability characteristics (Khanvilkar et al., 2001).

1.3.2 Pathological factors

Many diseases affect the thickness of the buccal epithelium, its turnover rate and mucin turnover rate resulting in alteration of the barrier properties of the mucosa and affecting the performance of mucoadhesive drug delivery systems. Some diseases or treatments may also influence the secretion and properties of the mucus and saliva (Khanvilkar et al., 2001). Changes at the mucosal surface due to these pathological conditions complicates the application and retention of bioadhesive delivery device. Therefore, understanding the nature of mucosa under relevant disease conditions is necessary for designing an effective buccal delivery system. In addition, drugs with the potential of changing physiological conditions of the oral cavity are unsuitable for buccal delivery. Cancer patients often develop oral candidosis and a substantial decrease in salivary flow after irradiation treatment. The bioadhesive buccal tablet containing miconazole has been shown to be effective in treatment of oral candidosis, but its efficacy was substantially influenced by low saliva secretion in cancer patients (Bouckaert et al., 1996).

1.3.3 Pharmacological factors

A buccal dosage form may be designed for systemic or local action. For local action, the residence time and local concentration of the drug in the mucosa are important considerations. For a systemic effect, the amount of drug transported across the mucosa into the circulatory system is an important factor (Hau and Heng, 2003).

1.3.4 Pharmaceutical/ Physicochemical factors

The drug must be released from the delivery system and should permeate through buccal mucosa (if necessary) to exert its pharmacological action. Poor drug solubility in saliva significantly retards drug release from the dosage form and hence leads to poor drug absorption. Cyclodextrins have been used to solubilize and increase the absorption of poorly water-soluble drugs delivered via buccal mucosa (Jain et al., 2002). Apart from these physicochemical characteristics required for desirable drug release and absorption, organoleptic properties of the drug or the delivery device are of utmost importance as buccal delivery systems are exposed to a highly developed sensory organ.

Some excipients are generally incorporated to enhance the effectiveness and acceptability of the dosage forms. Selection of formulation excipients is a very important consideration, since acidic compounds can stimulate the secretion of saliva, which enhances not only drug dissolution, but also drug loss by involuntary swallowing. Addition of a separate additive for each function could complicate and enlarge the dosage form, which

might be problematic for buccal applications. Therefore, additives with multiple functions need to be selected (Miller et al., 2005).

Important additives that can be incorporated into the delivery system are permeation enhancers, polymers that retard the drug release (when controlled or sustained delivery of drug is desirable) and polymers that help in retaining the delivery system for prolonged duration time at the site of application by mucoadhesion. In the next few sections, permeation enhancers and mucoadhesive polymers will be dealt with in detail.

1.4 Buccal Permeation Study

Prior to formulating a buccal delivery system for a drug, buccal absorption/permeation studies must be conducted to determine the feasibility of this route of administration for the candidate drug. The developed delivery systems should also be evaluated for drug permeation to establish safety and efficacy. These studies involve methods that would examine in vitro and/or in vivo buccal permeation profile and absorption kinetics of the drug.

1.4.1 In vitro methods

Most of the in vitro studies reported for examining drug transport across buccal mucosa have used buccal tissues from animal models and Franz Type diffusion cell (Giannola et al., 2006, 2007). Animals are sacrificed immediately before the start of an experiment. Buccal mucosa with underlying connective tissue is surgically removed from the oral cavity, after which the connective tissue is carefully removed and the buccal mucosal membrane is isolated. The membranes are then stored in ice-cold (4°C) buffer until mounted between a diffusion cell for the in vitro permeation experiments (Mashru et al., 2005; Vishnu et al., 2007).

The major issue in these in vitro models is the viability and integrity of the dissected tissue. The dissected tissue needs to be preserved properly as this directly affects the results of the study. There are no standard means by which the viability or the integrity of the dissected tissue can be assessed. Dowty et al. (1992a) have studied tissue viability by monitoring ATP levels in rabbit buccal mucosa. Using ATP levels as an indicator for tissue viability is not an accurate measure, as they reported that a 50% drop in the tissue ATP concentration did not result in change of tissue permeability. Therefore, a decrease in ATP levels does not assure a drop in permeability characteristics of the tissue. The most meaningful method to assess tissue viability is the actual permeation experiment itself. If the drug permeability does not change during the course of the study under specific

experimental conditions of pH and temperature, then the tissue is considered to be viable (Dowty et al., 1992a).

Buccal cell cultures have also been suggested as useful in vitro models for buccal drug permeation studies (Tavakoli-Saberi et al., 1989; Leipold and Quadros, 1993; Jacobsen et al., 1999). However, to utilize these cell cultures for buccal drug transport, the number of differentiated cell layers and the lipid composition of the barrier layers must be well characterized and controlled. This has not yet been reported with buccal cell cultures.

1.4.2 In vivo methods

In vivo methods were first reported with the buccal absorption test (Beckett and Triggs, 1967). Using this method, the kinetics of drug absorption was measured. The methodology involved swirling of a 25 ml sample of the test solution for up to 15 minutes by human subjects followed by expulsion of the solution. The amount of drug remaining in the expelled volume was then determined in order to assess the amount of drug absorbed. The drawbacks of this method include salivary dilution of the drug, accidental swallowing of a portion of the sample solution, and inability to localize the drug solution within a specific site (buccal, sublingual, or gingival) of the oral cavity. Various modifications of the buccal absorption test have been carried out (Schurmann and Turner, 1978; Barsuhn et al., 1988; Tucker, 1988) for correcting salivary dilution and accidental swallowing, but these modifications also suffer from the inability of site localization.

Other in vivo methods include those carried out using a small perfusion chamber attached to the upper lip of anesthetized dogs (Yamahara and Lee, 1993). Perfusion chamber is attached to the tissue by cyanoacrylate cement. The drug solution is circulated through the device for a predetermined period of time and sample fractions are then collected from the perfusion chamber (to determine the amount of drug remaining in the chamber) and blood samples are taken to determine amount of drug absorbed across the mucosa.

The best feasible approach is to carry out in vivo pharmacokinetic study in an appropriate animal model or healthy human subjects. Pharmacokinetic parameters can then be calculated from the plasma concentration versus time profile (Hosny et al., 2002; Jain et al., 2002; Nafee et al., 2004a; Perioli et al., 2004; Langoth et al., 2006).

1.4.3 Experimental animal species

Apart from the methodology of permeation studies, special attention should be paid to the animal species used for experimentation. For in vivo investigations, many researchers

have used small animals including rats (Siegel and Gordon, 1985; Aungst et al., 1988; Aungst and Rogers, 1989) and hamsters (Tanaka et al., 1980; Kurosaki et al., 1988; Aungst, 1994b) for permeability studies. However, such choices seriously limit the value of the data obtained since most laboratory animals have an oral lining that is totally keratinized. Rats have a buccal mucosa that is very thick and keratinized. Rabbit is the only laboratory animal that has non-keratinized mucosal lining similar to human tissue and has therefore been extensively utilized in experimental studies (Dowty et al., 1992a,b; Li et al., 1997; Cui and Mumper, 2002a,b). The oral mucosa of larger experimental animals like monkeys (Mehta et al., 1991), dogs (Tiwari et al., 1999; Hosny et al., 2002; Jain et al., 2002; Degim et al., 2006) and pigs (Perioli et al., 2004; Langoth et al., 2006) has been used for permeability and pharmacokinetic studies. Due to the difficulties associated with maintenance of monkeys, they are not very practical animal models. Instead, dogs are much easier to maintain and considerably less expensive than monkeys and their buccal mucosa is non-keratinized and has a close similarity to that of the human buccal mucosa. Pigs also have non-keratinized buccal mucosa similar to that of humans. In fact, the oral mucosa of pigs resembles human buccal mucosa more closely than any other animal in terms of structure and composition (Collins et al., 1981; Squier and Wertz, 1996).

1.5 Methods to Improve Permeability through Buccal Mucosa

Membrane permeation is the limiting factor for many drugs in the development of buccal adhesive delivery devices. Sometimes, epithelium that lines the buccal mucosa acts as a very effective barrier to the absorption of drugs. Consequently permeation enhancers are used to improve permeation of drugs through buccal mucosa (Chattarjee and Walker, 1995). The selection of enhancer and its efficacy depends on the physicochemical properties of the drug, site of administration, nature of the vehicle and other excipients (Nicolazzo et al., 2005a). The efficacy of enhancer is different at different sites of body and is dependent upon cellular morphology, membrane thickness, enzymatic activity and lipid composition of the membrane or mucosa. Effective penetration enhancers for transdermal or intestinal drug delivery may not have similar effects on buccal mucosa because of structural differences; however, enhancers used to improve drug permeation in other absorptive mucosae may improve drug penetration through buccal mucosa (Nicolazzo et al., 2005a). These permeation enhancers should be safe and non-toxic, pharmacologically and chemically inert, non-irritant, and non-allergic (Aungst, 1994a).

Buccal mucosal permeation of drugs can be improved by using permeation enhancers or other substances that have been postulated to improve absorption by following mechanisms (Nicolazzo et al., 2005a).

a. Changing mucus rheology: Mucus forms visco-elastic layer of varying thickness that affects drug absorption. Saliva covering the mucus layers also hinders absorption. Some permeation enhancers overcome this barrier by reducing the viscosity of mucus and saliva.
b. Increasing the fluidity of lipid bilayer membrane: These enhancers disturb the intracellular lipid packing by interaction with either lipid packing or protein components and also reduce the resistance for transcellular absorption of drugs.
c. Acting on the components at tight junctions: These enhancers act on tight intercellular junctions thereby increasing drug absorption by paracellular route.
d. Overcoming the enzymatic barrier: These act by inhibiting the various enzymes present within buccal mucosa, thereby overcoming the enzymatic barrier.
e. Increasing the thermodynamic activity of drugs: These enhancers increase the solubility of drug resulting in enhanced thermodynamic activity of drug leading to better absorption.
f. Increased retention of drug at mucosal surface: Few enhancers like bioadhesive polymers increase permeation by increasing the contact time of delivery system with buccal mucosa.

Table 1.1 gives list of different penetration enhancers reported and their proposed mechanism of action. Irritation and toxicity have always been an area of concern with penetration enhancers, although the buccal mucosa is more resistant to damage than other mucosal membranes (Gandhi and Robinson, 1994; Veuillez et al., 2001). The information available on buccal absorption enhancement is much less than that for transdermal enhancement. The relationships among structure, irritation, and enhancement effect of the enhancer have not been clearly elucidated. Very few penetration enhancers are available for buccal delivery systems and they have not been used in marketed buccal delivery systems owing to the lack of a satisfactory profile with respect to irritation and effectiveness (Robinson and Yang, 2001; Veuillez et al., 2001).

1.6 Importance of Mucoadhesion/ Bioadhesion

The term bioadhesion can be defined as the attachment of a synthetic or natural macromolecule to mucus and/or an epithelial surface (Longer and Robinson, 1986). In

general it is defined as adherence of a polymeric material to a biological surface (bioadhesion) or to a mucosal surface (mucoadhesion).

1.6.1 Theories of mucoadhesion

The mucus layer covering mucosal surfaces is composed primarily of mucins. Mucins are highly glucosylated glycoproteins with a large peptide backbone and oligosaccharide side chains. Their protein backbone is characterized by the presence of repeating sequences rich in serine, threonine and proline residues. As a result, mucins are negatively charged at physiological pH (Gandhi and Robinson, 1994).

Mechanisms of polymer attachment to mucosal surfaces are not yet fully understood. However, certain theories of bioadhesion have been proposed. It has been postulated that mucoadhesion might occur via physical entanglement and/or chemical interactions, such as electrostatic, hydrophobic, hydrogen bonding, and Van der Waals interactions.

Five theories have been suggested to play a major role in bioadhesion, namely, adsorption, diffusion, electronic, fracture and wetting theories. In the adsorption theory, primary and secondary chemical bonds of the covalent and non-covalent (electrostatic and Van der Waals forces, hydrogen, and hydrophobic bonds) type are formed upon initial contact between the mucus and the polymer (Peppas and Buri, 1985; Ahuja et al., 1997; Gu et al., 1998; Huang et al., 2000).

The diffusion theory suggests that chain entanglement between glycoproteins of the mucus and the mucoadhesive polymer is responsible for adhesion. Diffusion of polymeric chain into the mucus creates an entangled network between the two. The factors affecting entanglement are polymer chain flexibility, adequate exposure for surface contact, similarity in chemical structures and diffusion coefficient of the bioadhesive polymer (Peppas and Buri, 1985; Ahuja et al., 1997; Gu et al., 1998; Huang et al., 2000).

The electronic theory postulates that due to different electronic properties of the mucoadhesive polymer and the mucus glycoprotein, electron transfer between these two surfaces occurs, which results in forces of attraction and inter-diffusion of the two surfaces (Ahagon and Gent, 1975; Peppas and Buri, 1985; Ahuja et al., 1997; Gu et al., 1998; Huang et al., 2000).

The fracture theory relates the force required for detachment of polymers from the mucus to the strength of their adhesive bond. It has been found that the detachment force and work for detachment is greater when the network strands are longer or the degree of

cross-linking is reduced (Peppas and Buri, 1985; Ahuja et al., 1997; Gu et al., 1998; Huang et al., 2000).

Finally, the wetting theory describes the ability of a bioadhesive polymer to spread on biological surfaces. This theory is predominantly applicable to liquid bioadhesive systems. Moderately wettable polymers have been shown to exhibit optimal adhesion to human endothelial cells (Peppas and Buri, 1985; Van Wachem et al., 1985; Ahuja et al., 1997; Gu et al., 1998; Huang et al., 2000).

As bioadhesion occurs between inherently different mucosal surfaces and variety of formulations like solids, semi-solids and liquids, it is unlikely that a single, universal theory will account for all types of adhesion observed. Mostly all the mechanisms co-exist, with one predominant over another depending upon nature of the polymer and mucosal surface.

1.6.2 Factors affecting mucoadhesion in buccal cavity

Mucoadhesive characteristics are a factor of the adhesive polymer and environment (mucosal surface) in which the polymer resides. Extent of mucoadhesion is generally determined by both these factors.

1.6.2.1 Polymer-related factors

a. Molecular weight: It has been reported that the bioadhesive strength of a polymer increases with molecular weight. Direct correlation between the bioadhesive strength of polyoxyethylene polymers and their molecular weights, in the range of 200,000 to 7,000,000, has been demonstrated (Tiwari et al., 1999).
b. Flexibility: Bioadhesion starts with the diffusion of the polymer chains and their entanglement with glycoprotein chains of mucin. Therefore, it is important that the polymer chains contain a substantial degree of flexibility in order to achieve the desired entanglement with the mucus. It has been demonstrated that the use of tethered polyethylene glycol–polyacrylic acid hydrogels and their copolymers improved mucoadhesive properties (Huang et al., 2000). The increased chain interpenetration was attributed to the increased structural flexibility of the polymer upon incorporation of polyethylene glycol.
c. Hydrogen bonding capacity: Hydrogen bonding is another important factor in mucoadhesion of polymers. For mucoadhesion to occur, desired polymers must have functional groups that are able to form hydrogen bonds. Polymers such as polyvinyl alcohol, hydroxylated methacrylate, and polymethacrylic acid have good hydrogen bonding capacity (Peppas and Buri, 1985).

d. Cross-linking density: It has been reported that increasing density of cross-linking leads to slow diffusion of water into the polymer network, which in turn causes an insufficient swelling of the polymer and a decreased rate of interpenetration between polymer and mucin (Gu et al., 1998).
e. Concentration: Development of a strong adhesive bond with the mucus is dependent upon the polymer chain length available for penetration into the mucus layer. When concentration of the polymer is too low, the number of penetrating polymer chains per unit volume of the mucus is small resulting in lesser interaction between polymer and mucus (Peppas and Buri, 1985). A concentrated polymer generally results in a longer penetrating chain length and better adhesion. However, above a critical concentration, the polymer produces a significantly coiled structure. Due to this, the accessibility of solvent to the polymer decreases, and chain penetration of the polymer drastically reduces. Therefore, higher concentrations of polymers do not necessarily improve and, in some cases, actually diminish mucoadhesive properties (Solomonidou et al., 2001).
f. Hydration (Swelling): Swelling is important for a mucoadhesive polymer to expand and create a proper macromolecular mesh (Gu et al., 1998) of sufficient size. Swelling ensures that polymeric chains get entangled with mucus resulting in adhesion (Peppas and Buri, 1985).

1.6.2.2 Environmental factors

The mucoadhesion of a polymer not only depends on its molecular properties, but also on the environmental factors adjacent to the polymer. Saliva, as a dissolution medium, affects the behavior of the polymer. The pH of microenvironment surrounding the mucoadhesive polymer alters the ionization state of polymer and hence the adhesive properties of the polymer. Mucin turnover rate is another very important environmental factor. The residence time of dosage forms is limited by the mucin turnover time, which has been calculated to range between 47 and 270 min in rats (Lehsr et al., 1991) and 12–24 h in humans (Forstner, 1978). Movement of the buccal tissues while eating, drinking, and talking, is another concern, which should be considered when designing a dosage form for the oral cavity. Movements within the oral cavity continue even during sleep, and can potentially lead to detachment of the dosage form (Ho et al., 1992).

1.6.3 Buccal adhesive polymers

Bioadhesive formulations use polymers as the adhesive component. These formulations when in a dry form take up water from the biological surface and swell to form a viscous sticky gel like material resulting in adhesion to the mucosal surface.

1.6.3.1 Desired characteristics

The polymer-related factors have been discussed in the previous section. Some of the necessary structural characteristics for bioadhesive polymers include strong hydrogen bonding groups, strong anionic or cationic charges, high molecular weight, chain flexibility and appropriate wetting property favoring spreading on the mucus layer (Lee et al., 2000).

1.6.3.2 Classification

Bioadhesive polymers reported in the literature can broadly be classified based upon origin as natural, semi-synthetic and synthetic bioadhesive polymers. Naturally occurring bioadhesive macromolecules are structurally similar to synthetic polymers. They are generally linear polymers with high molecular weight, containing a substantial number of hydrophilic charged functional groups and forming three-dimensional expanded networks (Gu et al., 1998). Chitosan (CH) and various gums, such as guar and hakea, are classified as semi-natural/natural bioadhesive polymers. Polyacrylic acid, cellulose ester derivatives, and poly methacrylate derivatives are examples of synthetic bioadhesive polymers.

Bioadhesive polymers are sometimes also classified based upon water solubility as water-soluble and water-insoluble polymers. A classification system dependent upon the charge of the mucoadhesive polymer is also commonly used. The charged polymers are divided into cationic and anionic polymers, such as chitosan and polycarbophil, respectively. Hydroxy propyl cellulose is however an example of non-ionic bioadhesive polymer (Lee et al., 2000).

The classification of various polymers used for the purpose of buccal adhesive delivery has been extensively reviewed recently. The exhaustive classification and review carried out by these researchers (Miller et al., 2005) is presented in Table 1.2.

1.6.4 New generation mucoadhesive polymers

A special class of polymers called as thiomers have recently been reported for mucoadhesive property. These are hydrophilic macromolecules containing free thiol groups on the polymeric backbone. Thiolated derivatives of existing polymers like polyacrylic acid and chitosan resulted in marked improvement in their mucoadhesive property (Hornof et al.,

2003; Langoth et al., 2003). These polymers form disulphide bonds with mucus glycoproteins covering mucosal membranes (Marschutz and Bernkop-Schnurch, 2002; Soo et al., 2002). By immobilization of thiol groups, the mucoadhesive properties of polyacrylic acid and chitosan improved 100 to 250 times (Allen et al., 1999; Kast and Bernkop-Schnurch, 2001; Langoth et al., 2003). It has recently been reported that zinc dependent proteases such as aminopeptidases and carboxypeptidases are also inhibited by these thiolated polymers. The mechanism is based on the capability of these polymers to bind zinc ions. They also exhibit permeation-enhancing effects for the paracellular uptake of drugs (Kast et al., 2003).

A detailed literature review revealed few other mucoadhesive agents like milk protein and lectin based polymers. Milk protein concentrate containing a minimum of 85% of proteins at concentration of 15% to 50% in a bioadhesive tablet showed good bioadhesive property (Aiache et al., 2001). In recent years, lectin based mucoadhesive polymers have been reported as specific adhesives for drug delivery in the oral cavity (Smart, 2004). Lectins adhere to specific sugar residues on mucosal surface thereby resulting in mucoadhesion (Lehr, 2000; Smart et al., 2002). Moreover, lamellar and cubic liquid crystalline phases of glyceryl monooleate have shown mucoadhesive properties and feasibility to be used as carriers for buccal delivery of peptides (Lee and Kellaway, 2002).

1.7 Buccal Mucoadhesive Dosage Forms

Retentive buccal mucoadhesive formulations prove to be a good alternative to the conventional oral medications as they can be readily attached to the buccal cavity, retained for a prolonged duration of time and removed at any time. Buccal mucoadhesive dosage forms are categorized into three types based on drug release pattern. Type I is a single layer device with multidirectional drug release. This dosage form suffers from significant drug loss due to swallowing. In Type II devices, an impermeable backing layer is superimposed on top of the drug-loaded bioadhesive layer, creating a double-layered device and preventing drug loss from the top surface of the dosage form into the oral cavity. Type III is a unidirectional release device, from which drug loss is minimal, since the drug is released only from the side adjacent to the buccal mucosa. This is achieved by coating every face of the dosage form, except the one in contact with the buccal mucosa (Miller et al., 2005; Rossi et al., 2005; Sudhakar et al., 2006).

Buccal dosage forms can also be classified as either reservoir or matrix type. In the reservoir type, the drug is present in a reservoir surrounded by a polymeric membrane, which controls the release rate. In the matrix type systems, the drug is uniformly dispersed

in the polymer matrix, and drug release is controlled by diffusion through the polymer network. Different drug delivery systems intended for buccal administration have been developed and reported in literature. Buccal adhesive drug delivery systems using matrix tablets, films, layered systems, discs, microspheres, ointments and hydrogel systems have been studied and reported in literature (Miller et al., 2005; Rossi et al., 2005; Sudhakar et al., 2006).

In general, dosage forms designed for buccal drug delivery should be small and flexible enough to be acceptable for patients, non-irritating and should not interfere with normal functions such as talking, drinking and eating. Other desired characteristics of a buccal mucoadhesive dosage form include high drug loading capacity, controlled drug release, unidirectional release, good bioadhesive properties, smooth surface, tastelessness, and convenient application. Erodible formulations are considered better because they do not require system retrieval at the end of desired dosing interval.

Numerous important considerations should be taken into account while formulating buccal mucoadhesive drug delivery systems. Biocompatibility, reliability, durability; stability, accuracy, delivery scalability and permeability should be given importance while formulating the delivery system. The properties such as ease of application, spreadability, hardness, residence time affect the ultimate performance of delivery systems and their acceptance by patients should also be considered (Jones et al., 1997).

1.7.1 Mucoadhesive tablets formulations

Tablets have been the most commonly investigated dosage form for buccal drug delivery (Table 1.3). Buccal tablets are small, flat or oval, with a diameter of approximately 5–8 mm (Rathbone et al., 1994). Buccal tablets get hydrated and adhere to the mucosa. They are retained in position until drug dissolution and/or release is complete. Successive tablets can be applied to alternate sides of the mouth. The major drawback of buccal bioadhesive tablets is their lack of physical flexibility, leading to poor patient compliance for long-term and repeated use (Miller et al., 2005). The drugs and polymers that have recently been used and reported for developing buccal mucoadhesive tablets are listed in Table 1.3.

Bioadhesive tablets are usually prepared by direct compression, but wet granulation techniques can also be used (Miller et al., 2005). Tablets intended for buccal administration by insertion into the buccal pouch may dissolve or erode slowly (Ikinci et al., 2004). Multilayered tablets can also be prepared by sequentially adding and compressing the ingredients layer by layer (Park and Munday, 2002). If necessary, the drug may be

formulated in certain physical states, such as microspheres, prior to direct compression in order to achieve some desirable properties like enhanced activity and prolonged drug release (Giunchedi et al., 2002).

1.7.2 Mucoadhesive patches/ films

These are the most recently developed dosage form for buccal administration. Buccal films are sometimes preferred over adhesive tablets in terms of flexibility and comfort. They also overcome the relatively short residence time of oral gels on the mucosa (Rossi et al., 2005). Moreover, in the case of local delivery for oral diseases, the films also help protect the wound surface thus helping to reduce pain and treat the disease more effectively. An ideal film should be flexible, elastic and soft, yet adequately strong to withstand breakage due to stress from mouth movements. It must also possess good bioadhesive strength in order to be retained in the mouth for the desired duration of action. These are laminates consisting of an impermeable backing layer, a drug containing reservoir layer from which the drug is released in a controlled manner, and a bioadhesive surface for mucosal attachment (Li, 2003). Two methods have been reported for preparation of adhesive films namely solvent casting and direct milling. In the solvent casting method, the intermediate sheet from which patches are punched is prepared by casting the solution of the drug and polymer(s) onto a backing layer sheet, and subsequently allowing the solvent(s) to evaporate. In the direct milling method, formulation constituents are homogeneously mixed and compressed to the desired thickness, and patches of predetermined size and shape are then cut or punched out. An impermeable backing layer is generally applied to control the direction of drug release and prevent drug loss. The backing layer even minimizes deformation and disintegration of the device after application (Veuillez et al., 2001). The solvent casting method is simple, but suffers from some disadvantages, including long processing time, high cost, poor content uniformity and environmental concerns due to the solvents used. These drawbacks can be overcome by the hot-melt extrusion method recently reported by Repka et al. (2002). The drugs and polymers that have recently been used and reported for developing buccal mucoadhesive patches/ films are listed in Table 1.4.

1.7.3 Mucoadhesive semi-solid formulations

Semi-solid dosage forms, such as gels and ointments, have the advantage of easy dispersion throughout the oral mucosa but drug dosing from semi-solid dosage forms may not be as accurate as from tablets, patches or films. Poor retention of gels at the site of

application has been overcome by using bioadhesive semi-solid formulations containing entrapped drug molecules that are released by diffusion or erosion (Martin et al., 2003).

Bioadhesive semi-solids have not been described in the literature as extensively as other dosage forms, especially when compared to tablets and patches. Hydroxy propyl methyl cellulose (HPMC) has been used as an adhesive gel ingredient (Ahuja et al., 1997). A highly viscous gel has been reported using carbopol (CP) and hydroxy propyl cellulose as gelling agents, for semi-solid dosage forms that could be maintained on the buccal mucosa for up to 8 h (Ishida et al., 1983).

A major application of adhesive semi-solids is the local delivery of medicinal agents for the treatment of periodontitis. Mucoadhesive polymers were found to be useful for treatment of periodontitis, when incorporated in formulations containing antimicrobial agents (Jones et al., 2000b; Vinholis et al., 2001; Ikinci et al., 2002). Mucoadhesion ensures that the formulation is retained within the pocket. The drugs and polymers that have recently been used and reported for developing buccal mucoadhesive semi-solid formulations are listed in Table 1.5.

1.7.4 Miscellaneous mucoadhesive dosage forms

Innovative drug delivery systems, such as lipophilic gel, buccal spray and phospholipid vesicles have recently been proposed to deliver peptides via the buccal route. Researchers have proposed the use of cubic and lamellar liquid crystalline phases of glyceryl monooleate as buccal drug carrier for peptide drugs (Lee and Kellaway, 2000).

Phospholipid deformable vesicles, transferosomes, have been devised for delivery of insulin in the buccal cavity. They are morphologically similar to liposomes but respond to external stresses by rapid shape transformations requiring low energy. This high deformability allows them to deliver drugs across epithelial barriers. These formulations have resulted in significantly higher bioavailability of insulin in rabbits (Yang et al., 2002).

1.7.5 Commercial status of buccal mucoadhesive drug delivery systems

The market share of buccal adhesive drug delivery systems is increasing in the American and European market with a steady growth rate of above 10%. Buccal adhesive formulations that have been marketed or in advanced developmental stage have been reviewed extensively (Rossi et al., 2005) and are listed in Table 1.6.

A novel liquid aerosol formulation (Oralin, Generex Biotechnology) has been recently developed and is now in clinical phase II trials (Modi et al., 2002). High levels of insulin in the mouth were achieved when compared to conventional technology. This oral

aerosol formulation is rapidly absorbed through the buccal mucosal epithelium, and it provides the plasma insulin levels necessary to control postprandial glucose rise in diabetic patients.

Striant® a testosterone buccal delivery system has also recently been approved by the United States Food and Drug Administration (USFDA). It is indicated for replacement therapy in males for conditions associated with a deficiency or absence of endogenous testosterone. The tablet is applied to the gum region twice daily.

1.8 Evaluation of Buccal Mucoadhesive Dosage Forms

Evaluation (in vitro and in vivo) of buccal mucoadhesive dosage forms differ markedly from other dosage forms as the conditions prevalent in buccal cavity need to be simulated. Apart from routine evaluation tests like weight variation, friability, hardness, thickness, content uniformity, swelling (for buccal tablets), thickness, content uniformity, tensile strength, film endurance, swelling (for buccal patches/films) and content uniformity, viscosity (for buccal semi-solids), specialized evaluation tests like drug release rate, drug permeability, mucoadhesive strength, residence time, acceptability need to be carried out.

1.8.1 Mucoadhesion measurement

A majority of the quantitative mucoadhesion measurement methods found in literature are based upon measuring the force required to break the adhesive bond between model membrane and the delivery system. Depending on the direction in which the adhesive is being separated from the substrate, peel, shear, and tensile forces can be measured as shown in Figure 1.3 (Sudhakar et al., 2006).

1.8.1.1 Determination of peel strength

The peel adhesion tests are mainly used for buccal and transdermal patches (Lee et al., 2000). The test is based on calculation of energy required to detach dosage form from the substrate material (usually excised buccal mucosa) in the direction shown in Figure 1.3 (Sudhakar et al., 2006).

1.8.1.2 Determination of shear strength

Shear stress measures the force that is required for causing the delivery system to slide with respect to the mucus layer in a direction parallel to their plane of contact as shown in Figure 1.3. Researchers have studied the mucoadhesive strength of calcium polycarbophil, sodium CMC, HPMC using homogenized mucus from pig intestine as model

substrate (Lehr et al., 1991). Two glass plates were coated with this homogenized mucus and the delivery system was allowed to hydrate between these two mucus coated plates. The force required to pull these plates apart was used for calculation of shear strength. Similarly, mucoadhesive strength of carbopol, CMC, HPMC, gelatin, polyvinyl pyrollidine (PVP), acacia, polyethylene glycol (PEG), pectin, tragacanth and sodium alginate gels has been measured by the force required to pull the mucus coated plate out of the gel under constant experimental conditions (Ishida et al., 1981; Gurney et al., 1984; Smart and Johnson, 1996).

1.8.1.3 Determination of tensile strength

Tensile strength calculates the force required to detach the delivery system from mucosal membrane when a vertical force is applied (Figure 1.3). This is the most widely reported method for determination of mucoadhesion. This method for determination of mucoadhesion can be extended to a variety of dosage forms like solids, semi-solids and liquids (Wong et al., 1999a).

Many researchers have used Texture Analyzer for measuring bioadhesion. The force required to detach the delivery system from the mucosal surface can be accurately measured and work of adhesion can also be calculated using this technique (David et al., 1997; Jones et al., 1999; Wong et al., 1999a,b; Eouania et al., 2001; Michael et al., 2005; Owens et al., 2005). This is by far the most sensitive and validated technique reported in literature for determination of mucoadhesion (Wong et al., 1999a).

Many researchers have studied mucoadhesion of dosage forms using buccal mucosa as substrate by using modified pan balance (Lehr et al., 1991; Ahuja et al., 1997; Desai and Pramod Kumar, 2005). Few other methods like colloidal gold staining method (Park and Park, 1989) and direct staining methods (Koclkisch et al., 2001) have also been reported.

1.8.2 In vitro release studies

Novel dosage forms present problems in the development of in vitro release technologies simply because of the physicochemical properties of formulations and the unique physiological environment in which they should release their content (Siewert et al., 2003). An ideal in vitro release study should provide valuable information regarding in vivo performance of the delivery system. Currently, the United States Pharmacopoeia (USP) is working to increase the prevalence of USP performance testing, moving beyond solid oral dosage forms. The goal is to have a fully functional set of USP performance tests for all kinds of dosage forms. USP apparatus 4, apparatus 7 and modifications of the official

apparatuses have shown great potential and value for in vitro release for novel dosage forms (Williams and Foster, 2004).

USP allows use of disintegration test for ergoloid mesylate and ergotamine tartrate sublingual tablets and apparatus 2 with water as dissolution medium for isosorbide dinitrate sublingual tablets (US Pharmacopoeia, 2003). However, in vivo dissolution is limited for these tablets by the amount of saliva present within the mouth. As a result, dissolution tests using standard USP apparatuses and large volumes of liquids might not produce results that simulate actual in vivo dissolution of drug.

Therefore, several studies have been reported to investigate drug dissolution in smaller dissolution media volume or using different apparatuses to simulate conditions prevalent in buccal cavity. USP apparatus 3 at a rate of 20 strokes/min for conducting in vitro dissolution studies of hydrocortisone hemisuccinate mucoadhesive buccal tablets has been reported (Fabregas and Garcia, 1995).

Researchers have recently used a system comprising of continuous flow-through cell with a dip tube to remove finely divided solid particles (Hughes, 2003). The volume of liquid in the cell is small (10 ml) and the fluid is pumped through, to give a short residence time with almost complete removal in about 8 min. The cell is filled and flow rates are set up and allowed to reach steady state before the dosage form is introduced. The collected samples are filtered and analyzed. Simulated salivary fluid was used as dissolution media (Davis et al., 1971; Tavss et al., 1984).

Dor and Fix (2000) developed a special disintegration test using texture analyzer to accurately determine the rate of drug release from sublingual/buccal dosage forms. In this method, the tablet is attached to a cylindrical probe and then submerged under a constant force into a small volume (20 ml) of dissolution media. The time for complete tablet disintegration versus distance traveled is determined. The disadvantage of this method is that one side of the tablet cannot interact with immersion medium, due to the adhesive attaching the tablet to probe; whereas the tablet is moistened on all sides in the oral cavity which enhances disintegration. To compensate for this, Abdelbary et al. (2005) placed the tablet in a perforated grid, and then allowed the probe to be lowered onto the tablet until the desired pressure was created.

Drug release studies for buccal tablets is mostly reported using USP apparatus 2 (Ceschel et al., 2001; Rambali et al., 2001; Jain et al., 2002; Jug and BecirevicLacan, 2004). However some authors wanted to mimic the intended drug release in one direction only (buccal mucosa) and proposed use of intrinsic dissolution apparatus to analyze the drug released from one surface only (Parodi et al., 1996; Cilurzo et al., 2003; Akbari et al., 2004;

ElGindy, 2004). In order to expose a single face with constant area to the medium, they coated all surfaces except one using a water impermeable coating.

Ikinci et al. (2004) used an alternative method to study the release of nicotine from buccal tablets. They used modified Franz diffusion cells for this purpose. The dissolution medium was 22 ml phosphate buffer saline. Uniform mixing of the medium was provided by stirring. To provide unidirectional release, each bioadhesive tablet was embedded into paraffin wax which was placed on top of a bovine buccal mucosa as membrane. Mohammed and Khedr (2003) used an easier method to perform the in vitro drug release study by introducing a tablet in a beaker containing 10 ml of phosphate buffer (pH 6.8). The beaker was shaken horizontally at 50 RPM in a water bath. Samples were withdrawn at predetermined time intervals and replaced with fresh medium.

Mumtaz and Chang (1995) introduced another method for studying the dissolution of buccal tablets. The device that they used was based on the circulation of pre-warmed dissolution medium through a cell. The buccal tablet was attached onto chicken pouches. Samples were removed at different time intervals for drug content analysis.

1.8.3 Residence time

1.8.3.1 In vitro residence time

The method most widely reported for estimation of in vitro residence time uses modified USP disintegration test apparatus. The disintegration medium generally used is 800 ml isotonic phosphate buffer pH 6.7 maintained at 37 °C. A segment of rabbit intestinal mucosa is glued to the surface of a glass slab, vertically attached to the apparatus. The delivery system is hydrated from one surface using isotonic phosphate buffer and then the hydrated surface is brought into contact with the mucosal membrane. The glass slab is vertically fixed to the apparatus and allowed to move up and down. The time taken for complete erosion or detachment of the delivery system from the mucosal surface is recorded (Nafee et al., 2004b; Patel et al., 2006).

1.8.3.2 In vivo residence time

Reported in vivo residence time experiments have all been generally conducted on healthy human subjects between 25 to 50 years of age. Placebo bioadhesive delivery system is placed on the mucosal lining of buccal epithelium. The subjects then monitor the time for which the system is retained on the mucosa (Nafee et al., 2004b; Desai and Pramod Kumar, 2005).

1.8.4 Permeation studies

Prior to formulating a buccal delivery system for a drug, buccal absorption/permeation studies must be conducted to determine the feasibility of this route of administration for the candidate drug. Commonly reported in vitro, in vivo techniques including the animals models have already been discussed at length in section 1.4 of this chapter.

1.8.5 Acceptability studies

Acceptability and compatibility studies of placebo delivery systems have been reported in healthy human subjects between 25 to 50 years of age. Placebo bioadhesive delivery system is placed on the mucosal lining of buccal epithelium. The subjects are asked to record any complaints such as discomfort, bad taste, dry mouth or increase of salivary flux, difficulty in speaking, irritation or mucosal lesions (Nafee et al., 2004b; Desai and Pramod Kumar, 2005).

1.9 Buccal Delivery of Cardiovascular Drugs

Buccal delivery of cardiovascular drugs like metoprolol (Wong et al., 1999b), propranolol (Patel et al., 2006,2007), glyceryl trinitrate (Rossi et al., 2005; Sudhakar et al., 2006), verapamil (Sawicki and Janicki, 2002), diltiazem (Nafee et al., 2004a), nifedipine (Varshosaz and Dehghan, 2002) has been reported in the literature.

Buccal mucoadhesive delivery systems can be prepared for cardiovascular drugs that are not absorbed orally or undergo high first pass metabolism. Buccal delivery of cardiac drugs leads to quicker onset of action. Maintenance of drug concentration in the plasma for longer time duration is possible by controlling drug release, thereby reducing dosing frequency and increasing patient compliance. The route is also suitable for drugs absorbed poorly and erratically from GI tract (Varshosaz and Dehghan, 2002). Drugs administered by buccal route bypass the first pass effect and harsh GI environment resulting in higher bioavailability of drugs prone to first pass metabolism and degradation within the GI tract (Varshosaz and Dehghan, 2002).

1.10 Objectives of the Research

Lercanidipine hydrochloride (LER) is a third generation dihydropyridine calcium channel antagonist with a bulky bis-phenylalkylamine side chain. This drug is preferably used in the treatment of hypertension, because of its selectivity and specificity on the

smooth vascular cells. The drug is administered orally in a dose of 10-20 mg daily as its hydrochloride salt, reducing the blood diastolic pressure significantly after a single dose.

Formulations currently available in market (conventional tablets) show erratic oral absorption due to interaction with food and poor oral bioavailability (10-20%) due to extensive first pass metabolism with high inter- and intra-patient variability. Moreover the drug shows saturable first pass metabolism. Alternative routes of administration are being explored for such drugs to overcome above-mentioned problems for better therapeutic efficacy and patient compliance.

Present research endeavor aimed at designing better and alternative delivery systems to overcome above-mentioned problems associated with currently marketed formulations of LER. Buccal delivery of LER at controlled rate will help in achieving desired plasma concentration of drug quickly and for extended period. The venous drainage of buccal route is not subjected to hepatic first pass metabolism so bioavailability problems can be eliminated resulting in probable dose reduction and predictable plasma profile.

The present research work was thus targeted at preparation of buccal mucoadhesive controlled drug delivery systems of lercanidipine hydrochloride using various mucoadhesive and rate controlling polymers either alone or in combination for increasing bioavailability. Research work was carried out in following stages for achieving this broad objective.

- Selection of appropriate formulation additives on the basis of preformulation studies.
- Optimization of process variables and physical characteristics of formulations such as size, shape, thickness, hardness, friability and surface pH.
- Evaluation and optimization of designed formulations for in vitro release character and in vitro mucoadhesive property.
- Assessment of acceptability of drug free formulations in human subjects.
- In vivo pharmacokinetic, bioavailability and permeation studies in rabbits.

Formulation development, evaluation and optimization need suitable and sensitive analytical method(s) for analysis of drugs in variety of samples like bulk powders, formulations, in vitro release samples, stability samples and biosamples. The current research endeavor also aimed at developing and validating suitable analytical methods for estimation of drug in variety of samples using techniques like UV spectrophotometry and HPLC.

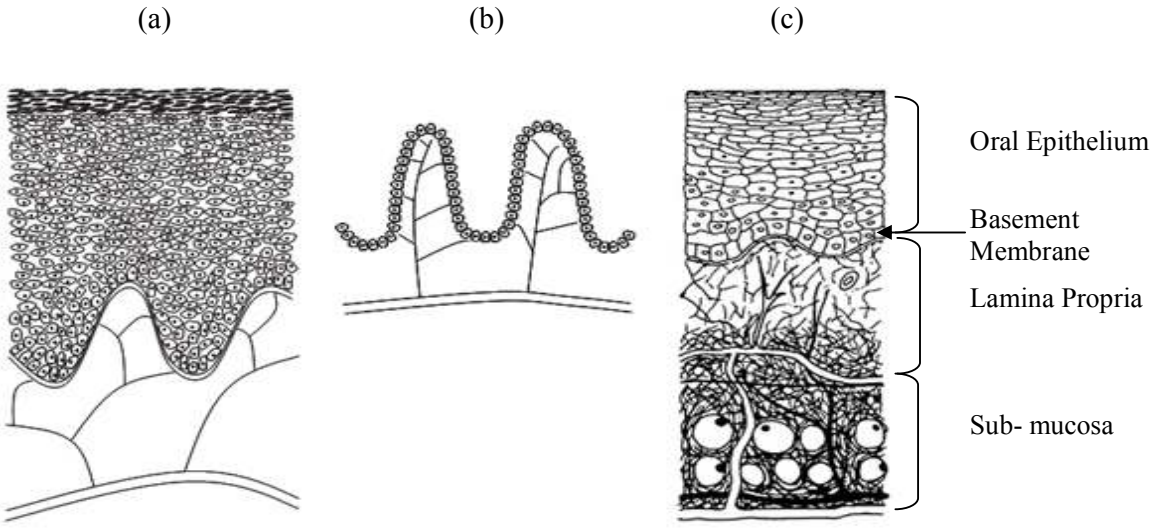

Figure 1.1: A structural comparison of the skin (a), small intestine (b) and buccal mucosa (c) (Miller et al., 2005; Nicolazzo et al., 2005a)

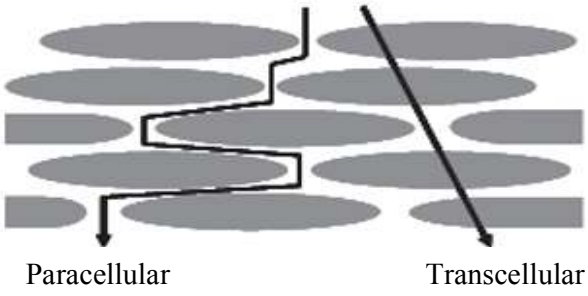

Figure 1.2: A schematic representation of buccal epithelium with both the routes of drug transport (Nicolazzo et al., 2005a)

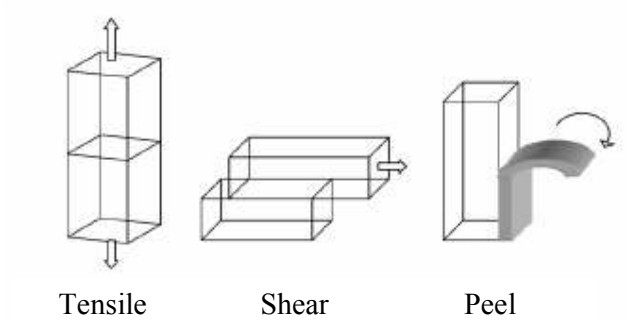

Figure 1.3: A schematic representation of tensile, shear and peel forces (Sudhakar et al., 2006)

Table 1.1: List of various permeation enhancers with their proposed mechanism(s) of action

Category	Examples	Mechanism(s)	References
Surfactants and Bile Salts	Sodium glycodeoxycholate Sodium dodecyl sulphate Sodium lauryl sulphate Polysorbate 80	Acting on the components at tight junctions; Increasing the fluidity of lipid bilayer membrane; By overcoming the enzymatic barrier	(Froebe et al., 1990; Steward et al., 1994; Borras-Blasco et al., 1997; Deneer et al., 2002; Xiang et al., 2002; Nicolazzo et al., 2004a)
Fatty Acids	Oleic acid Cod liver oil Capric acid Lauric acid	Increasing the fluidity of lipid bilayer membrane	(Coutel-Egros et al., 1992; Morishita et al., 2001; Tsutsumi et al., 2002)
Polymers and Polymer Derivatives	Chitosan Trimethyl chitosan Chitosan-4-thiobutylamide	Increasing the fluidity of lipid bilayer membrane; Increased retention of drug at mucosal surface	(Martin et al., 2003; Park and Munday, 2004; Sandri et al., 2004,2005,2006)
Others	Ethanol Azone® Octisalate Padimate Menthol Cyclodextrins	Acting on the components at tight junctions; Increasing the fluidity of lipid bilayer membrane	(Squier et al., 1986a; Coutel-Egros et al., 1992; Turunen et al., 1994; Du et al., 2000; Howie et al., 2001; Sciubba, 2001; Jain et al., 2002; Nicolazzo et al., 2004b,2005b,c)

Table 1.2: Classification of mucoadhesive polymers (Miller et al., 2005)

Criteria	Category	Examples
Source	Natural/ Semi Natural	Agarose, Chitosan, Gelatin, Hyaluronic acid Various Gums (Guar, Xanthan, Gellan, Carragenan, Pectin, Sodium Alginate)
	Synthetic	Cellulose Derivatives (Carboxy methyl cellulose (CMC), Thiolated CMC, Hydroxy ethyl cellulose, Hydroxy propyl cellulose, Hydroxy propyl methyl cellulose, Methyl cellulose, Methyl hydroxy ethyl cellulose)
		Polyacrylic acid based Polymers (Carbopol, Polycarbophil, Polyacrylates, Polymethacrylate, copolymers of acrylic acid and Polyethylene glycol)
Aqueous Solubility	Water Soluble	Carbopol, Hydroxy ethyl cellulose, Hydroxy propyl cellulose, Hydroxy propyl methyl cellulose, Sodium CMC, Sodium alginate, Polyethylene oxide, Chitosan, Polycarbophil
	Water Insoluble	Ethyl cellulose
Charge	Cationic	Chitosan, Aminodextran
	Anionic	Carbopol, Polycarbophil, Sodium alginate, Sodium CMC, CMC, Chitosan-EDTA
	Non ionic	Polyvinyl alcohol, Hydroxy propyl cellulose, Polyethylene oxide
Potential Bioadhesive Forces	Covalent	Cyanoacrylate
	Hydrogen Bond	Carbopol, Polycarbophil, Polyvinyl alcohol, Acrylates
	Electrostatic	Chitosan

Table 1.3: Recently reported drugs and polymers for developing buccal mucoadhesive tablets

Active Ingredient	Polymer Used	References
Acyclovir	HPC, CP	(Degim et al., 2006)
Buspirone	CP, HPMC	(Du et al., 2002)
Carbamazepine	HPMC, CP	(Giannola et al., 2006)
Cetylpyridinium	Sodium CMC, HPMC	(Ali et al., 2002)
Chlorhexidine	HPMC, CP	(Carlo et al., 2006)
Chlorpheniramine	Hakea gum	(Alur et al., 1999)
Diltiazem	CP, HPMC, Sodium CMC	(Nafee et al., 2004a)
Ergotamine tartrate	Carboxy vivyl polymer, HPC	(Tsutsumi et al., 2002)
Fluoride	Sodium CMC, CP	(Owens et al., 2005)
Hydrocortisone	HPMC, CP, PC	(Ceschel et al., 2001)
Insulin	CP, HPMC, HPC	(Hosny et al., 2002)
Lidocaine	HPC, HPMC	(Michael et al., 2005)
Metaclopromide	CP, HPMC, Sodium CMC	(Nafee et al., 2004a)
Metoprolol	Eudragit, HPMC, CP	(Wong et al., 1999b)
Metronidazole	HEC, HPC, HPMC, CP, PC	(Perioli et al., 2004)
Miconazole	Spray dried starch, CP	(Ameye et al., 2005b)
Nicotine	Chitosan, CP	(Ikinci et al., 2006)
Nifedipine	CMC, CP	(Varshosaz and Dehghan, 2002)
Nystatin	Spray dried starch, CP	(Ameye et al., 2005a)
Omeprazole	HPC, Sodium alginate	(Choi and Kim, 2000)
Piroxicam	HPMC, CP	(Jug and BecirevicLacan, 2004)
Prednisolone	HPMC, Sodium CMC, CP	(Mohammadi-Samani et al., 2005)
Propranolol	EC, CP, Sodium CMC	(Patel et al., 2007)
Testosterone	Spray dried starch, CP	(Ameye et al., 2005b)
Theophylline	Starch acrylic acid graft copolymers	(Geresh et al., 2004)
Verapamil	HPC, CP	(Sawicki and Janicki, 2002)

Table 1.4: Recently reported drugs and polymers for developing buccal mucoadhesive patches/ films

Active Ingredient	Polymer Used	References
Acyclovir	Chitosan, Polyacrylic acid	(Rossi et al., 2003)
Cetylpyridinium	PVA, HEC, Chitosan	(Nafee et al., 2003a)
Chlorhexidine	Chitosan	(Li et al., 1997)
Lidocaine	HPC	(Okamoto et al., 2002)
Miconazole	Sodium CMC, Chitosan, PVA, HEC, HPMC	(Nafee et al., 2003b)
Nicotine	PC, HPMC	(Garg and Kumar, 2007)
Plasmid DNA	PC, Eudragit	(Cui and Mumper, 2002a)
Propranolol	Chitosan	(Patel et al., 2006)
Testosterone	PC, Eudragit	(Jay et al., 2002)
Thiocolchicoside	Gelatin and CMC	(Artusi et al., 2003)
Triamcinolone	CP, HPMC, Poloxamer	(Chun et al., 2003)

Table 1.5: Recently reported drugs and polymers for developing buccal mucoadhesive semi-solid formulations

Active Ingredient	Polymer Used	References
Chlorhexidine	HEC, PVP, PC	(Jones et al., 2000a)
Denbufylline	Palmitoyl glycol chitosan	(Martin et al., 2003)
Lidocaine	PEG, CP, PVP	(Tan et al., 2000)
Recombinant human epidermal growth factor	PC	(Park et al., 2003)
Triamcinolone	Poloxamer, CP	(Shin and Kim, 2000)

Table 1.6: Buccal adhesive formulations marketed or in advanced developmental stage for both mucosal (local) or transmucosal (systemic) administration (Rossi et al., 2005)

Brand Name	Active Ingredient	Effect	Functional Agent	Company
Aphtach (Tablet)	Triamcinolone acetonide	Local	HPC	Teijin
Buccastem Buccal (Tablet)	Prochlorperazine	Systemic	Xanthan gum	Reckitt Benkiser
Oralin-Generex (Solution)	Insulin	Systemic	Unknown	Generex (Phase II Trials)
Lauriad (Tablet)	Miconazole	Local	Unknown	BioAlliance Pharma (Phase II Trials)
Striant SR Buccal (Tablet)	Testosterone	Systemic	CP, HPMC, PC	Ardana Bioscience
Suscard Buccal (Tablet)	Glyceryl trinitrate	Systemic	HPMC	Forest Laboratories

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Chapter 2
Drug Profile:
Lercanidipine Hydrochloride

2.1.1 Chemistry

Lercanidipine hydrochloride (LER) is chemically 2-[(3,3-diphenylpropyl) methylamine]- 1, 1-dimethyl ethyl methyl 1, 4- dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5 pyridine carboxylic ester hydrochloride (Figure 2.1) with a molecular weight of 648.2. The molecular weight of free base is 611.7. LER is a third generation dihydropyridine calcium channel antagonist with a bulky bis-phenylalkylamine side chain, which makes it more lipophilic than most other drugs in its class like nifedipine, felodipine and amlodipine (Luscher and Cosentino, 1998). It is a racemate due to the presence of a chiral carbon atom at position 4 of the 1,4-dihydropyridine ring (Herbette et al., 1997). LER is a odorless, yellow powder. The synthetic procedure was first reported in 1987 (Nardi et al., 1987).

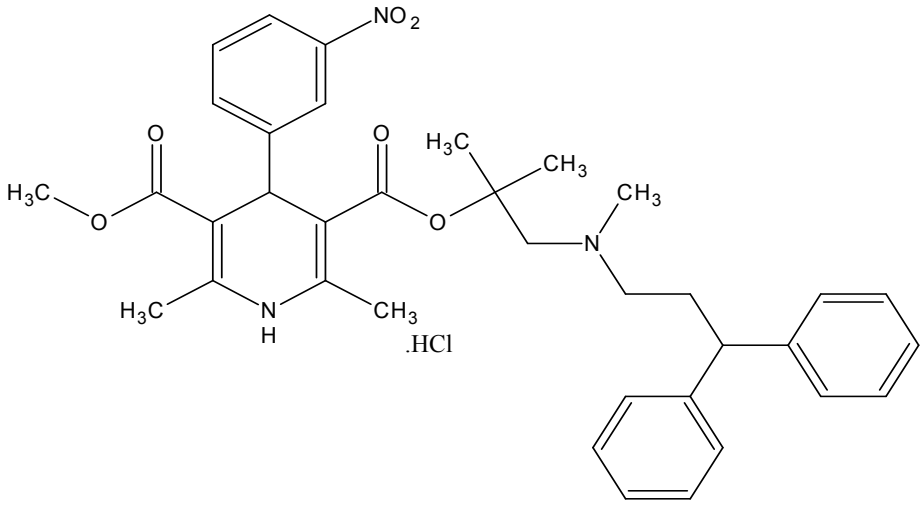

Figure 2.1: Structure of lercanidipine hydrochloride

2.1.2 Solubility

LER is poorly soluble in water but readily soluble in organic solvents like methanol and chloroform with a theoretical partition coefficient (Log P) of 4.3 (Vander Lee et al., 2000).

2.1.3 Polymorphism

Lercanidipine hydrochloride exhibits polymorphism (Form I and Form II) and crystallizes in specific polymorphic forms depending upon the synthetic route followed and

solvents used for crystallization. The two polymorphs have distinct chemical and physical properties (Bonifacio et al., 2005).

Both the crystalline forms of LER exhibit good stability. Form I is characterized by a paler yellow color, smaller crystal size, higher solubility in aqueous media (in comparison to Form II), and a melting point within the range of about 197-201^{o}C. Form II is characterized by a more pronounced yellow color, larger crystal size, slightly lower solubility in aqueous media, and a melting point within the range of about 207-211^{o}C. Form I and form II have also been reported to show distinct IR spectra (Bonifacio et al., 2005).

2.1.4 Stereoisomerism

LER exists as *(R)*- or *(S)*-enantiomer or as a racemate. The *(S)*-enantiomer and the racemate of LER, both possess antihypertensive activity, and can be used for treatment of hypertension and atherosclerotic diseases. On the other hand, *(R)*-enantiomer is reported to have minimal antihypertensive activity. This enantiomer can be used for treating atherosclerotic diseases without any concomitant cardiovascular effect. This enantiomer is useful for treatment of atherosclerotic patients for whom reduction of blood pressure would be undesirable (Leonardi and Motta, 1999).

2.1.5 Pharmacodynamic profile

The pharmacodynamic effects of LER have been evaluated in vitro, in animal models and in patients with essential hypertension with or without diabetes mellitus.

2.1.5.1 Mechanism of action

LER competitively binds to the di hydro pyridine (DHP) site of L-type calcium channels in cardiac and vascular smooth muscle cells, inhibiting transmembrane influx of calcium ions resulting in muscle relaxation (Guarneri et al., 1996; Leonardi et al., 1997; Herbette et al., 1998). The antihypertensive activity is primarily attributed to the *(S)*-enantiomer and racemic form of LER (Leonardi and Motta, 1999). The antihypertensive effect of LER results from peripheral vasodilation and decreased total peripheral resistance as demonstrated in canine models (Testa et al., 1997).

Due to its high lipophilicity, LER is soluble in phospholipid bilayer of cell membranes. This leads to accumulation and storage of LER in cell membranes resulting in longer duration of action. It has been demonstrated that LER inhibits contractile response of rat aorta to potassium ions. This inhibition persisted even after repeated washouts and accounts for longer duration of action of LER (Guarneri et al., 1996; Herbette et al., 1998).

2.1.5.2 Vascular selectivity

Literature shows that, unlike other DHP calcium channel antagonists, LER is highly selective for the vascular smooth muscle over other smooth muscle types. The relaxant potency of LER in vascular smooth muscles (rat aorta) was found to be 177-fold higher than in the rat bladder and 8.5-fold higher than in the rat colon. However, other DHP calcium channel antagonists (felodipine, nitrendipine, lacidipine and amlodipine) have been reported to have similar activity in these three tissues (Guarneri et al., 1996). Due to its vasoselectivity, LER has a weak cardiodepressant (negative ionotropic) activity as compared to other DHP calcium channel blockers (CCB) like felodipine, nitrendipine, lacidipine and amlodipine (Guarneri et al., 1996; Angelico et al., 1999).

2.1.5.3 Antihypertensive effects

LER has a slow onset and a long lasting antihypertensive effect, despite its short plasma half-life. This has been demonstrated in various studies using isolated rat aortic strips (Guarneri et al., 1996; Leonardi et al., 1997), stimulated isolated rabbit aorta (Angelico et al., 1999) and human subcutaneous arteries (Vander Lee et al., 2000). The antihypertensive activity of LER has been also been demonstrated in vivo in spontaneously hypertensive rats and dogs (Sironi et al., 1997; Testa et al., 1997). The gradual onset and prolonged duration of action has been significantly correlated with degree of lipophilicity ($p < 0.05$) for all DHP calcium channel blockers (Vander Lee et al., 2000).

Administration of LER results in blood pressure (BP) reduction that persist over 16 h after a single dose of 10 mg (Omboni and Zanchetti, 1998) or 20mg (Ambrosioni and Circo, 1997) in patients with mild-to-moderate hypertension. This combination of short plasma half-life, gradual onset of action and long duration of action account for the favorable safety and tolerability profile of LER (Epstein, 2001).

Plasma half-life of LER has been reported in literature as 3 to 5 h, yet it acts as a long-lasting CCB. This property has been related to the presence of a lipophilic anchor group, which causes LER to bind to the tissue wall compartment, and a protonated amine group, which allows exchange of LER between plasma components and the tissue wall compartment (Herbette et al., 1998).

2.1.5.4 Other effects

LER has antiatherogenic potential apart from its BP-lowering effects, and may protect end-organ damage. Moreover, the drug does not interfere with normal cardiac

excitation and conduction when used at therapeutic doses in patients with hypertension (Guarneri et al., 1996; Angelico et al., 1999).

LER has been reported to favorably affect lipid metabolism in patients with essential hypertension (Notarbartolo et al., 1999) and Type 2 diabetes (Rachmani et al., 2002). LER is also reported to have a nephroprotective effect in rats as it improves glomerular capillary pressure because of dilation of both the afferent and efferent glomerular arterioles (Sabbatini et al., 2000). Moreover, LER appears to causes less peripheral oedema than nifedipine (Fogari et al., 2000) or amlodipine (Lund-Johansen et al., 2003) in patients with hypertension.

2.1.6 Pharmacokinetic profile

The pharmacokinetics of LER have been examined and reported in healthy subjects and patients with hypertension, including elderly patients and patients with renal or hepatic impairment. A summary of pharmacokinetic properties of single dose LER (10 mg and 20 mg) is given in Table 2.1.

2.1.6.1 Absorption

LER is administered as a racemic mixture of *(R)*- and *(S)*-lercanidipine and after oral administration, it is completely absorbed from the gastro-intestinal tract (Barchielli et al., 1997). Absolute bioavailability is reduced to approximately 10% because of extensive first pass metabolism (Bang et al., 2003).

Following oral administration LER demonstrates nonlinear pharmacokinetics (Barchielli et al., 1997). After administration of a single oral dose of LER 10, 20 or 40 mg the mean maximum plasma concentration (C_{MAX}) values of *(S)*- lercanidipine in healthy subjects were in the ratio 1:3:8 and the area under the plasma concentration-time curve (AUC) values in the ratio 1:4:18 were obtained (Barchielli et al., 1997). Hence plasma levels and the area under curve are not linearly related to dose, indicating saturable first pass metabolism.

The absorption of LER increases in the presence of food. C_{MAX} for *(S)*- lercanidipine after a single oral dose of LER 20 mg increased from 3.20 µg/L when fasting to 10.21 µg/L after a high fat meal in 12 healthy subjects (Barchielli et al., 1997). Hence, LER is generally recommended to be taken before meals.

2.1.6.2 Distribution

LER accumulates in the lipid bilayer of cell membranes in the arterial wall and shows high serum protein binding (~98%). The apparent volume of distribution of LER is reported to be 2–2.5 L/kg after a 15-minute intravenous infusion in healthy subjects, reflecting the high lipophilicity of the drug (Barchielli et al., 1997).

2.1.6.3 Metabolism and elimination

After absorption, oral LER undergoes extensive first-pass metabolism to largely inactive metabolites (Barchielli et al., 1997). LER is metabolized by the hepatic enzyme cytochrome P450 (CYP) 3A4 and has the potential for interactions with drugs metabolized by this pathway (Bang et al., 2003). LER is eliminated to a similar extent in the urine and faeces, following biotransformation (Table 2.1). No unchanged drug is excreted in urine. Administration of a single dose of LER 10 mg and 20 mg to fasting healthy subjects or patients with hypertension resulted in mean terminal plasma elimination half-lives ($t_{1/2}$) of 2.8 and 4.4 h respectively (Barchielli et al., 1997). However, with the use of more sensitive assays in hypertensive patients and in those with angina pectoris, the $t_{1/2}$ for LER 10 and 20 mg was 8.0 or 10.5 h respectively (Bang et al., 2003).

2.1.7 Dosage and administration

Oral LER is approved for the treatment of mild-to-moderate hypertension in most of Europe (including the UK), Asia, Australia and South America. LER therapy should be initiated at 10 mg/day. The dosage can be gradually titrated to 20 mg/day in patients who do not respond satisfactorily (Bang et al., 2003). Dosage adjustments are not required in the elderly or in patients with mild-to-moderate renal or hepatic dysfunction. LER is not recommended for use in patients with severe hepatic or renal dysfunction, nor in patients below 18 years of age (Bang et al., 2003). LER is contraindicated during pregnancy and lactation, and in women of child bearing potential unless effective contraception is used (Bang et al., 2003). LER should not be co-administered with inhibitors of cytochrome P450 (CYP) 3A4 or cyclosporin or grapefruit juice. Furthermore, caution should be exercised when administering LER with inducers or other substrates of cytochrome P450 (CYP) 3A4 (McClellan and Jarvis, 2000).

2.1.8 Commercially available formulations

For the first time, Recordati introduced LER tablets in The Netherlands under the proprietary name Zanidip® (10 mg) for treatment of hypertension. Subsequently in 2003,

20 mg strength of Zanidip® was launched in the market. In India, Glenmark Pharmaceuticals Limited launched the product under the brand name Lerez®, subsequently Microlabs (Landip®), Nicholas Piramal India Limited (Lerka®) and Lupin Laboratories Limited (Lerva SC®) also launched tablets of 10 mg strength in Indian market. Recently, fixed dose combinations of LER with atenolol (Lerez-AT®) and enalparil (Zanipress®) have also been launched.

Table 2.1: Summary of pharmacokinetic properties of single dose lercanidipine in healthy subjects and patients with mild to moderate hypertension (Bang et al., 2003)

Dosage	C_{MAX} (μg/l)[a]	t_{max} (h)[b]	AUC (μg.h/l)[c]	$t_{1/2}$ (h)[d]	Elimination of Metabolites (%)	
					Urine	Faeces
LER 10 mg	1.75	2.3	4.55	8.0	NR	NR
LER 20 mg	4.09	3.3	16.36	10.5	43.8	50.4

[a] Maximum plasma concentration

[b] Time to reach maximum concentration

[c] Area under the plasma concentration-time curve

[d] Half-life

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Chapter 3
Analytical Method Development

3.1 Introduction

Analysis is an important and integral component in the formulation development of any drug molecule and characterization of the developed formulations. Suitable and validated analytical methods are imperative for estimation of the drug in variety of samples like bulk powders, formulations, in vitro release samples, stability samples and biological samples. If a suitable method for specific need is not reported it becomes essential to develop and validate a need based, sensitive, simple, rapid and cost effective method for estimation of the drug in various samples.

3.2 Analytical Methods for Estimation of Lercanidipine Hydrochloride

Lercanidipine Hydrochloride (LER) is a third generation dihydropyridine calcium channel antagonist. Considering that LER is a new drug, official methods for its estimation have not been incorporated in any of the leading Pharmacopeias. Few analytical methods have been reported for the estimation of LER in variety of study samples like bulk, formulation, stability and bio samples.

An extractive spectrophotometric method has been reported for estimation of LER in bulk and formulations and is based upon formation of colored chloroform extractable ion pair complexes with bromothymol blue and bromocresol green (Erk, 2003). A differential pulse voltammetric assay has also been reported for estimation in tablets (Alvarez-Lueje et al., 2002). A selective reversed phase HPLC method using UV and electrochemical detection of LER has also been reported (Alvarez-Lueje et al., 2003) for estimation in tablets. A capillary electrophoresis method has been reported for assay of both enantiomer and diasteromer of LER in bulk samples (Christians et al., 1999). Recently stability indicating HPLC and LCMS methods have been reported for estimation of LER in formulations (Fiori et al., 2006). Simultaneous estimation of dihydropyridine calcium channel antagonists (amlodipine, nitrendipine, felodipine, lacidipine and lercanidipine) in pharmaceutical formulations has been reported as well (Baranda et al., 2004, 2005a). Several methods have been reported for estimation of LER in human plasma by LCMS technique (Jabor et al., 2003; Salem et al., 2004; Baranda et al., 2005b; Kalovidouris et al., 2006). A clinical pharmacokinetic study based on data obtained by HPLC-UV detection has also been published (Barchielli et al., 1997).

An exhaustive survey of literature revealed that none of the reported methods were suitable for routine analysis of LER in formulations and in in vitro release samples of present research endeavor. In general, the method of analysis should be simple, cost effective and less time consuming apart from being sensitive, accurate, precise and stability

indicating. Simple spectrophotometric methods are found to be very suitable for routine analysis of formulations for content uniformity and analysis of in vitro release samples. Extractive spectrophotometric method (Erk, 2003) and differential pulse voltammetric method (Alvarez-Lueje et al., 2002) reported for analysis of drug content in bulk and formulations suffer from drawback of tedious sample preparation. Reported HPLC methods (Alvarez-Lueje et al., 2003; Fiori et al., 2006) although seem to be sensitive and precise but use of HPLC methods for the routine analysis of drug samples, especially from in vitro release studies of controlled release formulations, involving multiple time points seems to be cumbersome, costly and time consuming.

The methods reported for estimation of LER in biosamples, although found to be very sensitive, precise and accurate but mostly uses LCMS technique (Salem et al., 2004; Baranda et al., 2005b; Jabor et al., 2003; Kalovidouris et al., 2006). Such methods are not practical in laboratories with relatively modest infrastructure. Moreover, extensive literature survey did not reveal any method for estimation of LER in rabbit serum. As rabbit was selected as animal model for in vivo studies of developed formulations, it was planned to develop a simple, sensitive and accurate HPLC method for estimation of LER in rabbit serum.

Hence, the present investigation, in the first instance, aimed at the development of a simple, sensitive, accurate, reproducible and economical spectrophotometric analytical method for estimation of LER in bulk and formulations. It was decided to develop ultraviolet spectrophotometric method for estimation of LER in in vitro release samples. Liquid chromatographic methods were developed for estimation of drug content in stability samples and biosamples. All developed methods were validated according to the standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2003). Suitable statistical tests were performed to validate the developed methods (Bolton and Bon, 2004). These developed and validated methods were used for estimation of LER in bulk, formulations, in vitro release samples, stability samples and bio samples.

3.3. Materials

Racemic form of LER was obtained as a gift sample from Glenmark Pharmaceuticals Limited, Mumbai. Analytical grade potassium dihydrogen orthophosphate, sodium hydroxide, polysorbate 80, acetonitrile (HPLC grade) and orthophosphoric acid were purchased from Merck, India. Triple distilled water (TDW) produced using all quartz glass apparatus was used for all the methods. Buffers and TDW were filtered through 0.22 μm filters (Millipore, USA).

Four commercially available LER tablet formulations (Lerez® by Glenmark Pharmaceuticals Limited, India; Landip®10 by Microlabs Limited, India; Lerka® by Nicholas Piramal India Limited, India; Lerva SC® by Lupin Laboratories Limited, India) were purchased from local market.

In case of method development for estimation of LER in rabbit serum by HPLC, blood was collected and harvested so as to generate a drug free (blank) serum pool.

3.4 Reagents

Phosphate buffer (pH 6.8): Potassium dihydrogen orthophosphate (6.8 g) and sodium hydroxide (0.896 g) were dissolved in TDW and volume was made up to 1000 ml using TDW.

Phosphate buffer (pH 6.8) with 2.5 % v/v polysorbate 80: Polysorbate 80 (25 ml) was taken in 1000 ml volumetric flask and volume was made up to 1000 ml using phosphate buffer (pH 6.8).

Ortho phosphoric Acid (0.1 M): Ortho phosphoric acid (6.78 ml) was diluted to 1000 ml using TDW.

Phosphate buffer 10 mM (pH 4.0): Potassium dihydrogen orthophosphate (1.36 g) was dissolved in TDW and volume was made up to 1000 ml. The pH of solution was adjusted to 4.0 using 0.1 M ortho phosphoric acid.

3.5 Analytical Method 1

Ultraviolet (UV) Spectrophotometric Method for Estimation of LER in Bulk and Formulations

3.5.1 Instrumentation

A UV-VIS-NIR spectrophotometer (Jasco V570, Japan) with automatic wavelength correction and a wavelength accuracy of 0.5 nm with 10 mm matched quartz cells was used for all absorbance measurements. The instrument was connected to a computer loaded with Spectramanager software for computational purpose.

3.5.2 Selection of media

Various media were investigated to develop a suitable UV-spectrophotometric method for analysis of LER in bulk and formulations. For selection of media the criteria employed were stability of the drug, solubility of the drug, sensitivity of the method and

cost of the solvents in the order of priority. The media finally selected was acetonitrile-pH 6.8 phosphate buffer (50:50 v/v).

3.5.3 Calibration curve

A stock solution of LER was prepared by dissolving 10 mg of drug in 100 ml of acetonitrile-pH 6.8 phosphate buffer (50:50 v/v) to get a final concentration of 100 μg/ml. The λ_{max} of LER was determined by scanning a suitable dilution of the stock using spectrophotometer. From this stock solution, suitable dilutions were made to obtain solutions of concentrations 10, 20, 30, 40, 50 and 60 μg/ml, and absorbance was measured for all dilutions at the λ_{max} (354 nm) of the drug. To establish linearity of the proposed method, eight separate calibration sets were prepared and analyzed. Least square regression analysis was carried out for the obtained data and calibration equation was developed. The stability of drug solution during analysis was assessed by analyzing samples at different time intervals on the same day and the subsequent day by storing at 25 ± 2^{o}C. All the solutions were protected from light by using amber colored glassware. An analysis of variance test (one-way) was performed based on the absorbance observed for each pure drug concentration during the replicate measurement of the standard solutions.

3.5.4 Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2003; Bolton and Bon, 2004). Various validation parameters of the developed method were determined as per standard guidelines.

Specificity and selectivity of the method was assessed by scanning a solution with drug concentration of 20 μg/ml from pure drug stock and commercial sample stock in selected medium. The two spectra were compared for any change in absorbance pattern of LER in presence of excipients. Drug solutions with and without various commonly used excipients (mannitol, lactose, talc, magnesium stearate, hydroxy propyl methyl cellulose, chitosan, carbopol 934P, polycarbophil, polyethylene oxide) in formulations were prepared and analyzed for any change in the absorbance spectra of LER.

For determining the accuracy of the proposed method, different quality control (QC) levels of drug concentrations [lower quality control samples (LQC) = 15 μg/ml, medium quality control samples (MQC) = 35 μg/ml, and higher quality control samples (HQC) = 55 μg/ml] were prepared independently from stock solution and analyzed (n = 6). Accuracy was assessed by calculating mean percentage recovery and percentage bias (% bias). % bias

was calculated as, % bias = [(Predicted conc.-Nominal Conc.)/ Nominal Conc.] x 100. Further, different concentrations of pure drug (10, 20 and 30 µg/ml) were added to a known pre-analyzed formulation sample and analyzed using the proposed method (n = 5) to check analytical recovery. The percent analytical recovery of the added pure drug was calculated as, % Analytical Recovery = [(Cv – Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard addition, Cu is the drug concentration in the formulation, and Ca is the drug concentration added to the formulation solution.

Repeatability was determined by analyzing different QC levels of drug concentrations (n= 6) as mentioned in accuracy. Inter- and intra-day variation was studied to determine intermediate precision of the proposed method. Different QC levels of drug concentrations in triplicates were prepared twice in a day and studied for intra-day variation (n= 6). The same protocol was followed for three different days to study inter-day variation (n = 18). The percentage relative standard deviation (% RSD) of the predicted concentrations from the regression equation was taken as precision.

The limit of detection (LOD) and limit of quantitation (LOQ) of LER by the proposed method were calculated using standard deviation (SD) of intercept and the slope of regression equation based upon replicate measurement. Experiments were then performed to determine the actual concentration that can be experimentally quantified using the proposed method.

Robustness of the developed method was determined by varying the pH of the phosphate buffer by ± 0.5 unit and by changing the concentration of acetonitrile by ± 1% in the selected media.

3.5.5 Estimation of drug content in commercial tablets

Four commercially available tablet brands of LER (containing 10 mg of drug) were taken for estimation of total drug content per tablet. For each brand, 20 tablets were weighed, finely powdered and mixed. An accurately weighed aliquot amount (equivalent to 10 mg of LER) was transferred to a series of 100 ml volumetric flasks (5 in each case) and dissolved in acetonitrile-pH 6.8 phosphate buffer (50:50 v/v) by sonication and volume was made up to 100 ml. The resulting solution was filtered through Whatman filter paper no. 40. An aliquot of this solution was diluted suitably to a concentration of 20 µg/ml with the selected media and the samples were analyzed using proposed method.

3.5.6 Results and discussion

3.5.6.1 Selection of media

Solubility of the LER was studied in series of solvent like acetonitrile, methanol, acetonitrile-pH 6.8 phosphate buffer (50:50 v/v) and methanol-pH 6.8 phosphate buffer (50:50 v/v). Absorbance of the drug was found to be stable at least for 24 h at λ_{max} of 354 nm in acetonitrile-pH 6.8 phosphate buffer (50:50 v/v). The absorbance value of LER was stable only for 16 h in methanol and acetonitrile. Moreover, the absorbance value changed after 20 h in methanol-pH 6.8 phosphate buffer (50:50 v/v). Absorbance for the drug was found to be maximum in acetonitrile-pH 6.8 phosphate buffer (50:50 v/v). Finally, acetonitrile-pH 6.8 phosphate buffer (50:50 v/v) was selected as solvent system on the basis of sensitivity and stability.

3.5.6.2 Calibration curve

The spectrum of LER showed a distinct λ_{max} at 354 nm. Figure 3.1 shows overlaid spectra of LER and blank. The absorbance at 354 nm was found to be stable for at least 24 h at 25 ± 2^{o}C, indicating stability of the drug in the selected media. Absorbance values for different drug concentrations are shown in Table 3.1. At all concentration levels the SD was low and the % RSD did not exceed 1.02. The predicted concentrations were nearly matching with nominal concentrations. Linearity range was found to be 10-60 μg/ml. The linear regression equation obtained was Absorbance= [0.0100 x Concentration in μg/ml] + 0.0016; with excellent regression coefficient of 0.9999. Individual values of slopes and intercepts obtained from replicate measurements were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (1.89×10^{-5}), standard error of intercept (2.52×10^{-3}), standard error of estimate (3.38×10^{-1}) and MSSR (1.06×10^{-5}) indicated high precision of the proposed method. Lower calculated *F*-value [calculated *F* value (7, 40) of 3.88×10^{-4} and critical *F*-value of 2.24 at $p = 0.05$] further confirmed precision of the method.

3.5.6.3 Analytical method validation

Figure 3.2 shows overlaid spectra of solution of pure drug and solution containing drug and hydroxy propyl methyl cellulose 4000 cPs (HPMC K4M) in 1:1 ratio in the selected media. Estimation of LER in formulations and comparison of pure drug spectrum with that of drug spectrum in presence of common excipients used in formulations

confirmed lack of interference at the wavelength used (354 nm) in this method. Absence of interference confirmed selectivity and specificity of the proposed method.

All three QC levels (LQC, MQC, HQC) showed an accuracy (% bias) ranging from -0.20 to 1.72. The high (nearly 100%) mean percent recovery values and their low SD values (SD < 1.20) represented the accuracy of the method (Table 3.2). In the standard addition method, the mean percentage analytical recoveries (± SD) for 10, 20 and 30 μg/ml concentrations were found to be 101.04 (± 1.20), 101.03 (± 1.28) and 99.59 (± 0.69) respectively. This result further established the validity and reliability of the proposed method.

In repeatability study, the % RSD ranged from 0.56 to 1.16 (Table 3.2). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 1.87% and inter-day variation less than 1.25% (Table 3.3). Lower % RSD values indicated the repeatability and intermediate precision of the method.

LOD and LOQ were found to be 2.35 μg/ml and 7.03 μg/ml respectively. The mean percentage recovery (± SD) of 7.5 μg/ml (LOQ) in triplicate was found to be 99.37 (± 2.21) representing the accuracy and precision of the method. The method was found to be robust as variation of pH of the selected media by ± 0.5 units and variation of concentration of acetonitrile by ± 1% did not affect absorbance significantly.

The results of the estimation of LER in pharmaceutical formulations by the proposed method ranged from 99.50 to 100.20% of the claimed amount with maximum SD of 0.66 (Table 3.4). This indicated absence of interference of excipient matrix in estimation of LER by the proposed method. The estimated drug content with low values of SD further established precision of the proposed method (Table 3.4).

3.6 Analytical Method 2

UV Method for Estimation of LER in In vitro Release Sample

3.6.1 Instrumentation

Instrument used was same as mentioned in analytical method 1.

3.6.2 Calibration curve

Release media selected for carrying out in vitro release studies was pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80. The rational for selection of pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80 as in vitro release media has been discussed in subsequent chapters. A stock solution of LER was prepared by dissolving 10 mg of drug

in 100 ml of pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80 to get a final concentration of 100 μg/ml. The λ_{max} of LER was determined by scanning a suitable dilution of the stock using spectrophotometer. From this stock solution, suitable dilutions were made to obtain solutions of concentrations 10, 20, 40, 60, 80, 100 μg/ml, and absorbance was measured for all dilutions at the λ_{max} of the drug. To establish linearity of the proposed method, eight separate calibration sets were prepared and analyzed. Least square regression analysis was carried out for the obtained data and calibration equation was developed. The stability of drug solution during analysis was assessed by analyzing samples at different time intervals on the same day and the subsequent day by storing at 25 ± 2^{o}C. All the solutions were protected from light by using amber colored glassware. An analysis of variance test (one-way) was performed based on the absorbance observed for each pure drug concentration during the replicate measurement of the standard solutions.

3.6.3 Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2003; Bolton and Bon, 2004). Various validation parameters of the developed method were determined as per standard guidelines.

Specificity and selectivity of the method was assessed by scanning a solution with drug concentration of 20 μg/ml from pure drug stock and commercial sample stock in selected medium. The two spectra were compared for any change in absorbance pattern of LER in presence of excipients. Drug solutions with and without various commonly used excipients (mannitol, lactose, talc, magnesium stearate, hydroxy propyl methyl cellulose, chitosan, carbopol 934P, polycarbophil, polyethylene oxide) in formulations were prepared and analyzed for any change in the absorbance spectra of LER.

For determining the accuracy of the proposed method, different QC levels of drug concentrations [lower quality control samples (LQC) = 15 μg/ml, medium quality control samples (MQC) = 55 μg/ml, and higher quality control samples (HQC) = 95 μg /ml] were prepared independently from stock solution and analyzed (n = 6). Accuracy was assessed by calculating mean percentage recovery and % bias. % bias was calculated as, % bias = [(Predicted conc.-Nominal Conc.)/ Nominal Conc.] x 100. Further, different concentrations of pure drug (20, 30 and 40 μg/ml) were added to a known pre-analyzed formulation sample and analyzed using the proposed method (n = 5) to check analytical recovery. The percent analytical recovery of the added pure drug was calculated as, % Analytical Recovery = [(Cv – Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard

addition, Cu is the drug concentration in the formulation, and Ca is the drug concentration added to formulation solution.

Repeatability was determined by analyzing different QC levels of drug concentrations (n= 6) as mentioned in accuracy. Inter- and intra-day variation was studied to determine intermediate precision of the proposed method. Different QC levels of drug concentrations in triplicates were prepared twice in a day and studied for intra-day variation (n= 6). The same protocol was followed for three different days to study inter-day variation (n = 18). The % RSD of the predicted concentrations from the regression equation was taken as precision.

The limit of detection (LOD) and limit of quantitation (LOQ) of LER by the proposed method were calculated using SD of intercept and the slope of regression equation based upon replicate measurement. Experiments were then performed to determine the actual concentration that can be experimentally quantified using the proposed method.

Robustness of the developed method was determined by varying the pH of the phosphate buffer by ± 0.5 units.

3.6.4 Estimation of drug content in commercial tablets

Four commercially available tablet brands of LER (containing 10 mg of drug) were taken for estimation of total drug content per tablet. For each brand, 20 tablets were weighed, finely powdered and mixed. An accurately weighed aliquot amount (equivalent to 10 mg of LER) was transferred to a series of 100 ml volumetric flasks (5 in each case) and dissolved in pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80 by sonication and volume was made up to 100 ml. The resulting solution was filtered through Whatman filter paper no. 40. An aliquot of this solution was diluted suitably to a concentration of 20 μg/ml with the selected media and the samples were analyzed using proposed method.

3.6.5 Results and discussion

3.6.5.1 Calibration curve

The spectrum of LER showed a distinct λ_{max} at 354 nm. Figure 3.3 shows overlaid spectra of LER and blank. The absorbance at 354 nm was found to be stable for at least 24 h at 25 ± 2^{o}C, indicating stability of the drug in the media used. Absorbance values for different drug concentrations are shown in Table 3.5. At all concentration levels the SD was low and the % RSD did not exceed 2.79. The predicted concentrations were nearly matching with nominal concentrations. Linearity range was found to be 10-100 μg/ml. The

linear regression equation obtained was Absorbance = [0.0103 x Concentration in μg/ml] - 0.0044; with excellent regression coefficient of 0.9996. Individual values of slopes and intercepts obtained from replicate measurements were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (3.24×10^{-5}), standard error of intercept (2.67×10^{-3}), standard error of estimate (2.53) and MSSR (6.42×10^{-4}) indicated high precision of the proposed method. Lower calculated *F*-value [calculated *F* value (7, 40) of 3.0×10^{-4} and critical *F*-value of 2.24 at $p = 0.05$] further confirmed precision of the method.

3.6.5.2 Analytical method validation

Figure 3.4 shows overlaid spectra of solution of pure drug and solution containing drug and HPMC K4M in 1:1 ratio in the media. Estimation of LER in formulations and comparison of pure drug spectrum with that of drug spectrum in presence of common excipients used in formulations confirmed lack of interference at the wavelength used (354 nm) in this method. Absence of interference confirmed selectivity and specificity of the proposed method.

All three QC levels (LQC, MQC, HQC) showed an accuracy (% bias) ranging from -1.14 to 0.15 (Table 3.6). The high (nearly 100%) mean % recovery values and their low SD values (SD < 0.90) represented the accuracy of the method. In the standard addition method, the mean percentage analytical recoveries (± SD) for 20, 30 and 40 μg/ml concentrations were found to be 98.48 (± 1.73), 99.03 (± 0.86) and 100.10 (± 2.08) respectively. This result further established the validity and reliability of the proposed method.

In repeatability study, the % RSD ranged from 0.24 to 0.87 (Table 3.6). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 2.08% and inter-day variation less than 1.15% (Table 3.7). Lower % RSD values indicated the repeatability and intermediate precision of the method.

LOD and LOQ were found to be 2.40 μg/ml and 7.26 μg/ml respectively. The mean percentage recovery (± SD) of 7.5 μg/ml in triplicate was found to be 100.22 (± 1.94) representing the accuracy and precision of the method. The method was found to be robust as variation of pH of the selected media by ± 0.5 did not affect absorbance significantly.

The results of the estimation of LER in pharmaceutical formulations by the proposed method ranged from 100.88 to 101.55% of the claimed amount with maximum SD of 0.45 (Table 3.8). This indicated absence of interference of excipient matrix in estimation of LER by the proposed method. The estimated drug content with low values of SD further established precision of the proposed method (Table 3.8).

3.7 Analytical Method 3

Liquid Chromatographic Method for Estimation of LER in Bulk and Formulations

3.7.1 Instrumentation

The liquid chromatography system employed was Shimadzu HPLC (Shimadzu, Japan) with solvent delivery system of two pumps (Model LC 10AT VP Shimadzu LC, Shimadzu, Japan), autoinjector (Model SIL HT A Shimadzu autosampler, Shimadzu, Japan) and UV-VIS detector (Model SPD 10A VP Shimadzu, Shimadzu, Japan). Data collection and integration was accomplished using LC Solutions software.

3.7.2 Chromatographic conditions

An endcapped C18 reverse phase column (Lichrospher®, 125 mm long and 4.6 mm internal diameter, particle size 5 µm, E. Merck, Germany) equipped with a guard column of same packing material was used for the study. Mobile phase consisted of an aqueous phase (10 mM potassium dihydrogen phosphate buffer in TDW, pH adjusted to 4.0 using 0.1 M ortho phosphoric acid) and acetonitrile (40:60 v/v). The buffer was filtered through 0.22 µm Millipore® filtration membrane. The HPLC system was run for minimum 1 h at 1 ml/min flow rate for system equilibration through baseline monitoring, prior to actual analysis. LER was monitored at wavelength of 240 nm with mobile phase flow rate of 1 ml/min. The injection volume was 100 µl.

3.7.3 Selection of mobile phase

For mobile phase optimization various buffers of different pH and in varying combination with acetonitrile or methanol were investigated. Main purpose was to develop a simple, precise, sensitive and selective HPLC method for quantitation of LER in bulk, dosage forms and stability samples. Mobile phase finally selected consisted of an aqueous phase (10 mM potassium dihydrogen phosphate buffer in TDW, pH adjusted to 4.0 using 0.1 M ortho phosphoric acid) and acetonitrile (40:60 v/v). For the selection of mobile phase, the criteria employed were peak properties (retention time and asymmetric factor), sensitivity (height and area), ease of sample preparation, and applicability of the method for various purpose.

3.7.4 Preparation of calibration curve

Primary stock of LER was prepared by dissolving 5 mg of LER in acetonitrile and making up the volume to 50 ml to obtain a concentration of 100 µg/ml. Secondary stock of 10 µg/ml concentration was prepared by appropriate dilution of primary stock by mobile phase. From the secondary stock solution, calibration standards of 25, 50, 100, 250, 500, 750, 1000 ng/ml concentrations were made by suitable dilution with mobile phase for the purpose of calibration curve. All the solutions were protected from light by using amber colored glassware. 100 µl of each concentration was injected, and the area of the peak at 240 nm was determined. To establish linearity of the proposed method, eight separate calibrations sets were prepared and analyzed. Least square regression analysis was carried out for the obtained data and calibration equation was developed. An analysis of variance test (one-way) was performed based on the peak area observed for each pure drug concentration during the replicate measurement of the standard solutions.

3.7.5 Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2003; Bolton and Bon, 2004). Various validation parameters of the developed method were determined as per standard guidelines.

To study selectivity of the method, LER stock solutions (100 µg/ml) were separately prepared in a mobile phase with and without common excipients (mannitol, lactose, talc, magnesium stearate, hydroxy propyl methyl cellulose, chitosan, carbopol 934P, polycarbophil, polyethylene oxide). All the solutions were diluted suitably with the mobile phase to get a drug concentration of 250 ng/ml and were analyzed. A blank solution containing only excipients was also injected and interference near the drug peak was checked.

For determining the accuracy of the proposed method, different QC levels of drug concentrations [lower quality control samples (LQC) = 30 ng/ml, medium quality control samples (MQC) = 400 ng/ml, and higher quality control samples (HQC) = 900 ng /ml] were prepared independently from stock solution and analyzed (n = 6). Accuracy was assessed by calculating mean percentage recovery and percentage bias (% bias). % bias was calculated as, % bias = [(Predicted conc.-Nominal Conc.)/ Nominal Conc.] x 100. Further, different concentrations of pure drug (50, 100 and 500 ng/ml) were added to a known pre-analyzed formulation sample and analyzed using the proposed method (n = 5) to check analytical recovery. The percent analytical recovery of the added pure drug was calculated as,

% Analytical Recovery = [(Cv – Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard addition, Cu is the drug concentration in the formulation, and Ca is the drug concentration added to formulation solution.

Repeatability was determined by analyzing different QC levels of drug concentrations (n= 6) as mentioned in accuracy. Inter- and intra-day variation was studied to determine intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared twice in a day and studied for intra-day variation (n= 6). The same protocol was followed for three different days to study inter-day variation (n= 18). The % RSD of the predicted concentrations from the regression equation was taken as precision.

The limit of detection (LOD) and limit of quantitation (LOQ) of LER by the proposed method were calculated using SD of intercept and the slope of regression equation based upon replicate measurement. Experiments were then performed to determine the actual concentration that can be experimentally quantified using the proposed method.

Robustness of the developed method was determined by varying the pH of the media by ± 0.2 units. Benchtop and stock solution stability of LER was established by storing the samples at controlled room temperature (CRT) of 25 ± 2 ^{o}C for 24 h.

3.7.6 Estimation of drug content in commercial tablets

Four commercially available tablet brands of LER (containing 10 mg of drug) were taken randomly for estimation of total drug content per tablet. For each brand, 20 tablets were weighed, finely powdered and mixed. An accurately weighed aliquot amount (equivalent to 5 mg of LER) was transferred to a series of 50 ml volumetric flasks (5 in each case) and dissolved in acetonitrile by sonication and volume was made up to 50 ml. The resulting solution was filtered through Whatman filter paper no. 40. An aliquot of this solution was diluted suitably with the mobile phase to obtain a secondary stock of concentration 10 μg/ml. An aliquot of secondary stock was suitably diluted with mobile phase to obtain a concentration 500 ng/ml and the samples were analyzed using the proposed method.

3.7.7 Results and discussion

3.7.7.1 Selection of mobile phase

Optimization of mobile phase consisting of aqueous phase (10 mM potassium dihydrogen phosphate buffer, pH adjusted to 4.0 using 0.1 M ortho phosphoric acid) and

acetonitrile (40:60 v/v), was based on peak properties (retention time and asymmetric factor) and sensitivity (height and area). With optimized mobile phase retention time of LER was found to be 9.27 ± 0.31 min with an asymmetric factor of 1.09 ± 0.17 (Figure 3.5). The retention time of LER increased to 17.92 min with decrease in proportion of acetonitrile from 60% v/v to 50% v/v in the mobile phase. However, there was no effect on peak area, peak height and asymmetric factor. Use of methanol (60% v/v) instead of acetonitrile (60% v/v) in the mobile phase increased retention time of LER to 16.18 min. Change in pH of aqueous phase on either side of 4.0 resulted in reduction of peak area and peak height and increase in asymmetric factor of peak. Thus, aqueous phase (10 mM potassium dihydrogen phosphate buffer, pH adjusted to 4.0 using 0.1 M ortho phosphoric acid) and acetonitrile (40:60 v/v) was finally selected as mobile phase.

3.7.7.2 Calibration curve

Different concentrations and their corresponding area at 240 nm are shown in the Table 3.9. At all the concentration levels, the SD of the area was low and the % RSD did not exceed 1.76. Overlaid chromatograms of blank and 500 ng/ml are shown in Figure 3.5. Retention time of LER was 9.27 ± 0.31 min in selected mobile phase. Peak was having good resolution with asymmetric factor of 1.09 ± 0.17. Total run time for single injection was 12 minutes for the proposed method. The predicted concentrations were nearly matching with the nominal concentrations. The linear regression equation obtained was Peak Area = [247.86 x Concentration in ng/ml] + 471.87; with excellent regression coefficient of 0.9999. Individual values of slopes and intercepts obtained from replicate measurements were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (9.97×10^{-1}), standard error of intercept (162.55), standard error of estimate (7.54) and MSSR (2.43×10^{-5}) indicated high precision of the proposed method. Lower calculated *F*-value [calculated *F* value (7, 48) of 5.07×10^{-4} and critical *F*-value of 2.20 at $p = 0.05$] further confirmed precision of the method.

3.7.7.3 Analytical method validation

Figure 3.6 shows the overlaid chromatograms of pure LER and combination of LER with HPMC K4M in 1:1 proportion. Estimation of LER in formulations and comparison of pure drug peak with that of drug peak in presence of common excipients used in formulations confirmed lack of interference at the retention time of LER. The blank samples of excipients did not show any interference near the drug peak. In the presence of excipients, peak characteristics of the drug (retention time, area, and asymmetric factor)

were not affected. This indicated that there is no significant interference of excipients in the estimation of the drug by the proposed method. This confirmed the specificity and selectivity of the method.

All three QC levels showed an accuracy (% bias) ranging from 0.15 to 1.04 (Table 3.10). The high (nearly 100%) mean % recovery values and their low SD values (SD < 1.6) represented the accuracy of the method. In the standard addition method, the mean percentage analytical recoveries (± SD) for 50, 100 and 500 ng/ml concentrations were found to be 100.49 (± 0.59), 99.77 (± 1.96) and 99.94 (± 0.45), respectively. This result further established the validity and reliability of the proposed method.

In repeatability study, the % RSD ranged from 0.55 to 1.53 (Table 3.10). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 1.82% and inter-day variation less than 1.35% (Table 3.11). Lower % RSD values indicated the repeatability and intermediate precision of the method.

LOD and LOQ were found to be 6.12 and 18.55 ng/ml respectively. The mean percentage recovery (± SD) of 20 μg/ml (LOQ) in triplicate was found to be 100.08 (± 1.38) representing the accuracy and precision of the method. The method was found to be robust as variation of pH of the selected media by ± 0.2 unit did not have any significant effect on retention time, peak height, peak area and asymmetric factor. Different concentrations of bench-top LER solutions and stock solutions of LER showed % RSD values less than 1.69%, indicating stability of LER. These solutions exhibited no change in chromatographic characters (retention time, asymmetric factor, and area) at least until 24 h at room temperature. During this period no extra peaks were observed in the chromatograms across all concentrations.

The results of the estimation of LER in pharmaceutical formulations by the proposed method ranged from 99.75 to 100.82% of the claimed amount with maximum SD of 1.63 (Table 3.12). Assay values of formulations were very close to the label claim. This indicated absence of interference of excipient matrix in estimation of LER by the proposed method. The estimated drug content with low values of SD further established precision of the proposed method (Table 3.12).

3.8 Analytical Method 4

Liquid Chromatographic Method for Estimation of LER in Rabbit Serum

3.8.1 Instrumentation and chromatographic conditions

Liquid chromatographic instrument and chromatographic condition used were same as mentioned in analytical method 3.

Other instruments used in the method development and validation include cyclo mixer (Remi, India), sonicator (Bransonic Cleaning Company, USA), Millipore® filtration assembly (Waters, USA), vacuum concentrator-Maxi Dry Lyo 230v (Heto-holten, Denmark), refrigerated centrifuge (Model CPR 20, Remi, India) and deep freeze (Vestfrost, Australia).

3.8.2 Collection of blood and separation of serum

Blood was collected from marginal ear vein of male New Zealand white rabbits weighing between 2.0-2.5 kg. Collection of blood was carried out with permission of Institutional Animal Ethics Committee (Protocol approval no. IAEC/RES/7/4). The blood collected was harvested for 45 min at room temperature and centrifuged at 2000 RPM for 20 min. The clear supernatant serum layer was collected to generate a drug free serum pool.

3.8.3 Selection of mobile phase

For mobile phase optimization, various buffers of different pH and in varying combination with acetonitrile or methanol were investigated. Main purpose was to develop a simple, precise, sensitive and selective HPLC method for quantitation of LER in rabbit serum. Mobile phase finally selected consisted of an aqueous phase (10 mM potassium dihydrogen phosphate buffer, pH adjusted to 4.0 using 0.1 M ortho phosphoric acid) and acetonitrile (40:60 v/v). For the selection of media, the criteria employed were peak properties (retention time and asymmetric factor), sensitivity (height and area), ease of sample preparation, non interference from the bio matrix and applicability of the method for in vivo studies in rabbits.

3.8.4 Calibration curve

Primary stock of LER was prepared by dissolving 5 mg of LER in acetonitrile and making up the volume to 50 ml to obtain a concentration of 100 µg/ml. Secondary stock of 10 µg/ml concentration was prepared by appropriate dilution of primary stock by mobile phase. Serum standards were prepared by spiking appropriate amount of secondary stock of

LER in rabbit serum to obtain solutions of 25, 50, 100, 250, 500, 750, 1000 ng/ml concentration. To study absolute drug recovery from serum standards, analytical standards of 25, 50, 100, 250, 500, 750, 1000 ng/ml concentrations were made by spiking appropriate amount of secondary stock of LER in mobile phase. All the samples prepared were protected from light using amber colored glassware.

A simple and efficient one-step process was employed to isolate LER from rabbit serum. To aliquot of 500 µl of spiked serum samples, 1.5 ml of acetonitrile was added and vortex mixed for 1 min. Samples were then kept for 10 min on bench top condition to ensure complete precipitation. Subsequently samples were again vortex mixed for 1 min and centrifuged at 10000 RPM at 4^{o}C for 20 min. Supernatant of the centrifuged samples was transferred to 5 ml clean and dry conical tubes and evaporated to dryness in vacuum concentrator maintained at 30^{o}C. Vacuum dried residues were reconstituted in 500 µl of mobile phase, vortex mixed for 1 min and centrifuged at 10000 RPM at 4^{o}C for 10 min. The clear supernatant was then transferred to clean and dry auto-sampler vials. Serum and analytical standards (100 µl) were injected on to the column for analysis. The peaks obtained for both serum and analytical standards were integrated and peak area was calculated for each concentration. To establish linearity of the proposed method, eight separate sets of serum standards were prepared and analyzed. Percent absolute drug recovery from serum sample was calculated by using the formula [(Peak area of serum standard/ peak area of analytical standard of same concentration) x 100]. Least square regression analysis was performed for the obtained calibration data. An analysis of variance test (one-way) was performed based on the peak area observed for each concentration during the replicate measurement of the serum standards.

3.8.5 Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996; US Pharmacopoeia, 2003; Bolton and Bon, 2004). Various validation parameters of the developed method were determined as per standard guidelines.

Selectivity of the method can be defined as non-interference at the retention time of LER by the proteins and other impurities present in the bio matrix. Blank serum samples were processed as described above and analyzed by proposed method to demonstrate specificity and selectivity.

For determining the accuracy of the proposed method, different quality control (QC) levels of drug concentrations in serum [lower quality control samples (LQC) = 30 ng/ml,

medium quality control samples (MQC) = 400 ng/ml, and higher quality control samples (HQC) = 900 ng /ml] were prepared independently and analyzed (n = 6). Accuracy was assessed by calculating mean percentage recovery and percentage bias (% bias). % bias was calculated as, % bias = [(Predicted conc.-Nominal Conc.)/ Nominal Conc.] x 100.

Repeatability was determined by analyzing three QC levels of drug concentrations (n = 6) as mentioned in accuracy. Inter- and intra-day variation was studied to determine intermediate precision of the proposed method. Three QC levels of drug concentrations in triplicates were prepared twice in a day and studied for intra-day variation (n=6). The same protocol was followed for three different days to study inter-day variation (n = 18). The % RSD of the predicted concentrations from the regression equation was taken as precision.

Limit of quantitation (LOQ) is defined as minimum concentration of LER in serum sample that can be quantified with less than 20% RSD (International Conference on Harmonization, 1996). In order to determine LOQ, three independent serum samples containing 20 ng/ml of LER were prepared and analyzed using developed method. The peaks were integrated and concentrations were back calculated using calibration equation. Mean concentration and % RSD for these three values was determined.

Freeze thaw stability of LER in rabbit serum was determined by preparing three QC samples (LQC, MQC and HQC). Total of four sets were prepared in triplicates and one set of the prepared concentrations was analyzed on the day of preparation (no freeze thaw cycle) and the remaining three sets were frozen at -20^{o}C for 24 h. Frozen samples were thawed by keeping the sealed tubes at room temperature 25 ± 2^{o}C for at least 60 min. One set in triplicate was analyzed and the remaining two sets were kept at -20^{o}C for freezing and were analyzed after two and three freeze thaw cycles. The percentage deviation from the mean concentrations observed on day of preparation was calculated. Post extraction stability of the processed samples of LER in rabbit serum was investigated by preparing five sets of QC Samples (LQC, MQC and HQC) in triplicates. Processed samples were kept in the sample rack of auto-injector (25 ± 2^{o}C) and samples were analyzed in triplicates every 6 h for 24 h on the day of preparation. The percentage deviation from the mean concentrations observed at zero time was calculated. Long term stability of LER in rabbit serum was determined by preparing three QC Samples (LQC, MQC and HQC). Total of four sets were prepared in triplicates and one set of the prepared concentrations was analyzed on the day of preparation. The remaining three sets were frozen at -20^{o}C. One set each of stored samples was analyzed after 7, 14 and 30 days of sample preparation by thawing them at room temperature. The percentage deviation from the mean concentrations observed on day of preparation was calculated.

3.8.6 Results and discussion

3.8.6.1 Selection of mobile phase

Optimization of mobile phase consisting of aqueous phase (10 mM potassium dihydrogen phosphate buffer, pH adjusted to 4.0 using 0.1 M ortho phosphoric acid) and acetonitrile (40:60 v/v), was based on peak properties (retention time and asymmetric factor), sensitivity (height and area) and separation of peak from protein impurities present in serum . With optimized mobile phase retention time of LER was found to be 9.21 ± 0.24 min with an asymmetric factor of 1.27 ± 0.10. The retention time of LER increased to 18.34 min with decrease in proportion of acetonitrile from 60% v/v to 50% v/v in the mobile phase. However, there was no effect on peak area, peak height and asymmetric factor. Use of methanol (60% v/v) instead of acetonitrile (60% v/v) in the mobile phase increased retention time of LER to 16.40 min. Change in pH of aqueous phase on either side of 4.0 resulted in reduction of peak area and peak height and increase in asymmetric factor of peak. Thus, aqueous phase (10 mM potassium dihydrogen phosphate buffer, pH adjusted to 4.0 using 0.1 M ortho phosphoric acid) and acetonitrile (40:60 v/v) was finally selected as mobile phase.

3.8.6.2 Calibration curve

Different concentrations and their corresponding areas are shown in the Table 3.13. At all the concentration levels, the SD of the area was acceptable and the % RSD did not exceed 7.80. Overlaid chromatograms of blank serum, serum standard (500 ng/ml) and in vivo test sample are shown Figure 3.7. Retention time of LER was found to be 9.21 ± 0.24 min (Figure 3.7) in the selected mobile phase. Peak was having good resolution with asymmetric factor of 1.27 ± 0.10. Total run time for single injection was 15 min for the proposed method. The linearity range in the selected mobile phase was found to be 25–1000 ng/ml. According to a linear regression analysis, the slope (± standard error) and intercept (± standard error) were found to be 234.11 (± 4.04) and -525.81 (± 193.37), respectively with a regression coefficient value of 0.9998. Individual values of slopes and intercepts obtained from replicate measurements were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of estimate (7.54) and MSSR (2.43×10^{-5}) indicated high precision of the proposed method. Lower calculated F-value (7, 48) of 1.49×10^{-2} in comparison to critical F-value of 2.20 at $p = 0.05$, further confirmed precision of the method. The absolute recovery of LER from the spiked rabbit serum samples when compared with analytical standards of same concentration, were

within 92.13 to 97.52% with maximum SD of 7.72 (Table 3.13). Thus, the proposed protein precipitation technique was found to be accurate and precise with high recovery values precluding the use of internal standard.

3.8.6.3 Analytical method validation

Simple and efficient one-step precipitation technique was used to separate LER from rabbit serum. The technique was found to be suitable for estimation of LER from bio matrix with no interference from endogenous protein impurities. The metabolites of LER are expected to be more hydrophilic than the parent drug and are proposed to be eluted along with other protein impurities. In case of test sample of pilot in vivo studies, no additional peaks resulting from metabolism or degradation of the drug were observed in the near vicinity of drug peak (Figure 3.7). Blank serum sample also showed absence of any interference near the retention time of the drug (Figure 3.7). Thus, the proposed method is specific and selective for the estimation of LER in rabbit serum.

All three quality control samples [lower quality control samples (LQC) = 30 ng/ml, medium quality control samples (MQC) = 400 ng/ml, and higher quality control samples (HQC) = 900 ng /ml] showed an accuracy (% bias) ranging from -1.68% to -0.16% (Table 3.14). The high (nearly 100%) mean percent recovery values and low SD values (SD < 5.0) further established the accuracy of the method (Table 3.14).

In repeatability study, the % RSD ranged from 2.22 to 4.87 (Table 3.14). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 7.86% and inter-day variation less than 8.85% (Table 3.15). Lower % RSD values indicated the repeatability and intermediate precision of the method.

The mean concentration of three independent samples of 20 ng/ ml, calculated using calibration equation was found to be 18.31 ng/ml with % RSD value of 13.86. Hence, the concentration of 20 ng/ml was considered as limit of quantitation for the proposed method.

The stability of LER in rabbit serum was evaluated using QC samples under different stress conditions and the results obtained are shown in Figure 3.8. In freeze thaw stability, no significant degradation of LER was observed up to three cycles over a period of three days. The deviation from the zero time concentration was found to be less than 8% at the end of three freeze thaw cycles as shown in Figure 3.8a. In post-extraction stability study of the processed samples, LER was found to be stable for 24 h, with a maximum deviation of 6.27% from the zero time concentration as shown in Figure 3.8b. In long term stability studies, LER was found to be stable for 30 days when stored at -20^{o}C. The deviation in recoveries of LER after analysis at 7, 14 and 30 days of sample preparation was

found to be within acceptable limits (Figure 3.8c). The results of this study indicated that storage temperature of -20^{o}C was adequate for storing the samples for at least 30 days.

3.9 Conclusions

The developed analytical methods were found to be accurate, precise, sensitive and suitable for estimation of LER in bulk, formulations, in vitro release samples as well as in biological matrix. The UV method was found to be simple, quicker and cheaper than reported methods and suitable for estimation of LER in bulk and formulations. UV spectroscopic method for determination of LER in in vitro release samples was successfully employed for drug content estimation in release samples.

Proposed HPLC method for estimation of LER in bulk and formulations was found to be highly sensitive (low LOQ values) as compared to earlier reported methods using same instrument. The proposed method was found to be specific because of non-interference of the common excipients used in formulations. Proposed HPLC method for estimation of LER in bio samples was found to be highly sensitive (low LOQ value). The sensitivity and selectivity of this method was helpful in conducting pharmacokinetic study of developed formulations in rabbits.

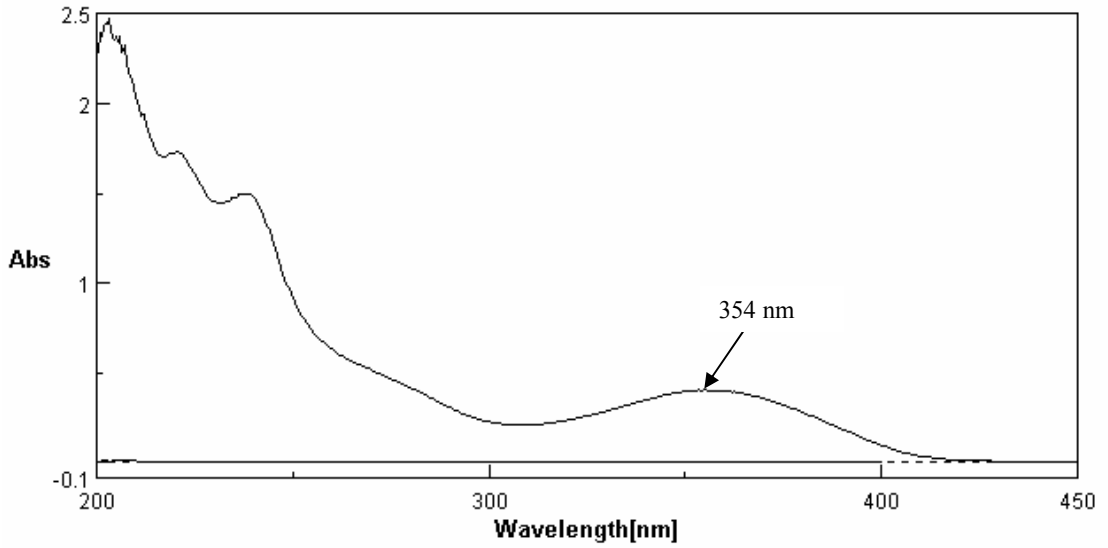


Figure 3.1: Overlaid UV absorption spectra of LER and blank for analytical method 1

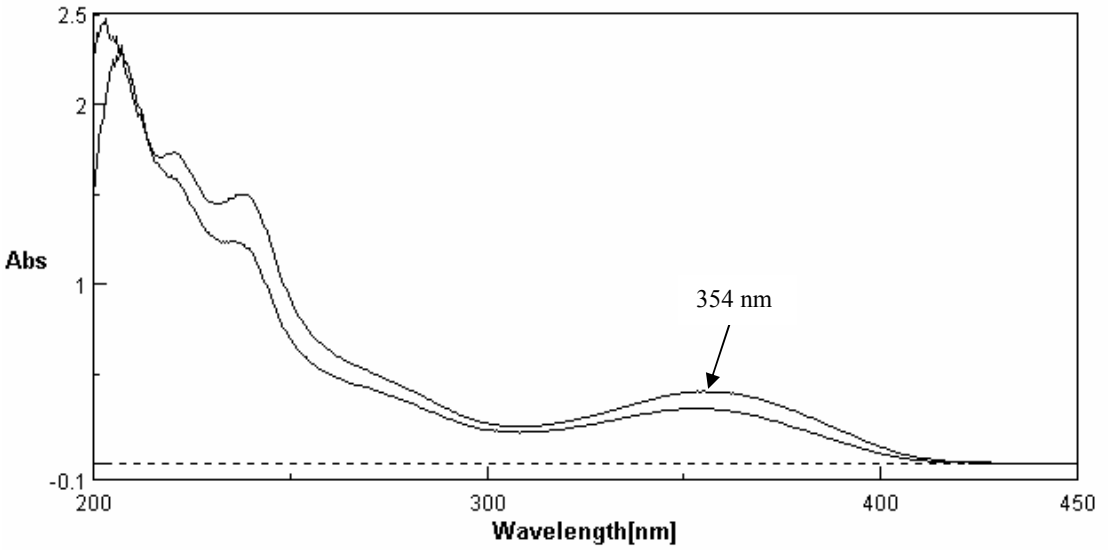


Figure 3.2: Overlaid spectra of pure drug solution and solution containing drug and HPMC K4M in 1:1 ratio obtained using analytical method 1

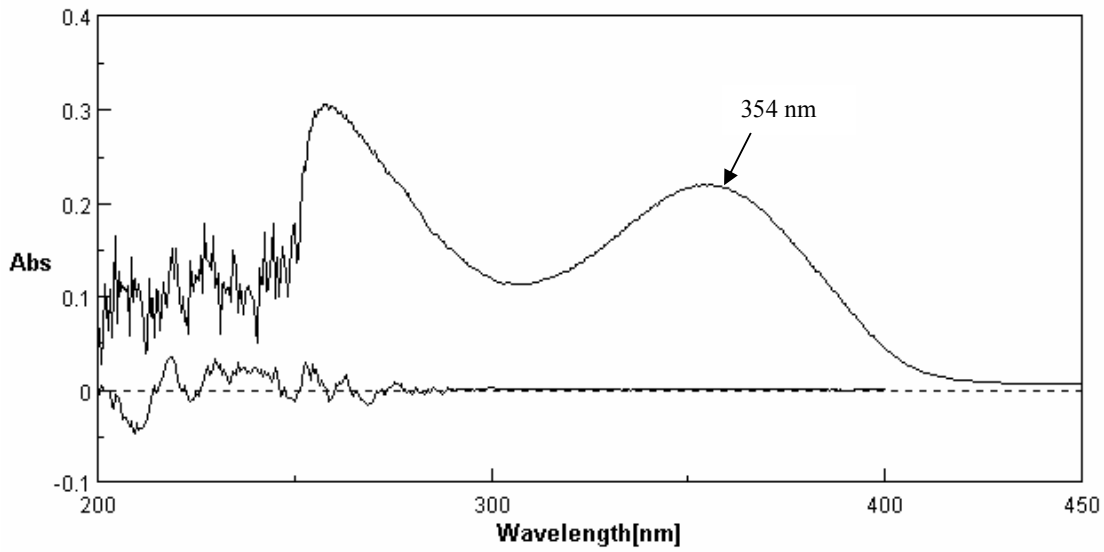


Figure 3.3: Overlaid UV absorption spectra of LER and blank for analytical method 2

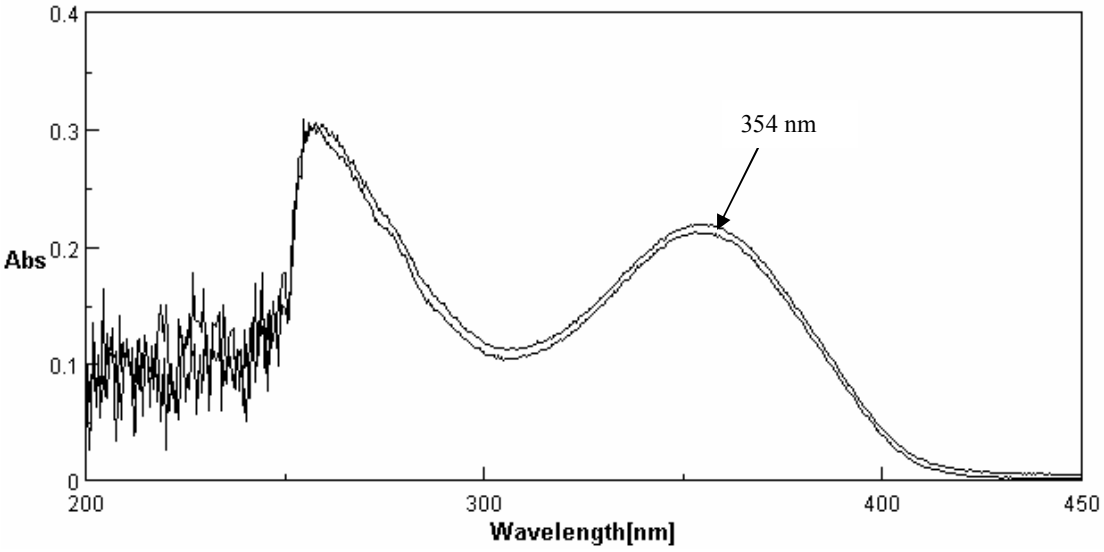


Figure 3.4: Overlaid spectra of pure drug solution and solution containing drug and HPMC K4M in 1:1 ratio obtained using analytical method 2

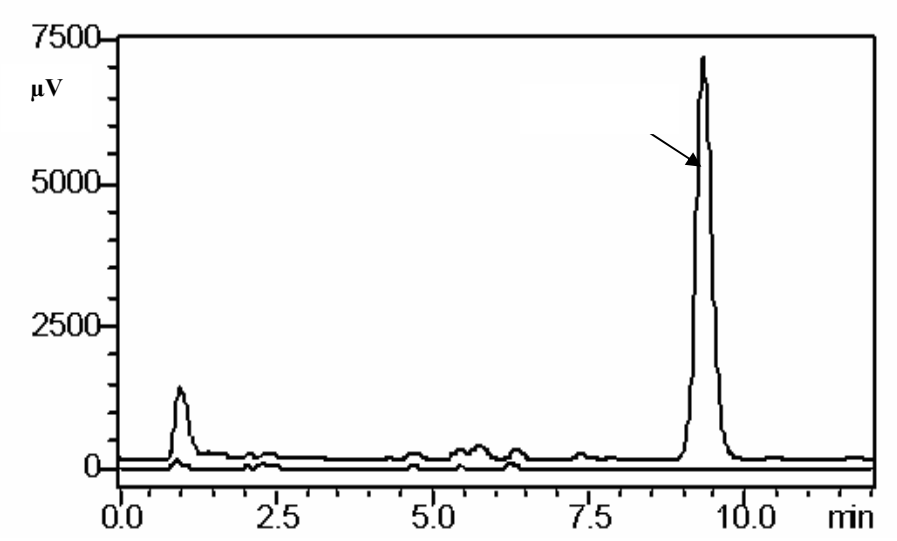


Figure 3.5: Overlaid chromatograms of blank (mobile phase) and pure LER (500 ng/ml)

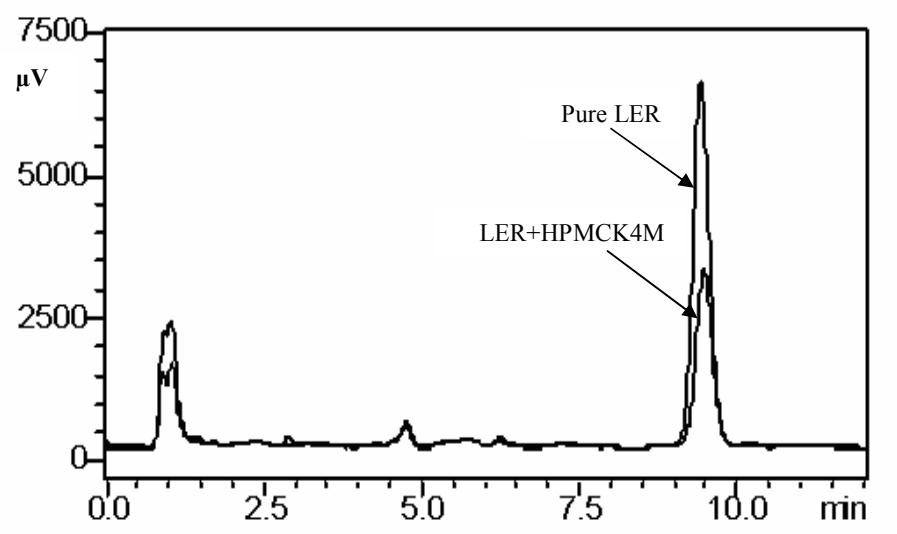


Figure 3.6: Overlaid chromatograms of pure LER (500 ng/ml) and combination of LER (250 ng/ml) with HPMC K4M in 1:1 proportion

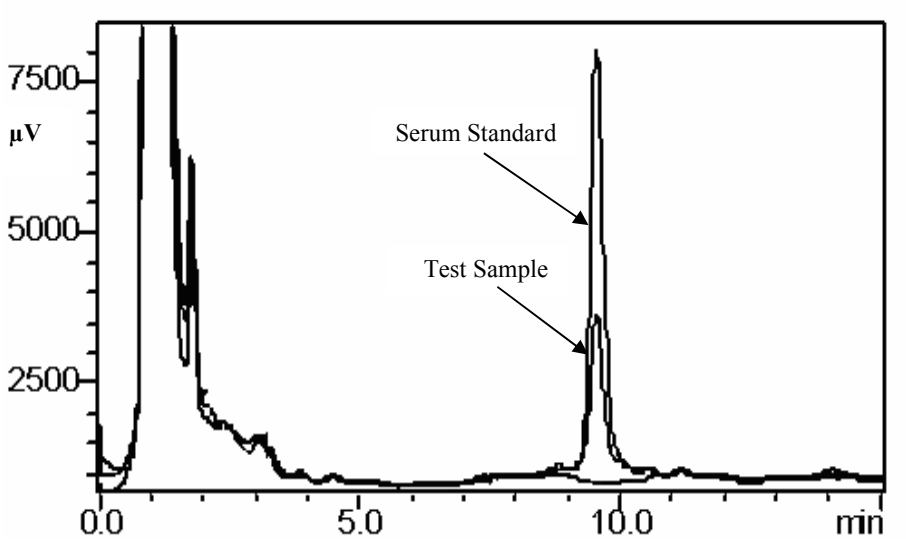
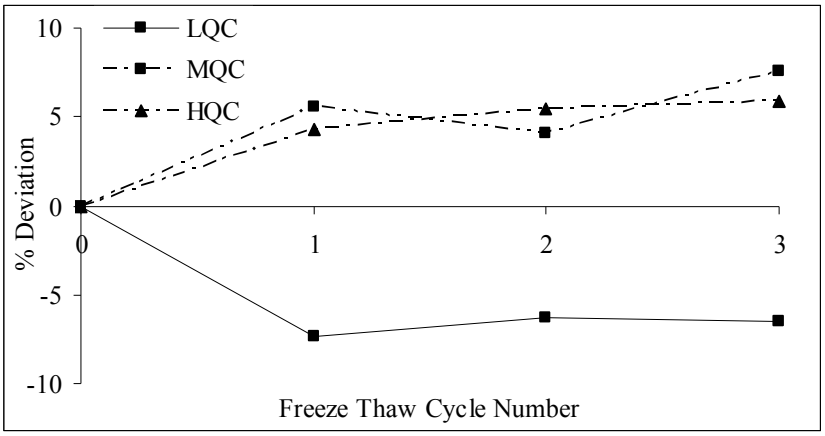
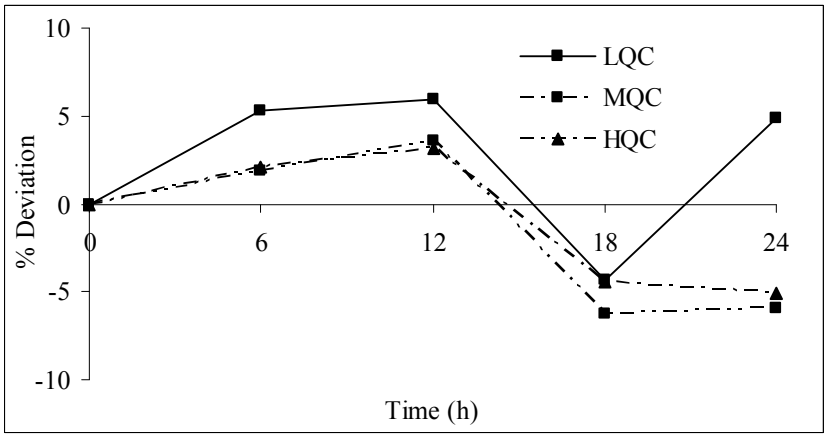


Figure 3.7: Overlaid chromatograms of blank serum, serum standard (500 ng/ml) and in vivo test sample (230 ng/ml)

(a)



(b)



(c)

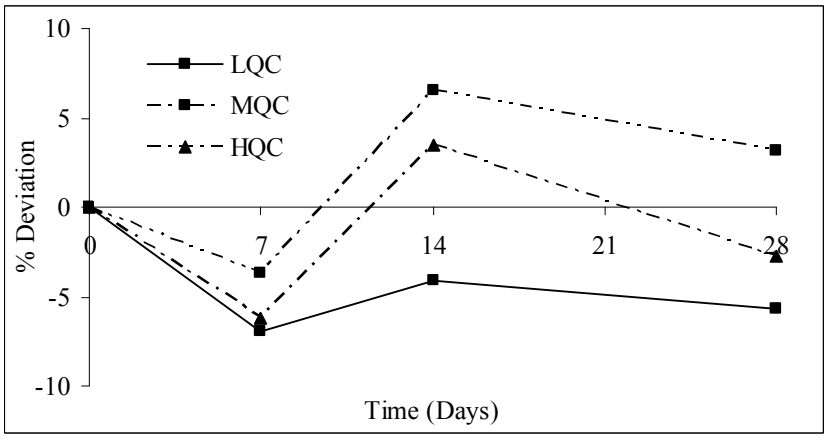


Figure 3.8: Stability study of LER in rabbit serum. (a) freeze thaw stability; (b) post extraction stability; (c) long term stability

Table 3.1: Calibration data for estimation of LER by analytical method 1

Conc. (µg/ml)	Mean Absorbance[a] (± SD)	% RSD[b]	Predicted Conc.[c] (µg/ml)
10	0.1010 ± 0.0008	0.82	9.94
20	0.2024 ± 0.0009	0.44	20.08
30	0.3023 ± 0.0031	1.02	30.07
40	0.4034 ± 0.0021	0.52	40.18
50	0.4984 ± 0.0048	0.97	49.68
60	0.6036 ± 0.0038	0.63	60.20

[a] Each value is mean of eight independent determinations
[b] Percentage relative standard deviation
[c] Predicted concentration is calculated from the regression equation

Table 3.2: Accuracy and precision data for analytical method 1

Level	Predicted Conc.[a] (µg/ml)			Mean % Recovery[b] (± SD)	% Bias[c]
	Range	Mean[b] (±SD)	% RSD		
LQC	14.87 - 15.12	14.97 ± 0.08	0.56	99.80 ± 0.56	-0.20
MQC	35.14 - 36.04	35.60 ± 0.41	1.14	101.72 ± 1.14	1.72
HQC	54.99 - 56.51	55.79 ± 0.65	1.16	101.43 ± 1.16	1.43

[a] Predicted concentration is calculated from the regression equation
[b] Each value is mean of six independent determinations
[c] Accuracy is given in % Bias

Table 3.3: Results of intermediate precision study for analytical method 1

Level	Intra-day repeatability (% RSD) (n=3)			Inter-day repeatability (% RSD) (n=18)
	Day 1	Day 2	Day 3	
LQC	0.37	0.89	1.59	1.06
	0.66	1.43	0.23	
MQC	0.75	1.13	1.87	1.20
	1.12	1.68	0.17	
HQC	0.83	0.64	0.37	1.20
	0.11	0.09	1.51	

Table 3.4: Determination of LER in marketed formulations using analytical method 1

Commercial Products	Mean Amount Found[a] (mg) (± SD)	% Assay[a] (± SD)
Lerez Tablets (10 mg)	9.95 ± 0.07	99.54 ± 0.66
Landip 10 Tablets (10 mg)	9.95 ± 0.03	99.50 ± 0.35
Lerka Tablets (10 mg)	10.02 ± 0.02	100.20 ± 0.23
Lerva SC Tablets (10 mg)	9.97 ± 0.04	99.70 ± 0.39

[a] Each value is mean of five independent determinations

Table 3.5: Calibration data for estimation of LER by analytical method 2

Conc. (µg/ml)	Mean Absorbance[a] (± SD)	% RSD[b]	Predicted Conc.[c] (µg/ml)
10	0.1030 ± 0.0029	2.79	10.43
20	0.2041 ± 0.0033	1.60	20.24
40	0.4040 ± 0.0026	0.65	39.65
60	0.6038 ± 0.0021	0.35	59.05
80	0.8123 ± 0.0078	0.96	79.29
100	1.0341 ± 0.0127	1.23	100.83

[a] Each value is mean of eight independent determinations
[b] Percentage relative standard deviation
[c] Predicted concentration is calculated from the regression equation

Table 3.6: Accuracy and precision data for analytical method 2

Level	Predicted Conc.[a] (µg/ml)			Mean % Recovery[b] (± SD)	% Bias[c]
	Range	Mean[b] (± SD)	% RSD		
LQC	14.79 - 14.88	14.83 ± 0.04	0.24	98.86 ± 0.24	-1.14
MQC	54.64 - 55.98	55.04 ± 0.48	0.87	100.06 ± 0.87	0.06
HQC	94.70 - 95.94	95.14 ± 0.53	0.56	100.15 ± 0.56	0.15

[a] Predicted concentration is calculated from the regression equation
[b] Each value is mean of six independent determinations
[c] Accuracy is given in % Bias

Table 3.7: Results of intermediate precision study for analytical method 2

Level	Intra-day repeatability (% RSD) (n=3)			Inter-day repeatability (% RSD) (n=18)
	Day 1	Day 2	Day 3	
LQC	0.33	0.08	1.20	0.77
	0.04	0.76	0.49	
MQC	0.15	1.33	0.34	0.91
	1.33	1.16	0.40	
HQC	0.52	1.01	1.07	1.10
	0.07	1.28	2.08	

Table 3.8: Determination of LER in marketed formulations using analytical method 2

Commercial Products	Mean Amount Found[a] (mg) (± SD)	% Assay[a] (± SD)
Lerez Tablets (10 mg)	10.10 ± 0.03	100.99 ± 0.28
Landip 10 Tablets (10 mg)	10.09 ± 0.05	100.91 ± 0.45
Lerka Tablets (10 mg)	10.09 ± 0.03	100.88 ± 0.31
Lerva SC Tablets (10 mg)	10.15 ± 0.04	101.55 ± 0.37

[a] Each value is mean of five independent determinations

Table 3.9: Calibration data for estimation of LER by analytical method 3

Conc. (ng/ml)	Mean Area[a] (µv-sec) (± SD)	% RSD[b]	Predicted Conc.[c] (ng/ml)
25	6323.88 ± 111.59	1.76	23.61
50	12477.38 ± 143.59	1.15	48.44
100	25033.13 ± 300.89	1.20	99.09
250	62903.38 ± 685.60	1.09	251.88
500	124748.88 ± 1705.96	1.37	501.40
750	187889.63 ± 1672.79	0.89	756.14
1000	246944.88 ± 3882.98	1.57	994.40

[a] Each value is mean of eight independent determinations
[b] Percentage relative standard deviation
[c] Predicted concentration is calculated from the regression equation

Table 3.10: Accuracy and precision data for analytical method 3

Level	Predicted Conc.[a] (ng/ml)			Mean % Recovery[b] (± SD)	% Bias[c]
	Range	Mean[b] (± SD)	% RSD		
LQC	29.82 - 30.78	30.32 ± 0.46	1.53	101.05 ± 1.54	1.04
MQC	399.42 - 407.93	401.19 ± 3.35	0.84	100.30 ± 0.84	0.30
HQC	897.56 - 911.19	901.38 ± 4.94	0.55	100.15 ± 0.55	0.15

[a] Predicted concentration is calculated from the regression equation
[b] Each value is mean of six independent determinations
[c] Accuracy is given in % Bias

Table 3.11: Results of intermediate precision study for analytical method 3

Level	Intra-day repeatability (% RSD) (n=3)			Inter-day repeatability (% RSD) (n=18)
	Day 1	Day 2	Day 3	
LQC	1.43	1.59	1.32	1.34
	1.63	0.56	1.82	
MQC	0.19	0.73	0.49	0.70
	0.22	1.05	1.05	
HQC	0.09	0.67	0.03	0.59
	0.19	0.98	0.28	

Table 3.12: Determination of LER in marketed formulations using analytical method 3

Commercial Products	Mean Amount Found[a] (mg) (± SD)	% Assay[a] (± SD)
Lerez Tablets (10 mg)	10.07 ± 0.07	100.73 ± 0.67
Landip 10 Tablets (10 mg)	9.97 ± 0.16	99.75 ± 1.63
Lerka Tablets (10 mg)	10.06 ± 0.07	100.61 ± 0.68
Lerva SC Tablets (10 mg)	10.08 ± 0.08	100.82 ± 0.80

[a] Each value is mean of five independent determinations

Table 3.13: Calibration data for estimation of LER by analytical method 4

Conc. (ng/ml)	Mean Area[a] (µv-sec) (± SD)	% RSD[b]	% Recovery[c] (± SD)
25	5931.38 ± 462.65	7.80	93.84 ± 7.72
50	11526.50 ± 628.04	5.45	92.39 ± 5.19
100	21908.75 ± 1189.93	5.43	97.52 ± 4.47
250	58641.13 ± 4245.36	7.24	93.24 ± 6.94
500	116440.75 ± 7733.67	6.64	93.31 ± 5.62
750	173155.63 ± 12493.88	7.22	92.13 ± 6.08
1000	234959.75 ± 10327.17	4.40	95.15 ± 4.08

[a] Each value is mean of eight independent determinations
[b] Percentage relative standard deviation
[c] Percent drug recovery = [(Peak area of serum standard/ peak area of analytical standard of same concentration) x 100]

Table 3.14: Accuracy and precision data for analytical method 4

Level	Predicted Conc.[a] (ng/ml)			Mean % Recovery[b] (± SD)	% Bias[c]
	Range	Mean[b] (± SD)	% RSD		
LQC	27.91 - 32.07	29.75 ± 1.45	4.87	99.18 ± 4.87	-0.82
MQC	381.21 - 400.43	393.26 ± 8.72	2.22	98.32 ± 2.22	-1.68
HQC	858.95 - 920.17	898.60 ± 25.75	2.87	99.84 ± 2.87	-0.16

[a] Predicted concentration is calculated from the regression equation
[b] Each value is mean of six independent determinations
[c] Accuracy is given in % Bias

Table 3.15: Results of intermediate precision study for analytical method 4

Level	Intra-day repeatability (% RSD) (n=3)			Inter-day repeatability (% RSD) (n=18)
	Day 1	Day 2	Day 3	
LQC	3.72	7.24	2.73	6.47
	5.43	5.40	3.58	
MQC	2.46	2.54	5.80	8.82
	2.47	7.86	5.04	
HQC	3.43	6.62	6.60	6.02
	2.73	4.74	4.91	

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Chapter 4

Preformulation Studies

4.1 Introduction

Prior to any formulation development program, preformulation study is very critical and important for the understanding of pharmaceutically significant physicochemical properties of the selected drug. The goal of preformulation study is to investigate critical physicochemical properties and parameters of the drug and generate a thorough understanding of stability of drug under various conditions that are useful for designing an appropriate formulation. Preformulation data confirm the presence or absence of potential barriers to the development of optimally bioavailable and stable formulation for a drug substance thereby helping in developing a clinically effective formulation. An adequate understanding of these properties of the drug substance minimizes problems in formulation stages and helps in selection of compatible excipients and development of appropriate dosage form for the drug substance (Wadke and Jacobson, 1980; Fiese and Hagen, 1987, Ravin and Radebaugh, 1990). For drugs with poor and erratic oral availability, the variability in the bioavailability in most of the cases can be traced down to their physicochemical properties (D'Incalci et al., 1982; Harvey et al., 1985). This knowledge can help to decide logical and effective approaches to design suitable dosage form for better availability of the drug.

Preformulation studies generally include physicochemical characterization of drug like determination of solubility, stability, dissociation constant, partition coefficient and particle size of the drug substance. Thorough understanding of stability of drug in pure form and in physical mixture with proposed excipients under various conditions of temperature, light and humidity is important for identification of potential drug excipient incompatibility problems.

Physicochemical properties of lercanidipine hydrochloride (LER) like pH solubility, pH stability and partition coefficient have not been reported in scientific literature. So determination of these physicochemical properties with a knowledge of stability of drug in pure form and in physical mixture with proposed excipients was considered imperative for deciding strategy or steps in formulation of buccal mucoadhesive controlled delivery systems. Polymorphic form of LER used for formulation was also characterized.

4.2 Experimental

4.2.1 Materials

Racemic form of LER was obtained as a gift sample from Glenmark Pharmaceuticals Limited, Mumbai. Hydroxy propyl methyl cellulose (HPMC) of various viscosity grades were purchased from Sigma Aldrich Chemicals, USA. Polycarbophil (PC) and carbopol 934P (CP) were obtained as gift samples from Noveon Inc., USA and Cadila Pharmaceuticals Ltd., India respectively. Polyethylene oxide (Polyox WSR 1105; 900KDa) and chitosan (CH) were obtained as gift samples from Ranbaxy Laboratories Ltd., India. All other chemical used were of analytical grade and purchased from Qualigens, Mumbai. Triple distilled water (TDW) from all quartz glass apparatus was used for preparation of various aqueous phases used in the study.

4.2.2 Equipments/Instruments

A constant temperature water bath shaker (MAC Instruments, India) was used for solubility studies and partition coefficient determination. All pH measurements were carried out using pH meter (Elico, India) equipped with glass electrode filled with potassium chloride gel and with auto temperature compensation probe. Frost-free-200 L refrigerator (Godrej, India) was used for stability studies at refrigerated conditions. A humidity chamber (MAC Instruments, India) was used to maintain accelerated conditions (40 ± 2^{o}C; 75 ± 5% RH). Thermal analysis was performed using differential scanning calorimeter (Shimadzu, Japan; model: DSC-60; integrator: TA-60WS thermal analyzer; integrating software: TA-60WS collection monitor version 1.51; analysis software: TA60; principle: heat flux type; temperature range: -150-600^{o}C; heat flow range: ± 40 mW; temperature program rate: 0–99^{o}C per min; atmosphere: inert nitrogen at 30 ml/min). A five digit analytical balance (Mettler Toledo, Switzerland) was used for all weighing purposes. Polymorphic form characterization and compatibility studies were carried out using Fourier Transform Infrared (FTIR) Spectrophotometer (Shimadzu, Japan; model-IR Prestige-21). The software used for IR data processing and plotting was IRSolutions, version 1.0. Eluted Thin Layer Chromatographic (TLC) plates were checked in UV-Fluorescence chamber (Superfit, India). Analytical instruments mentioned in chapter 3 were used for all sample analysis.

4.3 Methods

Analytical method 1 mentioned in chapter 3 was used during solubility and stability studies. Analytical method 3 of chapter 3 was used for analysis of photostability and partition coefficient samples.

4.3.1 Characterization of polymorphic form

LER exhibits polymorphism (two forms namely, Form I and Form II), different polymorphic forms are obtained depending upon the synthetic route followed and solvents used for crystallization. Polymorphic forms have been reported to show distinct chemical and physical properties (color, crystal size, melting point and solubility). Form I and Form II have also been reported to show distinct IR spectrum (Bonifacio et al., 2005).

Hence, Differential Scanning Calorimetric (DSC) and Fourier Transform Infrared Spectroscopic (FTIR) analysis of pure LER was carried out for confirmation of polymorphic form. The infrared (IR) spectrum and DSC thermogram obtained for pure drug were compared with that reported in literature (Bonifacio et al., 2005) for characterization of polymorphic form of LER.

For DSC studies, pure LER (3.76 mg) was taken and sealed in a standard aluminum pan with lid. The temperature range used was 35^{o}C to 300^{o}C with a heating rate of 10^{o}C per min. Nitrogen (inert gas) was purged at a rate of 30 ml/min. For FTIR study, LER was appropriately diluted with dried potassium bromide and IR spectrum was acquired in the range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The data was processed using Kubelka Munk method.

4.3.2 Determination of solubility

Solubility study was carried out by shake flask method in TDW (measured pH 7.0), unbuffered and buffered solutions with pH ranging from 1.2 to 9.0. Solubility of LER was also determined in the proposed dissolution media (pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80). Excess amount of LER was added to 25 ml volumetric flasks containing 20 ml of TDW/ proposed dissolution media/ unbuffered/ buffered pH solution. The volumetric flasks were agitated in constant temperature water bath shaker maintained at 37 ± 2^{o}C for 24 h. Stability of drug in various media was assessed before carrying out solubility studies. At various time points these containers were checked for presence of insoluble drug. Obtained aqueous samples (after 24 h) were filtered through Whatman filter paper (No.40). Clear solutions obtained after filtration, were diluted appropriately and analyzed using analytical method 1 in chapter 3. All the measurements were carried out in triplicate. All samples were protected from light using amber colored glassware.

4.3.3 Determination of partition coefficient

Literature suggests use of n-octanol/ TDW system for determining Log P of drugs for buccal permeation (Mashru et al., 2005). Partition coefficient of LER was determined in n-octanol/ TDW system by shake flask method. n-octanol was pre-saturated with TDW by shaking it with TDW on rotary flask shaker for 24 h at room temperature (25 ± 2^{o}C). n-octanol saturated with TDW was separated using glass separating funnel and used for further experimentation. To 5 ml of this n-octanol, 30 ml of 50 µg/ml solution of drug in TDW (initial drug concentration in aqueous phase was determined using analytical method 3 mentioned in chapter 3) was added and kept for shaking on rotary flask shaker at room temperature. At the end of 24 h, one ml of aqueous phase was taken and centrifuged at 4000 RPM for 10 min. After equilibration at room temperature for 15 min, aqueous phase was collected, diluted appropriately and analyzed by analytical method 3 mentioned in chapter 3. The entire experiment was carried out in triplicate. Apparent partition coefficient was calculated using the equation given below and Log P was calculated by taking logarithm to the base 10 of partition coefficient. Samples were protected from light during the study.

$$P_{o/w} = (A_i - A_f)6/A_f$$

Where, $P_{o/w}$ = Apparent partition coefficient; A_i = Initial amount of drug in aqueous phase; A_f = Final amount of drug in aqueous phase; Factor of 6 was multiplied as volume of aqueous phase was six times that of organic phase.

4.3.4 Determination of stability

4.3.4.1 Solution state stability

Solution state stability profile of LER was established in various buffered solutions of varying pH (pH 1.2, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.4, 8.0, 9.0). A stock solution (1 mg/ml) of LER was prepared in acetonitrile. 625 µl of this stock solution was added to buffered solutions of varying pH and volume was made up to 25 ml to achieve a final concentration of 25 µg/ml. All samples were kept at 25 ± 2^{o}C in closed containers. The entire experiment was carried out in triplicates. Samples were protected from light during the study. Samples were withdrawn at different time points and were analyzed by analytical method 1 of chapter 3. At different time points the solutions were spotted on a TLC plate and eluted using chloroform:methanol (90:10) for assessing stability of the drug. LER dissolved in acetone was used as control in all TLC studies.

To establish thermal stability and photostability, 25 µg/ml solution of LER was made in pH 7.0 buffer as mentioned above. The samples were exposed to different conditions of temperature (25 ± 2^{o}C; 40 ± 2^{o}C; and 60 ± 2^{o}C) in closed containers. For

photostability, the samples were exposed to natural sunlight. The entire experiment was carried out in triplicates. Samples were withdrawn at different time points and were analyzed by analytical method 1 of chapter 3. At different time points the solutions were spotted on a TLC plate and eluted using chloroform:methanol (90:10) for assessing stability of the drug. LER dissolved in acetone was used as control in all TLC studies. Samples were protected from light during thermal studies.

4.3.4.2 Solid state stability

Solid state stability of LER and compatibility with various excipients short-listed for preparing buccal mucoadhesive controlled drug delivery systems was studied. Excipients used for the study were lactose, mannitol, CP, PC, CH, PEO, HPMC (HPMC K4M, HPMC K15M, HPMC K100M), magnesium stearate and talc.

DSC study was carried out for pure LER, individual excipient and combination of LER with different excipients (mixed in 1:1 ratio). Mixed samples of drug and excipient were analyzed by analytical method 1 of chapter 3 for content uniformity. Around 2-4 mg of sample (pure LER, individual excipient and combination of LER with excipient) was taken and sealed in standard aluminum pan with lid. DSC studies were carried out as mentioned previously in section 4.3.1. Endothermic peaks recorded in the thermograms are directed downwards. All the samples were stored at controlled room temperature (CRT: 25 ± 2^{o}C and 60 ± 5 % RH) protected from light for 12 months and the study was repeated.

FTIR study was also carried out for pure LER, individual excipient and combination of LER with excipient (mixed in 1:1 ratio) as mentioned earlier in section 4.3.1. All samples were stored at CRT (25 ± 2^{o}C and 60 ± 5 % RH) protected from light for 12 months and the study was repeated.

LER (passed through 80 #) and various excipients were physically mixed in 1:10 ratio. The physical mixtures were prepared carefully with geometric mixing and analyzed for content uniformity using analytical method 1 of chapter 3. The prepared mixtures were filled in vials and kept at different temperature conditions like refrigerated temperature (FT: 5 ± 2^{o}C), controlled room temperature (CRT: 25 ± 2^{o}C/ 60 ± 5 % RH) and at accelerated condition (AT: 40 ± 2^{o}C/ 75 ± 5 % RH). At predetermined time intervals, samples (in triplicates) were taken and analyzed for drug content by analytical method 1 of chapter 3 after suitable dilution. At different time points the samples (dissolved in acetone) were spotted on a TLC plate and eluted using chloroform:methanol (90:10) for assessing stability of the drug. LER dissolved in acetone was used as control in all TLC studies. Samples were protected from light during the study.

4.3.4.3 Determination of solid state photostability

To study the solid state photostability, LER was spread in thin layer in petriplates (n=9). One set of three petriplates was exposed to UV light (254 nm) in a UV chamber. Another set of three petriplates was stored at CRT (25 ± 2^{o}C/ 60 ± 5 % RH) unprotected from light. Samples were taken at predetermined time intervals, suitably diluted and analyzed for drug content using analytical method 3 of chapter 3. The drug content of exposed samples was compared to the drug content of last set of three petriplates stored at CRT (25 ± 2^{o}C/ 60 ± 5 % RH) and protected from light using aluminum foil. These three petriplates acted as control for the entire experiment.

4.3.5 Hygroscopicity

Moisture sorption property of LER was studied by exposing drug to various conditions of humidity. Initial moisture content of LER was determined by spreading accurately weighed drug in petriplates (n=3), and drying the samples at 105 ^{o}C to a constant weight. For studying hygroscopicity, accurately weighed quantity of drug was spread in petriplates (n=6). One set of three petriplates was exposed at 30 ± 5 % RH for 48 h and percentage moisture content was determined by weighing the exposed samples. Another set of three petriplates was exposed at 75 ± 5 % RH for 48 h and moisture content was determined as mentioned earlier.

4.4 Results and Discussion

4.4.1 Characterization of polymorphic form

DSC thermogram of LER showed a distinct melting endotherm of drug at 192.64^{o}C (Figure 4.1). Review of literature revealed that LER exhibits polymorphism and exists in two crystalline forms namely Form I and Form II. Form I is characterized by a melting point (DSC peak) within the range of about 190-199^{o}C, more specifically, about 193.7^{o}C and Form II shows melting endotherm in range of 207-211^{o}C (Bonifacio et al., 2005). This confirms that Form I of LER was used for this study.

IR spectrum of LER showed few important IR bands as mentioned in Table 4.1. Literature suggests that IR spectrum of Form II shows two distinct IR bands at 1675 cm^{-1} and 1705 cm^{-1}. However, in IR spectrum of Form I only one IR band corresponding to 1675 cm^{-1} is observed (Bonifacio et al., 2005). In the IR spectrum obtained for pure LER

used in this study, only one band at 1668 cm^{-1} was observed because of C=O stretching as shown in Figure 4.2. This result further confirmed that Form I of LER was used for this study.

4.4.2 Determination of solubility

The solubility of LER at 37 ± 2°C in TDW and various buffered and unbuffered solutions of pH ranging from 1.2 to 9.0 is given in Table 4.2. The pH solubility profiles of LER in both buffered and unbuffered systems are shown in Figure 4.3. LER was found to be poorly soluble drug, with maximum solubility of 86.09 and 86.55 µg/ml (both at acidic pH of 1.2) in buffered and unbuffered solutions respectively. In general with increasing pH, solubility decreased. This profile is in compliance with the weakly basic nature of LER. The solubility profile of LER in both buffered and unbuffered systems was found to be almost identical. It can be said that solubility of LER is dependent of pH with a marginal reduction in solubility as pH increases from 1.2 to 9.0.

In presence of 2.5% v/v polysorbate 80 the solubility of LER increased drastically to 2.56 mg/ml in pH 6.8 phosphate buffer at 37 ± 2°C. This result shows that pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80 can be used as in vitro release media for evaluating drug release pattern from the designed controlled release drug delivery systems of LER (Brian, 2001; Shah et al., 2002).

Insufficient aqueous solubility of drugs has been reported as one of the reasons for poor and erratic absorption of drug with large inter- and intra-subject variations in blood levels (Horter and Dressman, 2001). The low aqueous solubility of LER might be responsible for its poor and erratic oral absorption. LER has high melting point, which is indicative of strong crystal lattice energy. This high melting point and crystal lattice energy might be one of the factors responsible for poor solubility of LER.

4.4.3 Determination of partition coefficient

The equilibrium partition coefficient of LER was determined in n-octanol/ TDW system by shake flask method. The partition coefficient of drug was found to be 2960.973 ± 26.165. Log P of the drug was found to be 3.471 ± 0.004. The reported computational value of Log P is 4.3 (Vander Lee et al., 2000). This difference between computational (theoretical) and experimental value of Log P may be attributed to sensitivity of the method for determination of Log P (shake flask method). Log P value of LER indicates hydrophobic nature of the drug and can be correlated to poor aqueous solubility of the drug.

4.4.4 Determination of stability

4.4.4.1 Solution state stability

LER was found to be extremely sensitive to pH. The log percent remaining to be degraded (% RTD) versus time profiles were linear for all the plots across all pH values indicating first order degradation kinetics (Figure 4.4). First order degradation rate constants obtained from slope of the curves were used to determine $t_{90\%}$ at various pH values (Table 4.3). Low MSSR values and regression coefficient values (R^2) close to 1 further established linear relationship between log % RTD versus time (Table 4.3).

Degradation of LER was found to be pH sensitive. Towards acidic pH degradation was found to be higher when compared to neutral and basic pH. Degradation rate constant (K_{deg}) values obtained were ranging from 9.01 x 10^{-4} h^{-1} (pH 7.0) to 19.71 x 10^{-4} h^{-1} (pH 1.2) and $t_{90\%}$ values obtained were ranging from 2.23 to 4.87 days (Table 4.3). From Figure 4.5 it becomes further evident that the drug has minimal degradation at neutral pH range of 6 to 7.4 with higher degradation at acidic pH.

The retention factor (R_f) for LER was found to be 0.63 in the given elution phase (chloroform:methanol in 90:10 v/v ratio). There was no difference in the R_f values of freshly prepared pure drug solution and drug in buffers of varying pH at zero time. In near neutral pH range (6.0-7.4) only one spot corresponding to drug was observed till 48 h indicating higher stability of drug in this range of pH. In acidic pH of 1.2 and 2.0 one extra spot other than that of pure drug was observed under UV wavelength of 254 nm after 24 h of the study. In all other buffers one extra spot other than that of pure drug was observed under UV wavelength of 254 nm after 48 h of the study.

In thermal stability (25 ± 2^oC; 40 ± 2^oC and 60 ± 2^oC) and photostability studies of LER in pH 7.0 buffer, the log % RTD versus time profiles were linear for all the plots indicating first order degradation kinetics. First order degradation rate constants obtained from slope of the curves were used to determine $t_{90\%}$ values at various conditions. Low MSSR values and R^2 close to 1 established linear relationship between log % RTD versus time (Table 4.4). In thermal stability studies (at 25^oC, 40^oC and 60^oC), the degradation rate was found to be dependent upon temperature. K_{deg} values obtained were ranging from 9.01 x 10^{-4} h^{-1} (at 40^oC) to 12.16 x 10^{-4} h^{-1} (at 60^oC) and $t_{90\%}$ values obtained were ranging from 3.61 to 4.87 days for thermal degradation studies. In photostability studies, the drug was found to be very sensitive to light with K_{deg} value of 20.28 x 10^{-4} h^{-1} and $t_{90\%}$ value of 2.16 days (Table 4.4).

The retention factor (R_f) for LER was found to be 0.63 in the given elution phase. At 60^{o}C and in photostability study one extra spot other than that of pure drug was observed under UV wavelength of 254 nm after 48 h of the study. At temperature of 25^{o}C and 40^{o}C only one spot corresponding to drug was observed till 48 h indicating higher stability of drug at these temperature conditions.

4.4.4.2 Solid state stability

DSC study was carried out for pure LER, individual excipient and combination of LER with various excipients (mixed in 1:1 ratio). The content uniformity of all the samples was found to be in range of 98.76 to 101.19 % with maximum SD of 1.14.

DSC thermogram of LER showed a distinct melting endotherm of drug at 192.64^{o}C (Figure 4.1) with an enthalpy value of -56.16 J/g (Table 4.5). Figure 4.6 to 4.16 represent thermograms of LER, excipients and physical mixtures of LER with different excipients selected for the study. Melting endotherm of drug was well preserved in most of the cases. However, a slight change in peak shape with little broadening and shifting to higher or lower temperature was observed in some physical mixtures, which could be attributed to the mixing process that lowers the purity of each component of the mixture (Verma and Garg, 2004). Similar results were obtained when the study was repeated on the samples stored at CRT for 12 months.

In DSC thermogram of physical mixture of LER and lactose, melting endotherm of drug was well preserved with an enthalpy value of -59.86 J/g (Table 4.5). For pure lactose a sharp endothermic peak was observed at 146.80^{o}C which can be attributed to loss of bound water (Araujo et al., 2003) followed by melting endotherm at around 217.46^{o}C (Figure 4.6). All the peaks were retained in the physical mixture of drug and lactose indicating lack of interaction between drug and lactose.

In DSC thermogram of pure mannitol, a sharp endothermic peak was observed at 168.13^{o}C (Figure 4.7) very near to that of the drug with an enthalpy value of -207.31 J/g (Table 4.5). In physical mixture a single, wide endothermic peak was observed which can be attributed to both drug and mannitol because of their similar melting points. The enthalpy value of single peak observed in physical mixture of drug and mannitol was found to be -263.02 J/g, which is almost equal to summation of individual enthalpy values of drug (-56.16 J/g) and mannitol (-207.31 J/g) (Table 4.5). On basis of this observation it can be concluded that drug is stable in presence of mannitol (Verma and Garg, 2004).

In DSC thermograms of PC, CP, CH, talc and various viscosity grades of HPMC (HPMC K4M, HPMC K15M, HPMC K100M) no peaks were observed (Figure 4.8 to 4.14).

For physical mixtures, in all the cases melting endotherm of drug was well preserved (Figure 4.8 to 4.14) with little or no change in enthalpy value of drug (Table 4.5) indicating compatibility.

The DSC thermogram of PEO showed an endothermic peak at 67.13^{o}C (Figure 4.15). Endothermic peaks of both PEO and LER were retained in physical mixtures with little or no change in enthalpy value of drug (Table 4.5) indicating absence of interaction.

In case of DSC thermogram of magnesium stearate, an endothermic peak was obtained at 110.85^{o}C. Endothermic peak of magnesium stearate was retained in physical mixture but the drug peak shifted to 178.72 ^{o}C (Figure 4.16) with an enthalpy value of -46.96 J/g (Table 4.5). However, in FTIR study of drug and magnesium stearate all the IR bands pertaining to drug were retained. Moreover, stability study of drug and magnesium stearate at CRT and AT indicated lack of interaction. So, considering the results of all three studies it can be concluded that drug is compatible with magnesium stearate.

In FTIR study, the IR bands that can be attributed to drug are presented in Table 4.1. In all the drug-excipient mixtures studied, these bands were retained representing absence of chemical interaction between the drug and excipients. FTIR study further established that there is no chemical interaction between drug and excipients studied. Similar results were obtained when the study was repeated on the samples stored at CRT for 12 months.

Physical mixture of LER prepared in 1:10 ratio with various excipients showed good content uniformity between 98.23 to 102.14 % with maximum SD of 1.83. Table 4.6 gives the degradation kinetics of drug alone and combination of drug with various excipients. At refrigerated temperature (FT: 5 ± 2^{o}C), pure drug and all drug excipient combinations were stable for entire study period (12 months). At controlled room temperature (CRT: 25 ± 2^{o}C/ 60 ± 5 % RH) and at accelerated conditions (AT: 40 ± 2^{o}C/ 75 ± 5 % RH), the log % RTD versus time profiles were linear indicating first order degradation kinetics. Low MSSR values and R^2 value close to 1 further established linear relationship between log % RTD versus time (Table 4.6). The degradation rate constant (K_{deg}) for pure drug was found to be 27.27 x 10^{-4} and 87.01 x 10^{-4} month^{-1} at CRT and AT respectively. The $t_{90\%}$ of drug at CRT and AT was found to be 38.65 and 12.11 months respectively.

The K_{deg} values for all the mixtures were ranging from 20.22 x 10^{-4} to 34.59 x 10^{-4} month^{-1} when stored at CRT. The highest degradation rate constant was observed with magnesium stearate and lowest degradation rate constant was observed with HPMC K100M. At these storage condition $t_{90\%}$ were ranging from 30.46 to 52.12 months. LER alone and in combination with various excipients was stable for at least 12 months at this condition.

When stored at accelerated condition, the K_{deg} values for all the drug excipient mixtures were ranging from 58.91 x 10^{-4} to 89.13 x 10^{-4} $month^{-1}$. The highest degradation rate constant was observed with CP and lowest degradation rate constant was observed with CH. At these storage condition $t_{90\%}$ were ranging from 11.82 to 17.89 months. LER alone and in combination with various excipients was stable for at least 6 months at this condition.

At both the storage conditions, the degradation rate constants of combination of drug with various excipients did not differ much from that of pure drug indicating solid state stability and compatibility. Extra spots were not observed in TLC studies as compared to control for entire duration of study at FT (12 months), CRT (12 months) and AT (6 months).

4.4.4.3 Determination of solid state photostability

In photostability study, the drug was found to be extremely photosensitive with first order degradation rate constant of 60.11 x 10^{-4} $days^{-1}$ when exposed to UV light (254 nm) in a UV chamber with $t_{90\%}$ value of 17.53 days. The degradation rate constant and $t_{90\%}$ value when drug was stored at CRT (unprotected from light) were found to be 15.66 x 10^{-4} $days^{-1}$ and 67.29 days respectively. Degradation was not observed in control samples of LER stored at CRT (protected from light) using aluminum foil indicating photo-instability problem of LER.

4.4.5 Hygroscopicity

Average (± SD) initial percentage moisture content of LER was found to be 0.2483 (± 0.0078). Average (± SD) percentage moisture content of the samples after 48 h of exposure at 30 ± 5 % and 75 ± 5 % RH was found to be 0.2778 (± 0.0045) and 0.5200 (± 0.0068) respectively. So, it can be concluded that LER is almost non-hygroscopic at RH of 30 ± 5 % and therefore formulations should be processed preferably below this RH.

4.5 Conclusions

DSC and FTIR data confirmed that polymorphic Form I has been used for this study. Preformulation studies of LER indicated poor solubility of LER with high partition coefficient. Solubility of drug decreased marginally with increasing pH.

LER followed first order degradation kinetics in solution state with good stability in neutral and near neutral pH range when compared to acidic pH. LER was found to be photosensitive in solution and solid state photostability studies with first order degradation

kinetics. Drug was found to be compatible and stable with all the proposed excipients in solid state stability studies. The results of these studies were helpful in design and development of buccal mucoadhesive formulation of LER.

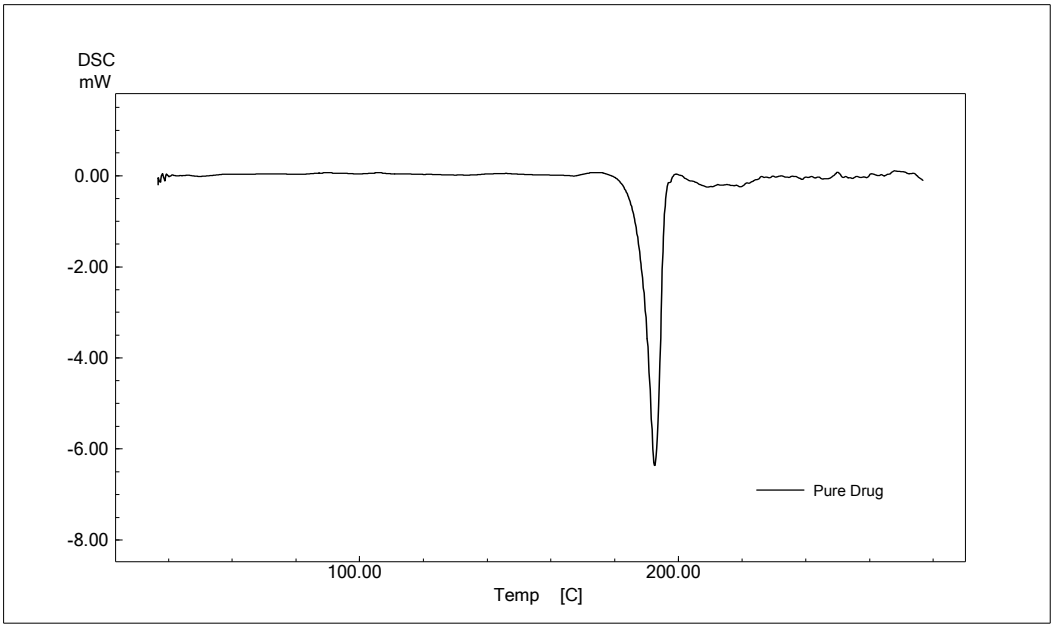

Figure 4.1: DSC thermogram of pure LER

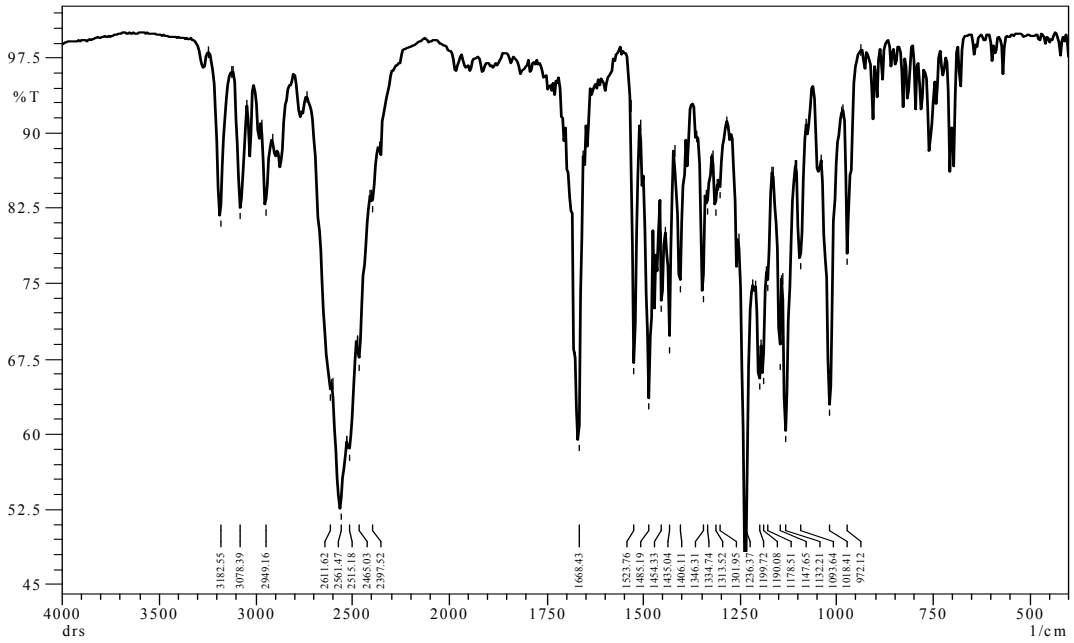

Figure 4.2: IR spectrum of LER in potassium bromide

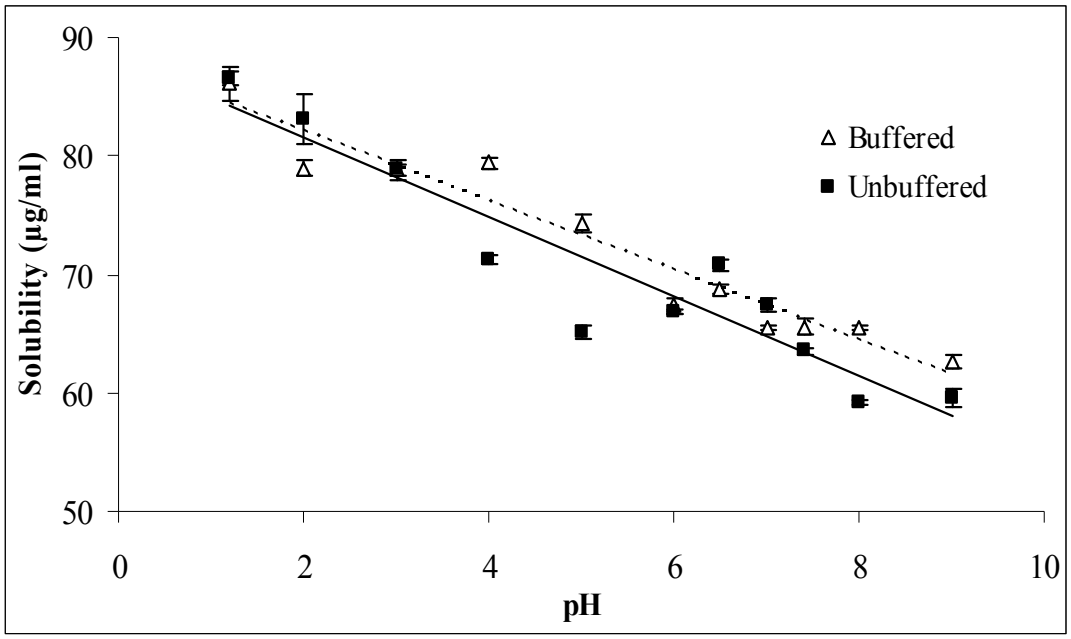

Figure 4.3: pH solubility profile of LER in various buffered and unbuffered solutions of varying pH at 37 ± 2°C

(Each point represents mean of 3 independent determinations with standard deviation)

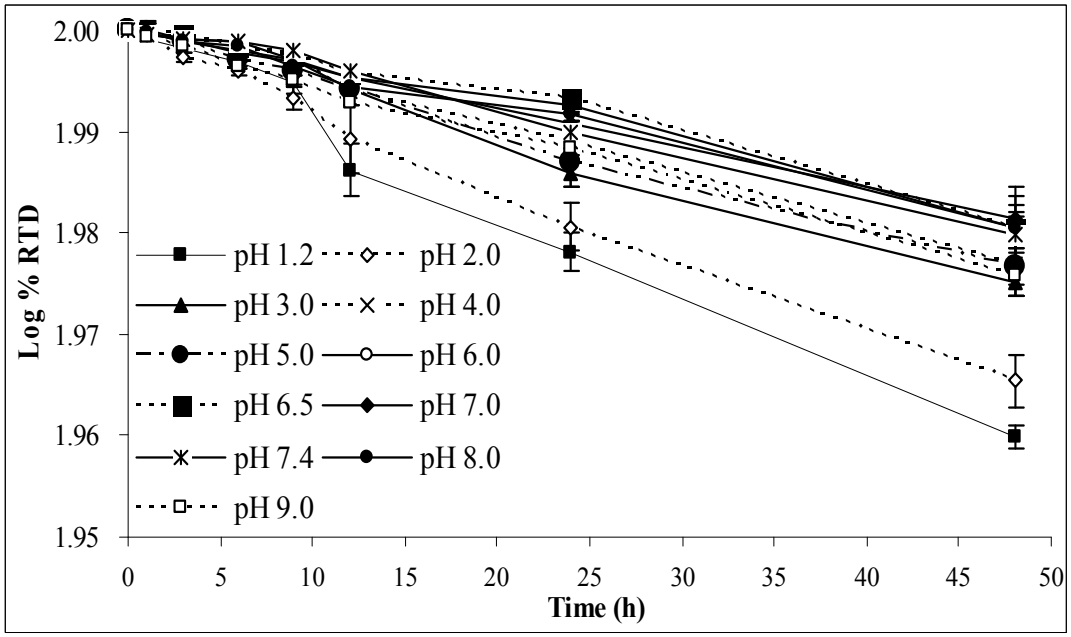

Figure 4.4: Log % RTD versus time profile for pH stability of LER at 25 ± 2°C

(Each point represents mean of 3 independent determinations with standard deviation)

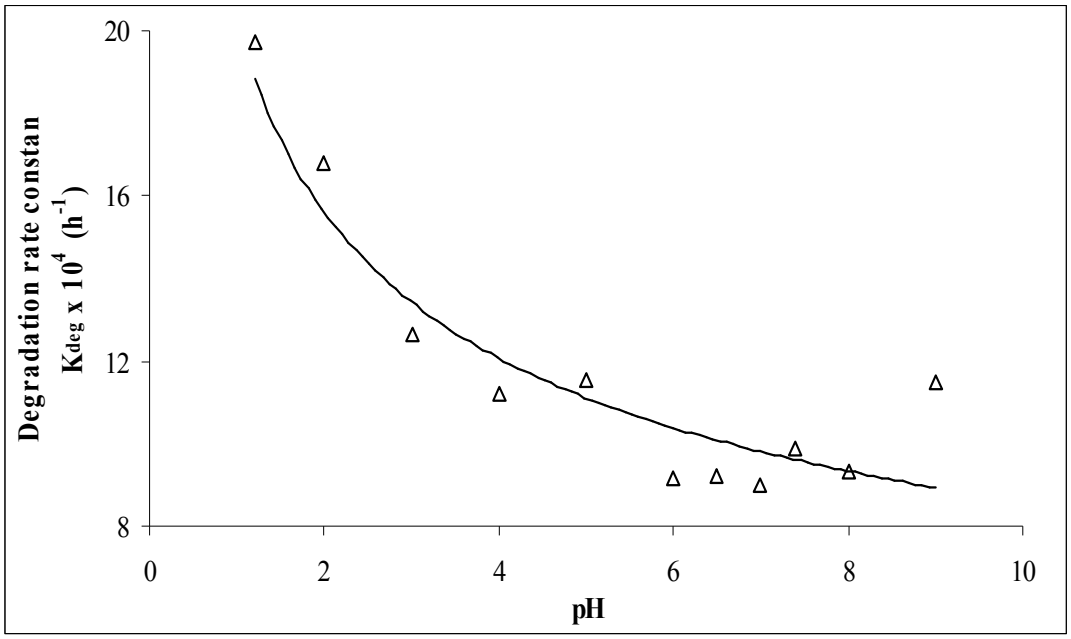

Figure 4.5: Plot of degradation rate constant versus pH for LER at 25 ± 2°C

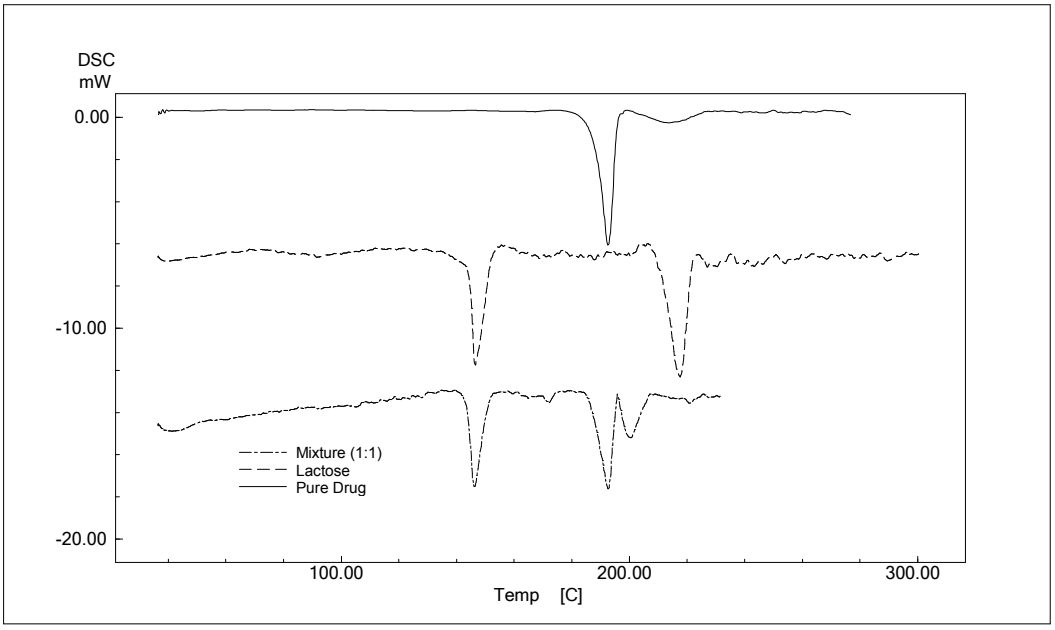

Figure 4.6: DSC thermogram of pure LER, lactose and 1:1 physical mixture

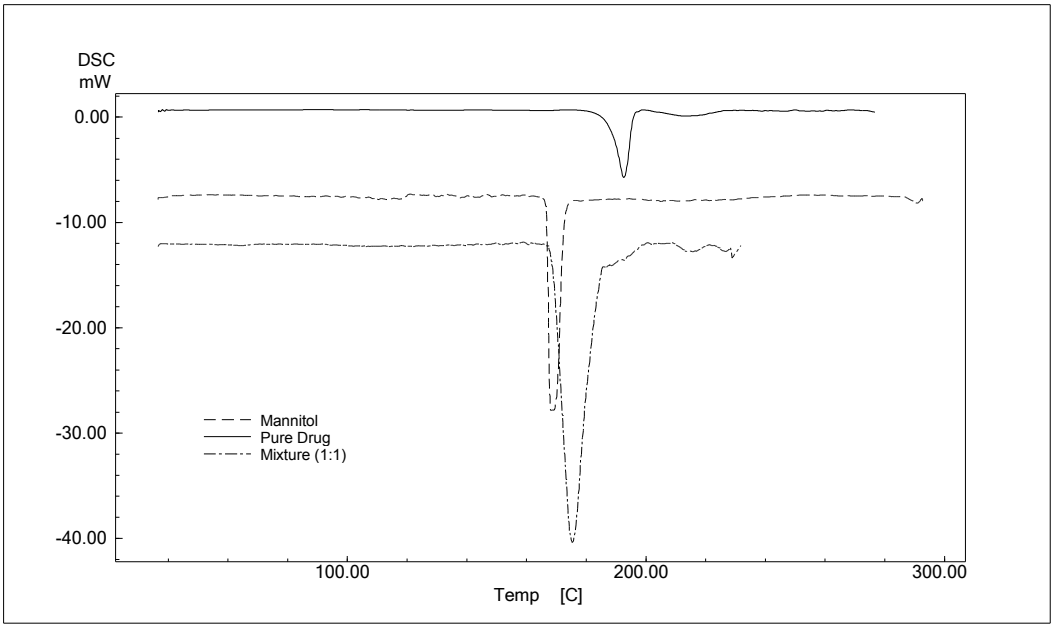

Figure 4.7: DSC thermogram of pure LER, mannitol and 1:1 physical mixture

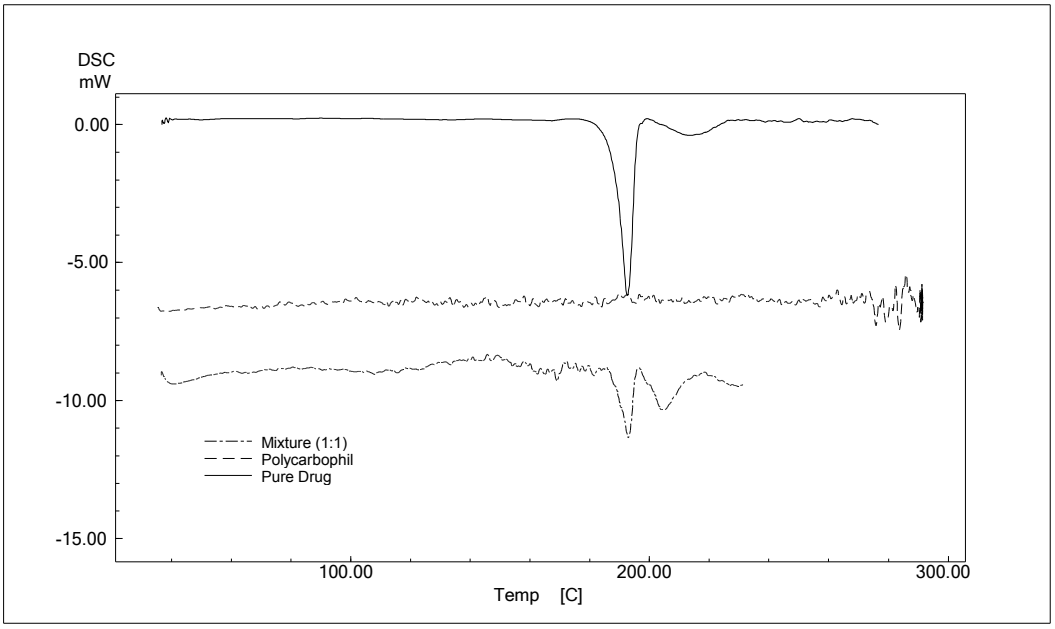

Figure 4.8: DSC thermogram of pure LER, PC and 1:1 physical mixture

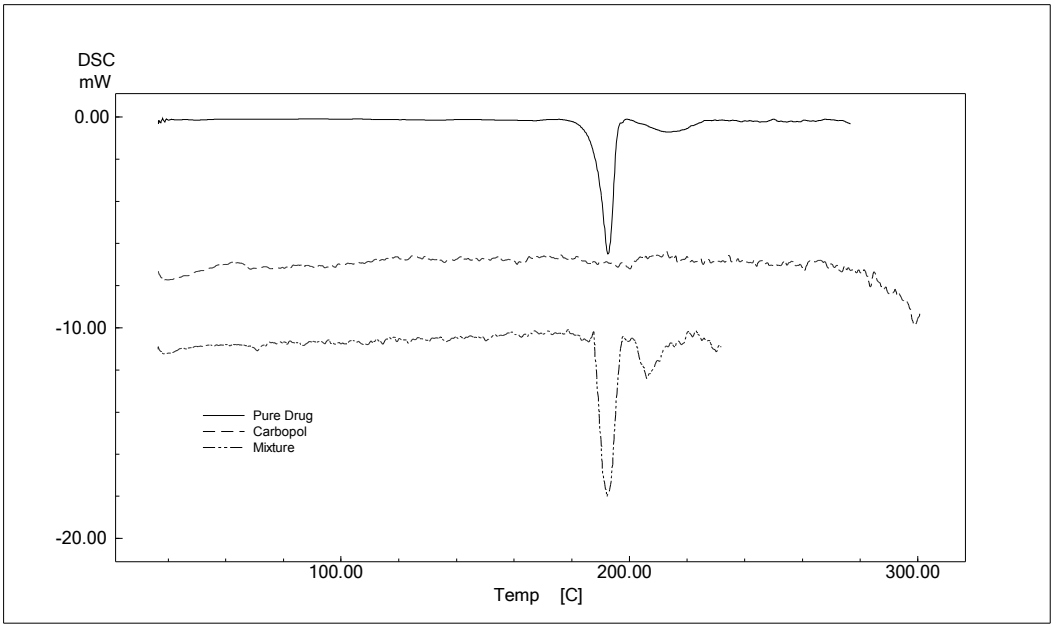

Figure 4.9: DSC thermogram of pure LER, CP and 1:1 physical mixture

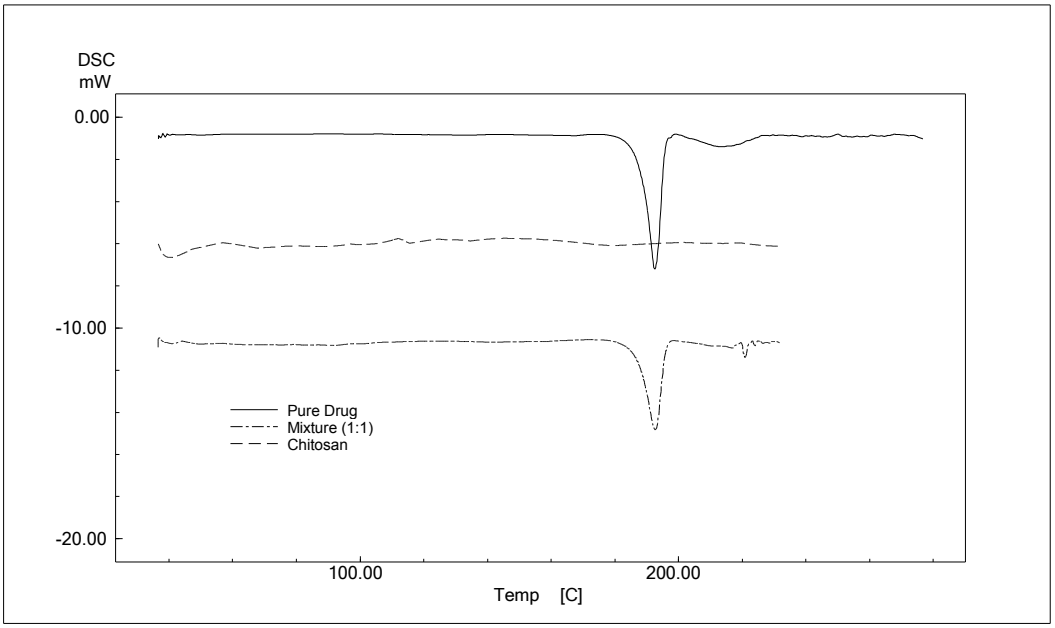

Figure 4.10: DSC thermogram of pure LER, CH and 1:1 physical mixture

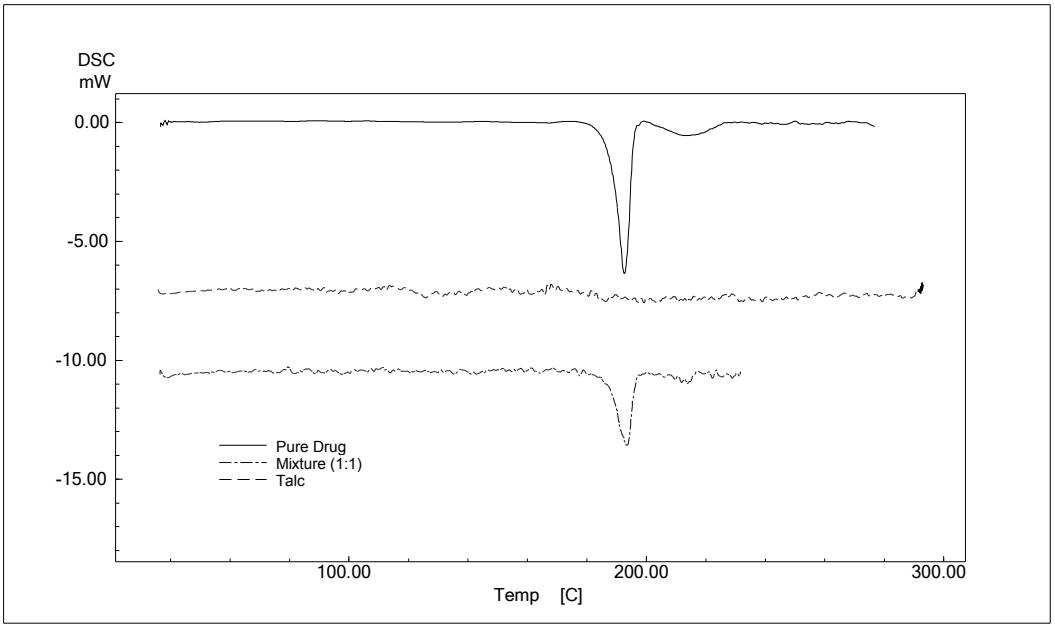

Figure 4.11: DSC thermogram of pure LER, talc and 1:1 physical mixture

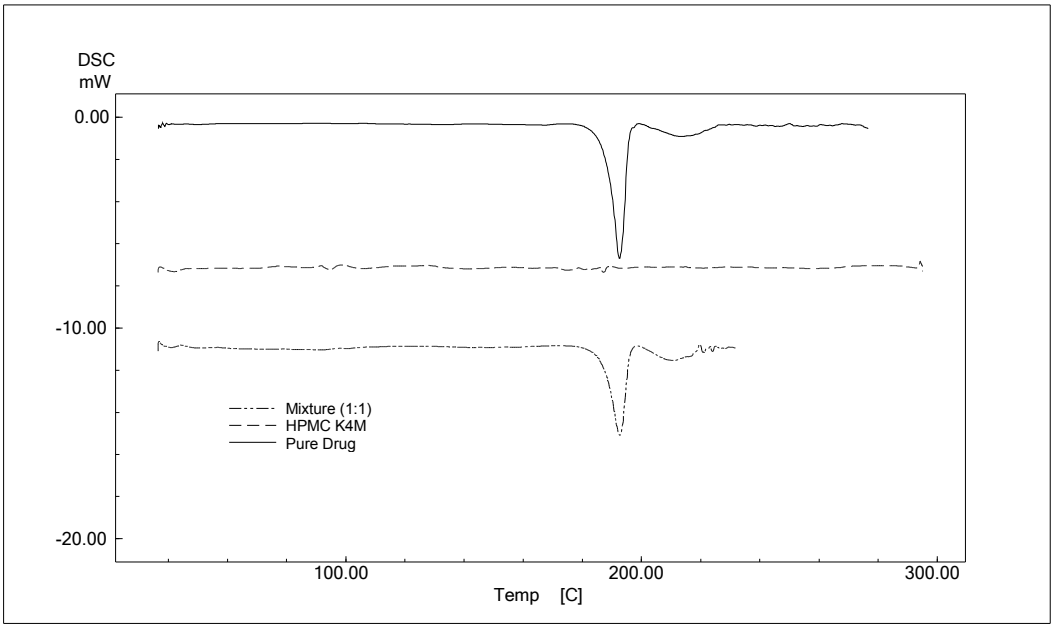

Figure 4.12: DSC thermogram of pure LER, HPMC K4M and 1:1 physical mixture

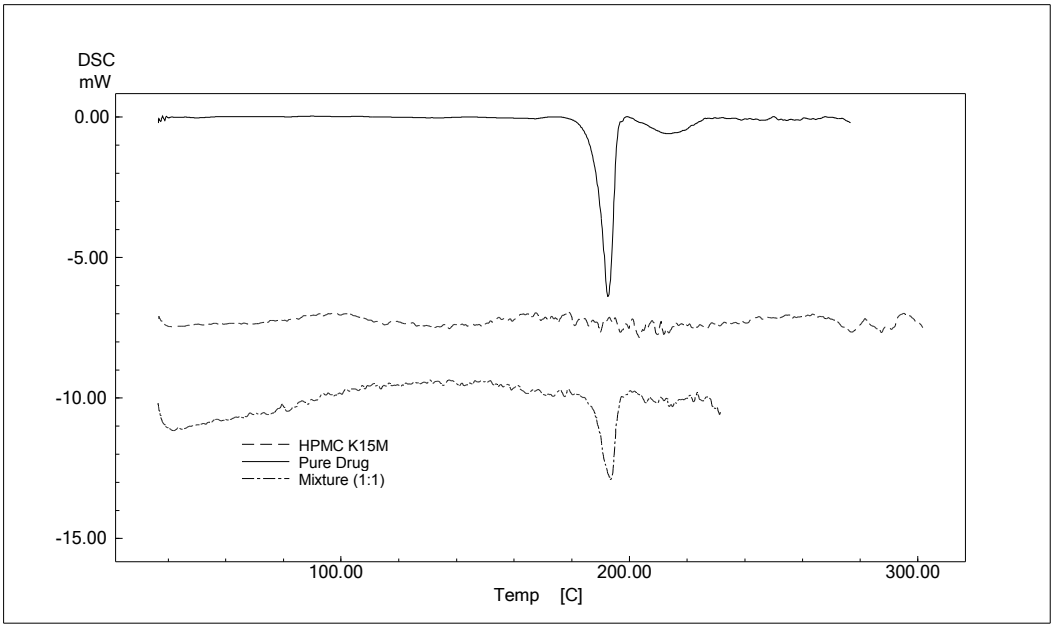

Figure 4.13: DSC thermogram of pure LER, HPMC K15M and 1:1 physical mixture

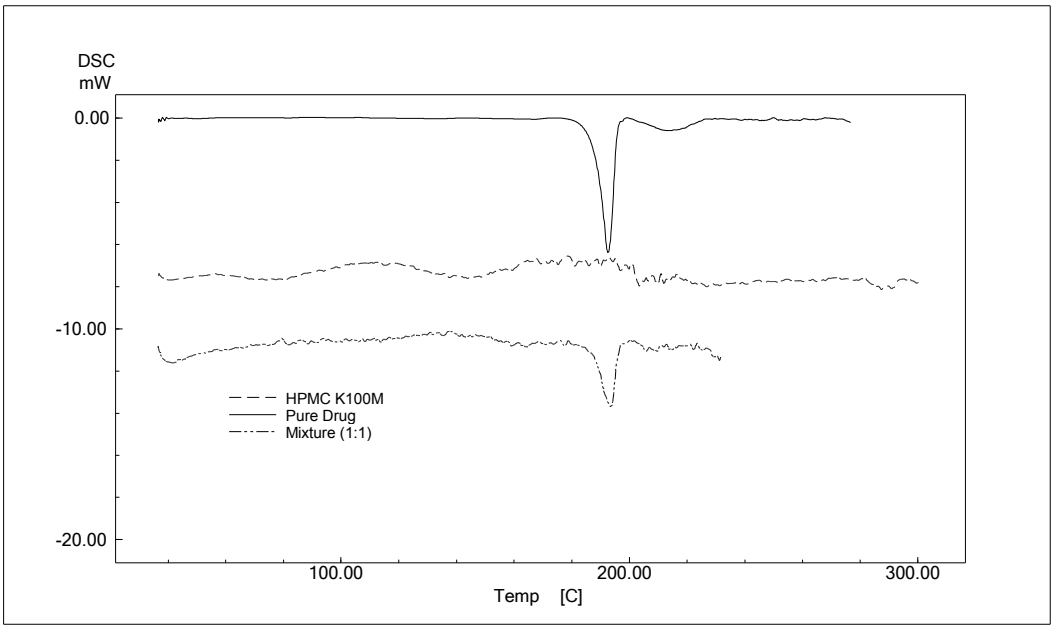

Figure 4.14: DSC thermogram of pure LER, HPMC K100M and 1:1 physical mixture

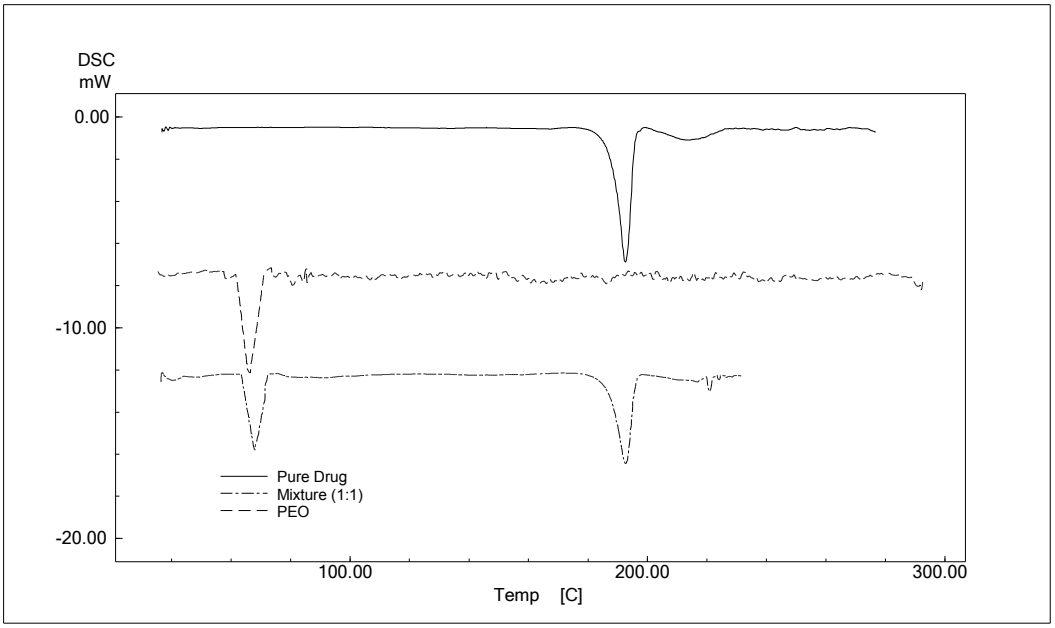

Figure 4.15: DSC thermogram of pure LER, PEO and 1:1 physical mixture

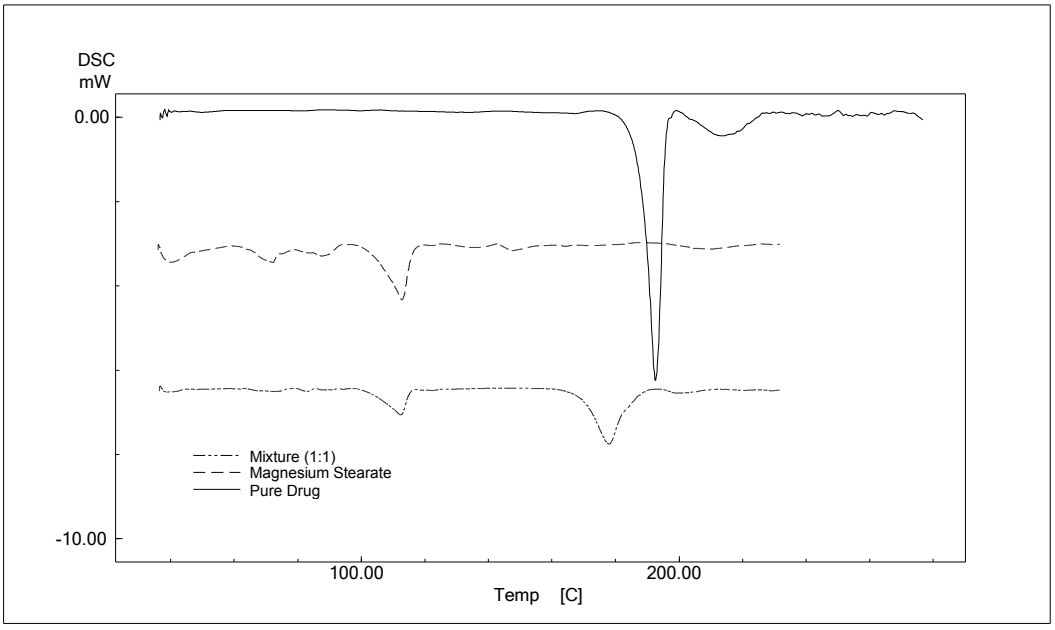

Figure 4.16: DSC thermogram of pure LER, magnesium stearate and 1:1 physical mixture

Table 4.1: Wavelength attribution of IR spectrum of LER in potassium bromide

Wavelength (cm^{-1})	**Attribution**
3182	NH stretching
3100-2800	Alkyl and phenyl stretching
2531	N^+ stretching
1668	C=O stretching
1523, 1346	Assymetric and symmetric stretching of NO_2 group
1406, 1384	Bending of geminal methyl group
795-696	Out of plane bending of 5 and 3 adjacent hydrogen on aromatic ring

Table 4.2: Solubility of LER in TDW and various buffered and unbuffered solutions of varying pH at 37 ± 2°C

Media/pH	**Solubility[a] (µg/ml) Mean ± SD**		
	TDW	**Buffered Systems**	**Unbuffered Systems**
TDW	69.91 ± 1.67	----	----
1.2	----	86.09 ± 1.41	86.55 ± 0.66
2.0	----	78.99 ± 0.70	83.18 ± 2.10
3.0	----	78.83 ± 0.79	78.81 ± 0.52
4.0	----	79.45 ± 0.49	71.28 ± 0.39
5.0	----	74.35 ± 0.79	65.18 ± 0.60
6.0	----	67.40 ± 0.66	66.76 ± 0.19
6.5	----	68.74 ± 0.33	70.79 ± 0.48
7.0	----	65.55 ± 0.20	67.46 ± 0.60
7.4	----	65.52 ± 0.67	63.50 ± 0.33
8.0	----	65.49 ± 0.25	59.23 ± 0.24
9.0	----	62.69 ± 0.54	59.60 ± 0.73

[a] Each value is mean of three independent determinations

Table 4.3: First order degradation kinetics of LER in buffered media of varying pH at 25 ± 2°C

pH	Degradation Rate Constant K_{deg}x 10^4 (h^{-1})	$t_{90\%}$ (Days)	R^2	MSSR
1.2	19.71	2.23	0.9813	4.74×10^{-6}
2.0	16.80	2.61	0.9934	5.08×10^{-6}
3.0	12.62	3.48	0.9863	1.67×10^{-6}
4.0	11.22	3.91	0.9923	5.33×10^{-1}
5.0	11.54	3.80	0.9953	1.25×10^{-6}
6.0	9.18	4.78	0.9865	8.07×10^{-7}
6.5	9.22	4.76	0.9784	1.37×10^{-6}
7.0	9.01	4.87	0.9995	1.09×10^{-6}
7.4	9.90	4.43	0.9906	1.04×10^{-6}
8.0	9.30	4.72	0.9903	1.40×10^{-6}
9.0	11.48	3.83	0.9974	6.10×10^{-7}

Table 4.4: First order kinetics of thermal and photolytic degradation of LER in pH 7.0

Condition	Degradation rate constant K_{deg}x 10^4 (h^{-1})	$t_{90\%}$ (Days)	R^2	MSSR
25°C	9.01	4.87	0.9995	1.09×10^{-6}
40°C	9.73	4.51	0.9821	1.00×10^{-6}
60°C	12.16	3.61	0.9448	1.22×10^{-2}
Natural Light	20.28	2.16	0.9389	1.50×10^{-4}

Table 4.5: Thermal properties of drug alone, excipient alone and physical mixtures (1:1)

Sample	Peak	Onset (^{o}C)	Peak (^{o}C)	Endset (^{o}C)	Heat (J/g)
LER	Endothermic	185.19	192.64	196.56	-56.16
Lactose	Endothermic	144.70	146.80	151.93	-53.96
	Endothermic	211.89	217.46	221.63	-87.25
LER + Lactose	Endothermic	187.22	192.61	197.52	-59.86
Mannitol	Endothermic	165.80	168.13	173.41	-207.31
LER + Mannitol	Endothermic	167.26	177.37	187.19	-263.02
Polycarbophil	---	---	---	---	---
LER + Polycarbophil	Endothermic	188.65	194.88	198.65	-53.07
Carbopol 934P	---	---	---	---	---
LER + Carbopol 934P	Endothermic	186.37	193.45	196.14	-52.13
Chitosan	---	---	---	---	---
LER + Chitosan	Endothermic	187.42	193.65	197.21	-53.29
Talc	---	---	---	---	---
LER + Talc	Endothermic	185.04	195.49	198.07	-52.83
HPMC K4M	---	---	---	---	---
LER + HPMC K4M	Endothermic	185.60	193.70	196.82	-53.39
HPMC K15M	---	---	---	---	---
LER + HPMC K15M	Endothermic	185.45	193.65	197.71	-57.04
HPMC K100M	---	---	---	---	---
LER + HPMC K100M	Endothermic	186.51	193.68	197.51	-57.46
PEO	Endothermic	62.28	67.13	71.78	-100.61
LER + PEO	Endothermic	185.32	191.47	196.20	-59.28
Magnesium Stearate	Endothermic	104.18	110.85	117.62	-14.62
LER + Magnesium Stearate	Endothermic	174.23	178.72	183.21	-46.96

Table 4.6: First order reaction kinetics of incompatibility study of LER with different excipients

LER + Excipient (1:10)	CRT: 25 ± 2°C/60 ± 5 % RH				AT (40 ± 2°C/75 ± 5 % RH)			
	K_{deg} x 10^4 ($month^{-1}$)	$t_{90\%}$ (month)	R^2	MSSR	K_{deg} x 10^4 ($month^{-1}$)	$t_{90\%}$ (month)	R^2	MSSR
LER	27.27	38.65	0.9744	1.53×10^{-6}	87.01	12.11	0.9600	4.12×10^{-6}
LER + Lactose	23.24	45.35	0.9543	1.75×10^{-6}	70.08	15.04	0.9822	$2.04x\ 10^{-6}$
LER + Chitosan	30.70	34.33	0.9622	1.83×10^{-6}	58.91	17.89	0.9999	3.88×10^{-7}
LER + Carbopol 934P	23.65	44.95	0.9531	2.18×10^{-5}	89.13	11.82	0.9966	6.02×10^{-6}
LER + HPMC K4M	28.53	36.93	0.9764	1.53×10^{-5}	87.15	12.09	0.9619	2.01×10^{-4}
LER + HPMC K15 M	26.44	39.86	0.9521	1.33×10^{-6}	68.72	15.33	0.9777	7.77×10^{-6}
LER + HPMC K100 M	20.22	52.12	0.9435	1.81×10^{-6}	78.60	13.41	0.9973	3.25×10^{-7}
LER + PC	31.00	34.00	0.9684	2.02×10^{-6}	88.73	11.88	0.9936	8.88×10^{-7}
LER + PEO	32.70	32.22	0.9559	1.19×10^{-5}	73.10	14.42	0.9416	3.97×10^{-6}
LER + Mannitol	26.99	39.04	0.9675	1.09×10^{-6}	62.99	16.73	0.9761	1.20×10^{-1}
LER + Magnesium stearate	34.59	30.46	0.9760	1.18×10^{-6}	81.02	13.01	0.9777	2.96×10^{-6}
LER + Talc	22.75	46.31	0.9644	9.02×10^{-7}	83.94	12.55	0.9508	1.47×10^{-4}

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Chapter 5
Formulation Development and In vitro Characterization

5.1 Introduction

The buccal mucosa of the oral cavity is an attractive region for administration of the drugs with poor oral bioavailability. Buccal delivery involves administration of the drug through the buccal mucosal membrane lining of the oral cavity. The buccal route as a site for drug administration has already been discussed at length in chapter 1.

To achieve required concentration of drug in the body continuously with predictable bioavailability and better patient compliance, several researchers have developed controlled release buccal mucoadhesive drug delivery systems (Ali et al., 2002; Varshosaz and Dehghan, 2002; Diarra et al., 2003; Narendra et al., 2005; Owens et al., 2005). Buccal drug delivery systems remain in contact with buccal mucosa for prolonged duration of time and can release drug in a controlled way to achieve steady plasma drug concentration with higher drug bioavailability thereby reducing total dose and dosing frequency (Sawicki and Janicki, 2002; Rossi et al., 2003; Patel et al., 2006,2007).

Various polymers that have been used and reported in literature for development of buccal mucoadhesive systems have been extensively reviewed in chapter 1 and the list of these polymers with classification is presented in Table 1.2 of chapter 1.

In this chapter, studies involving development and evaluation of controlled release buccal mucoadhesive tablets of lercanidipine hydrochloride (LER), prepared by matrix embedding technique have been presented. The mucoadhesive tablets were prepared using various polymers either alone or in combination by direct compression. Effect of polymer type, polymer proportion and polymer combination on in vitro release behavior and mucoadhesion was studied. Various quality control tests were carried out for developed formulations. Stability of developed formulations was assessed at various conditions of temperature and humidity. Batch reproducibility of the developed formulations was also assessed.

5.2 Materials and Reagents

Drug, materials and reagents used were same as mentioned in chapter 3 and 4.

5.3 Equipments

A 16 station tablet compression machine (Cadmach, India) equipped with 10 mm punches was used for manufacturing tablets. A five digit analytical balance (Mettler Toledo, Switzerland) was used for all weighing purposes. Friability of the designed tablets was studied using friability test apparatus (Campbell, India). In vitro release studies were carried out using in-house modified USP Type I Dissolution Apparatus (Electrolab TDT-06P,

India). Mucoadhesion was tested using in-house modified Double Pan Analytical Balance (E. Roy and Company, India). Hardness, mucoadhesion and swelling behavior were studied using Texture Analyzer TA-XT2 (Stable Micro Systems, Surrey available at Scientific and Digital Systems, New Delhi) fitted with 30 kg load cell. The machine had a force resolution of 0.1 g with force measurement accuracy of 0.001% and a distance resolution of 0.001 mm. Sonicator (Bransonic Cleaning Company, USA) and pH meter (Elico, India) were other minor instruments used for the study. Frost-free-200 L (Godrej, India) refrigerator was used for stability studies at refrigerated conditions. A humidity chamber (MAC Instruments, India) was used to maintain accelerated conditions (40 ± 2^{o}C; 75 ± 5% RH). Analytical instruments mentioned in chapter 3 were used for all sample analysis.

5.4 Methods

5.4.1 Preparation of buccal mucoadhesive controlled release tablets

Controlled release buccoadhesive tablets were prepared by direct compression technique using different polymers like carbopol 934P (CP), polycarbophil (PC), hydroxy propyl methyl cellulose of different viscosity grades (HPMC K4M, HPMC K15M, HPMC K100M), polyethylene oxide (PEO) and chitosan (CH) either alone or in combination. Drug (100#), polymer (100#) and other excipients (80#) were carefully mixed using geometrical technique and compressed using 10 mm punches. The prepared tablets were packed into sealed airtight cellophane packets and stored at CRT (25 ± 2^{o}C/ 60 ± 5 % RH) protected from light. Three batches of tablets for each formulation were prepared to check for batch reproducibility. Composition of all the designed formulations is given in Table 5.1a to 5.1e.

5.4.2 Effect of various formulation parameters

Tablets were designed using varying proportions of polymers like CP, PC, PEO, CH, HPMC (various viscosity grades) to study the effect of polymer type and polymer proportion on in vitro drug release pattern and release mechanism and in vitro mucoadhesion. Effect of nature of polymer on in vitro drug release and mucoadhesion was also studied by selecting cationic (CH), anionic (CP, PC) and non-ionic (PEO, HPMC) polymers. To assess influence of combination of polymers on in vitro drug release and mucoadhesive behavior, formulations were also prepared using combination of polymers (CP and HPMC; PC and HPMC; PEO and HPMC; CH and HPMC).

5.4.3 Evaluation of buccal mucoadhesive controlled release tablets

5.4.3.1 Physical characteristics

For each batch 20 tablets were weighed for assessing weight variation. Thickness and diameter were determined using vernier caliper. Friability was determined by subjecting 20 tablets to falling shocks in friabilator for 4 min at 25 RPM. Percentage friability was calculated using initial and final weights of 20 tablets taken for testing.

5.4.3.2 Drug content

To determine the drug content of each batch, 20 tablets were weighed and finely powdered. An aliquot of this powder equivalent to 10 mg of drug was accurately weighed and dissolved in acetonitrile-pH 6.8 phosphate buffer (50:50 v/v). The resultant solution was suitably diluted and analyzed using analytical method 1 of chapter 3.

5.4.3.3 Crushing strength/ Hardness

Use of texture analyzer for determination of hardness has been reported in the literature (Lamey at al, 2003). Crushing strength (hardness) of the tablets was determined using texture analyzer fitted with a 30 kg load cell and a 3 mm diameter probe (stainless steel cylindrical flat bottom probe - SMS P/3). The probe was set to penetrate a distance of 5 mm in the tablet with a speed of 0.1 mm/sec and the probe was withdrawn at a speed of 10 mm/sec. The force required to break the tablet by applying diametrical compression force was determined in triplicate for each batch. Hardness of the tablet was recorded as the maximum force required (in N) for breaking the tablet.

5.4.3.4 Surface pH

The prepared tablets were first allowed to swell in contact with 5 ml of TDW (pH 7.0) for 2 h in petriplates. The surface pH was measured by bringing glass electrode of pH meter in contact with the surface of tablets and allowing it to equilibrate for 1 min. The surface pH of the tablets was determined in order to investigate the possibility of any side effect in oral cavity. As acidic or alkaline pH may lead to irritation in oral cavity, surface pH near neutral region is always preferable (Bottenberg et al., 1991).

5.4.3.5 In vitro drug release studies

Different surface-active agents (sodium lauryl sulphate and polysorbate 80) in varying proportions (1 to 2.5%) in pH 6.8 phosphate buffer were tested as in vitro release

media. Dissolution of commercially available immediate release tablets of LER (Lerez® by Glenmark Pharmaceuticals, India) and pure drug were carried out using in-house modified apparatus in all the test media for selection and optimization of the release media. The media in which drug release was not dissolution limited was selected as in vitro release media. Stability of the drug in the media and non-interference of the surface-active agent used with drug analysis were other criteria for selection of dissolution media.

In vitro release studies were carried out using in-house modified USP Type I dissolution test apparatus. The jars of standard dissolution apparatus were replaced with in-house fabricated perplex plates with a cavity in centre to accommodate 60 ml glass beakers concentric with the shaft of the dissolution apparatus. Dissolution media used was 50 ml of pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80 to simulate buccal pH. The volume of media was kept low to simulate conditions prevalent in buccal cavity. Polysorbate 80 was used to enhance drug solubility and maintain sink conditions. Temperature was maintained at 37 ± 1 ^{o}C with a stirring rate of 25 RPM. Samples (5 ml) were collected and replaced with fresh dissolution media at predetermined time intervals. Volume of sample withdrawn was kept high so as to maintain the sink conditions within the dissolution media. The samples collected were diluted appropriately with the dissolution media (if needed) and analyzed using analytical method 2 of chapter 3.

The release data was mathematically treated using the power equation (Ritger and Peppas, 1987) to investigate the mechanism of drug release from the designed formulations. The values of release rate constant (K), diffusion exponent (n), time required for 50% drug release ($t_{50\%}$), regression coefficient (R^2) and mean sum of square residuals (MSSR) were calculated using the power equation for all the designed formulations.

Furthermore, the kinetics of drug release from all the designed formulations was inferred based upon R^2 values obtained from the plots for zero order, first order and Higuchi's square root kinetics (Hiremath, 2005).

5.4.3.6 Mucoadhesion studies

Mucoadhesion of designed formulations was evaluated using in-house modified double pan balance and texture analyzer.

a. Mucoadhesion studies using modified balance method

The experimental setup for determination of mucoadhesive strength of tablets was a modification of the earlier reported method (Yong et al., 2001). Freshly excised porcine buccal mucosa was obtained from the local slaughterhouse. The animals were sacrificed by

decapitation. The tissue was then removed, placed in simulated salivary fluid (SSF) and stored in refrigerated condition till further usage. The tissue was thawed at the time of use by immersion in a bath of SSF at ambient temperature.

The left pan of the balance was replaced with a teflon block (upper block) of 5 cm total length and 6.2 cm diameter. The schematic diagram of setup is shown in Figure 5.1. The upper block had a downward protrusion of 2.5 cm with a diameter of 1.5 cm. The upper block was suspended using torsion-less, non-elastic wire. Designed buccal tablet was attached to the downward protrusion of upper block using adhesive glue. An empty pre-weighed beaker was kept on the right pan of the balance. Weights were added on the right pan of the balance so as to balance both the sides.

Concentric with the upper block, a lower block (8 cm in height with a diameter of 6.2 cm) was kept on the platform of the balance with an upward protrusion (2.5 cm long with a diameter of 1.5 cm). The mucosal tissue was tied to lower block kept in a beaker containing SSF maintained at 37°C, so that the fluid was just in contact with the surface of mucosal tissue to keep it moistened (Figure 5.1). The entire assembly was designed so that the protrusion of upper and lower block just touch each other when the balance is in raised position (ensuring contact between the tablet and buccal mucosa).

Upon contact of tablet with buccal mucosa 40 g weight was applied for 10 min to ensure adhesion. After 10 min, water was added drop wise to the beaker in right pan using micropipette at a rate of 5 ml/min until the two supports separated due to breaking of the adhesive bond. Bioadhesive strength was determined for three tablets per batch. Bioadhesive strength was represented in terms of force required per unit area (N/cm^2) to detach the tablet from buccal tissue.

b. Mucoadhesion studies using texture analyzer

Texture analyzer has recently been extensively reported for determination of mucoadhesive strength of solid and semi solid bioadhesive formulations (Eouani et al., 2001). The experimental set up used for determination of bioadhesive strength of designed buccal tablets was similar to that of the earlier reported methods (Eouani et al., 2001; Repka et al., 2005). Schematic representation of the experimental set up is shown in Figure 5.2. The effect of instrument variables such as contact force and contact time of the probe on force of detachment and work of adhesion was also studied.

Freshly excised porcine buccal mucosa was processed as discussed earlier. The mucosal tissue was held using clips on a holder immersed in SSF maintained at 37°C, so that the fluid is just in contact with the surface of the mucosal tissue (Figure 5.2). The probe

used for the study was stainless steel cylindrical probe (SMS P/10) with a diameter of 10 mm. The designed buccal mucoadhesive tablet was attached to the probe using adhesive glue (Figure 5.2). The probe was lowered at a pre-test speed of 0.1 mm/sec so that the tablet comes in contact with the hydrated mucosal tissue. Upon contact of tablet and mucosal tissue a constant force of 0.4 N was applied for 30 sec for mucoadhesion to occur. After 30 sec, the probe was withdrawn at a speed of 0.5 mm/sec. Peak detachment force and area under the force-time curve were used to establish mucoadhesive strength and work of adhesion respectively of designed mucoadhesive formulations. Bioadhesive strength was determined for three tablets per batch.

To study the effect of contact time, four different contact times (10, 30, 120 and 300 sec) were used with a contact force of 0.4 N for formulations containing CP, PC, HPMC, PEO and CH and effect on peak detachment force and work of adhesion was studied. Other test parameters were kept unchanged.

To study the effect of contact force, four different contact forces (0.2, 0.4, 1.0 and 1.5 N) were applied to the same formulations and effect on peak detachment force and work of adhesion was studied. A contact time of 30 sec was used without changing any other test parameters.

5.4.3.7 Swelling studies

The swelling behavior of the formulations was also investigated using texture analyzer. Tablets were placed in the glass beakers under conditions identical to those described above for in vitro drug release. The hydrated tablets were removed at predetermined time intervals and subjected to textural profiling after gently removing excess water with a tissue paper. All measurements were carried out in triplicates at each time point using different tablets. The force-displacement-time profile associated with the penetration of a 3 mm round-tipped stainless steel probe (cylindrical probe SMS P/3) into the swollen matrices was monitored at a data acquisition rate of 200 points per second as previously described (Jamzed et al., 2005). Probe approached the sample at pretest speed of 0.1 mm/sec. Once a trigger force of 5 g was detected (upon contact of the probe with tablet) the probe advanced into the sample at a test speed of 0.1 mm/sec until the maximum force of 30 g was reached. After the set load was reached the probe was withdrawn at a 0.5 mm/sec. Swollen thickness was determined by measuring the total probe penetration value recorded and by the observation of textural profiles. Percent axial swelling was calculated according to the following equation: Axial swelling (%) =

[Swollen thickness − Original thickness] × 100/ [Original thickness]. The original thickness of the tablets was determined using vernier caliper before commencement of the study.

5.4.3.8 Batch reproducibility

To study batch reproducibility, three batches of each formulation were manufactured and evaluated in the same way. Triplicate samples from each batch were evaluated for all quality parameters discussed above.

5.4.3.9 Stability studies

One best formulation from each lot showing desired release and mucoadhesion characteristic was packed in airtight cellophane packets and stored at ambient as well as accelerated storage conditions as per ICH guidelines (International Conference on Harmonization, 1996). Formulations were kept at different conditions of temperature and humidity like refrigerated temperature (FT: 5 ± 2^{o}C), room temperature (CRT: 25 ± 2^{o}C/ 60 ± 5 % RH) and at accelerated condition (AT: 40 ± 2^{o}C/ 75 ± 5 % RH). Samples in triplicate were withdrawn from each batch at predetermined time intervals (0, 0.5, 1, 3 and 6 months for AT condition; 0, 1, 3, 6, 12 and 24 months for CRT and FT condition). All the quality control tests were carried out on aged samples to assess stability of developed formulations. Drug content of aged formulations was determined using same analytical method. The results of quality control tests of aged samples were compared with zero time results. The percentage drug remaining to be degraded (%RTD) was plotted against time and the degradation rate constant (K_{deg}) and $t_{90\%}$ value was calculated at different storage conditions for all the formulations.

5.5 Results and Discussion

5.5.1 Buccal mucoadhesive controlled release formulations

Buccal mucoadhesive controlled release formulations of LER were prepared using excipients like lactose, mannitol, magnesium stearate and talc. The composition of all the batches prepared is shown in Table 5.1a to 5.1e. Lactose and mannitol were used as diluent and mannitol additionally functioned as a sweetener. Magnesium stearate and talc were used as manufacturing additives. Apart from these excipients, polymers reported for both mucoadhesive and controlled release purposes (CP, PC, PEO, CH, HPMC) (Table 5.1a to 5.1e) were used either alone or in combination in the formulations. To avoid complication and increase in total weight of tablets additives with multiple functions were selected.

5.5.2 Evaluation of buccal mucoadhesive controlled release formulations

The designed buccal mucoadhesive controlled release formulations containing LER were found to possess very good physical properties and the results are presented in Table 5.2a to 5.2e. The tablets prepared with CP, PC, HPMC and PEO were smooth and pale yellow in color. However, the tablets prepared with CH were smooth and brownish yellow in color.

Weight variation in all formulations was low (within ± 1.5% of theoretical tablet weight) as also indicated by the low SD values (maximum SD of 2.54 mg) (Table 5.2b). This falls well within the acceptance criteria. Friability in case of all the designed tablets was less than 1% w/w indicating suitability of the method used manufacturing good tablets.

The prepared tablets showed maximum thickness of 2.55 mm (Table 5.2d) with maximum SD of 0.17 (Table 5.2c). The % RSD for thickness did not exceed 7.0. Tablets prepared with CH and PEO were thicker relative to the tablets of same weight prepared using CP, PC and HPMC due to lower bulk density. The drug content of all the developed formulations was between 98 to 103% of the theoretical claim with maximum SD of 2.57 (Table 5.2a). This further indicated reliability and reproducibility of the manufacturing process.

All the tablets were found to posses good hardness. The hardness values for various formulations prepared using different polymers varied between 29.42 to 40.21 N. Tablets prepared using CH and PEO were less hard relative to the tablets of same weight prepared using CP, PC and HPMC when compressed at an identical compression force. This can be attributed to lower bulk density of CH and PEO. Surface pH of the tablets varied between 6.38 and 7.13 (data not shown). The near neutral surface pH of the tablets is essential for avoiding potential irritation to buccal mucosa due to continuous application of designed formulations.

5.5.3 In vitro drug release studies

Buccal dosage forms present problems in the development of in vitro release technologies simply because of the physicochemical properties of the formulations and the unique physiological environment in which they should release their content. An ideal in vitro release study should provide valuable information regarding in vivo performance of the delivery system. To simulate the conditions prevalent in the oral cavity, it was decided to carry out in vitro release studies in 50 ml of aqueous media with pH of 6.8. However, LER being a poorly soluble drug, it was decided to incorporate surface-active agent for promoting sink conditions. Hence, different surface-active agents (sodium lauryl sulphate

and polysorbate 80) in varying proportions (1 to 2.5%) in pH 6.8 phosphate buffer were tested as dissolution media (Brian, 2001; Shah et al., 2002).

In case of pH 6.8 buffer with 2% w/v SLS, almost 100% of drug from marketed formulations was released within 30 min. However, this high a concentration of SLS was making the solution very hazy and interfering with the analysis of LER. In case of pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80, almost 100% of drug was released from marketed formulation within 30 min (Figure 5.3). Similar result was obtained when dissolution study of pure drug was carried out in this media. The drug was stable for atleast 24 h in this media. Moreover, the media was not interfering with estimation of the drug (analytical method 2 of chapter 3). Hence, 50 ml of pH 6.8 phosphate buffer with 2.5% v/v polysorbate 80 was selected as final in vitro release media to simulate the conditions prevalent in buccal cavity and to maintain sink conditions.

The objective of this work was to develop formulations retarding drug release for 4-8 h so that steady plasma levels of drug can be maintained for long time. Moreover, release of 10-15% drug within first one hour would help in achieving the target concentration thereby precluding use of loading dose in formulations. A longer retardation of drug release was undesirable because acceptability of delivery system might reduce sharply if it is meant to be applied for prolonged duration of time.

In vitro release study indicated that duration of release of drug is dependent on the percentage of polymer used in the formulations for all the polymers studied. An increase in the polymer concentration causes increase in the viscosity of the gel leading to formation of gel layer with a longer diffusional path. This leads to a decrease in the diffusion of the drug and therefore a reduction in the drug release rate (Hiremath, 2005).

Mostly, the drug release mechanism was anomalous non-Fickian transport for all the formulations. The drug release was rapid initially which might help in achieving the minimum effective concentration quickly, precluding the use of loading dose in the formulations (Saha et al., 2001). In non-Fickian transport, a combination of polymer erosion, polymer swelling and diffusion of dissolved drug plays a role in release of drug from matrices. As polymer takes some time to swell after coming in contact with dissolution media, drug is released rapidly initially. Once polymer swelling is complete, the drug release rate decreases with time and is dependent upon polymer erosion and effective molecular diffusional area (Hosny, 1993).

Comparative cumulative percentage drug release (% CDR) profiles from tablets prepared using various polymers either alone or in combination are shown in Figure 5.4 to 5.22. The in vitro release data was mathematically treated using the power equation (Ritger

and Peppas, 1987) to investigate the mechanism of drug release from the designed formulations. The values of release rate constant (K), diffusion exponent (n), time required for 50% drug release ($t_{50\%}$), regression coefficient (R^2) and mean sum of square residuals (MSSR) were calculated and the results are presented in Table 5.3a to 5.3e.

A comparative plot of % CDR versus time for matrix embedded controlled release buccal mucoadhesive tablets prepared using varying proportions of CP is shown in Figure 5.4. The release of drug extended from 3 h to 12 h as the proportion of CP was increased from 25% to 200% w/w of the drug weight (Table 5.1a). The percent drug released for the first hour varied between 11-43% depending upon the polymer concentration and time taken by polymer layer to swell. However, in the later stages the release was slower and more controlled in the tablets with higher proportion of CP. The release rate constants according to power equation for formulations containing 25, 50, 100, 150 and 200 % w/w CP of the drug weight were 42.14 x 10^{-2} $h^{-0.8921}$, 35.27 x 10^{-2} $h^{-0.9814}$, 25.49 x 10^{-2} $h^{-0.7659}$, 22.94 x 10^{-2} $h^{-0.7143}$ and 12.65 x 10^{-2} $h^{-0.8484}$ respectively. The $t_{50\%}$ values were 1.22, 1.42, 2.40, 2.97 and 5.04 h respectively for these formulations according to Peppas power equation (Table 5.3a). The n value for all the formulations ranged from 0.7143 to 0.9814 indicating that the release mechanism was anomalous non-Fickian transport. The release data for all the formulations prepared using CP fitted best in zero order kinetic model. Formulations prepared using 100% w/w CP of drug, resulted in preferable drug release profile.

Figure 5.5 shows a comparative plot of % CDR versus time profile for tablets prepared using varying proportions of PC. When lower proportions of PC were used (25 and 50 % w/w of drug weight) the release was rapid with almost 100% drug release within initial 4 h, indicating insufficient polymer proportion to extend the release of drug beyond 4h. However, when the proportion of PC was further increased (100, 150 and 200 % w/w of drug weight) the release of drug extended up to almost 14 h. The initial percent drug released for first hour varied between 12–39%. The release rate of drug from the formulation decreased after the first hour. The release rate constants according to the Peppas power equation for the formulations containing 25, 50, 100, 150 and 200 % w/w PC of the drug weight (Table 5.1a) were 40.54 x 10^{-2} $h^{-0.7073}$, 38.98 x 10^{-2} $h^{-0.7313}$, 21.03 x 10^{-2} $h^{-0.7421}$, 15.89 x 10^{-2} $h^{-0.7753}$ and 12.07 x 10^{-2} $h^{-0.8221}$ respectively. The $t_{50\%}$ values for these formulations were 1.35, 1.47, 3.21, 4.38, 5.63 h respectively (Table 5.3a). The n values for all the formulations prepared using varying proportions of PC ranged from 0.7073 to 0.8221 indicating that the release mechanism was anomalous non-Fickian transport. The release data for all the formulations prepared using PC fitted best in zero order kinetic model.

Release rate of drug decreased after initial rapid release because of complete polymer swelling resulting in formation of stronger gel layer thereby retarding drug release.

Amongst tablets prepared with anionic polymers like PC and CP, PC retarded the drug release to greater extent at same proportion. The initial pattern of drug release (for first 1 h) was identical with both the polymers. However, after 1 h, PC being more hydrophobic polymer than CP, resulted in formation of a gel layer that erodes slowly resulting in slower drug release (Hosny, 1993).

Effect of polymer combination on release profile of the drug from tablets was studied by using anionic polymer (CP) in combination with nonionic polymers like HPMC K4M, HPMC K15M and HPMC K100 M (Figure 5.6 to 5.8). The ratio of polymer to drug in these tablets was 1:1. However, the composition of polymer layer was varied by mixing CP and HPMC (various viscosity grades) in the ratios 3:1, 1:1 and 1:3 (Table 5.1a to 5.1b). As the proportion of CP in the polymer layer (composed of CP in combination with HPMC K4M or HPMC K15M) was reduced, the release mechanism shifted progressively towards Fickian diffusion as indicated by the reduction in n value (Table 5.3a and 5.3b) and release rate increased. This may be because of increase in proportion of hydrophilic polymer (HPMC) in the tablet matrix. However, when CP was used in combination with higher viscosity grade of HPMC (HPMC K100M), the release rate and release mechanism was not getting affected when compared to formulations prepared using CP alone (Table 5.3a and 5.3b). This can be attributed to larger strength of gel formed by higher viscosity grades of HPMC. When CP was used in combination with HPMC K4M the release data fitted best in first order kinetic model and with higher viscosity grades of HPMC (HPMC K15M and HPMC K100M) zero order release was observed. So, with increase in viscosity grade of HPMC combined with CP, the release pattern shifted from first order to zero order. HPMC polymer forms a gelatinous layer on surface of tablets upon hydration. The thickness and durability of this layer depends upon the concentration and viscosity grade of the HPMC used in the matrix. Higher viscosity grades of HPMC form a stronger gel layer so polymer relaxation becomes predominant mechanism of drug release. This explains zero order release when higher viscosity grades of HPMC were used in combination with CP.

Effect of combination of PC with HPMC K4M, HPMC K15 and HPMC K100M, on release pattern of the drug from tablets was also studied (Figure 5.9 to 5.11). The ratio of polymer to drug in these tablets was 1:1. However, the composition of polymer layer was varied by mixing PC and HPMC (various viscosity grades) in the ratios 3:1, 1:1 and 1:3 (Table 5.1b). The release rate constants according to Peppas equation for formulations containing PC to HPMC K4M in 3:1, 1:1 and 1:3 ratio were 21.03 x 10^{-2} $h^{-0.7421}$,

25.49 x 10^{-2} $h^{-0.7629}$ and 27.04 x 10^{-2} $h^{-0.7583}$ respectively with $t_{50\%}$ values of 3.21, 2.40 and 2.24 h respectively. The release data fitted best in zero order kinetic model. The release rate constants for formulations containing PC to HPMC K15M in 3:1, 1:1 and 1:3 ratio were 25.49 x 10^{-2} $h^{-0.6105}$, 29.90 x 10^{-2} $h^{-0.7659}$ and 34.11 x 10^{-2} $h^{-0.5981}$ respectively with $t_{50\%}$ values of 2.43, 2.32 and 1.89 h respectively (Table 5.3b). The release rate constants according to Peppas equation for formulations containing PC to HPMC K100M in 3:1, 1:1 and 1:3 ratio were 14.17 x 10^{-2} $h^{-0.7630}$, 15.32 x 10^{-2} $h^{-0.7961}$ and 16.86 x 10^{-2} $h^{-0.7605}$ respectively with $t_{50\%}$ values of 4.57, 4.41 and 4.27 h respectively (Table 5.3b). Hence, HPMC (various viscosity grades) when used in combination with PC did not affect the mechanism of drug release when compared with tablets prepared using PC alone (Table 5.3a to 5.3b). As the proportion of HPMC in polymer matrix with respect to PC was increased, the release rate also increased. This can be because of more hydrophilic nature of HPMC as compared to PC (Hiremath, 2005).

A comparative plot of % CDR versus time for matrix embedded controlled release buccal mucoadhesive tablets prepared using varying proportions of HPMC K4M is shown in Figure 5.12. The release of drug extended up to 10 h as the proportion of HPMC K4M was increased from 50% to 200% w/w of the drug weight. The initial percent drug released for the first hour varied between 23-42%. However, in the later stages the release was slower and more controlled. This change in release rate was because of time taken by polymer to swell upon hydration. The release rate constants according to power equation for formulations containing 50, 100, 125, 150 and 200 % w/w HPMC K4M (Table 5.1b) of the drug weight were 41.97 x 10^{-2} $h^{-0..6221}$, 37.28 x 10^{-2} $h^{-0.6069}$, 34.53 x 10^{-2} $h^{-0.5846}$, 29.02 x 10^{-2} $h^{-0.5981}$ and 22.44 x 10^{-2} $h^{-0.6102}$ respectively. The $t_{50\%}$ value were 1.32, 1.62, 1.88, 2.48 and 3.71 h respectively for these formulations according to Peppas power equation (Table 5.3c). The n value for all the formulations ranged between 0.5846 and 0.6221 indicating that the release mechanism was anomalous non-Fickian transport. The release data for all the formulations prepared using HPMC K4M fitted best in first order kinetic model.

Figure 5.13 shows comparative % CDR versus time profile of tablets formulated using varying proportions of HPMC K15M. Release rate of drug from formulation was found to be inversely proportional to the amount of polymer in the tablet. Increasing the proportion of HPMC K15M from 25% to 200% w/w of drug weight (Table 5.1c) in the formulation resulted in extension of drug release from 4 to 12h. Initial drug release from all the formulations was rapid with more controlled and slower release in the later stages. The release data from all the formulations prepared using HPMC K15M fitted best in the first

order kinetic model. However, very high proportions of HPMC K15M (200% w/w of drug weight) resulted in zero order kinetics. As the proportion of HPMC K15M was increased, drug release was governed more predominantly by polymer swelling than polymer erosion and release rate decreased (Seng et al., 1985; Capan et al., 1991). The release rate constants according to power equation for formulations containing 25, 50, 100, 150 and 200% w/w HPMC K15M (Table 5.1c) of the drug weight were 44.90 x 10^{-2} $h^{-0.5669}$, 33.48 x 10^{-2} $h^{-0.6149}$, 30.64 x 10^{-2} $h^{-0.5847}$, 25.38 x 10^{-2} $h^{-0.5755}$ and 18.35 x 10^{-2} $h^{-0.6591}$ respectively with $t_{50\%}$ values of 1.20, 1.90, 2.30, 3.24 and 4.57 h respectively. The n values for the formulations ranged from 0.5669 to 0.6591 indicating that the release mechanism was anomalous non-Fickian transport (Table 5.3c).

Comparative release profiles of various formulations prepared using HPMC K100M is shown in Figure 5.14. The release data fitted best in the first order kinetic model. Very high proportions of HPMC K100M (200% w/w of drug weight) resulted in zero order release profile. Similar observation was made in tablets manufactured using high proportions of HPMC K15M. The release rate constants according to power equation for formulations containing 25, 50, 100, 150 and 200 % w/w HPMC K100M (Table 5.1c) of the drug weight were 40.04 x 10^{-2} $h^{-0.5631}$, 32.10 x 10^{-2} $h^{-0.5976}$, 27.32 x 10^{-2} $h^{-0.5369}$, 16.78 x 10^{-2} $h^{-0.6473}$ and 14.40 x 10^{-2} $h^{-0.6984}$ respectively with $t_{50\%}$ values of 1.48, 2.09, 3.08, 5.40 and 5.94 h respectively (Table 5.3c). The n values for the formulations ranged from 0.5369 to 0.6984 indicating that the release mechanism was anomalous non-Fickian transport.

For tablets prepared using different viscosity grades of HPMC, drug release extended from 6-10 h when the viscosity of HPMC used was increased from 4000 cPs (HPMC K4M) to 100000 cPs (HPMC K100M) keeping the proportion constant (100 % w/w of drug weight). The release rate was faster with lower viscosity grades (4000 cPs) of HPMC probably due to lesser polymer entanglement, lesser gel strength and larger effective molecular diffusional area when compared to higher viscosity grades (Kim and Fassihi, 1997; Hiremath and Saha, 2004).

The comparative release profiles of formulations prepared using PEO are shown in Figure 5.15. Formulations were prepared with 50, 100, 125, 150, 200 % w/w PEO of drug weight (Table 5.1c). PEO being a water soluble polymer led to rapid drug release when used in smaller proportion. Increasing PEO from 50 to 200% w/w of drug weight resulted in extension of drug release from 4 to 10 h. The drug release was rapid initially and slowly tapered off as the time progressed. The release data of all the formulations prepared using PEO fitted best in the first order kinetic model. The release rate constants according to

power equation for formulations containing 50, 100, 125, 150 and 200 % w/w PEO of the drug weight were 47.92 x 10^{-2} $h^{-0.5137}$, 43.09 x 10^{-2} $h^{-0.5175}$, 33.86 x 10^{-2} $h^{-0.5808}$, 26.61 x 10^{-2} $h^{-0.6333}$ and 19.96 x 10^{-2} $h^{-0.6699}$ respectively with $t_{50\%}$ values of 1.08, 1.33, 1.95, 2.70 and 3.93 h respectively (Table 5.3d). When lower proportions of PEO (50 and 100 % w/w of drug weight) were used n values close to 0.5 were obtained indicating Fickian diffusion as the mechanism of drug release. But in case of formulations containing higher proportions of PEO (125, 150 and 200% w/w of drug weight), the drug release was found to follow anomalous non-Fickian transport mechanism as indicated by the n values ranging from 0.5808 to 0.6699. At lower proportions of PEO, diffusion was predominant mechanism of drug release and as the proportion of PEO increased in the polymer matrix, release mechanism shifted progressively towards polymer relaxation (swelling and erosion). At higher polymer concentration, the polymeric chains entangle to a greater degree resulting in virtual cross-linking and therefore formation of a stronger gel layer.

Figure 5.16 shows comparative in vitro release profiles of buccal mucoadhesive controlled release tablets prepared using CH as rate controlling polymer. Formulations were prepared with 50, 100, 125, 150, 200 % w/w CH of drug weight (Table 5.1d). The rate of drug release from the matrix was inversely proportional to the polymer proportion. The drug release from all these tablets was initially rapid and reduced subsequently with passage of time. The release data from all the formulations fitted best in first order kinetic model. The release rate constants according to power equation for formulations containing 50, 100, 125, 150 and 200 % w/w CH of the drug weight were 44.22 x 10^{-2} $h^{-0.6098}$, 37.11 x 10^{-2} $h^{-0.6267}$, 26.14 x 10^{-2} $h^{-0.7465}$, 21.68 x 10^{-2} $h^{-0.7477}$ and 16.86 x 10^{-2} $h^{-0.7605}$ respectively with $t_{50\%}$ values of 1.22, 1.60, 2.38, 3.05 and 4.17 h respectively (Table 5.3d). The n values for the formulations ranged from 0.6098 to 0.7605 indicating that the release mechanism was anomalous non-Fickian transport.

Effect of combination of hydrophilic polymers on release pattern of the drug from tablets was studied using combination of PEO with HPMC K4M, HPMC K15M and HPMC K100M (Figure 5.17 to 5.19). The ratio of polymer to drug in all the tablets was 1:1. However, the composition of polymer layer was varied by mixing PEO and HPMC (various viscosity grades) in the ratios 3:1, 1:1 and 1:3 (Table 5.1d). Use of HPMC (various viscosity grades) in combination with PEO in tablet formulations did not significantly affect the release mechanism of drug from formulations as indicated by similar n values of formulations prepared using PEO or HPMC alone or in combination (Table 5.3c to Table 5.3e). The release mechanism in all the formulations prepared using combination of PEO and HPMC (various viscosity grades) was anomalous non-Fickian transport. As the

proportion of PEO in polymer matrix with respect to HPMC was increased, the release rate increased. This may be attributed to more hydrophilic nature of PEO as compared to HPMC.

Effect of combination of cationic and nonionic polymers on the release mechanism was studied by using combination of CH and HPMC (various viscosity grades) in the polymer matrix of designed tablets (Figure 5.20 to 5.22). The ratio of polymer to drug in these tablets was 1:1. However, the composition of polymer layer was varied by mixing CH and HPMC (various viscosity grades) in the ratios 3:1, 1:1 and 1:3 (Table 5.1e). Combination of HPMC (various viscosity grades) with CH did not affect the release mechanism from the formulations when compared with formulations prepared with HPMC (various viscosity grades) or CH alone as indicated by the n values. The release mechanism in all the formulations prepared using combination of CH and HPMC (various viscosity grades) was anomalous non-Fickian transport (Table 5.3c to 5.3e).

In formulations prepared using PEO (nonionic polymer), drug release rate was faster than formulations containing anionic polymers (PC, CP) which can be attributed to soluble nature of PEO. Similar results were obtained when cationic polymer (CH) was used in tablet formulations. So, it can be concluded that ionic nature of the polymer per se did not affect the mechanism and rate of drug release from the designed formulations. Rather, water solubility of the polymer was major determinant of release rate of studied drug from the designed formulations.

5.5.4 Mucoadhesion studies

5.5.4.1 Mucoadhesion studies using modified balance method

The hydration time (10 min) and weight applied (40 g) were optimized through series of experiments to increase the reproducibility and precision (data not shown). Bioadhesion testing of all the designed formulations was carried out using porcine buccal mucosa as model membrane (Chen et al., 2002; Mashru et al., 2005). The force per unit area (N/cm^2) to detach the formulation from the mucosa was determined. The data is presented in Table 5.2a to 5.2e and depicted in Figure 5.23 to 5.33. The values of detachment force ranged between 0.16 and 1.69 N/cm^2.

Detachment force for all the polymers (CP, PC, HPMC K4M, HPMC K15M, HPMC K100M, PEO and CH) was found to be directly proportional to the amount of polymer present in the formulation (Table 5.2a to 5.2e). When the concentration of polymer is low, the number of penetrating polymeric chains per unit volume of the mucus is low resulting in

weaker interaction (Peppas and Buri, 1985). In case of formulations prepared using HPMC K4M and HPMC K15 M alone, increasing polymer concentration beyond a critical concentration did not increase mucoadhesive strength significantly ($p<0.05$). So it can be said that for each polymer there exist a critical concentration, above which the mucoadhesive strength does not necessarily increase, due to formation of significantly coiled structure and reduced accessibility of the solvent in the polymer matrix (Miller et al., 2005).

Detachment force obtained for formulations prepared using varying proportions of CP is shown in Figure 5.23. The force required to detach the formulation from mucosa was directly proportional to the amount of CP in the formulation (Table 5.2a). Effect of combination of anionic and non ionic polymers on mucoadhesive strength was studied by using combination of CP and HPMC in tablet matrix (Figure 5.24). Reduction in mucoadhesive strength was observed, when a part of CP in polymer layer was replaced with HPMC K4M or HPMC K15M (Table 5.2a). However, when higher viscosity grade of HPMC (HPMC K100M) was used in combination of CP, almost identical detachment forces were obtained when compared to tablets prepared using CP alone (Table 5.2a and 5.2b). This can be attributed to higher strength of gel formed by HPMC K100M as compared to that of HPMC K4M and HPMC K15M resulting in stronger entanglement of polymeric chains with glycoprotein chains of mucus (Sudhakar et al., 2006). Similar results were obtained when formulations were prepared using PC (Figure 5.25) alone. The detachment force obtained using PC were significantly higher ($p<0.05$) when compared to CP (Table 5.2a). In formulations prepared using combination of PC with various viscosity grades of HPMC, identical results as that of combination of CP with HPMC were obtained (Figure 5.26). In case of both CP and PC, as the proportion of HPMC in polymer layer was increased with respect to CP or PC, detachment force reduced, indicating reduction in mucoadhesive property. This may be because of more hydrophilic nature of HPMC as compared to CP and PC (Table 5.2a to 5.2b).

Detachment force for tablets prepared using various viscosity grades of HPMC alone are shown in Figure 5.27 to 5.29. The mucoadhesive strength of the tablets was directly proportional to the proportion and viscosity grade of HPMC used in the formulations. These values were significantly lower ($p<0.05$) than the values obtained with CP and PC alone (Table 5.2c). This may be due to higher hydrophilicity and shorter polymeric chains of HPMC resulting in poor poorer entanglement of polymeric chains with glycoprotein chains of mucus.

Detachment force for formulations prepared using PEO alone and in combination with various viscosity grades of HPMC are shown in Figure 5.30 and 5.31 respectively. Formulations containing PEO alone showed superior mucoadhesion when compared with other hydrophilic polymers like HPMC K4M, HPMC K15M and HPMC K100M (Table 5.2c and 5.2d). This may be because of quicker swelling (Tiwari et al., 1999) and higher flexibility of polymeric chains of PEO (Huang et al., 2000) resulting in better interaction with mucin. However, CP and PC showed superior mucoadhesion as compared to PEO (Table 5.2b and 5.2d). This can be attributed to the higher swellability of PC and CP as compared to PEO resulting in better interaction with mucosal surface. Moreover, higher hydrophobicity of PC and CP results in slower erosion of gel formed after swelling, thereby increasing the time of interaction with mucin (Gu et al., 1998).

Reduction in mucoadhesive strength was observed, when a part of PEO in polymer layer was replaced with HPMC K4M or HPMC K15 M. This can be due to lesser chain length and chain flexibility of HPMC as compared to that of PEO. However, when higher viscosity grade (HPMC K 100M) was used in combination of PEO, almost identical detachment forces were obtained when compared to tablets prepared using PEO alone (Table 5.2d and 5.2e). Higher viscosity grades of HPMC resulted in formation of stronger gel layer that erodes slowly thereby increasing the contact time with mucosal tissue.

Detachment force for formulations prepared using CH alone and in combination with various viscosity grades of HPMC are shown in Figure 5.32 and 5.33 respectively. Formulations containing CH alone showed lesser adhesion than tablets prepared using CP, PC and PEO. Lesser swellability, poorer chain flexibility and lesser chain interpenetration of CH may be responsible for reduced interaction with mucin as compared to CP, PC and PEO (Rossi et al., 2003; Miller et al., 2005). Mucoadhesive strength of formulations containing CH was not affected when a part of CH in polymer matrix was replaced with either HPMC K4M or HPMC K15M. However, replacement of part of CH in polymer matrix with equal amount of HPMC K100M resulted in superior mucoadhesive character (Table 5.2d and 5.2e). Higher adhesion when used in combination with HPMC K100M can be attributed to better swellability and chain flexibility of HPMC K100M compared to that of CH.

So it can be concluded that mucoadhesion of designed formulations was affected by concentration, viscosity grade, hydrophilicity or hydrophobicity and swelling behavior of the polymer. Ionic nature of polymer used was not found to affect the mucoadhesive strength per se but may be a contributing factor to the above mentioned parameters.

5.5.4.2 Mucoadhesion studies using texture analyzer

Two parameters namely, work of adhesion and detachment force were measured using texture analyzer for assessing mucoadhesive strength of designed formulations. It has been reported that the work required to detach the bioadhesive system from the mucosa (equivalent to area under the force-time curve) is a more sensitive parameter than the detachment force (Lejoeux et al., 1988). The values of work of adhesion and detachment force for various formulations studied are given in Table 5.4 and depicted in Figures 5.34 to 5.36. Contact force (0.4 N), contact time (30 sec) and probe withdrawal speed (0.5 mm/sec) was optimized using a series of experiments (data not shown). Significantly higher detachment force values were obtained using texture analyzer when compared to data obtained using modified balance method at 5% level of significance. This may be because of higher sensitivity and more precision of texture analyzer. However, similar pattern of mucoadhesion was evident with formulations prepared with PC and HPMC K4M showing maximum and minimum detachment force and work of adhesion respectively (Table 5.4).

Figure 5.37 and 5.38 represent detachment force and work of adhesion of designed formulation as function of contact time, respectively. At lower contact time of 10 sec, formulations prepared with PEO were found to have maximum mucoadhesion with higher value of work of adhesion. This may be because of faster hydration of PEO based formulations. When contact time of 10 sec was used the order of mucoadhesive strength was found to be PEO > PC > CP > CH > HPMC K4M > HPMC K100M > HPMC K15M. When contact time was increased to 30 sec, a drastic increase in detachment force and work of adhesion was seen for formulations prepared using all the polymers (Table 5.5). The order of mucoadhesive strength at contact time of 30 sec was PC > CP > PEO > HPMC K100M > CH > HPMC K15M > HPMC K4M. This reversal in order may be because of longer time taken by formulations containing PC, CP and HPMC K100M for hydration. The same order was maintained when contact time was further increased to 120 sec and 300 sec. The work of adhesion and detachment force values increased at slower rate when the contact time was increased beyond 30 sec and ultimately almost reached a constant value at contact time of 300 sec. So, it can be said that a proper contact time is very important for polymer hydration and polymer swelling, which in turn affect the level of interaction of formulation with mucus and hence extent of bioadhesion. These results were in agreement with similar studies carried out by other research groups (Choy et al., 1999).

Effect of contact force on peak detachment force and work of adhesion of selected formulations was also studied and the results are shown in Figure 5.39 and 5.40 respectively. For all the formulations, increase in contact force increased the detachment

force and work of adhesion required to detach the adhered formulation from the mucus membrane with a contact time of 30 sec. The order of mucoadhesive strength obtained was PC > CP > PEO > HPMC K100M > CH > HPMC K15M > HPMC K4M at all contact forces. The rate of increase in work of adhesion and detachment force was decreasing with increase in contact force beyond 0.4 N. However, constant or almost constant values were not obtained even when contact force was increased beyond 1.0 N (Table 5.6). So, a certain contact force is necessary to develop a satisfactory intimate molecular contact between the formulation and mucus membrane. These results were in agreement with similar studies carried out by other research groups (Choy et al., 1999).

5.5.5 Swelling studies

Textural analysis for swelling behavior of formulations was carried out for further elucidation of in vitro release and mucoadhesion characteristics of the designed formulations. At the molecular level, drug release is determined by polymer swelling, drug dissolution, drug diffusion and matrix erosion. These phenomena depend upon the interaction between water, polymer matrix contents and the drug. Water has to penetrate the polymer matrix leading to polymer swelling and drug dissolution before the drug can diffuse out of the matrix. Penetration of water leads to transformation of glassy polymer into a rubbery mass enhancing the mobility of polymeric chains (Jamzad et al., 2005). This enhanced mobility is responsible for transport of water and dissolved drug (Nazzal et al., 2007). Moreover, higher the mobility, greater will be the flexibility of polymeric chains leading to better interaction with mucin resulting in superior mucoadhesion (Miller et al., 2005).

The profile of % axial swelling of studied formulations at different time points is given in Figure 5.41. Formulation prepared with PEO, HPMC K4M and HPMC K15M showed rapid swelling behavior with minimum 18% axial swelling within first 30 min of the study. This further explains rapid drug release from these formulations during in vitro drug release study. PEO showed maximum swelling behavior of all the polymers. Longer polymeric chains of PEO and quicker swelling of PEO based formulations, compared to that of HPMC (various viscosity grades), led to better interaction with glycoprotein chains of mucin and hence superior bioadhesion when compared to HPMC based formulations (Miller et al., 2005).

However, in case of formulations prepared with CP, PC and CH significantly lesser % axial swelling ($p<0.05$) as compared to that of PEO based formulations was obtained. This explains higher $t_{50\%}$ values for these formulations (Table 5.3a and 5.3d). This also

explains reduced mucoadhesion of CH based formulations as compared to that to PEO based formulations. However, in case CP and PC superior mucoadhesion was observed even with lesser swelling. This may be because of higher strength of gel formed by CP and PC compared to PEO, resulting in longer contact time between formulations and mucosa.

5.5.6 Batch reproducibility

Batch to batch variability and reproducibility of the manufacturing process was studied based on evaluation of the physical properties, mucoadhesive strength and release characteristic in triplicate from three batches of each of the designed formulations. Low values of standard deviation for drug content, weight variation, crushing strength and thickness for three independently prepared batches, indicated that the manufacturing process employed was reliable and reproducible (Table 5.2a to 5.2e). Insignificant difference was observed in the in vitro release profile and mucoadhesive strength as indicated by low SD values, confirming excellent batch-to-batch reproducibility (Table 5.3a to 5.3e).

5.5.7 Stability studies

Results of stability studies carried out on the designed formulations at different condition of temperature and humidity like refrigerated temperature (FT: 5 ± 2^{o}C), room temperature (CRT: 25 ± 2^{o}C/ 60 ± 5 % RH) and at accelerated condition (AT: 40 ± 2^{o}C/ 75 ± 5 % RH) are shown in Table 5.7. At refrigerated condition (FT: 5 ± 2^{o}C) all the designed formulations were stable for entire study period (24 months). Hence the data has not been given for this condition. The log percent drug remaining to be degraded versus time profiles were linear for all designed formulations at various storage conditions indicating first order degradation kinetics. Low values of MSSR and R^2 values close to 1 further established the first order kinetics of drug degradation (Table 5.7).

At accelerated condition, the maximum degradation rate constant for the drug was found to be 111.86 x 10^{-4} month^{-1} for formulations prepared using HPMC K4M with predicted $t_{90\%}$ values of 9.42 months. The minimum degradation rate constant of 70.89 x 10^{-4} month^{-1} was obtained for formulation prepared using combination of PC and HPMC K 15M with predicted $t_{90\%}$ value of 14.87 months. These values were almost comparable to degradation rate constant (87.01 x 10^{-4} month^{-1}) and $t_{90\%}$ (12.11 months) values of pure drug obtained during preformulation studies (section 4.4.4.2 of chapter 4). In vitro drug release profile from the aged samples was similar to zero time profiles for all the designed formulations (data not given). All the formulations were stable for entire study

duration (6 months) with no apparent change in physical characteristics and in vitro release and mucoadhesive behavior.

In the formulations stored at CRT, the maximum degradation rate constant for the drug was found to be 32.08 x 10^{-4} $month^{-1}$ with predicted $t_{90\%}$ value of 32.85 months for formulations prepared using PEO. The minimum degradation rate constant observed was 20.91 x 10^{-4} $month^{-1}$ with a predicted $t_{90\%}$ values of 50.39 months for formulation prepared using combination of PEO and HPMC K100M. These values were almost comparable to degradation rate constant (27.27 x 10^{-4} $month^{-1}$) and $t_{90\%}$ (38.65 months) values of pure drug obtained during preformulation studies (section 4.4.4.2 of chapter 4). All the formulations were stable for entire study duration (24 months) with no apparent change in physical characteristics and in vitro release and mucoadhesive behavior.

5.6 Conclusions

The designed buccal mucoadhesive controlled release tablets of LER were found to possess good physical characteristics indicating suitability of direct compression technique adopted for manufacturing the tablets. Weight variation and content uniformity of all the designed formulations was found to be highly satisfactory. Acceptable values of friability, hardness, thickness and low batch to batch variation further confirmed the suitability of the adopted method. The designed formulations were found to be stable for at least 2 years when stored at CRT. This indicated that excipients, process and packaging materials adopted were appropriate. The method used for manufacturing was found to be relatively simple and can easily be adopted in conventional formulation manufacturing units on a commercial scale.

Drug release from matrix embedded buccal mucoadhesive controlled release tablets was affected by polymer nature and proportion, polymer combination and swelling behavior of the polymer. In the present study, a series of formulations extending the release of LER from 4 -14 h were prepared. The release mechanism in almost all the designed formulations was anomalous non Fickian transport. Near zero order drug release kinetics were obtained in the tablets designed with polymers like CP and PC either alone or in combination with various viscosity grades of HPMC. Tablets designed using other polymers mostly resulted in drug release by first order kinetics. The release of LER from designed formulations was unaffected by the ionic nature of polymer used in the matrix.

The designed tablets were found to possess good mucoadhesive characteristics. Mucoadhesion of designed formulations was affected by concentration, viscosity grade,

hydrophilicity or hydrophobicity and swelling behavior of the polymer. Proper contact time and contact force were found to be important for appropriate mucoadhesion.

Formulations showing extension of drug release between 4-8 h with good bioadhesion values were considered for further studies. Formulations prepared using HPMC (various viscosity grades) resulted in lower mucoadhesive strength relative to other polymers. HPMC based formulations were not considered for further studies. Formulations prepared using combination of polymers did not offer definite advantage in terms of drug release retardation or mucoadhesion. Moreover, as formulations prepared using combination of polymers had an extra processing step they were not considered for further studies. The formulations finally selected for further studies were SBT/CP/10.0, SBT/PC/10.0, SBT/PEO/10.0 and SBT/CH/10.0.

Table 5.1a: Composition of designed buccal mucoadhesive controlled release tablets prepared using CP, PC and combination of CP with HPMC

Formulation Code	Formulation Composition (in mg/ tablet)									Theoretical Tablet Weight (mg)
	LER	Mannitol	Lactose	PC	CP	HPMC K4M	HPMC K15M	Talc	Magnesium Stearate	
SBT/CP/2.5	10.0	80.0	80.0	-	2.5	-	-	2.0	2.0	176.5
SBT/CP/5.0	10.0	80.0	80.0	-	5.0	-	-	2.0	2.0	179.0
SBT/CP/10.0	10.0	80.0	80.0	-	10.0	-	-	2.0	2.0	184.0
SBT/CP/15.0	10.0	80.0	80.0	-	15.0	-	-	2.0	2.0	189.0
SBT/CP/20.0	10.0	80.0	80.0	-	20.0	-	-	2.0	2.0	194.0
SBT/PC/2.5	10.0	80.0	80.0	2.5	-	-	-	2.0	2.0	176.5
SBT/PC/5.0	10.0	80.0	80.0	5.0	-	-	-	2.0	2.0	179.0
SBT/PC/10.0	10.0	80.0	80.0	10.0	-	-	-	2.0	2.0	184.0
SBT/PC/15.0	10.0	80.0	80.0	15.0	-	-	-	2.0	2.0	189.0
SBT/PC/20.0	10.0	80.0	80.0	20.0	-	-	-	2.0	2.0	194.0
SBT/CPHK4/5050	10.0	80.0	80.0	-	5.0	5.0	-	2.0	2.0	184.0
SBT/CPHK4/7525	10.0	80.0	80.0	-	7.5	2.5	-	2.0	2.0	184.0
SBT/CPHK4/2575	10.0	80.0	80.0	-	2.5	7.5	-	2.0	2.0	184.0
SBT/CPHK15/5050	10.0	80.0	80.0	-	5.0	-	5.0	2.0	2.0	184.0
SBT/CPHK15/7525	10.0	80.0	80.0	-	7.5	-	2.5	2.0	2.0	184.0
SBT/CPHK15/2575	10.0	80.0	80.0	-	2.5	-	7.5	2.0	2.0	184.0

Table 5.1b: Composition of designed buccal mucoadhesive controlled release tablets prepared using HPMC and combination of HPMC with CP or PC

Formulation Code	Formulation Composition (in mg/ tablet)										Theoretical Tablet Weight (mg)
	LER	Mannitol	Lactose	PC	CP	HPMC K4M	HPMC K15M	HPMC K100 M	Talc	Magnesium Stearate	
SBT/CPHK100/5050	10.0	80.0	80.0	-	5.0	-	-	5.0	2.0	2.0	184.0
SBT/CPHK100/7525	10.0	80.0	80.0	-	7.5	-	-	2.5	2.0	2.0	184.0
SBT/CPHK100/2575	10.0	80.0	80.0	-	2.5	-	-	7.5	2.0	2.0	184.0
SBT/PCHK4/5050	10.0	80.0	80.0	5.0	-	5.0	-	-	2.0	2.0	184.0
SBT/PCHK4/7525	10.0	80.0	80.0	7.5	-	2.5	-	-	2.0	2.0	184.0
SBT/PCHK4/2575	10.0	80.0	80.0	2.5	-	7.5	-	-	2.0	2.0	184.0
SBT/PCHK15/5050	10.0	80.0	80.0	5.0	-	-	5.0	-	2.0	2.0	184.0
SBT/PCHK15/7525	10.0	80.0	80.0	7.5	-	-	2.5	-	2.0	2.0	184.0
SBT/PCHK15/2575	10.0	80.0	80.0	2.5	-	-	7.5	-	2.0	2.0	184.0
SBT/PCHK100/5050	10.0	80.0	80.0	5.0	-	-	-	5.0	2.0	2.0	184.0
SBT/PCHK100/7525	10.0	80.0	80.0	7.5	-	-	-	2.5	2.0	2.0	184.0
SBT/PCHK100/2575	10.0	80.0	80.0	2.5	-	-	-	7.5	2.0	2.0	184.0
SBT/HK4/5.0	10.0	80.0	80.0	-	-	5.0	-	-	2.0	2.0	179.0
SBT/HK4/10.0	10.0	80.0	80.0	-	-	10.0	-	-	2.0	2.0	184.0
SBT/HK4/12.5	10.0	80.0	80.0	-	-	12.5	-	-	2.0	2.0	186.5
SBT/HK4/15.0	10.0	80.0	80.0	-	-	15.0	-	-	2.0	2.0	189.0
SBT/HK4/20.0	10.0	80.0	80.0	-	-	20.0	-	-	2.0	2.0	194.0

Table 5.1c: Composition of designed buccal mucoadhesive controlled release tablets prepared using HPMC and PEO

Formulation Code	Formulation Composition (in mg/ tablet)								Theoretical Tablet Weight (mg)
	LER	Mannitol	Lactose	PEO	HPMC K15M	HPMC K100 M	Talc	Magnesium Stearate	
SBT/HK15/2.5	10.0	80.0	80.0	-	2.5	-	2.0	2.0	176.5
SBT/HK15/5.0	10.0	80.0	80.0	-	5.0	-	2.0	2.0	179.0
SBT/HK15/10.0	10.0	80.0	80.0	-	10.0	-	2.0	2.0	184.0
SBT/HK15/15.0	10.0	80.0	80.0	-	15.0	-	2.0	2.0	189.0
SBT/HK15/20.0	10.0	80.0	80.0	-	20.0	-	2.0	2.0	194.0
SBT/HK100/2.5	10.0	80.0	80.0	-	-	2.5	2.0	2.0	176.5
SBT/HK100/5.0	10.0	80.0	80.0	-	-	5.0	2.0	2.0	179.0
SBT/HK100/10.0	10.0	80.0	80.0	-	-	10.0	2.0	2.0	184.0
SBT/HK100/15.0	10.0	80.0	80.0	-	-	15.0	2.0	2.0	189.0
SBT/HK100/20.0	10.0	80.0	80.0	-	-	20.0	2.0	2.0	194.0
SBT/PEO/5.0	10.0	80.0	80.0	5.0	-	-	2.0	2.0	179.0
SBT/PEO/10.0	10.0	80.0	80.0	10.0	-	-	2.0	2.0	184.0
SBT/PEO/12.5	10.0	80.0	80.0	12.5	-	-	2.0	2.0	186.5
SBT/PEO/15.0	10.0	80.0	80.0	15.0	-	-	2.0	2.0	189.0
SBT/PEO/20.0	10.0	80.0	80.0	20.0	-	-	2.0	2.0	194.0

Table 5.1d: Composition of designed buccal mucoadhesive controlled release tablets prepared using CH and combination of HPMC with PEO

Formulation Code	Formulation Composition (in mg/ tablet)										Theoretical Tablet Weight (mg)
	LER	Mannitol	Lactose	CH	PEO	HPMC K4M	HPMC K15M	HPMC K100 M	Talc	Magnesium Stearate	
SBT/CH/5.0	10.0	80.0	80.0	5.0	-	-	-	-	2.0	2.0	179.0
SBT/CH/10.0	10.0	80.0	80.0	10.0	-	-	-	-	2.0	2.0	184.0
SBT/CH/12.5	10.0	80.0	80.0	12.5	-	-	-	-	2.0	2.0	186.5
SBT/CH/15.0	10.0	80.0	80.0	15.0	-	-	-	-	2.0	2.0	189.0
SBT/CH/20.0	10.0	80.0	80.0	20.0	-	-	-	-	2.0	2.0	194.0
SBT/PEOHK4/5050	10.0	80.0	80.0	-	5.0	5.0	-	-	2.0	2.0	184.0
SBT/PEOHK4/7525	10.0	80.0	80.0	-	7.5	2.5	-	-	2.0	2.0	184.0
SBT/PEOHK4/2575	10.0	80.0	80.0	-	2.5	7.5	-	-	2.0	2.0	184.0
SBT/PEOHK15/5050	10.0	80.0	80.0	-	5.0	-	5.0	-	2.0	2.0	184.0
SBT/PEOHK15/7525	10.0	80.0	80.0	-	7.5	-	2.5	-	2.0	2.0	184.0
SBT/PEOHK15/2575	10.0	80.0	80.0	-	2.5	-	7.5	-	2.0	2.0	184.0
SBT/PEOHK100/5050	10.0	80.0	80.0	-	5.0	-	-	5.0	2.0	2.0	184.0
SBT/PEOHK100/7525	10.0	80.0	80.0	-	7.5	-	-	2.5	2.0	2.0	184.0
SBT/PEOHK100/2575	10.0	80.0	80.0	-	2.5	-	-	7.5	2.0	2.0	184.0

Table 5.1e: Composition of designed buccal mucoadhesive controlled release tablets prepared using combination of CH with HPMC

Formulation Code	Formulation Composition (in mg/ tablet)									Theoretical Tablet Weight (mg)
	LER	Mannitol	Lactose	CH	HPMC K4M	HPMC K15M	HPMC K100 M	Talc	Magnesium Stearate	
SBT/CHHK4/5050	10.0	80.0	80.0	5.0	5.0	-	-	2.0	2.0	184.0
SBT/CHHK4/7525	10.0	80.0	80.0	7.5	2.5	-	-	2.0	2.0	184.0
SBT/CHHK4/2575	10.0	80.0	80.0	2.5	7.5	-	-	2.0	2.0	184.0
SBT/CHHK15/5050	10.0	80.0	80.0	5.0	-	5.0	-	2.0	2.0	184.0
SBT/CHHK15/7525	10.0	80.0	80.0	7.5	-	2.5	-	2.0	2.0	184.0
SBT/CHHK15/2575	10.0	80.0	80.0	2.5	-	7.5	-	2.0	2.0	184.0
SBT/CHHK100/5050	10.0	80.0	80.0	5.0	-	-	5.0	2.0	2.0	184.0
SBT/CHHK100/7525	10.0	80.0	80.0	7.5	-	-	2.5	2.0	2.0	184.0
SBT/CHHK100/2575	10.0	80.0	80.0	2.5	-	-	7.5	2.0	2.0	184.0

Table 5.2a: Results of quality control tests carried out on designed buccal mucoadhesive tablets prepared using CP, PC and combination of CP with HPMC

Formulation Code	Mean Weight[a] ± SD (mg)	Friability[a] (% w/w)	Mean Thickness[b] ± SD (mm)	Mean Assay[b] ± SD (%)	Mean Crushing Strength[b] ± SD (N)	Mean Detachment Force[b] ± SD (N/cm^2)
SBT/CP/2.5	176.89 ± 1.25	0.69	2.40 ± 0.08	99.96 ± 2.57	33.56 ± 1.25	0.25 ± 0.03
SBT/CP/5.0	181.05 ± 0.56	0.72	2.38 ± 0.04	100.23 ± 1.64	35.14 ± 1.05	0.62 ± 0.02
SBT/CP/10.0	183.55 ± 1.18	0.58	2.39 ± 0.06	99.83 ± 1.23	37.83 ± 0.86	1.03 ± 0.04
SBT/CP/15.0	189.51 ± 1.59	0.63	2.42 ± 0.04	101.00 ± 0.11	36.42 ± 1.03	1.26 ± 0.02
SBT/CP/20.0	193.54 ± 1.98	0.71	2.48 ± 0.04	98.52 ± 1.08	34.61 ± 1.35	1.62 ± 0.02
SBT/PC/2.5	175.65 ± 2.01	0.60	2.39 ± 0.08	98.69 ± 0.97	38.74 ± 1.42	0.31 ± 0.04
SBT/PC/5.0	179.42 ± 1.65	0.55	2.44 ± 0.09	99.37 ± 1.25	35.25 ± 1.39	0.72 ± 0.05
SBT/PC/10.0	182.26 ± 1.25	0.68	2.42 ± 0.08	99.57 ± 0.76	33.54 ± 1.32	1.11 ± 0.05
SBT/PC/15.0	188.52 ± 1.33	0.79	2.43 ± 0.06	98.34 ± 1.58	39.41 ± 1.56	1.36 ± 0.08
SBT/PC/20.0	194.14 ± 0.98	0.70	2.47 ± 0.09	99.16 ± 0.98	38.02 ± 1.54	1.69 ± 0.03
SBT/CPHK4/5050	183.33 ± 1.24	0.89	2.40 ± 0.09	99.05 ± 0.94	40.21 ± 1.69	0.69 ± 0.05
SBT/CPHK4/7525	184.54 ± 1.54	0.83	2.42 ± 0.05	99.48 ± 0.56	38.54 ± 1.54	0.82 ± 0.06
SBT/CPHK4/2575	182.41 ± 1.35	0.75	2.40 ± 0.09	102.54 ± 0.97	39.54 ± 1.20	0.57 ± 0.04
SBT/CPHK15/5050	186.24 ± 1.27	0.80	2.41 ± 0.05	100.26 ± 1.69	38.59 ± 1.84	0.68 ± 0.06
SBT/CPHK15/7525	184.15 ± 1.98	0.74	2.43 ± 0.06	101.43 ± 1.11	35.24 ± 1.02	0.76 ± 0.12
SBT/CPHK15/2575	183.24 ± 1.78	0.68	2.39 ± 0.10	102.37 ± 2.05	36.51 ± 1.54	0.64 ± 0.02

[a] For each batch 20 tablets were taken
[b] Mean of three batches with triplicate determination per batch

Table 5.2b: Results of quality control tests carried out on designed buccal mucoadhesive tablets prepared using combination of HPMC with CP or PC

Formulation Code	Mean Weight[a] ± SD (mg)	Friability[a] (% w/w)	Mean Thickness[b] ± SD (mm)	Mean Assay[b] ± SD (%)	Mean Crushing Strength[b] ± SD (N)	Mean Detachment Force[b] ± SD (N/cm^2)
SBT/CPHK100/5050	182.41 ± 1.55	0.75	2.35 ± 0.07	99.84 ± 1.23	36.01 ± 1.48	0.93 ± 0.05
SBT/CPHK100/7525	185.96 ± 1.36	0.77	2.37 ± 0.10	99.09 ± 2.26	39.52 ± 1.54	1.10 ± 0.09
SBT/CPHK100/2575	184.22 ± 0.87	0.64	2.35 ± 0.05	100.07 ± 1.03	38.54 ± 1.36	0.84 ± 0.10
SBT/PCHK4/5050	183.74 ± 1.54	0.65	2.38 ± 0.05	101.39 ± 1.53	39.21 ± 1.58	0.86 ± 0.05
SBT/PCHK4/7525	185.29 ± 1.65	0.69	2.33 ± 0.09	99.16 ± 1.45	32.13 ± 1.35	0.97 ± 0.02
SBT/PCHK4/2575	185.63 ± 1.13	0.79	2.35 ± 0.08	98.54 ± 1.38	35.62 ± 1.32	0.77 ± 0.06
SBT/PCHK15/5050	184.71 ± 1.98	0.81	2.29 ± 0.10	99.22 ± 1.64	35.28 ± 1.21	0.84 ± 0.02
SBT/PCHK15/7525	185.41 ± 2.11	0.87	2.39 ± 0.04	98.65 ± 2.54	36.29 ± 1.97	1.01 ± 0.06
SBT/PCHK15/2575	184.29 ± 2.54	0.61	2.36 ± 0.07	102.31 ± 1.55	35.24 ± 1.56	0.79 ± 0.06
SBT/PCHK100/5050	183.57 ± 1.78	0.68	2.38 ± 0.10	99.54 ± 0.82	36.15 ± 1.28	1.06 ± 0.12
SBT/PCHK100/7525	186.04 ± 1.54	0.73	2.37 ± 0.09	99.62 ± 0.86	38.27 ± 1.57	1.27 ± 0.11
SBT/PCHK100/2575	184.51 ± 1.36	0.84	2.33 ± 0.09	98.65 ± 1.23	36.70 ± 1.68	0.96 ± 0.07

[a] For each batch 20 tablets were taken
[b] Mean of three batches with triplicate determination per batch

Table 5.2c: Results of quality control tests carried out on designed buccal mucoadhesive tablets prepared using HPMC

Formulation Code	Mean Weight[a] ± SD (mg)	Friability[a] (% w/w)	Mean Thickness[b] ± SD (mm)	Mean Assay[b] ± SD (%)	Mean Crushing Strength[b] ± SD (N)	Mean Detachment Force[b] ± SD (N/cm^2)
SBT/HK4/5.0	178.99 ± 1.41	0.68	2.38 ± 0.13	98.32 ± 1.69	36.43 ± 1.29	0.18 ± 0.04
SBT/HK4/10.0	184.28 ± 1.08	0.73	2.51 ± 0.04	99.46 ± 1.73	35.29 ± 1.84	0.37 ± 0.01
SBT/HK4/12.5	185.24 ± 0.53	0.84	2.42 ± 0.09	102.57 ± 1.11	36.47 ± 1.39	0.50 ± 0.05
SBT/HK4/15.0	188.52 ± 1.35	0.72	2.45 ± 0.04	98.26 ± 1.65	37.77 ± 1.81	0.55 ± 0.03
SBT/HK4/20.0	193.21 ± 1.47	0.59	2.44 ± 0.17	99.73 ± 1.05	38.54 ± 1.98	0.61 ± 0.04
SBT/HK15/2.5	176.21 ± 1.59	0.81	2.46 ± 0.10	98.26 ± 1.25	33.18 ± 0.75	0.21 ± 0.05
SBT/HK15/5.0	180.25 ± 1.54	0.75	2.35 ± 0.12	102.10 ± 1.16	35.14 ± 1.49	0.45 ± 0.05
SBT/HK15/10.0	183.64 ± 1.47	0.76	2.50 ± 0.10	100.42 ± 1.51	36.87 ± 1.24	0.53 ± 0.05
SBT/HK15/15.0	188.66 ± 1.69	0.62	2.33 ± 0.05	102.12 ± 1.22	35.29 ± 1.51	0.66 ± 0.03
SBT/HK15/20.0	193.34 ± 1.87	0.69	2.37 ± 0.07	99.68 ± 1.10	32.10 ± 1.36	0.71 ± 0.02
SBT/HK100/2.5	175.98 ± 1.95	0.66	2.30 ± 0.10	99.27 ± 1.09	35.26 ± 1.52	0.16 ± 0.04
SBT/HK100/5.0	177.98 ± 1.32	0.72	2.31 ± 0.03	98.53 ± 2.04	35.24 ± 1.73	0.40 ± 0.04
SBT/HK100/10.0	182.66 ± 2.14	0.73	2.43 ± 0.09	99.74 ± 1.87	35.93 ± 1.52	0.62 ± 0.04
SBT/HK100/15.0	187.65 ± 2.34	0.54	2.41 ± 0.03	99.61 ± 1.29	36.82 ± 1.06	0.80 ± 0.04
SBT/HK100/20.0	193.52 ± 2.05	0.59	2.40 ± 0.03	98.34 ± 0.53	36.97 ± 1.54	0.97 ± 0.06

[a] For each batch 20 tablets were taken
[b] Mean of three batches with triplicate determination per batch

Table 5.2d: Results of quality control tests carried out on designed buccal mucoadhesive tablets prepared using PEO, CH and combination of HPMC with PEO

Formulation Code	Mean Weight[a] ± SD (mg)	Friability[a] (% w/w)	Mean Thickness[b] ± SD (mm)	Mean Assay[b] ± SD (%)	Mean Crushing Strength[b] ± SD (N)	Mean Detachment Force[b] ± SD (N/cm^2)
SBT/PEO/5.0	178.44 ± 1.04	0.91	2.49 ± 0.04	101.24 ± 1.31	29.42 ± 1.20	0.49 ± 0.03
SBT/PEO/10.0	183.20 ± 1.54	0.84	2.53 ± 0.04	98.76 ± 1.30	30.28 ± 1.68	0.79 ± 0.04
SBT/PEO/12.5	185.23 ± 1.69	0.89	2.54 ± 0.08	100.42 ± 1.81	30.58 ± 1.63	0.92 ± 0.05
SBT/PEO/15.0	190.25 ± 1.34	0.94	2.55 ± 0.10	101.28 ± 1.48	31.04 ± 1.65	1.07 ± 0.04
SBT/PEO/20.0	194.56 ± 1.39	0.84	2.54 ± 0.05	102.14 ± 1.51	32.73 ± 1.84	1.23 ± 0.03
SBT/CH/5.0	178.41 ± 1.87	0.89	2.52 ± 0.06	100.35 ± 1.98	28.56 ± 0.54	0.42 ± 0.05
SBT/CH/10.0	184.52 ± 1.34	0.79	2.53 ± 0.10	98.71 ± 2.54	29.44 ± 1.21	0.61 ± 0.03
SBT/CH/12.5	186.59 ± 1.97	0.82	2.55 ± 0.05	99.43 ± 1.21	32.59 ± 0.99	0.66 ± 0.03
SBT/CH/15.0	190.25 ± 1.85	0.89	2.54 ± 0.05	102.24 ± 2.30	33.42 ± 0.59	0.80 ± 0.06
SBT/CH/20.0	195.04 ± 1.67	0.88	2.55 ± 0.08	98.67 ± 1.29	36.42 ± 1.42	0.99 ± 0.04
SBT/PEOHK4/5050	184.14 ± 1.54	0.75	2.52 ± 0.01	98.83 ± 1.46	33.28 ± 1.05	0.54 ± 0.01
SBT/PEOHK4/7525	184.00 ± 1.76	0.79	2.53 ± 0.09	100.54 ± 2.35	36.25 ± 1.15	0.70 ± 0.05
SBT/PEOHK4/2575	185.63 ± 1.87	0.82	2.50 ± 0.03	100.79 ± 1.59	32.19 ± 1.43	0.49 ± 0.04

[a] For each batch 20 tablets were taken
[b] Mean of three batches with triplicate determination per batch

Table 5.2e: Results of quality control tests carried out on designed buccal mucoadhesive tablets prepared using combination of HPMC with PEO or CH

Formulation Code	Mean Weight[a] ± SD (mg)	Friability[a] (% w/w)	Mean Thickness[b] ± SD (mm)	Mean Assay[b] ± SD (%)	Mean Crushing Strength[b] ± SD (N)	Mean Detachment Force[b] ± SD (N/cm^2)
SBT/PEOHK15/5050	184.2 ± 1.74	0.54	2.53 ± 0.01	100.26 ± 0.89	35.32 ± 1.49	0.59 ± 0.04
SBT/PEOHK15/7525	185.23 ± 1.52	0.68	2.55 ± 0.03	99.15 ± 0.98	39.84 ± 1.68	0.78 ± 0.04
SBT/PEOHK15/2575	184.69 ± 1.63	0.69	2.53 ± 0.01	102.36 ± 2.14	36.21 ± 1.51	0.55 ± 0.03
SBT/PEOHK100/5050	184.88 ± 1.55	0.76	2.51 ± 0.05	100.49 ± 2.00	35.28 ± 0.78	0.89 ± 0.07
SBT/PEOHK100/7525	185.73 ± 1.74	0.79	2.51 ± 0.09	98.25 ± 1.80	36.98 ± 1.88	0.91 ± 0.07
SBT/PEOHK100/2575	185.96 ± 1.26	0.68	2.52 ± 0.05	99.17 ± 1.27	37.54 ± 0.87	0.96 ± 0.08
SBT/CHHK4/5050	184.56 ± 1.11	0.51	2.54 ± 0.09	100.28 ± 2.42	38.59 ± 1.36	0.44 ± 0.05
SBT/CHHK4/7525	186.09 ± 1.56	0.56	2.53 ± 0.15	101.57 ± 1.46	29.62 ± 1.28	0.55 ± 0.04
SBT/CHHK4/2575	185.66 ± 1.65	0.68	2.51 ± 0.08	99.29 ± 1.30	30.26 ± 0.95	0.34 ± 0.07
SBT/CHHK15/5050	184.57 ± 1.32	0.57	2.52 ± 0.07	99.87 ± 0.54	36.45 ± 1.36	0.44 ± 0.02
SBT/CHHK15/7525	185.77 ± 1.46	0.69	2.53 ± 0.09	99.68 ± 2.06	38.24 ± 0.59	0.58 ± 0.04
SBT/CHHK15/2575	185.34 ± 1.59	0.67	2.51 ± 0.10	98.57 ± 1.64	36.47 ± 1.58	0.42 ± 0.02
SBT/CHHK100/5050	185.41 ± 1.74	0.59	2.50 ± 0.09	99.72 ± 1.08	35.21 ± 1.34	0.63 ± 0.02
SBT/CHHK100/7525	185.34 ± 1.65	0.51	2.52 ± 0.07	100.25 ± 2.11	39.54 ± 1.26	0.63 ± 0.02
SBT/CHHK100/2575	185.24 ± 1.44	0.62	2.51 ± 0.03	99.59 ± 1.36	29.64 ± 1.29	0.68 ± 0.05

[a] For each batch 20 tablets were taken
[b] Mean of three batches with triplicate determination per batch

Table 5.3a: Data of drug release kinetics study of formulations prepared using CP, PC and combination of CP with HPMC

Formulation Code	Peppas Model				
	n^a	Release Rate Constant K (h^{-n})	$t_{50\%}{}^b$ (h)	R^2	MSSR
SBT/CP/2.5	0.8921	42.14×10^{-2}	1.22	0.9806	1.58×10^{-3}
SBT/CP/5.0	0.9814	35.27×10^{-2}	1.42	0.9915	9.57×10^{-4}
SBT/CP/10.0	0.7659	25.49×10^{-2}	2.40	0.9987	7.63×10^{-5}
SBT/CP/15.0	0.7143	22.94×10^{-2}	2.97	0.9952	2.79×10^{-4}
SBT/CP/20.0	0.8484	12.65×10^{-2}	5.04	0.9947	5.27×10^{-4}
SBT/PC/2.5	0.7073	40.54×10^{-2}	1.35	0.9593	2.10×10^{-3}
SBT/PC/5.0	0.7313	38.98×10^{-2}	1.47	0.9542	2.00×10^{-3}
SBT/PC/10.0	0.7421	21.03×10^{-2}	3.21	0.9925	4.76×10^{-4}
SBT/PC/15.0	0.7753	15.89×10^{-2}	4.38	0.9848	1.16×10^{-3}
SBT/PC/20.0	0.8221	12.07×10^{-2}	5.63	0.9957	4.38×10^{-4}
SBT/CPHK4/5050	0.6105	29.90×10^{-2}	2.32	0.9916	3.57×10^{-4}
SBT/CPHK4/7525	0.6531	25.99×10^{-2}	2.72	0.9974	3.75×10^{-4}
SBT/CPHK4/2575	0.5981	34.11×10^{-2}	1.89	0.9999	3.62×10^{-6}

[a] Diffusion exponent indicative of release mechanism
[b] Time for 50% drug release

Table 5.3b: Data of drug release kinetics study of formulations prepared using combination of HPMC with CP or PC

Formulation Code	Peppas Model				
	n[a]	Release Rate Constant K (h^{-n})	$t_{50\%}$[b] (h)	R^2	MSSR
SBT/CPHK15/5050	0.6540	24.33×10^{-2}	3.00	0.9814	9.23×10^{-4}
SBT/CPHK15/7525	0.7690	19.59×10^{-2}	3.38	0.9891	7.43×10^{-4}
SBT/CPHK15/2575	0.6245	27.37×10^{-2}	2.62	0.9930	3.13×10^{-4}
SBT/CPHK100/5050	0.9021	12.08×10^{-2}	4.82	0.9968	2.46×10^{-4}
SBT/CPHK100/7525	0.9829	10.59×10^{-2}	4.84	0.9936	5.91×10^{-4}
SBT/CPHK100/2575	0.8836	13.48×10^{-2}	4.40	0.9955	3.33×10^{-4}
SBT/PCHK4/5050	0.7629	25.49×10^{-2}	2.40	0.9987	7.63×10^{-5}
SBT/PCHK4/7525	0.7421	21.03×10^{-2}	3.21	0.9925	4.76×10^{-4}
SBT/PCHK4/2575	0.7583	27.04×10^{-2}	2.24	0.9942	3.48×10^{-4}
SBT/PCHK15/5050	0.7659	29.90×10^{-2}	2.32	0.9987	7.63×10^{-5}
SBT/PCHK15/7525	0.6105	25.49×10^{-2}	2.43	0.9916	3.58×10^{-4}
SBT/PCHK15/2575	0.5981	34.11×10^{-2}	1.89	0.9999	3.62×10^{-6}
SBT/PCHK100/5050	0.7961	15.32×10^{-2}	4.41	0.9903	7.83×10^{-4}
SBT/PCHK100/7525	0.7630	14.17×10^{-2}	4.57	0.9784	1.77×10^{-3}
SBT/PCHK100/2575	0.7605	16.86×10^{-2}	4.27	0.9909	6.72×10^{-4}

[a] Diffusion exponent indicative of release mechanism
[b] Time for 50% drug release

Table 5.3c: Data of drug release kinetics study of formulations prepared using HPMC

Formulation Code	Peppas Model				
	n[a]	Release Rate Constant K (h^{-n})	$t_{50\%}$[b] (h)	R^2	MSSR
SBT/HK4/5.0	0.6221	41.97×10^{-2}	1.32	0.9997	1.20×10^{-5}
SBT/HK4/10.0	0.6069	37.28×10^{-2}	1.62	0.9988	4.44×10^{-5}
SBT/HK4/12.5	0.5846	34.53×10^{-2}	1.88	0.9956	1.56×10^{-4}
SBT/HK4/15.0	0.5981	29.02×10^{-2}	2.48	0.9993	2.70×10^{-5}
SBT/HK4/20.0	0.6102	22.44×10^{-2}	3.71	0.9844	7.45×10^{-4}
SBT/HK15/2.5	0.5669	44.90×10^{-2}	1.20	0.9986	4.76×10^{-5}
SBT/HK15/5.0	0.6149	33.48×10^{-2}	1.90	0.9960	1.57×10^{-4}
SBT/HK15/10.0	0.5847	30.64×10^{-2}	2.30	0.9944	2.18×10^{-4}
SBT/HK15/15.0	0.5755	25.38×10^{-2}	3.24	0.9973	1.11×10^{-4}
SBT/HK15/20.0	0.6591	18.35×10^{-2}	4.57	0.9934	3.96×10^{-4}
SBT/HK100/2.5	0.5631	40.04×10^{-2}	1.48	0.9970	9.54×10^{-5}
SBT/HK100/5.0	0.5976	32.10×10^{-2}	2.09	0.9771	8.66×10^{-4}
SBT/HK100/10.0	0.5369	27.32×10^{-2}	3.08	0.9922	2.87×10^{-4}
SBT/HK100/15.0	0.6473	16.78×10^{-2}	5.40	0.9744	1.63×10^{-3}
SBT/HK100/20.0	0.6984	14.40×10^{-2}	5.94	0.9512	3.71×10^{-3}

[a] Diffusion exponent indicative of release mechanism
[b] Time for 50% drug release

Table 5.3d: Data of drug release kinetics study of formulations prepared using PEO, CH and combination of HPMC with PEO

Formulation Code	Peppas Model				
	n[a]	Release Rate Constant K (h^{-n})	$t_{50\%}$[b] (h)	R^2	MSSR
SBT/PEO/5.0	0.5137	47.92×10^{-2}	1.08	0.9931	1.86×10^{-4}
SBT/PEO/10.0	0.5175	43.09×10^{-2}	1.33	0.9976	6.52×10^{-5}
SBT/PEO/12.5	0.5808	33.86×10^{-2}	1.95	0.9760	8.60×10^{-4}
SBT/PEO/15.0	0.6333	26.61×10^{-2}	2.70	0.9894	4.90×10^{-4}
SBT/PEO/20.0	0.6699	19.96×10^{-2}	3.93	0.9927	4.15×10^{-4}
SBT/CH/5.0	0.6098	44.22×10^{-2}	1.22	0.9942	2.22×10^{-4}
SBT/CH/10.0	0.6267	37.11×10^{-2}	1.60	0.9945	2.20×10^{-4}
SBT/CH/12.5	0.7465	26.14×10^{-2}	2.38	0.9986	8.37×10^{-5}
SBT/CH/15.0	0.7477	21.68×10^{-2}	3.05	0.9990	6.19×10^{-5}
SBT/CH/20.0	0.7605	16.86×10^{-2}	4.17	0.9909	6.72×10^{-4}
SBT/PEOHK4/5050	0.5631	40.04×10^{-2}	1.48	0.9970	9.54×10^{-5}
SBT/PEOHK4/7525	0.5669	44.90×10^{-2}	1.20	0.9986	4.76×10^{-5}
SBT/PEOHK4/2575	0.6149	33.48×10^{-2}	1.91	0.9960	1.57×10^{-4}

[a] Diffusion exponent indicative of release mechanism
[b] Time for 50% drug release

Table 5.3e: Data of drug release kinetics study of formulations prepared using combination of HPMC with PEO or CH

Formulation Code	Peppas Model				
	n[a]	Release Rate Constant K (h^{-n})	$t_{50\%}$[b] (h)	R^2	MSSR
SBT/PEOHK15/5050	0.7422	27.25×10^{-2}	2.26	0.9979	1.21×10^{-4}
SBT/PEOHK15/7525	0.6572	32.00×10^{-2}	1.97	0.9945	2.45×10^{-4}
SBT/PEOHK15/2575	0.7972	23.67×10^{-2}	2.75	0.9992	5.05×10^{-5}
SBT/PEOHK100/5050	0.5976	32.10×10^{-2}	2.09	0.9771	8.66×10^{-4}
SBT/PEOHK100/7525	0.5631	40.04×10^{-2}	1.48	0.997	9.54×10^{-5}
SBT/PEOHK100/2575	0.5847	30.43×10^{-2}	2.30	0.9944	2.19×10^{-4}
SBT/CHHK4/5050	0.7465	26.14×10^{-2}	2.38	0.9986	8.37×10^{-5}
SBT/CHHK4/7525	0.6267	37.11×10^{-2}	1.60	0.9945	2.20×10^{-4}
SBT/CHHK4/2575	0.5523	36.31×10^{-2}	1.78	0.9917	2.63×10^{-4}
SBT/CHHK15/5050	0.7659	25.43×10^{-2}	2.40	0.9987	7.63×10^{-5}
SBT/CHHK15/7525	0.6025	34.26×10^{-2}	1.87	0.9990	3.87×10^{-5}
SBT/CHHK15/2575	0.5979	32.10×10^{-2}	2.09	0.9771	8.66×10^{-4}
SBT/CHHK100/5050	0.7143	22.94×10^{-2}	2.97	0.9952	2.80×10^{-4}
SBT/CHHK100/7525	0.5847	30.64×10^{-2}	2.30	0.9944	2.19×10^{-4}
SBT/CHHK100/2575	0.5755	25.38×10^{-2}	3.24	0.9973	1.12×10^{-4}

[a] Diffusion exponent indicative of release mechanism
[b] Time for 50% drug release

Table 5.4: Results of mucoadhesion test conducted on prepared formulations using texture analyzer

Formulation Code	Detachment Force[a] ± SD (N/cm^2)	Work of Adhesion[a] ± SD ($N.sec/cm^2$)
SBT/CP/5.0	2.68 ± 0.06	2.22 ± 0.06
SBT/CP/10.0	3.52 ± 0.02	3.00 ± 0.03
SBT/CPHK4/5050	2.81 ± 0.02	1.90 ± 0.74
SBT/PC/5.0	3.25 ± 0.03	2.90 ± 0.04
SBT/PC/10.0	5.02 ± 0.06	4.31 ± 0.62
SBT/PCHK4/5050	3.44 ± 0.01	3.38 ± 0.04
SBT/HK4/5.0	1.08 ± 0.06	0.87 ± 0.03
SBT/HK4/10.0	1.62 ± 0.04	1.35 ± 0.05
SBT/HK15/5.0	1.38 ± 0.03	0.96 ± 0.04
SBT/HK15/10.0	2.00 ± 0.03	1.43 ± 0.03
SBT/HK100/5.0	1.93 ± 0.02	1.54 ± 0.03
SBT/HK100/10.0	2.53 ± 0.03	2.23 ± 0.04
SBT/PEO/5.0	2.22 ± 0.04	1.85 ± 0.03
SBT/PEO/10.0	3.00 ± 0.06	2.20 ± 0.69
SBT/PEOHK100/5050	2.58 ± 0.06	2.29 ± 0.06
SBT/CH/5.0	1.97 ± 0.07	1.42 ± 0.07
SBT/CH/10.0	2.47 ± 0.03	1.82 ± 0.02
SBT/CHHK15/5050	1.91 ± 0.01	1.32 ± 0.06

[a] Mean of 3 independent determinations

Table 5.5: Mucoadhesive performance of prepared formulations at varying contact times using texture analyzer

Formulation Code	Contact Time							
	10 Sec		30 Sec		120 Sec		300 Sec	
	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)
SBT/CP/10.0	2.10 ± 0.08	1.77 ± 0.10	3.52 ± 0.02	3.00 ± 0.03	4.08 ± 0.10	3.49 ± 0.10	4.19 ± 0.07	3.58 ± 0.22
SBT/PC/10.0	2.14 ± 0.06	1.77 ± 0.10	5.02 ± 0.06	4.31 ± 0.62	5.55 ± 0.07	4.80 ± 0.31	5.86 ± 0.04	4.94 ± 0.36
SBT/HK4/10.0	1.58 ± 0.06	1.33 ± 0.09	1.62 ± 0.04	1.35 ± 0.05	1.87 ± 0.06	1.55 ± 0.13	2.00 ± 0.05	1.71 ± 0.09
SBT/HK15/10.0	1.34 ± 0.06	1.13 ± 0.03	2.00 ± 0.03	1.43 ± 0.03	2.29 ± 0.04	1.92 ± 0.12	2.52 ± 0.03	2.28 ± 0.03
SBT/HK100/10.0	1.54 ± 0.07	1.36 ± 0.11	2.53 ± 0.03	2.23 ± 0.04	3.32 ± 0.09	3.08 ± 0.05	3.76 ± 0.06	3.54 ± 0.17
SBT/PEO/10.0	2.20 ± 0.08	2.02 ± 0.05	3.00 ± 0.06	2.20 ± 0.69	3.30 ± 0.05	3.09 ± 0.16	3.41 ± 0.06	3.01 ± 0.13
SBT/CH/10.0	1.66 ± 0.07	1.53 ± 0.12	2.47 ± 0.03	1.82 ± 0.02	2.95 ± 0.08	2.66 ± 0.14	2.99 ± 0.06	2.59 ± 0.14

[a] Mean of 3 independent determinations

Table 5.6: Mucoadhesive performance of prepared formulations at varying contact forces using texture analyzer

Formulation Code	Contact Force							
	0.2 N		0.4 N		1.0 N		1.5 N	
	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)	Detachment Force[a] (N/cm^2)	Work of Adhesion[a] ($N.sec/cm^2$)
SBT/CP/10.0	2.33 ± 0.09	2.03 ± 0.09	3.52 ± 0.02	3.00 ± 0.03	4.68 ± 0.02	4.12 ± 0.12	5.18 ± 0.07	4.45 ± 0.31
SBT/PC/10.0	3.14 ± 0.13	1.93 ± 1.36	5.02 ± 0.06	4.31 ± 0.62	6.26 ± 0.07	5.47 ± 0.18	7.12 ± 0.07	6.46 ± 0.16
SBT/HK4/10.0	1.12 ± 0.09	0.92 ± 0.09	1.62 ± 0.04	1.35 ± 0.05	2.36 ± 0.09	1.89 ± 0.07	2.79 ± 0.12	2.31 ± 0.10
SBT/HK15/10.0	1.27 ± 0.07	1.13 ± 0.04	2.00 ± 0.03	1.43 ± 0.03	2.67 ± 0.06	2.32 ± 0.09	3.41 ± 0.05	3.13 ± 0.13
SBT/HK100/10.0	1.66 ± 0.07	1.33 ± 0.10	2.53 ± 0.03	2.23 ± 0.04	3.21 ± 0.09	2.69 ± 0.14	3.81 ± 0.14	3.07 ± 0.12
SBT/PEO/10.0	2.34 ± 0.09	2.2 ± 0.06	3.00 ± 0.06	2.20 ± 0.69	3.40 ± 0.08	3.21 ± 0.17	3.92 ± 0.13	3.77 ± 0.07
SBT/CH/10.0	1.42 ± 0.06	1.22 ± 0.13	2.47 ± 0.03	1.82 ± 0.02	3.12 ± 0.07	2.63 ± 0.14	3.54 ± 0.08	3.14 ± 0.13

[a] Mean of 3 independent determinations

Table 5.7: First order degradation kinetic parameters of LER in prepared formulations

Formulation Code	CRT: 25 ± 2°C/60 ± 5 % RH				AT (40 ± 2°C/75 ± 5 % RH)			
	K_{deg}x 10^4 ($month^{-1}$)	$t_{90\%}$ (month)	R^2	MSSR	K_{deg} x 10^4 ($month^{-1}$)	$t_{90\%}$ (month)	R^2	MSSR
SBT/CP/10.0	22.57	46.69	0.9232	7.60×10^{-6}	99.54	10.59	0.9147	1.23×10^{-5}
SBT/PC/10.0	22.96	45.90	0.9541	6.01×10^{-6}	83.48	12.62	0.9754	2.30×10^{-6}
SBT/CPHK4/5050	24.30	43.37	0.9691	3.52×10^{-6}	93.59	11.26	0.9285	1.02×10^{-5}
SBT/CPHK15/5050	22.13	47.61	0.9343	1.01×10^{-5}	96.82	10.88	0.9683	3.76×10^{-6}
SBT/CPHK100/5050	28.83	36.55	0.9255	1.47×10^{-5}	111.60	9.44	0.9222	1.28×10^{-5}
SBT/PCHK4/7525	25.54	41.26	0.9510	2.23×10^{-6}	82.98	12.70	0.9446	6.59×10^{-6}
SBT/PCHK15/5050	25.43	41.45	0.9914	1.71×10^{-6}	70.89	14.87	0.9257	5.52×10^{-6}
SBT/PCHK100/5050	26.05	40.46	0.9851	3.17×10^{-6}	76.44	13.79	0.9205	6.21×10^{-6}
SBT/HK4/12.5	27.24	38.68	0.9394	1.00×10^{-5}	111.86	9.42	0.9841	5.95×10^{-6}
SBT/HK15/10.0	25.10	41.98	0.9397	1.84×10^{-6}	84.57	12.46	0.9071	1.30×10^{-6}
SBT/HK100/10.0	30.70	34.33	0.9622	1.89×10^{-6}	93.94	11.29	0.9638	3.81×10^{-6}
SBT/PEO/12.5	32.08	32.85	0.9684	2.89×10^{-6}	91.11	11.57	0.9936	8.05×10^{-6}
SBT/CH/10.0	22.91	45.99	0.9531	1.06×10^{-6}	86.78	12.14	0.9966	6.09×10^{-6}
SBT/PEOHK4/2575	22.75	46.31	0.9435	1.83×10^{-6}	97.97	10.76	0.9973	3.22×10^{-5}
SBT/PEOHK15/5050	25.29	41.67	0.9559	1.19×10^{-5}	94.98	11.10	0.9416	3.92×10^{-6}
SBT/PEOHK100/5050	20.91	50.39	0.9446	1.29×10^{-6}	88.76	11.87	0.9874	3.26×10^{-6}
SBT/CHHK4/5050	25.93	40.64	0.9841	2.97×10^{-5}	91.80	11.48	0.9356	1.47×10^{-5}
SBT/CHHK15/5050	30.42	34.64	0.9652	1.57×10^{-6}	86.04	12.25	0.9364	2.45×10^{-6}
SBT/CHHK100/5050	21.26	49.57	0.9455	2.98×10^{-6}	79.66	13.23	0.9562	8.59×10^{-5}

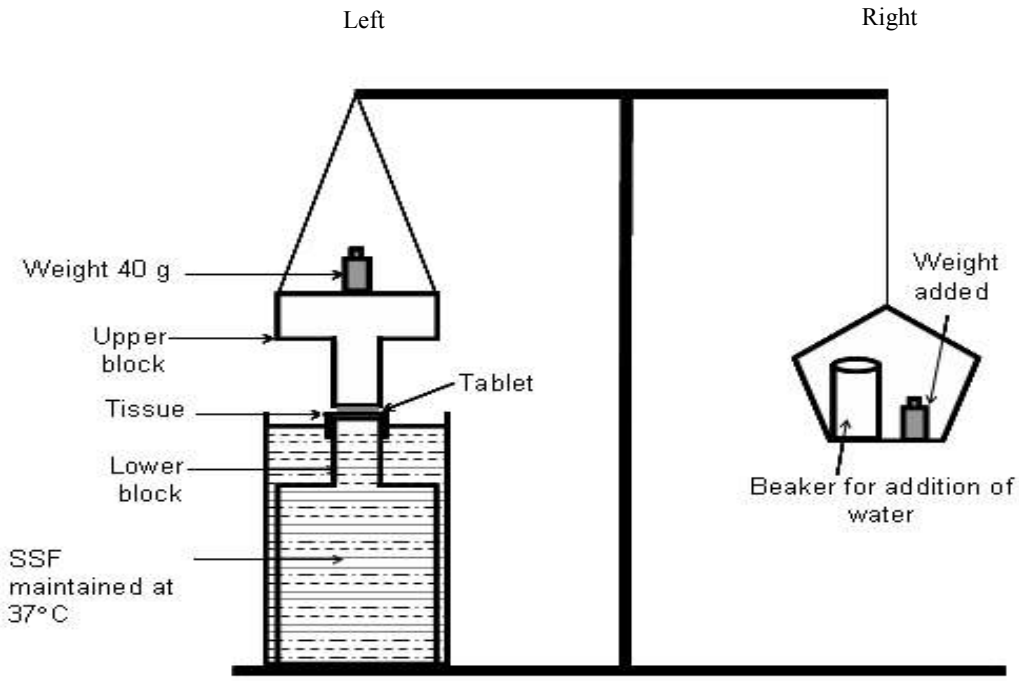

Figure 5.1: Schematic diagram for setup of conventional bioadhesion testing

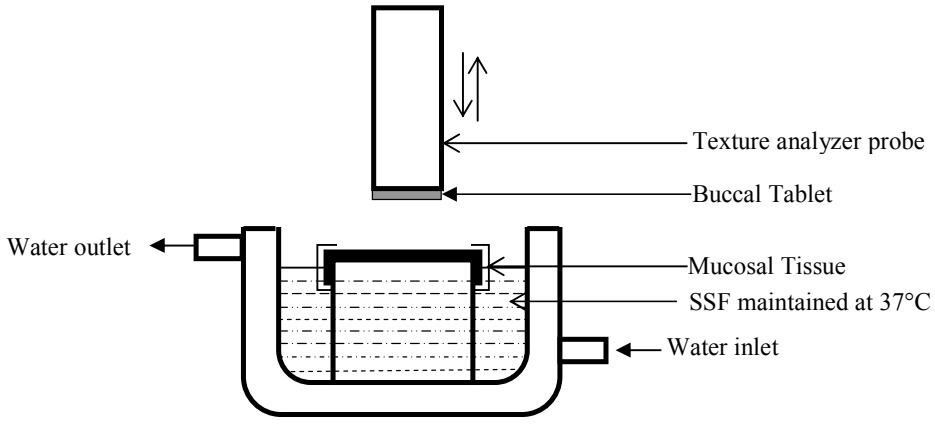

Figure 5.2: Schematic representation of bioadhesion testing using texture analyzer

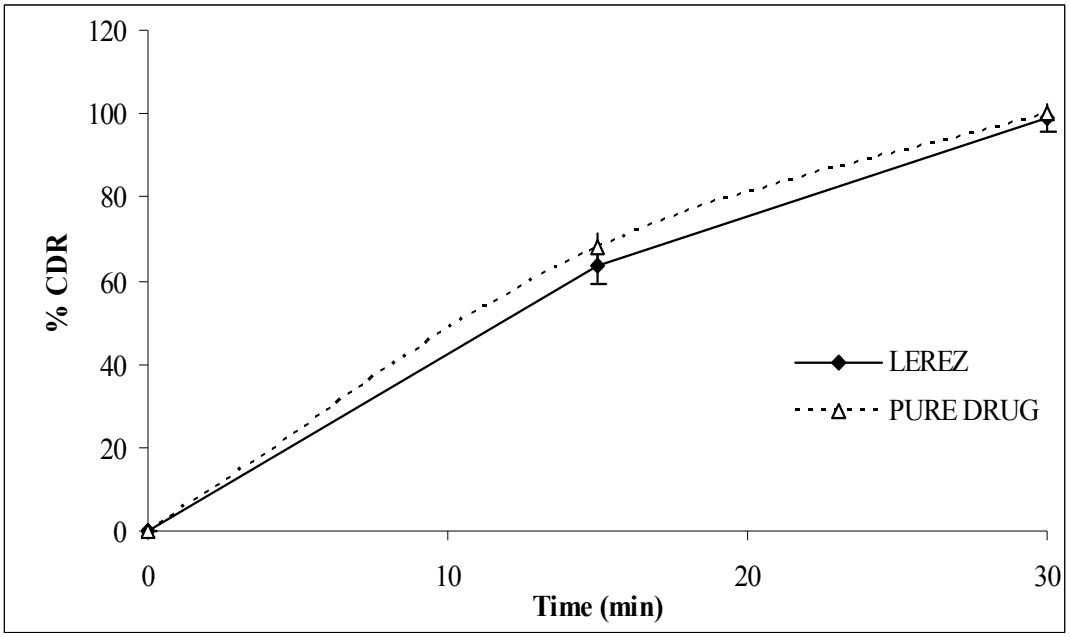

Figure 5.3: Comparative in vitro release profile of commercial tablets Lerez® and pure drug in the selected release media

(Each point represents mean of three independent determinations with standard deviation)

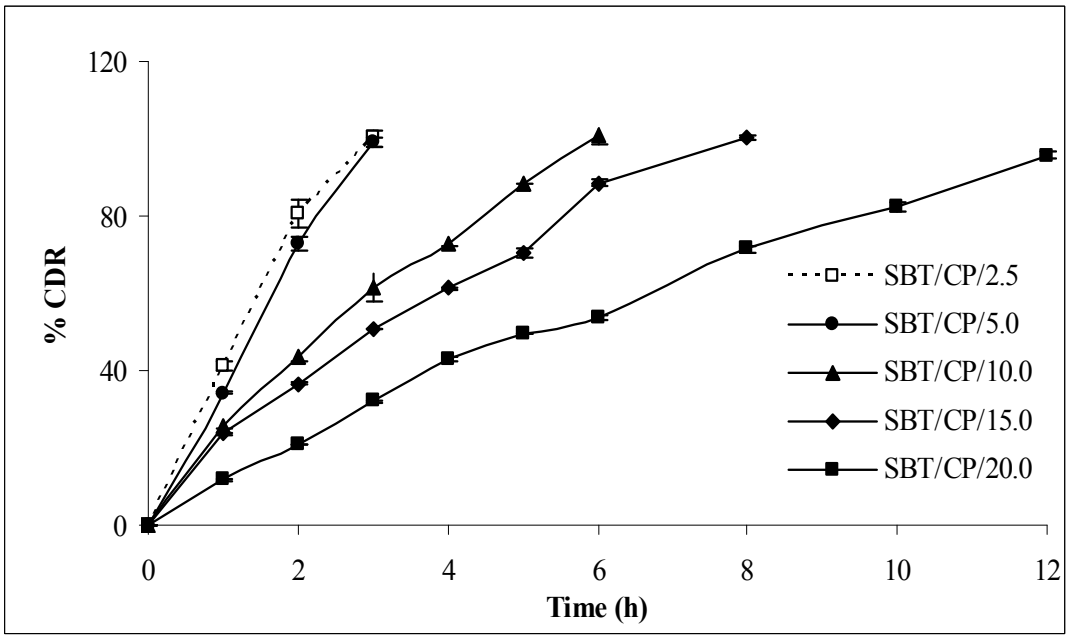

Figure 5.4: Comparative in vitro release profile of LER from tablet formulations prepared using varying proportions of CP

(Each point represents mean and SD of three batches with triplicate determination per batch)

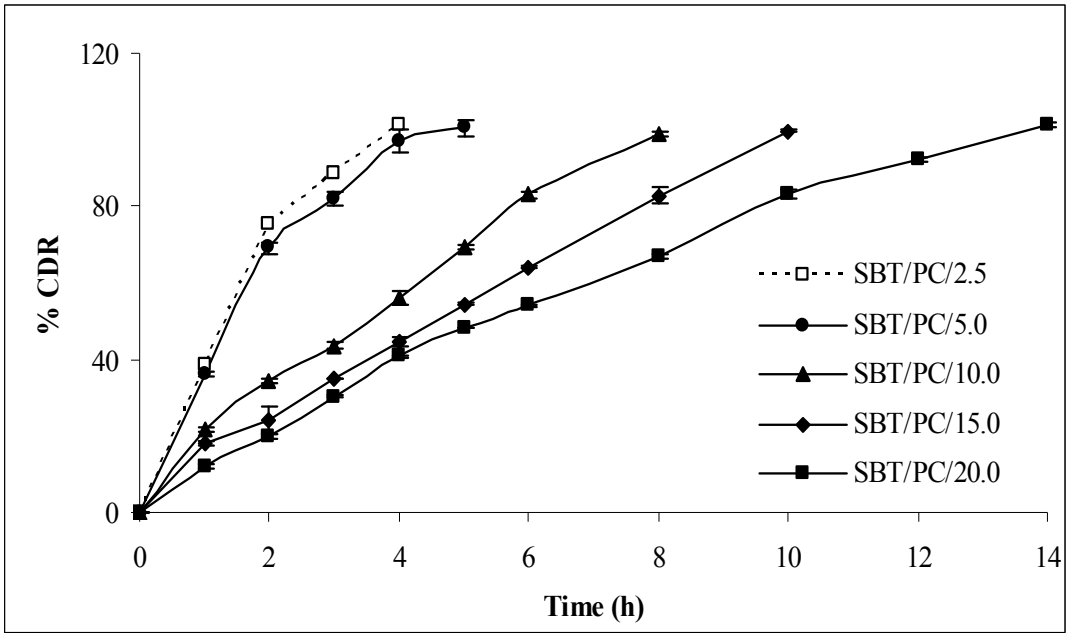

Figure 5.5: Comparative in vitro release profile of LER from tablet formulations prepared using varying proportions of PC

(Each point represents mean and SD of three batches with triplicate determination per batch)

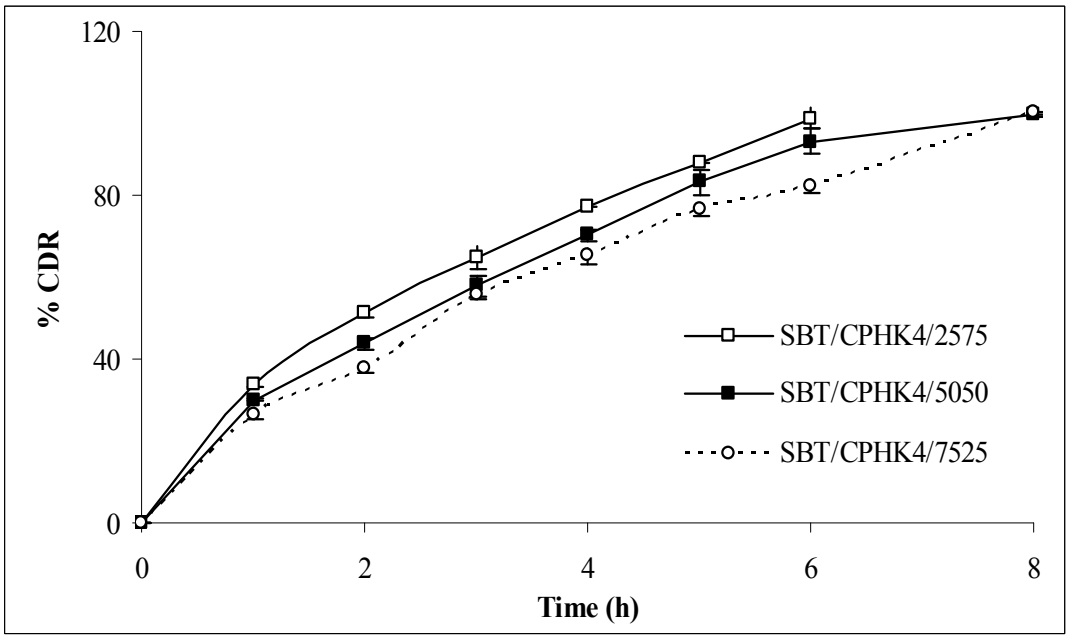

Figure 5.6: Comparative in vitro release profile of LER from tablet formulations prepared using combination of CP and HPMC K4M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

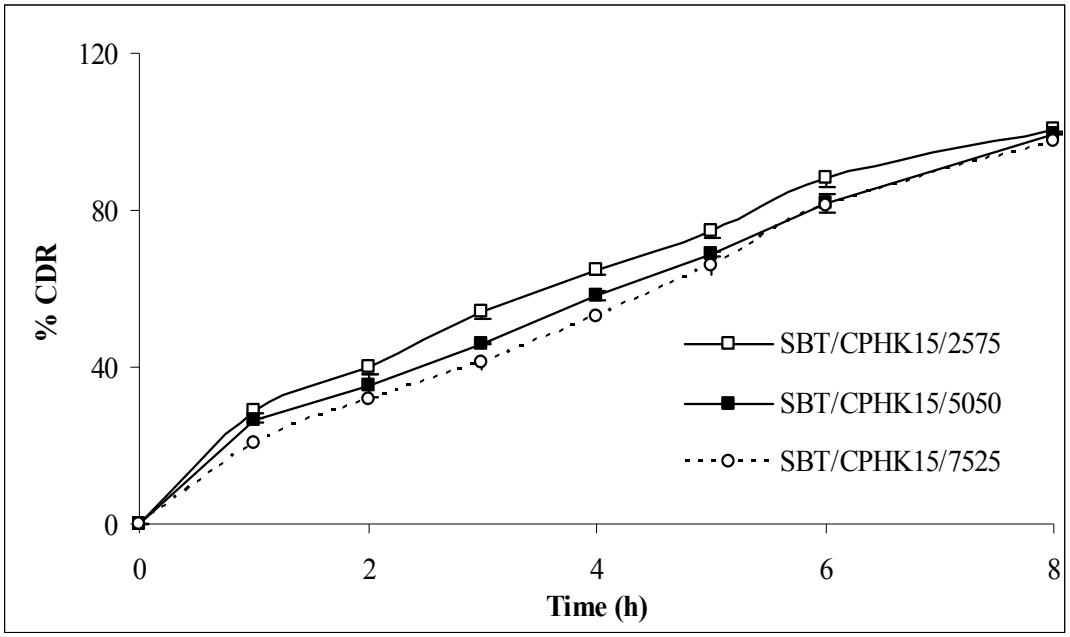

Figure 5.7: Comparative in vitro release profile of LER from tablet formulations prepared using combination of CP and HPMC K15M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

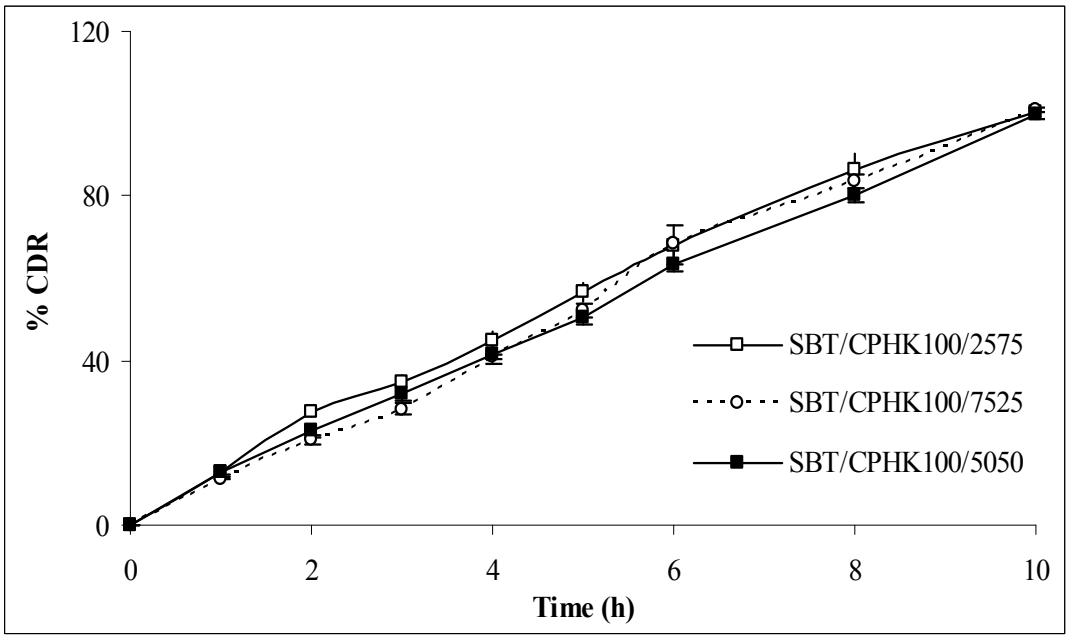

Figure 5.8: Comparative in vitro release profile of LER from tablet formulations prepared using combination of CP and HPMC K100M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

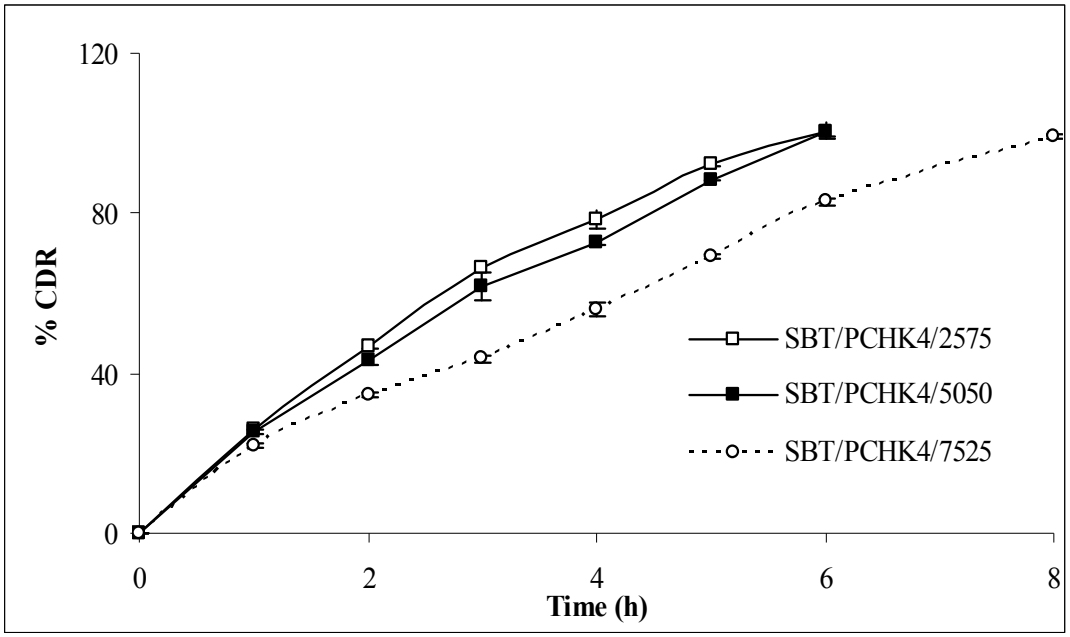

Figure 5.9: Comparative in vitro release profile of LER from tablet formulations prepared using combination of PC and HPMC K4M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

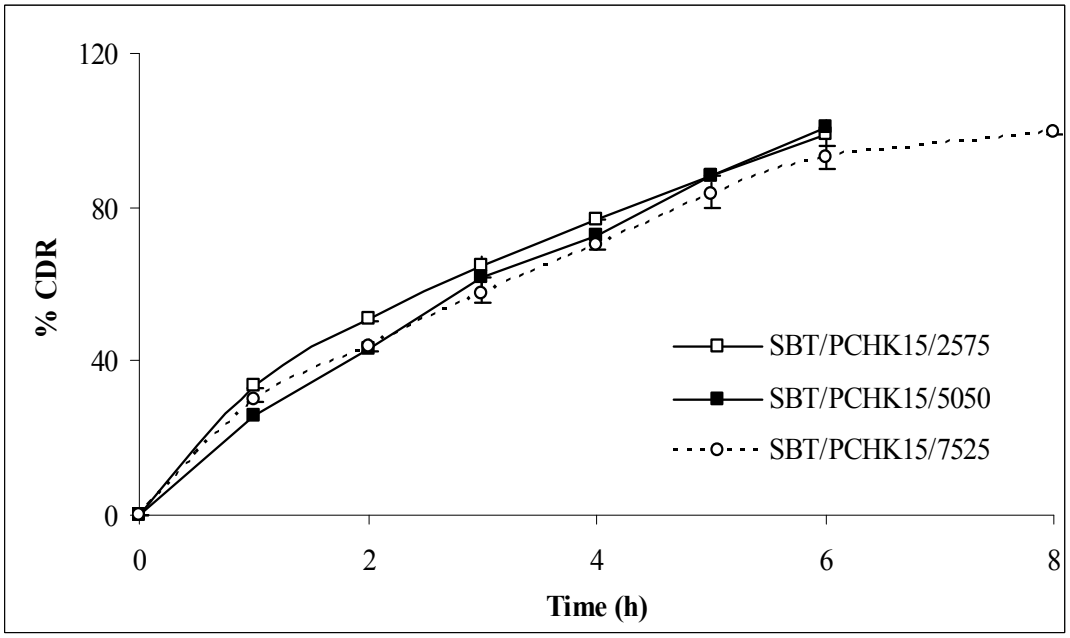

Figure 5.10: Comparative in vitro release profile of LER from tablet formulations prepared using combination of PC and HPMC K15M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

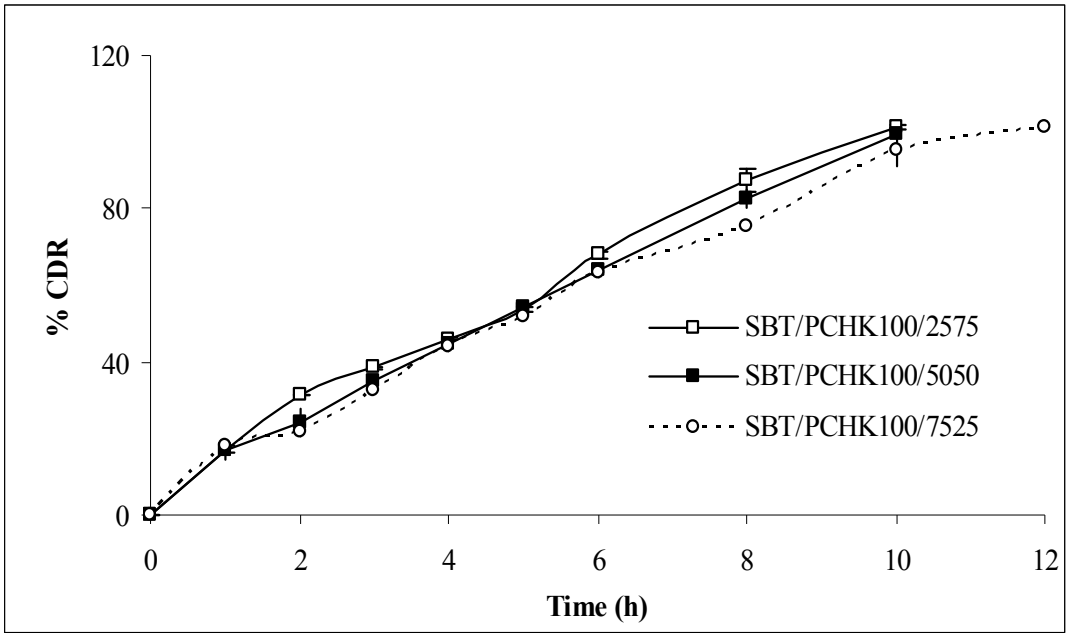

Figure 5.11: Comparative in vitro release profile of LER from tablet formulations prepared using combination of PC and HPMC K100M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

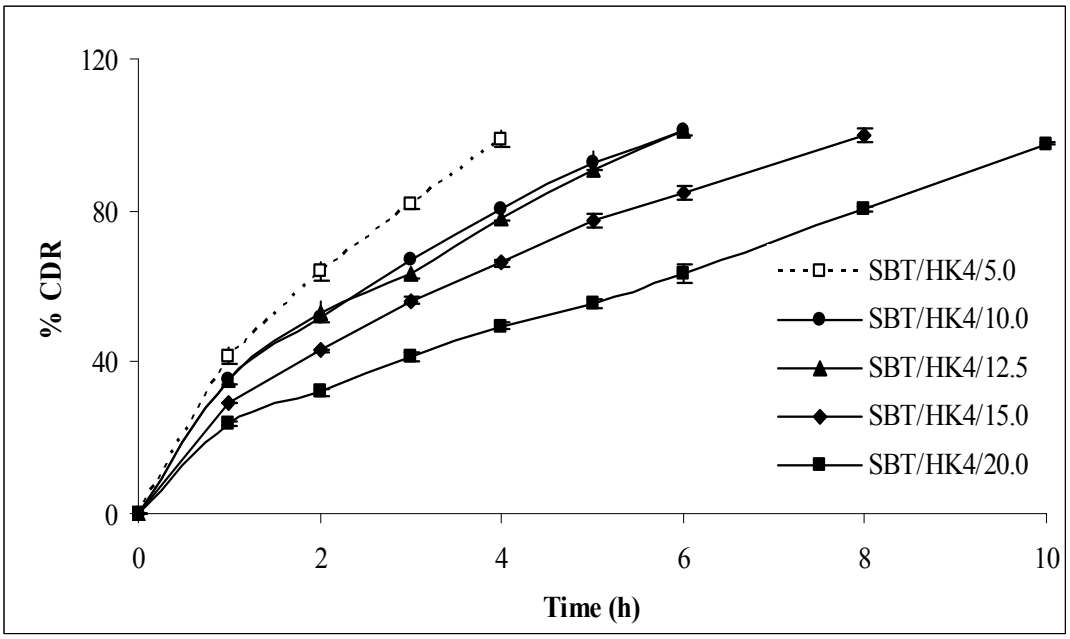

Figure 5.12: Comparative in vitro release profile of LER from tablet formulations prepared using varying proportions of HPMC K4M

(Each point represents mean and SD of three batches with triplicate determination per batch)

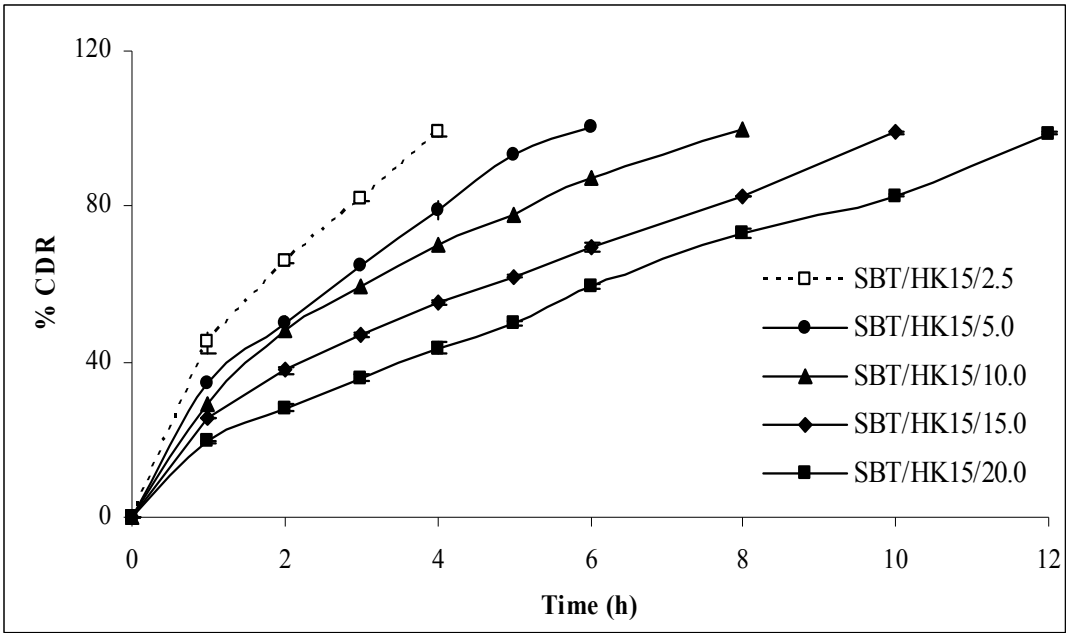

Figure 5.13: Comparative in vitro release profile of LER from tablet formulations prepared using varying proportions of HPMC K15M

(Each point represents mean and SD of three batches with triplicate determination per batch)

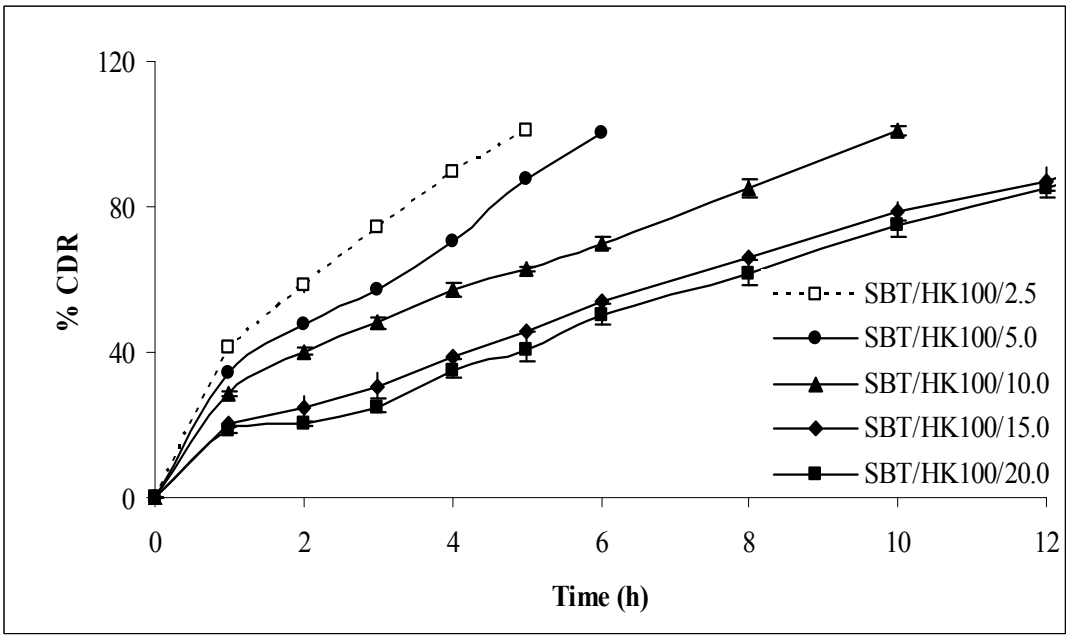

Figure 5.14: Comparative in vitro release profile of LER from tablet formulations prepared using varying proportions of HPMC K100M

(Each point represents mean and SD of three batches with triplicate determination per batch)

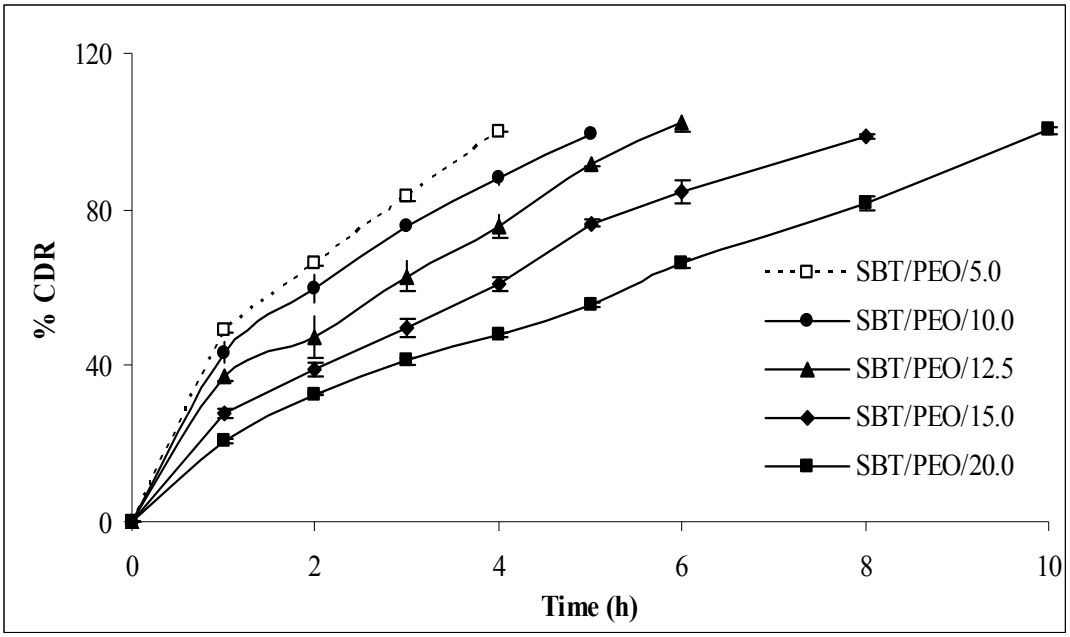

Figure 5.15: Comparative in vitro release profile of LER from tablet formulations prepared using varying proportions of PEO

(Each point represents mean and SD of three batches with triplicate determination per batch)

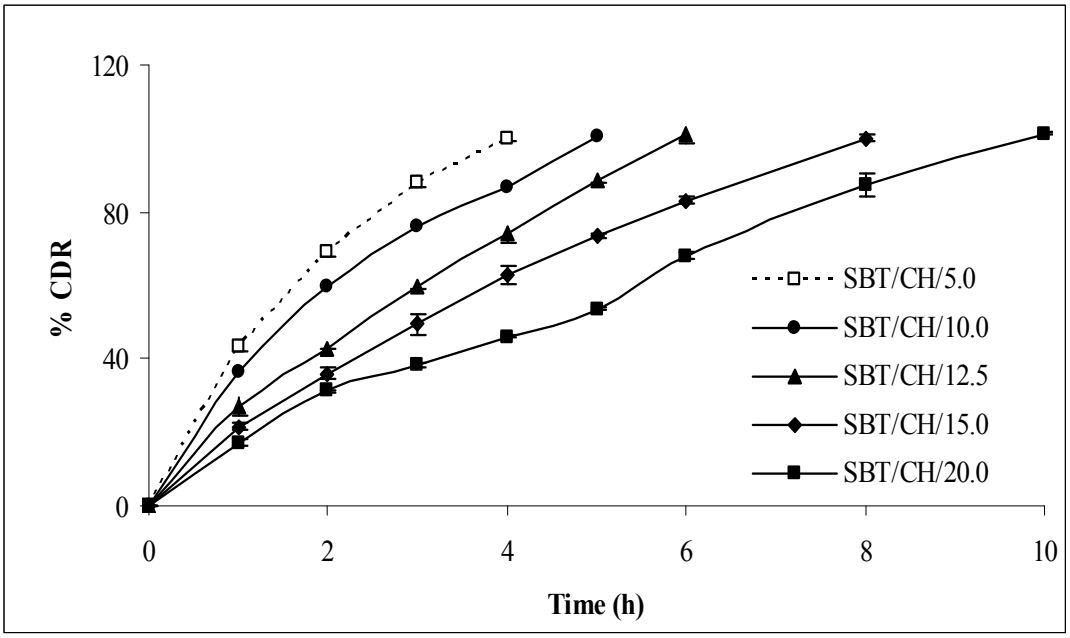

Figure 5.16: Comparative in vitro release profile of LER from tablet formulations prepared using varying proportions of CH

(Each point represents mean and SD of three batches with triplicate determination per batch)

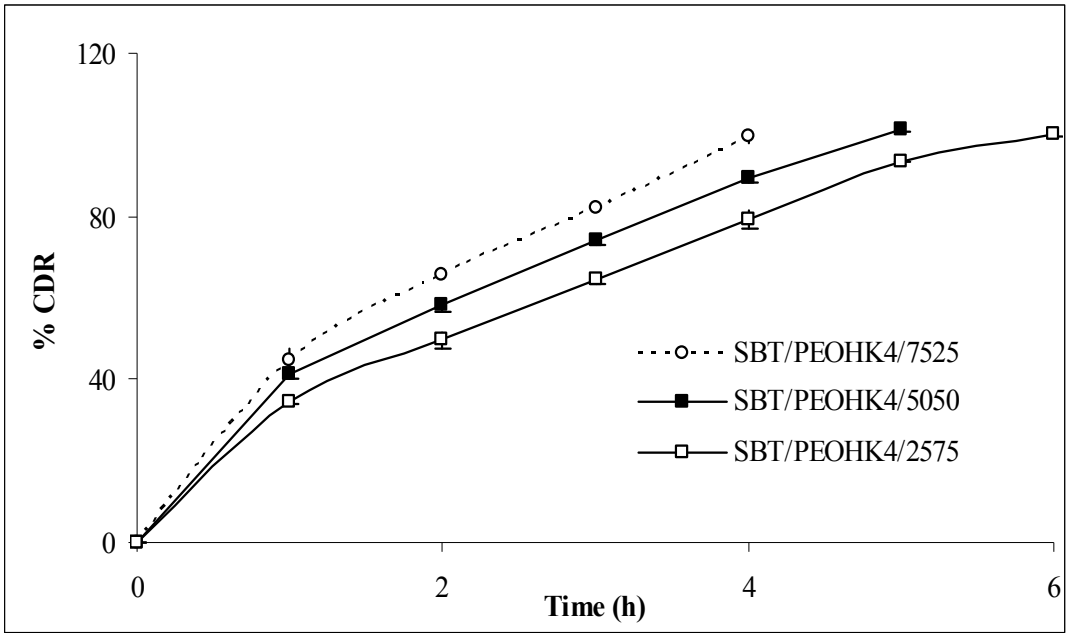

Figure 5.17: Comparative in vitro release profile of LER from tablet formulations prepared using combination of PEO and HPMC K4M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

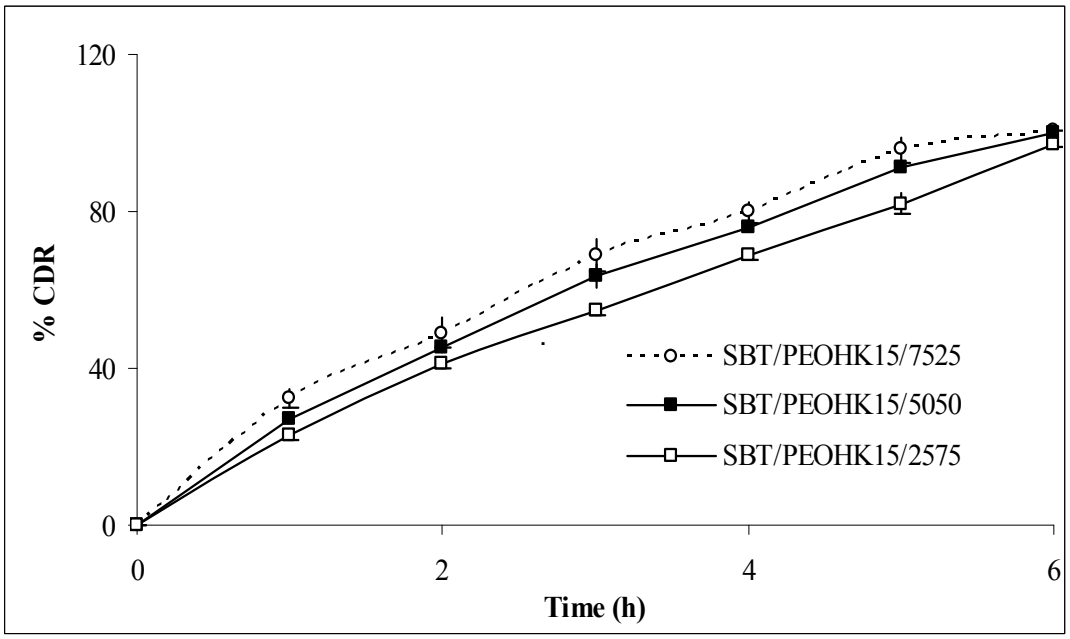

Figure 5.18: Comparative in vitro release profile of LER from tablet formulations prepared using combination of PEO and HPMC K15M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

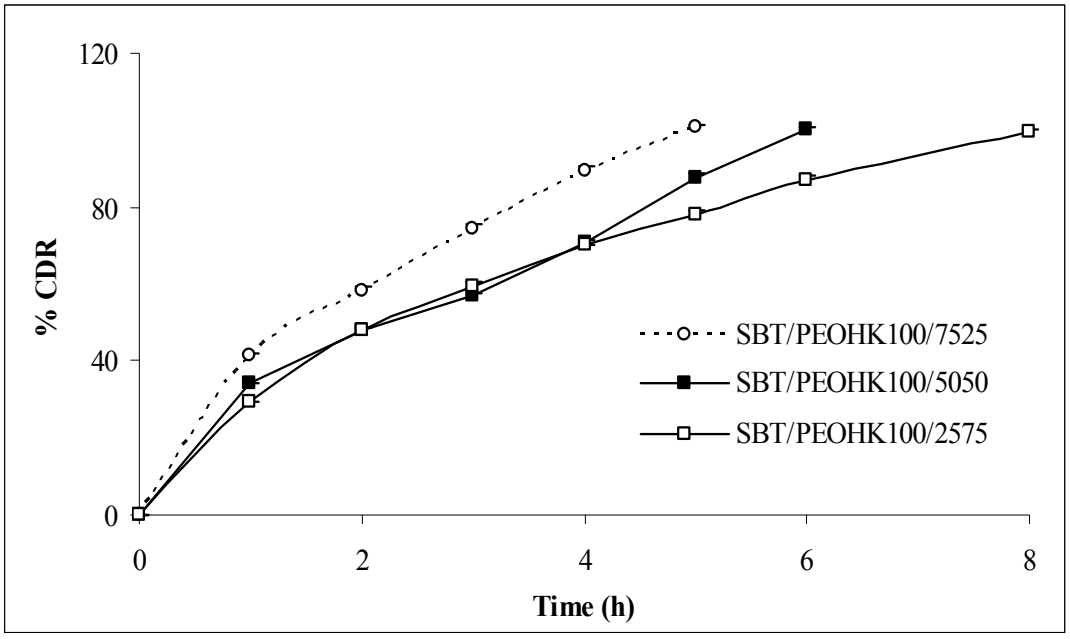

Figure 5.19: Comparative in vitro release profile of LER from tablet formulations prepared using combination of PEO and HPMC K100M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

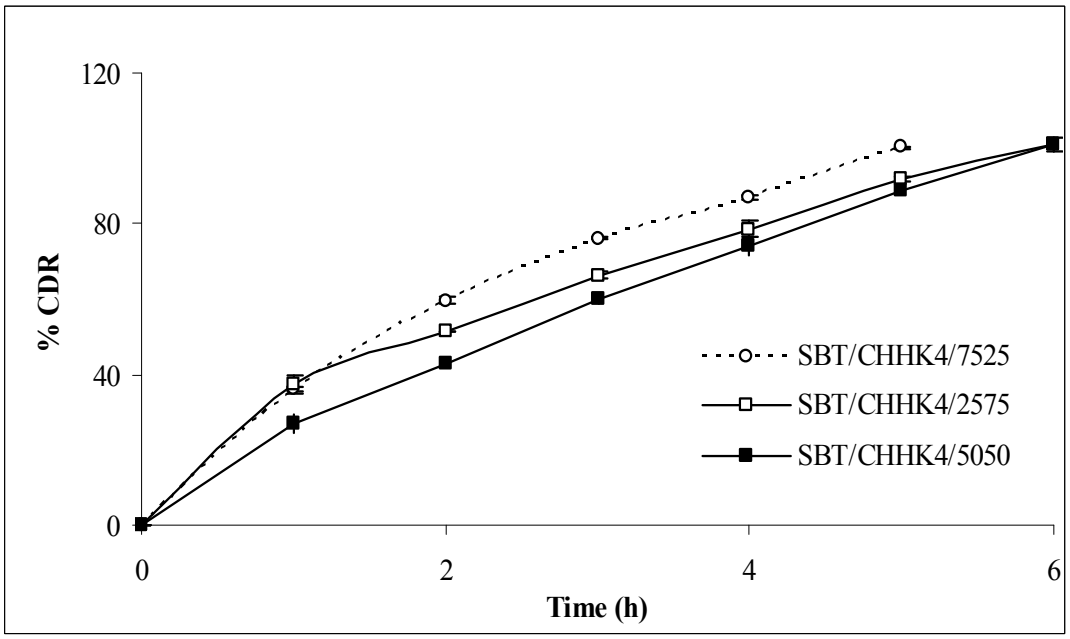

Figure 5.20: Comparative in vitro release profile of LER from tablet formulations prepared using combination of CH and HPMC K4M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

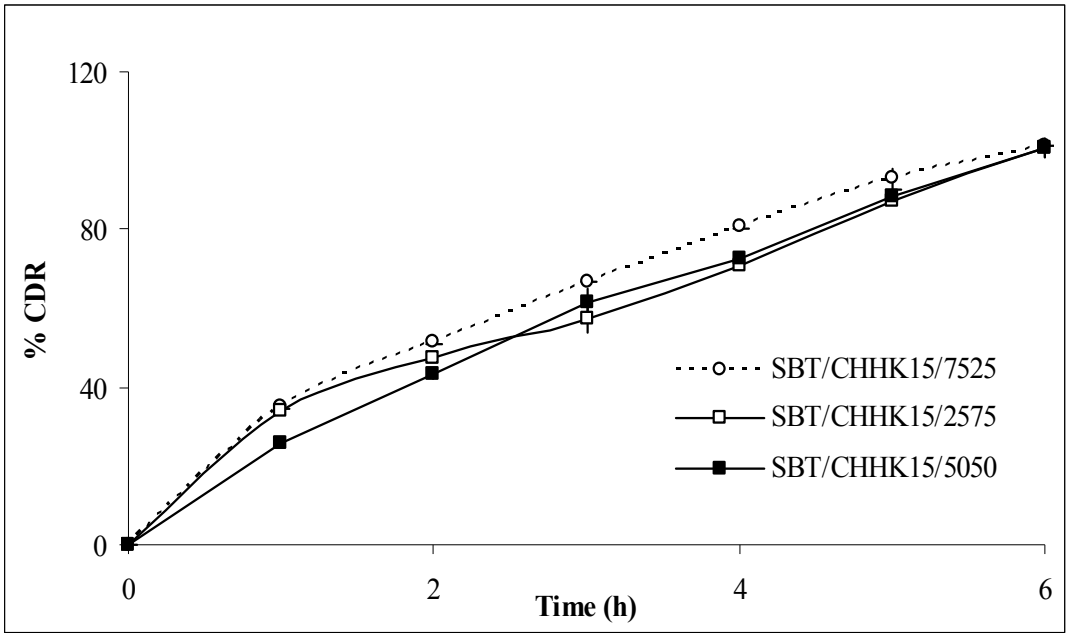

Figure 5.21: Comparative in vitro release profile of LER from tablet formulations prepared using combination of CH and HPMC K15M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

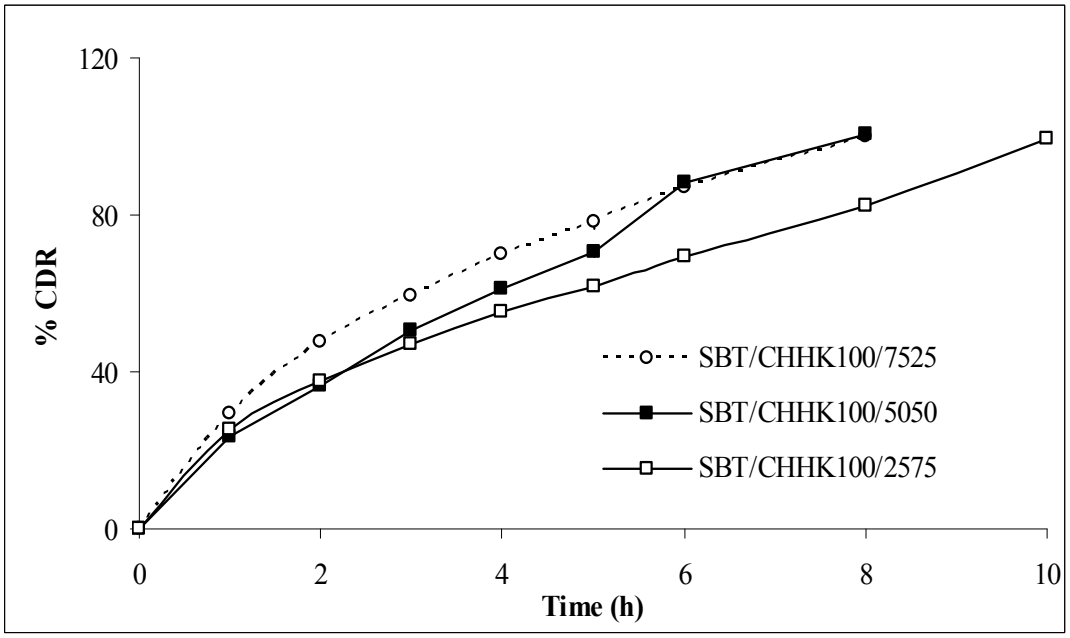

Figure 5.22: Comparative in vitro release profile of LER from tablet formulations prepared using combination of CH and HPMC K100M in varying proportions

(Each point represents mean and SD of three batches with triplicate determination per batch)

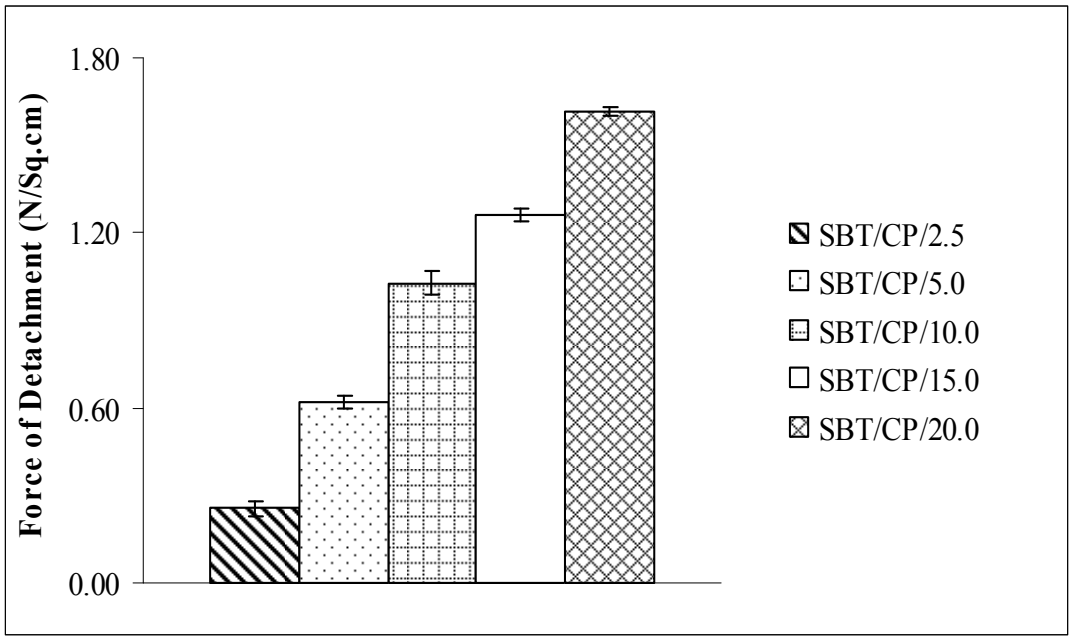

Figure 5.23: Results of in vitro mucoadhesion studies of tablet formulations prepared using varying proportions of CP using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

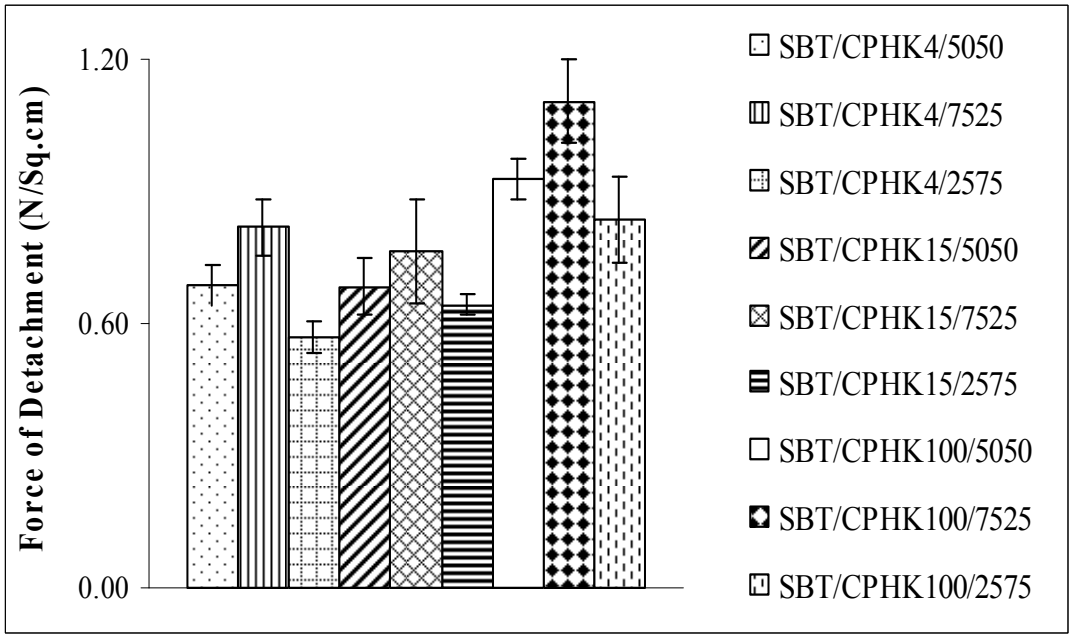

Figure 5.24: Results of in vitro mucoadhesion studies of tablet formulations prepared using combination of CP with HPMC K4M, HPMC K15M and HPMC K100M using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

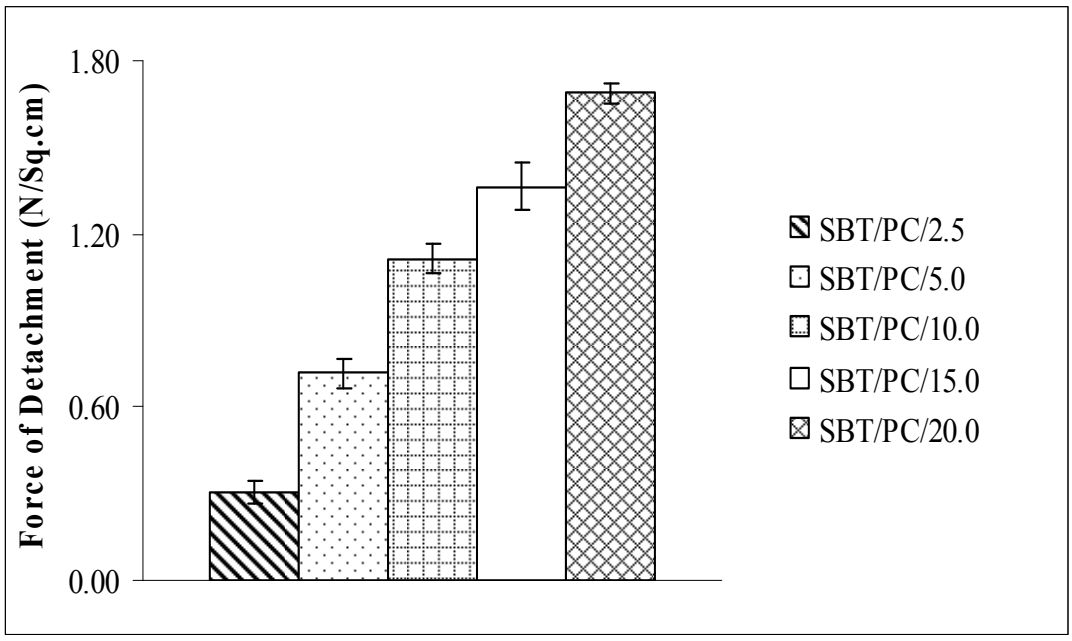

Figure 5.25: Results of in vitro mucoadhesion studies of tablet formulations prepared using varying proportions of PC using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

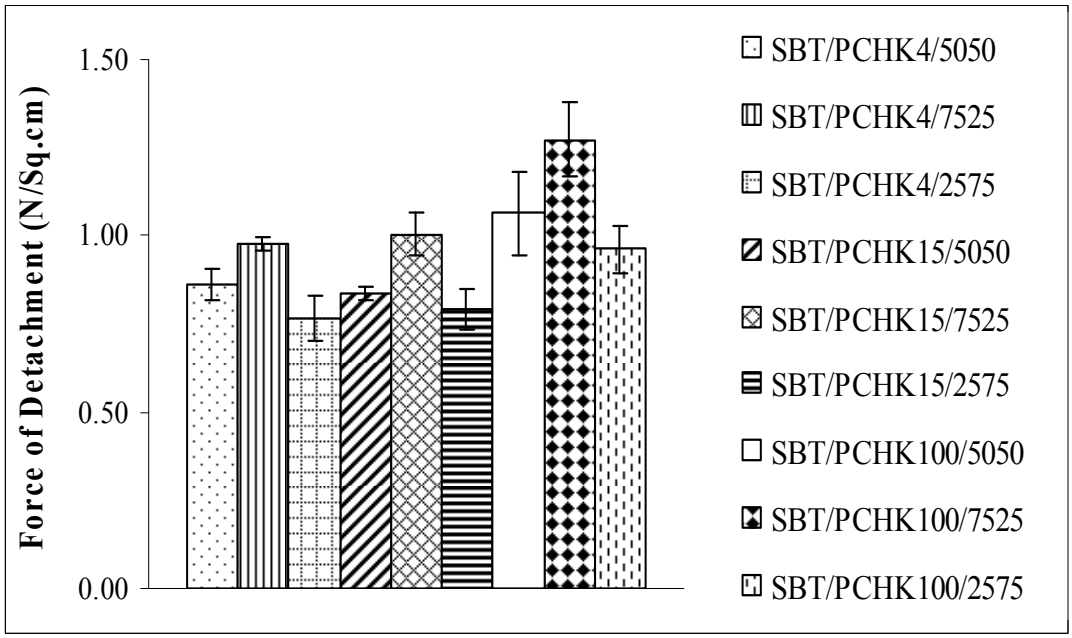

Figure 5.26: Results of in vitro mucoadhesion studies of tablet formulations prepared using combination of PC with HPMC K4M, HPMC K15M and HPMC K100M using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

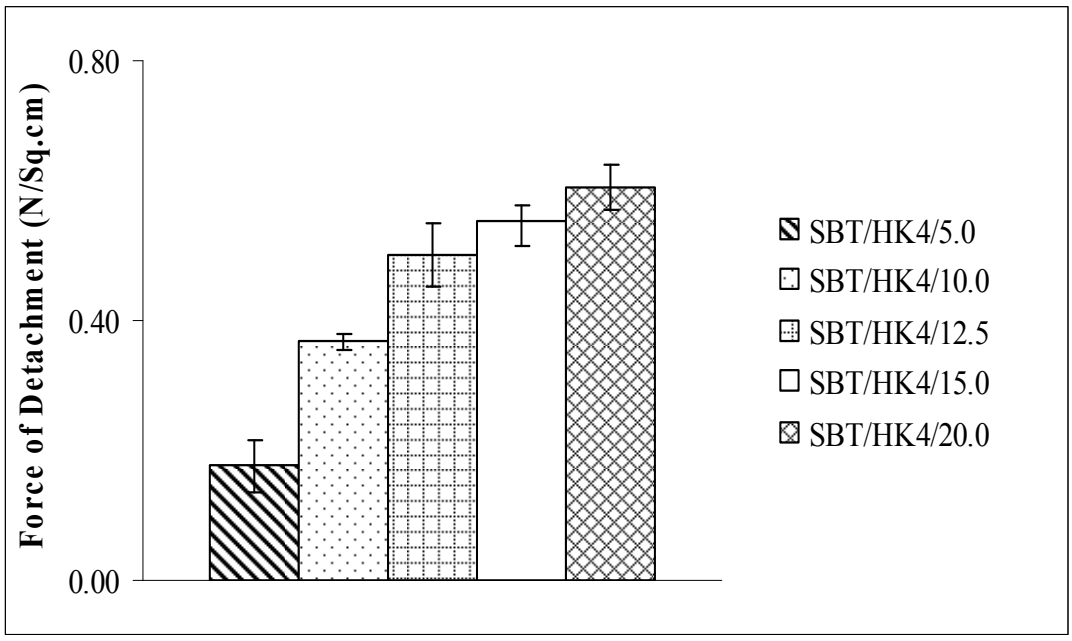

Figure 5.27: Results of in vitro mucoadhesion studies of tablet formulations prepared using varying proportions of HPMC K4M using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

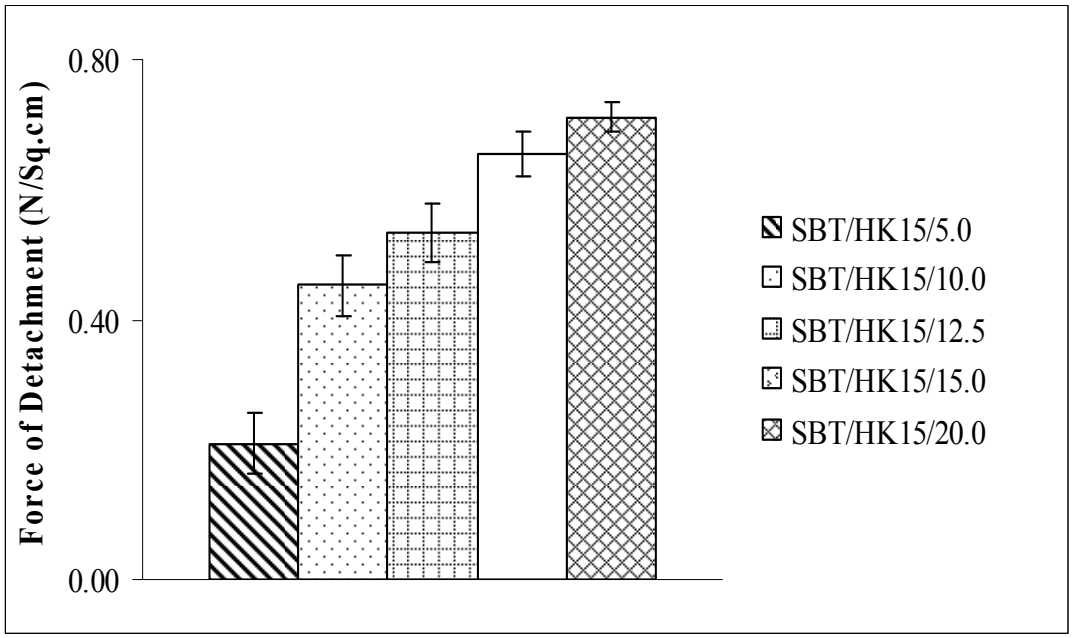

Figure 5.28: Results of in vitro mucoadhesion studies of tablet formulations prepared using varying proportions of HPMC K15M using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

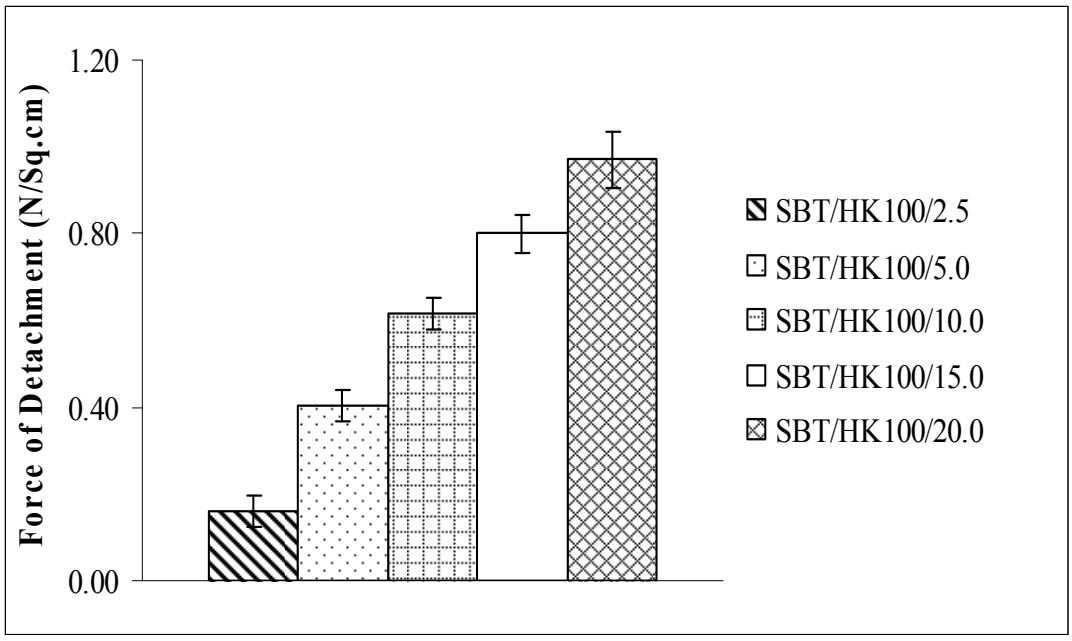

Figure 5.29: Results of in vitro mucoadhesion studies of tablet formulations prepared using varying proportions of HPMC K100M using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

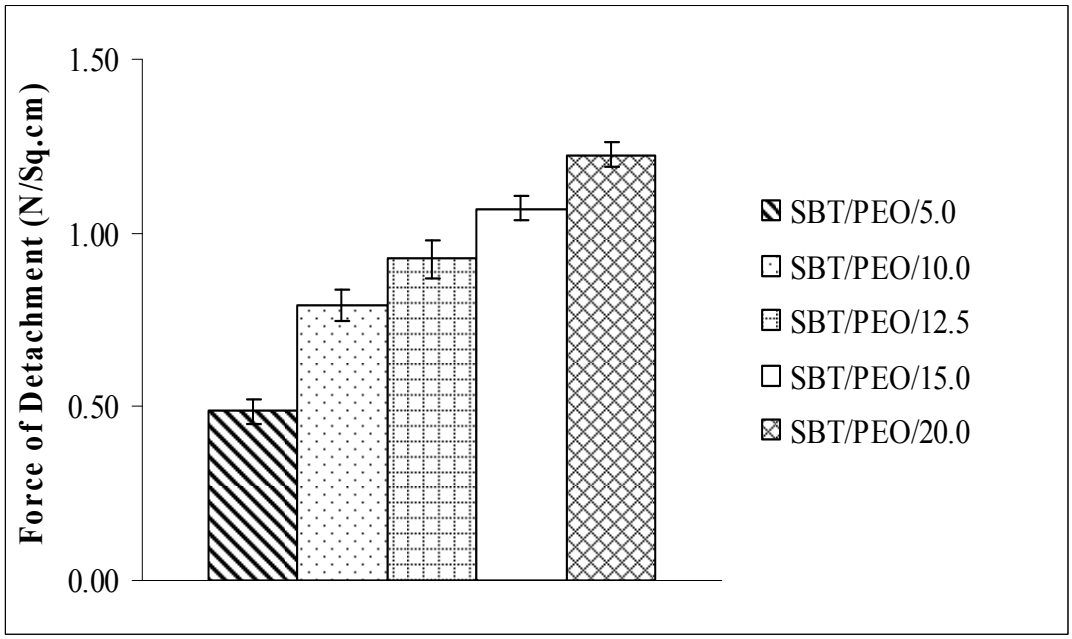

Figure 5.30: Results of in vitro mucoadhesion studies of tablet formulations prepared using varying proportions of PEO using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

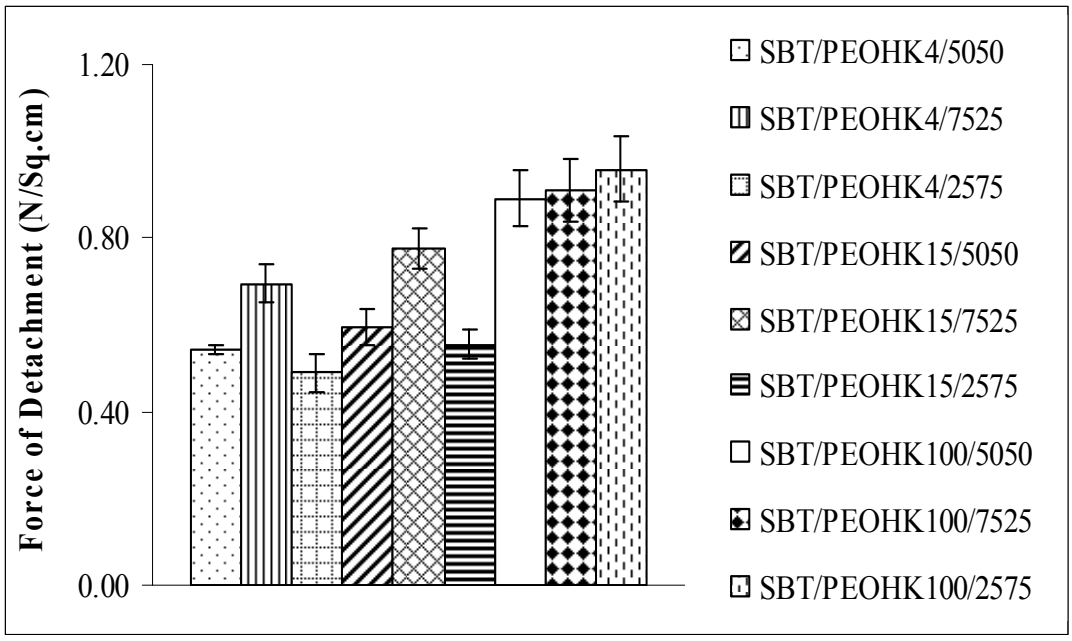

Figure 5.31: Results of in vitro mucoadhesion studies of tablet formulations prepared using combination of PEO with HPMC K4M, HPMC K15M and HPMC K100M using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

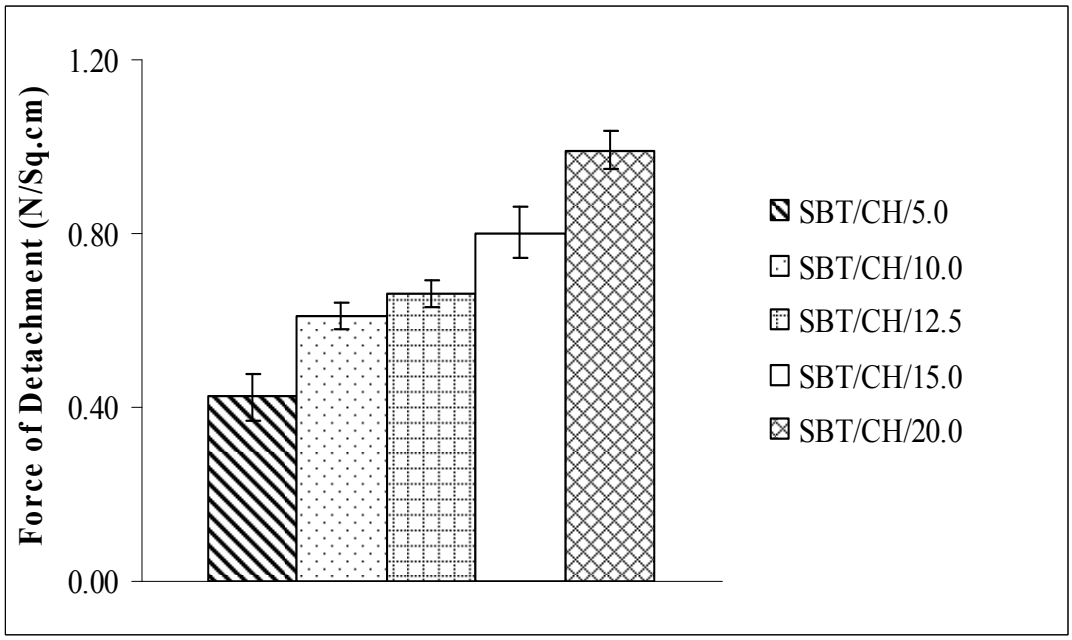

Figure 5.32: Results of in vitro mucoadhesion studies of tablet formulations prepared using varying proportions of CH using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

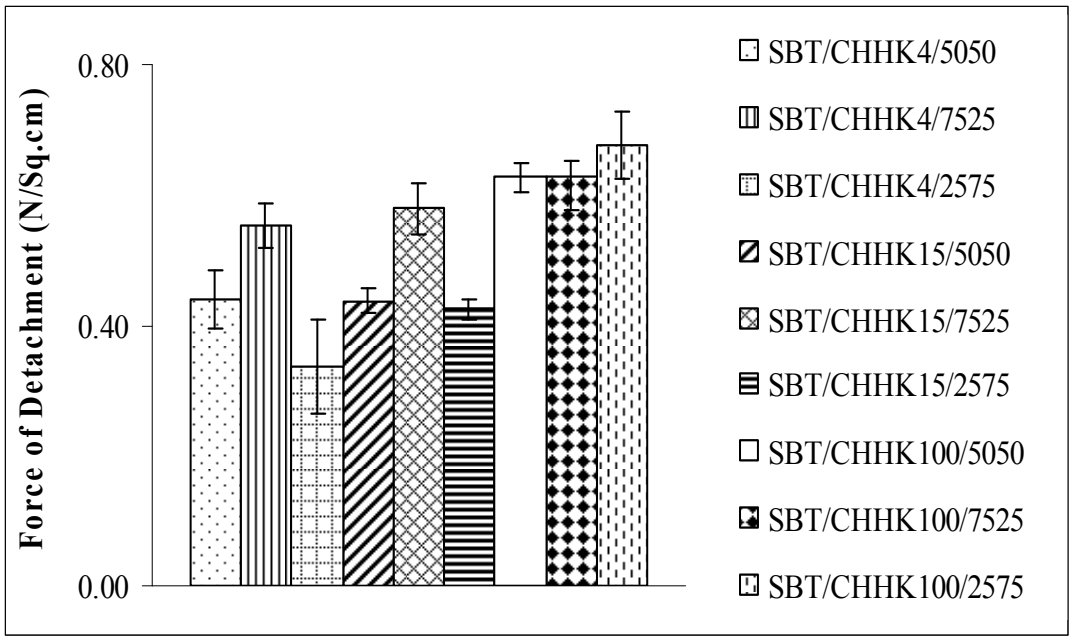

Figure 5.33: Results of in vitro mucoadhesion studies of tablet formulations prepared using combination of CH with HPMC K4M, HPMC K15M and HPMC K100M using modified balance method

(Each value represents mean and SD of three batches with triplicate determination per batch)

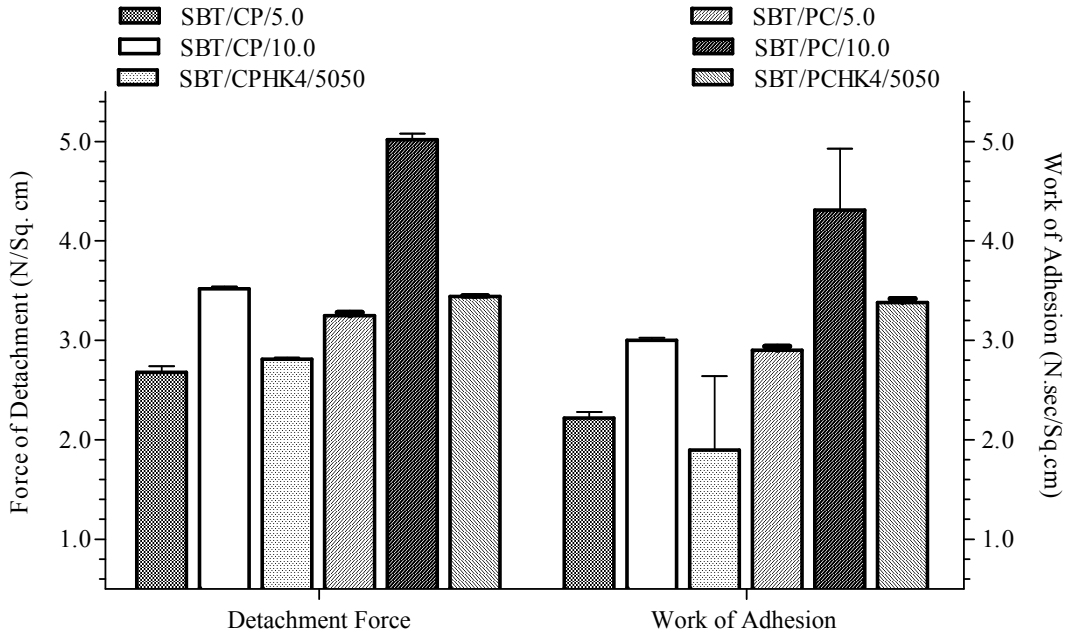

Figure 5.34: Results of mucoadhesion studies carried out using texture analyzer for tablets containing CP, PC and combination of HPMC with CP or PC

(Each value represents mean of three independent determinations with standard deviation)

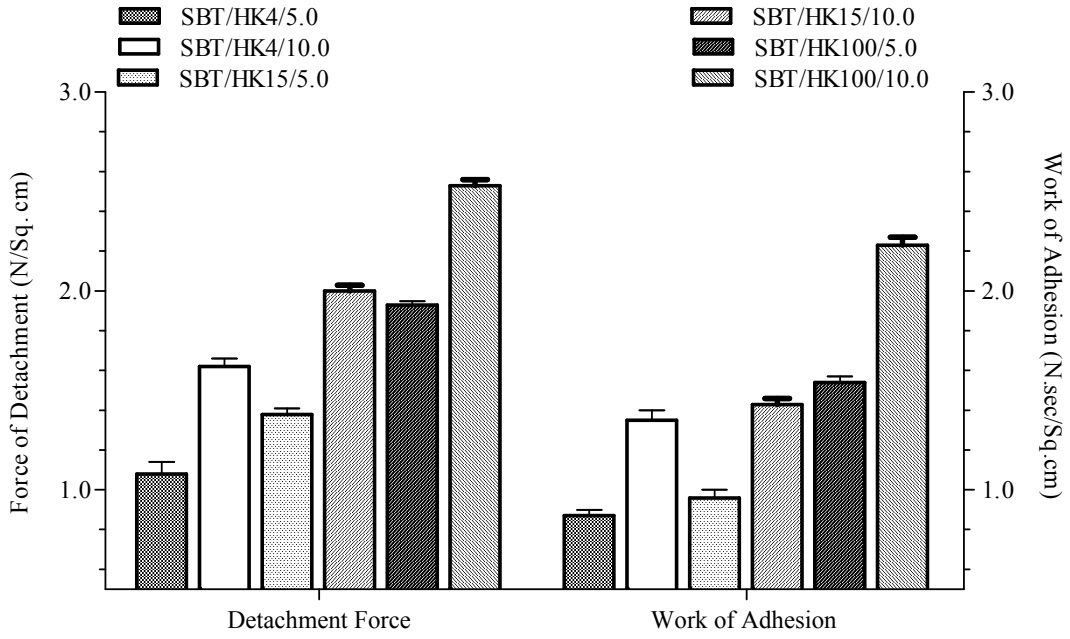

Figure 5.35: Results of mucoadhesion studies carried out using texture analyzer for tablets containing various viscosity grades of HPMC

(Each value represents mean of three independent determinations with standard deviation)

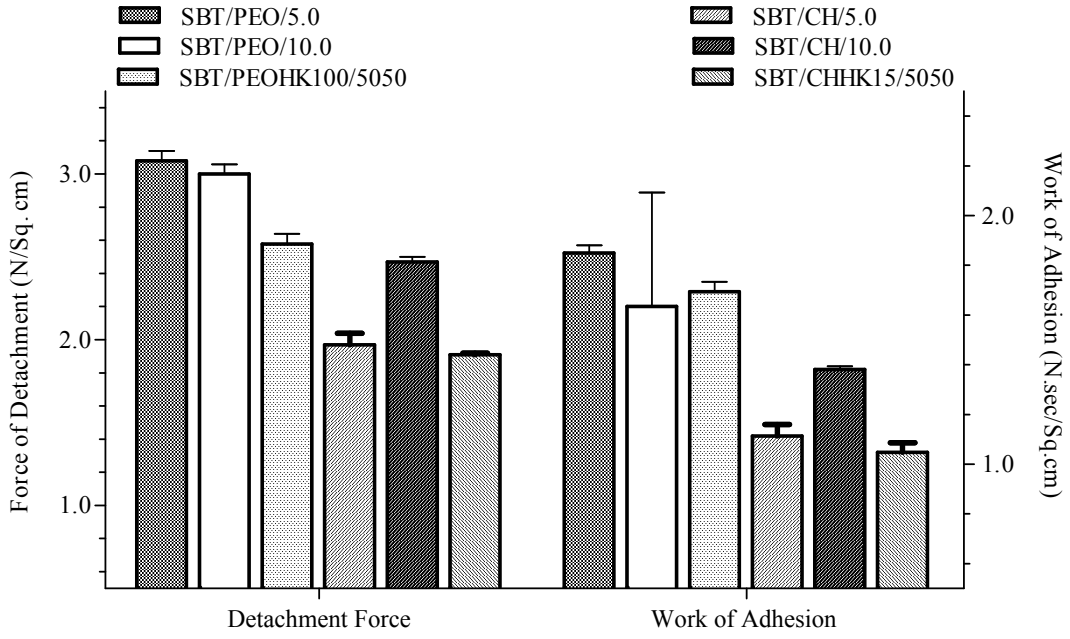

Figure 5.36: Results of mucoadhesion studies carried out using texture analyzer for tablets containing PEO, CH and combination of HPMC with PEO or CH

(Each value represents mean of three independent determinations with standard deviation)

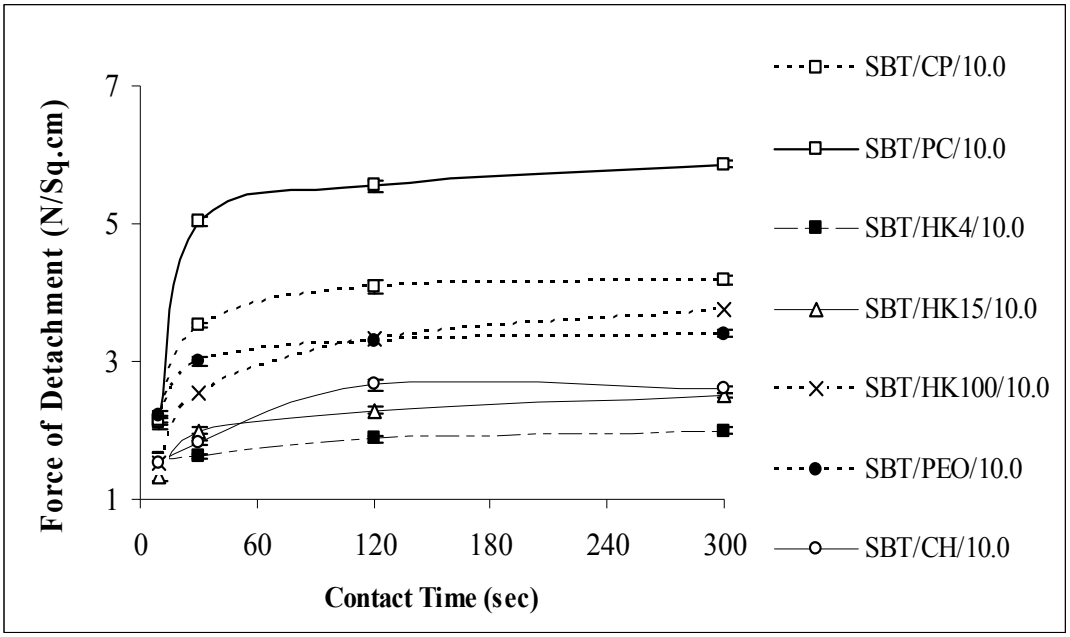

Figure 5.37: Force of detachment of tablet formulations at varying contact times
(Each value represents mean of three independent determinations with standard deviation)

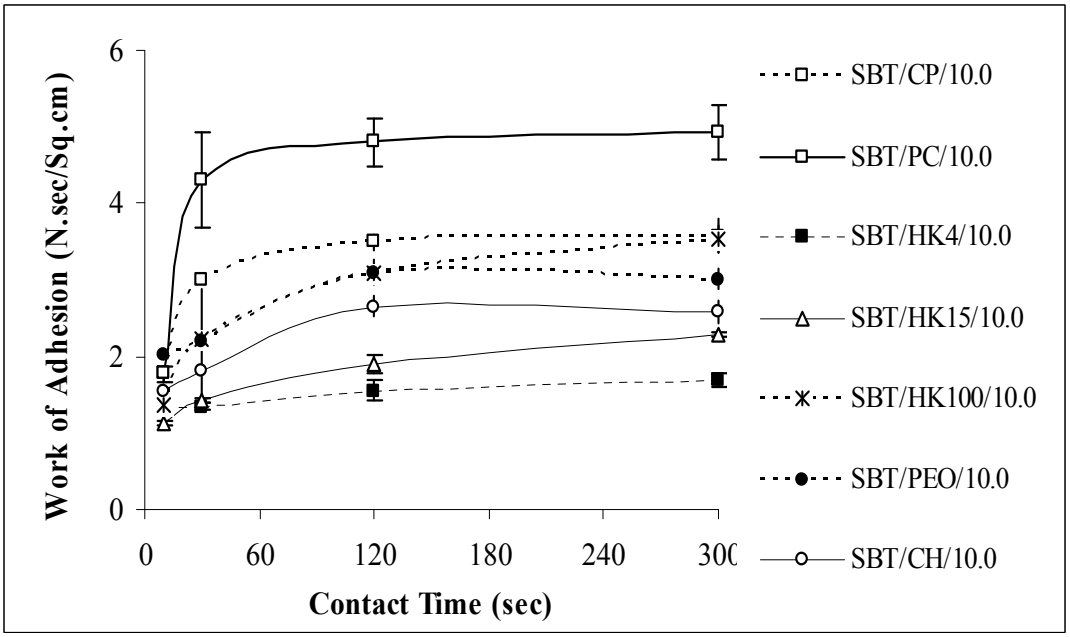

Figure 5.38: Work of adhesion of tablet formulations at varying contact times
(Each value represents mean of three independent determinations with standard deviation)

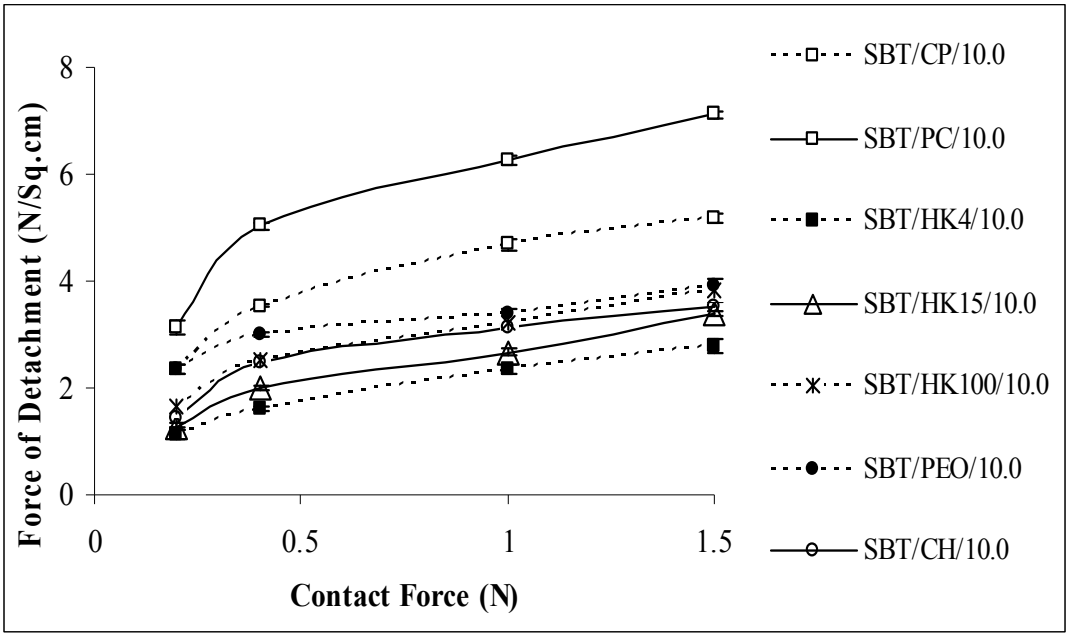

Figure 5.39: Force of detachment of tablet formulations at varying contact forces
(Each value represents mean of three independent determinations with standard deviation)

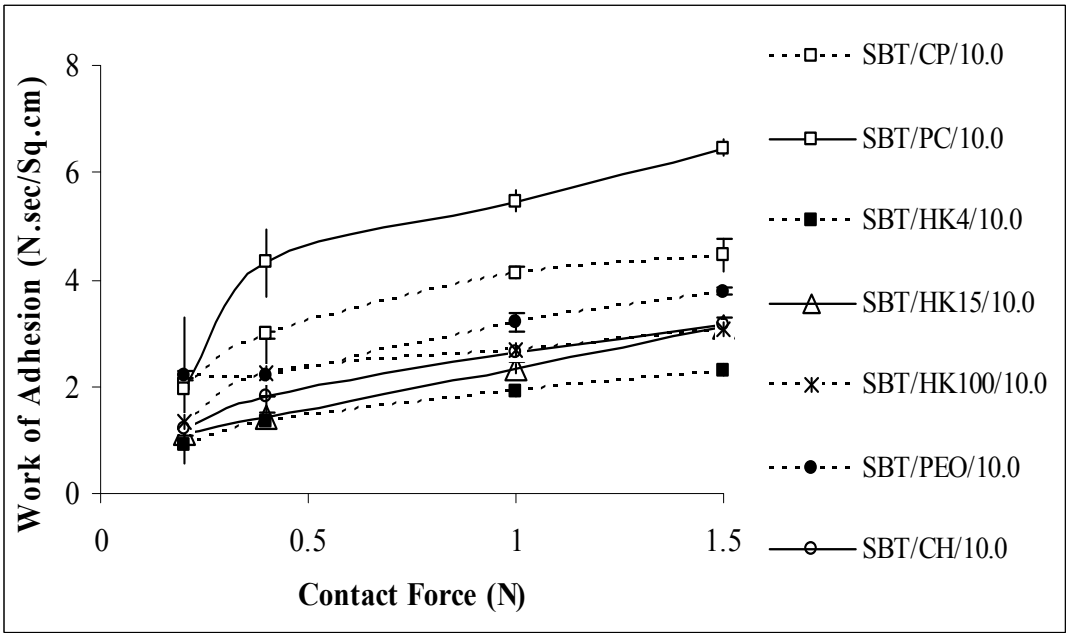

Figure 5.40: Work of adhesion of tablet formulations at varying contact forces
(Each value represents mean of three independent determinations with standard deviation)

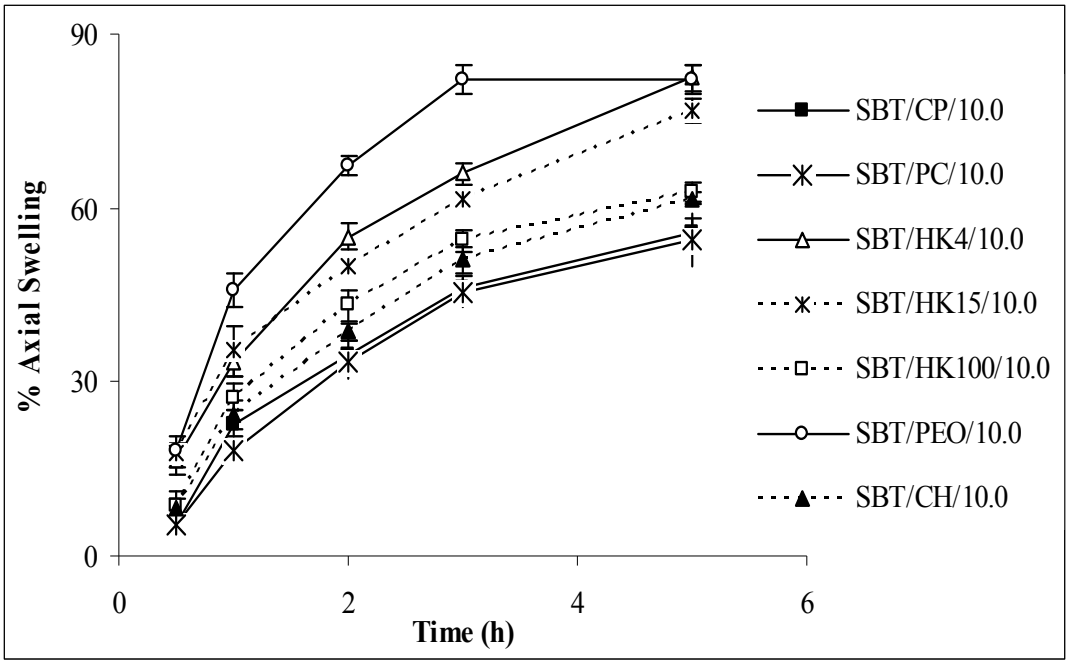

Figure 5.41: Percentage axial swelling of prepared formulations at different time points
(Each value represents mean of three independent determinations with standard deviation)

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Chapter 6
In vivo Studies

6.1 Introduction

It is essential that, in addition to in vitro evaluation, in vivo studies be carried out for developed delivery systems in appropriate animal models and/or human subjects to ascertain clinical efficacy and bioavailability. Apart from being clinically effective, an ideal buccal mucoadhesive drug delivery system should be non-irritating to buccal mucosa, should be comfortable and should not hinder with normal activities such as drinking, talking and eating. Bioavailability studies provide vital information regarding pharmacokinetic behavior and possible therapeutic efficacy of the drug and the delivery system. Buccal mucoadhesive drug delivery systems are mostly developed to enhance the bioavailability of drugs with poor oral bioavailability because of extensive first pass metabolism and degradation within GI tract. So, buccal availability of drug from developed formulation need to be compared with oral bioavailability to prove the clinical relevance of the developed formulations. Variety of animal models like rabbits (Cui and Mumper, 2002a,2002b), hamsters (Aungst, 1994), rats (Aungst and Rogers, 1989), pigs (Perioli et al., 2004; Langoth et al., 2006), dogs (Jain et al., 2002; Degim et al., 2006) and monkeys (Mehta et al., 1991) have been used and reported for carrying out bioavailability studies of buccal drug delivery systems. Advantages and disadvantages of each of these animal models have been extensively reviewed in chapter 1. Acceptability studies of buccal mucoadhesive drug delivery systems have been reported in human subjects (Desai and Pramod Kumar, 2005).

In this chapter, in vivo studies of developed buccal mucoadhesive controlled release formulations of lercanidipine hydrochloride (LER) or placebo formulations have been presented. Human acceptability studies of placebo formulations were carried out to assess the compatibility of developed formulations in human buccal mucosa. Buccal bioavailability and pharmacokinetics of LER from developed formulations was studied using male New Zealand white rabbit as an animal model. Bioavailability of LER from developed buccoadhesive formulations was compared with that of oral availability of LER from a solution formulation.

6.2 Materials

Racemic form of LER was obtained as a gift sample from Glenmark Pharmaceuticals Limited, India. Polyethylene glycol 400 (PEG 400) was purchased from Qualigens, India. Other materials and reagents used were same as mentioned in chapter 3 and chapter 4.

6.3 Human Acceptability Studies

The formulations selected for human acceptability studies were SBT/CP/10.0, SBT/PC/10.0, SBT/CH/10.0 and SBT/PEO/10.0. The complete composition of these formulations is given in Table 5.1a, 5.1c and 5.1d of chapter 5. Freshly prepared placebo formulations were used for the study. Placebo tablets of the selected formulations were prepared by replacing LER with equal quantity of lactose.

Studies were conducted with the approval of Institutional Human Ethics Committee (Protocol approval number: IHEC-02/05-06). Informed consent was obtained from human subjects selected for the study. Thorough information was given to all the selected subjects about the study and possible side effects of the study. Subjects were explained the importance of following the treatment regimen and other aspects like fasting. The study was conducted as per the guidelines given by the Institutional Human Ethics Committee and under the supervision of a registered medical practitioner. The study was conducted on 10 healthy human male subjects (aged 20 to 25 years) using placebo buccal mucoadhesive drug delivery systems. Before placing the tablet in buccal cavity, subjects were asked to wash the oral cavity using around 100 ml of TDW. Subjects were instructed to press the placebo tablets against the mucosal lining of cheek for 1 min. Water and food were not allowed for first 30 and 60 min respectively after application of tablets. Subjects were asked to record time of tablet placement and time and condition at the end of adhesion (erosion or dislodgement of tablets). Subjects were given a questionnaire to assess the acceptability of the designed tablets (Desai and Pramod Kumar, 2005). The study design was multiple parallel, so that each subject received each of the test formulations. Subjects were instructed to place successive tablet at a location opposite to the site of placement of the previous tablet.

Each formulation was tested on 10 human subjects. The percentage response of subjects for various parameters listed in questionnaire was calculated. A scoring system as shown in Table 6.1 was used to quantify subjective variables listed in the questionnaire. Average score for each formulation was calculated using summation of rating points given by each subject divided by the number of subjects. The formulation was rated as acceptable, suitable, tolerable, moderately unacceptable or unacceptable based upon the average score obtained by the formulation as shown in Table 6.2.

6.4 In vivo Pharmacokinetic Study in Rabbits

6.4.1 Animal model

New Zealand white male rabbits weighing between 2.0 to 2.5 kg were provided by the Central Animal Facility of Birla Institute of Technology and Science, Pilani. The mean weight of the animals selected for the study was 2.19 kg with a standard deviation of 0.12 kg. A prior approval from Institution Animal Ethics Committee was obtained for carrying out the study (Protocol approval number: IAEC/RES/7/4). The study was conducted as per the guidelines given by the Institutional Animal Ethics Committee and under the supervision of a registered veterinarian. Animals were housed in standard cages in light controlled room at 25 ± 2 ^{o}C and 50 ± 5% RH. Animals were issued and acclimatized 6 days prior to the actual experimentation. Animals were kept on standard pellet diet (Hindustan Lever Ltd., India) and water ab libitum during period of acclimatization. Animals were kept on fasting 8 h prior to the actual start of the experimentation. Food and water was not given to animals till 2 h after the start of the study.

6.4.2 Preparation of formulation

LER (50 mg) was accurately weighed and transferred to a 10 ml volumetric flask. To this 7 ml of 40% v/v PEG 400 in TDW was added and vortex mixed to dissolve the entire drug. The volume of the resultant solution was made up to 10 ml using 40% v/v PEG 400 in TDW to get a drug solution of 5 mg/ml. An aliquot of resultant solution was appropriately diluted and assayed using analytical method 1 of chapter 3.

Fresh batches of SBT/CP/10.0, SBT/PC/10.0, SBT/CH/10.0 and SBT/PEO/10.0 were prepared prior to animal experimentation containing 10 mg of LER. The complete composition of these formulations is given in Table 5.1a, 5.1c and 5.1d of chapter 5. Quality of the prepared formulations was evaluated by checking content uniformity, friability, crushing strength and thickness of prepared formulations. In vitro mucoadhesion and release studies were also carried out prior to using the formulations for animal studies. The methodology adopted for all these tests has been mentioned at length in chapter 5.

6.4.3 Dosing

To study the oral pharmacokinetics of LER, 2 ml of 5 mg/ml solution of LER was administered to rabbits using an oral catheter. The catheter was flushed with 5 ml of 40% v/v of PEG 400 in TDW to ensure complete dosing. The study was carried out in triplicates.

The designed buccal mucoadhesive controlled release tablet containing 10 mg LER was pre-moistened by dipping the tablet in TDW for 5 sec. The mouth of rabbit was opened using specially designed mouth restrainers and the pre-moistened tablet was placed in the buccal cavity using forceps. The tablet was pressed gently against mucosal lining of cheek for 1 min to ensure adhesion. The entire study was carried out in triplicates. Each rabbit was dosed with specific dose (10 mg) of LER without taking weight of the rabbit into consideration (Holm and Norling, 2007).

6.4.4 Blood sample collection and processing

For each study, blood samples (1 ml) were withdrawn from the marginal ear vein at 0.25, 0.50, 0.75, 1.00, 2.00, 4.00, 6.00, 9.00, 12.00, 18.00, 24.00 h post dosing using a 21 G needle in clean and dry glass centrifuge tubes. Blood sample was also collected prior to dosing from all the rabbits. The blood collected was harvested for 45 min at room temperature and centrifuged at 2000 RPM for 20 min. The clear supernatant serum layer was collected and stored at -20 ^{o}C until analysis.

6.4.5 Sample analysis

Frozen serum samples were thawed by keeping the sealed tubes at room temperature (25 ± 2 ^{o}C) for at least 60 min and analyzed using analytical method 4 of chapter 3. The serum drug concentration at various time points of the study was thus measured.

6.4.6 Data analysis

The serum drug concentration versus time data of LER obtained during various sets of studies was subjected to non-compartmental analysis using WinNonlin Standard edition, Version 2.1 (WinNonlin Scientific Consultants, USA) to acquire various pharmacokinetic parameters.

6.5 Results and Discussion

6.5.1 Human acceptability studies

The rating of formulations based upon average score is presented in Table 6.3. All the formulations were found to be acceptable, with maximum average score of 25.20 for tablets prepared using PEO. The percentage response of human subjects to various parameters of questionnaire for each of the formulations is presented in Table 6.4.

The mean time of adhesion of designed placebo formulations to human buccal mucosa is presented in Table 6.3 and depicted in Figure 6.1. These results were in accordance with results of in vitro evaluation using texture analyzer (section 5.5.4.2 of chapter 5). Tablets prepared using PC and CH showed maximum and minimum time of adhesion to human buccal mucosa respectively. None of the formulation showed dislodgement of designed tablet during course of the study indicating sufficient adherence (Table 6.4). However, problem of particle shredding was seen with formulations prepared using PEO and CH. None of the subjects reported significant discomfort after application of designed systems.

Based on these results it can be concluded that all of the designed formulations were non-irritating and acceptable with no apparent toxicity when used in human subjects.

6.5.2 In vivo pharmacokinetic study in rabbits

The serum concentration versus time profiles of LER following administration of 10 mg single dose by oral and buccal routes are given in Figure 6.2. Summary of pharmacokinetic parameters obtained using non-compartmental data analysis are listed in Table 6.5.

Following oral dose (10 mg), LER was rapidly absorbed resulting in maximum serum concentration of (C_{max}) of 140.07 ± 19.58 ng/ml, 1.00 h post dosing (Figure 6.2). The elimination rate constant was found to be 0.08 ± 0.02 h^{-1}. Serum concentration of LER was detectable up to 18 h post dosing. $AUC_{(0-\infty)}$ was found to be 1347.03 ± 63.06 ng h/ml (Table 6.5).

The pharmacokinetic parameters of designed buccal mucoadhesive formulations were compared to orally obtained parameters. For buccal tablets prepared with CP (SBT/CP/10.0), C_{max} of 226.67 ± 36.66 ng/ml was observed 2.00 h after dosing. LER was detectable till 24 h in serum after dosing with $AUC_{(0-\infty)}$ value of 2565.33 ± 419.60 ng h/ml (Table 6.5). The difference in C_{max} and $AUC_{(0-\infty)}$ values following administration of oral solution and SBT/CP/10.0 was found to be statistically significant at 5% level of significance. Bioavailability of LER following administration of SBT/CP/10.0 was found to be 1.90 times relative to bioavailability of oral solution (Table 6.5).

Buccal formulations prepared using PC (SBT/PC/10.0) resulted in C_{max} of 208.71 ± 33.48 ng/ml, 2.00 h post dosing. C_{max} and $AUC_{(0-\infty)}$ values following administration of SBT/PC/10.0 were significantly higher ($p<0.05$) compared to values of C_{max} and $AUC_{(0-\infty)}$ achieved after oral dosing. Relative bioavailability of 1.78 was obtained

compared to the oral route following administration of formulation prepared using PC (Table 6.5).

Formulation prepared using CH (SBT/CH/10.0) showed maximum bioavailability of all the formulations with C_{max}, $AUC_{(0-\infty)}$ and time to reach C_{max} (T_{max}) values of 273.79 ± 55.12 ng/ml, 3072.07 ± 430.92 ng h/ml and 2.00 h respectively. Almost two-fold increase in C_{max} was obtained for this formulation compared to oral solution of LER with relative bioavailability of 2.28. This increase in C_{max} and $AUC_{(0-\infty)}$ were significant at 5% level of significance (Table 6.5). Formulations prepared with PEO (SBT/PEO/10.0) also showed significant increase in C_{max} and $AUC_{(0-\infty)}$ at 5% level of significance compared to oral solution of LER with relative bioavailability of 1.85 (Table 6.5).

Formulations prepared with chitosan (CH) as adhesive and rate-controlling polymer resulted in maximum bioavailability. Literature suggests that chitosan acts as penetration enhancer by disrupting the intercellular lipid organization of the buccal epithelium (Senel et al., 2000; Portero et al., 2002; Martin et al., 2003; Nicolazzo et al., 2005). This explains higher bioavailability of chitosan based buccal formulations. Formulations prepared using CP, PC and PEO showed higher bioavailability when compared to orally administered solution. However, CH based formulations resulted in significantly (p<0.05) higher bioavailability when compared to CP, PC and PEO based formulations.

All the designed formulations showed higher C_{max} and $AUC_{(0-\infty)}$ values when compared to same dose of LER administered orally, indicating significant increase in bioavailability. LER was detectable in serum till 24 h post dosing when given by buccal route. This enhanced bioavailability can be attributed to reduced first pass metabolism of LER when administered via buccal route. Moreover, other disadvantages of LER like erratic oral absorption and interaction with food can also be potentially overcome by designed buccal drug delivery systems.

6.6 Conclusions

The designed buccal mucoadhesive tablets were found to be non-irritating and acceptable in human subjects. In vivo human acceptability studies demonstrated that the designed tablets adhered well for atleast 4 h without any discomfort to human subjects. In vivo studies of selected formulations (SBT/CP/10.0, SBT/PC/10.0, SBT/PEO/10.0 and SBT/CH/10.0) in rabbits demonstrated significant increase in bioavailability of lercanidipine hydrochloride due to reduction in the first pass metabolism. Polymers used in the tablet matrix also played an important role in enhancement of absorption and bioavailability. Formulation prepared using CH resulted in maximum increase of

bioavailability. So, it can be concluded that the designed buccal mucoadhesive tablet formulations are promising and may lead to substantial dose reduction, more predictable serum drug concentration profile and longer duration of action of LER as compared to conventional marketed preparations.

Table 6.1: Scoring system followed for evaluating acceptability of formulations in humans

Criteria	Rating Points
Irritation	
None	3
Slight	2
Moderate	1
Severe	0
Comfort	
Very Comfortable	4
Comfortable	3
Slightly Uncomfortable	2
Moderately Uncomfortable	1
Severely Uncomfortable	0
Taste	
Very Pleasant	4
Pleasant	3
Normal	2
Slightly Unpleasant	1
Very Unpleasant	0
Dryness of mouth	
None	3
Slight	2
Moderate	1
Severe	0
Heaviness of delivery system	
None	3
Slight	2
Moderate	1
Severe	0
Hindrance during drinking	
None	3
Slight	2
Moderate	1
Severe	0
Hindrance during eating	
None	3
Slight	2
Moderate	1
Severe	0
Dislodgement of the system during study	
No	1
Yes	0
Hindrance during speaking	
None	3
Slight	2
Moderate	1
Severe	0
Maximum possible rating points	**27**

Table 6.2: Rating given to formulations based upon average score

Rating	Score Range
Severely unacceptable	< 5
Moderately unacceptable	5 - 10
Tolerable	10 - 15
Suitable	15 – 20
Acceptable	> 20

Table 6.3: Results of human acceptability studies of the designed formulations

Formulation Code	Average Score[a]	Rating	Mean Adhesion Time[b] ± SD (h)
SBT/CP/10.0	23.90	Acceptable	5.01 ± 0.73
SBT/PC/10.0	24.00	Acceptable	5.25 ± 0.39
SBT/CH/10.0	23.70	Acceptable	4.27 ± 0.26
SBT/PEO/10.0	25.20	Acceptable	4.56 ± 0.37

[a] Average score was calculated by adding sum of ratings given by each subject divided by the number of subjects
[b] Mean of 10 subjects

Table 6.4: Percentage response of healthy human male subjects to various parameters

Criteria	Percentage Subject Response[a]			
	SBT/CP/10.0	SBT/PC/10.0	SBT/CH/10.0	SBT/PEO/10.0
Irritation				
None	80	70	70	90
Slight	20	20	30	10
Moderate		10		
Severe				
Comfort				
Very Comfortable	60	80	60	50
Comfortable	40	20	40	50
Slightly Uncomfortable				
Moderately Uncomfortable				
Severely Uncomfortable				
Taste				
Very Pleasant	10		10	40
Pleasant	50	60	30	50
Normal	40	40	50	10
Slightly Unpleasant			10	
Very Unpleasant				
Dryness of mouth				
None	80	70	60	90
Slight	20	30	40	10
Moderate				
Severe				
Heaviness of delivery system				
None	60	70	90	90
Slight	30	20	10	
Moderate	10	10		10
Severe				
Hindrance during drinking				
None	100	100	90	90
Slight			10	10
Moderate				
Severe				
Hindrance during eating				
None	60	70	70	90
Slight	30	30	30	10
Moderate	10			
Severe				
Dislodgement of system during study				
No	100	100	100	100
Yes				
Hindrance during speaking				
None	100	100	90	100
Slight			10	
Moderate				
Severe				

[a] Each formulation was given to 10 subjects

Table 6.5: Summary of pharmacokinetic parameters of LER following administration of single dose of LER (10 mg) by oral and buccal route (Mean ± SD for 3 rabbits)

Pharmacokinetic Parameter	**Oral Solution**	**SBT/CP/10.0***	**SBT/PC/10.0***	**SBT/CH/10.0***	**SBT/PEO/10.0***
C_{max}[a] (ng/ml)	140.07 ± 19.58	226.67 ± 36.66	208.71 ± 33.48	273.79 ± 55.12	216.69 ± 39.54
T_{max}[b] (h)	1.00	2.00	2.00	2.00	2.00
Elimination Rate Constant[c] (h^{-1})	0.08 ± 0.02	0.09 ± 0.00	0.09 ± 0.00	0.09 ± 0.01	0.08 ± 0.00
$AUC_{(0-\infty)}$[d] (ng h/ml)	1347.03 ± 63.06	2565.33 ± 419.60	2403.75 ± 354.39	3072.07 ± 430.92	2492.88 ± 461.61
$AUMC_{(0-\infty)}$[e] (ng h^2/ml)	15953.09 ± 2459.95	29366.48 ± 3883.49	27084.84 ± 2853.24	34943.72 ± 4364.23	29959.06 ± 5588.27
MRT[f] (h)	11.84 ± 2.40	11.45 ± 0.40	11.27 ± 0.55	11.37 ± 0.86	12.02 ± 0.04
F_r[g]	-	1.90	1.78	2.28	1.85

[a] C_{max}: Maximum serum concentration
[b] T_{max}: Time to reach C_{max}
[c] Elimination rate constant was calculated using MRT
[d] $AUC_{(0-\infty)}$: Area under the serum concentration-time curve
[e] $AUMC_{(0-\infty)}$: Area under the first moment curve
[f] MRT: Mean residence time
[g] Fr: Relative bioavailability with respect to oral solution
* $p < 0.05$

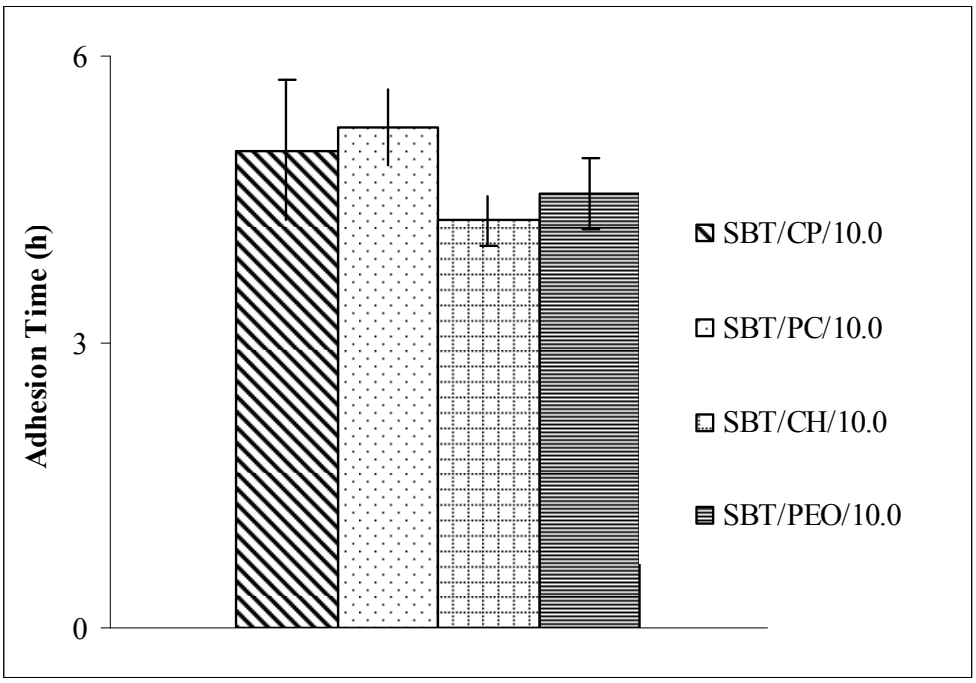

Figure 6.1: Results of mucoadhesion studies of tablet formulations in healthy human male subjects

(Each value represents mean of 10 independent determinations with standard deviation)

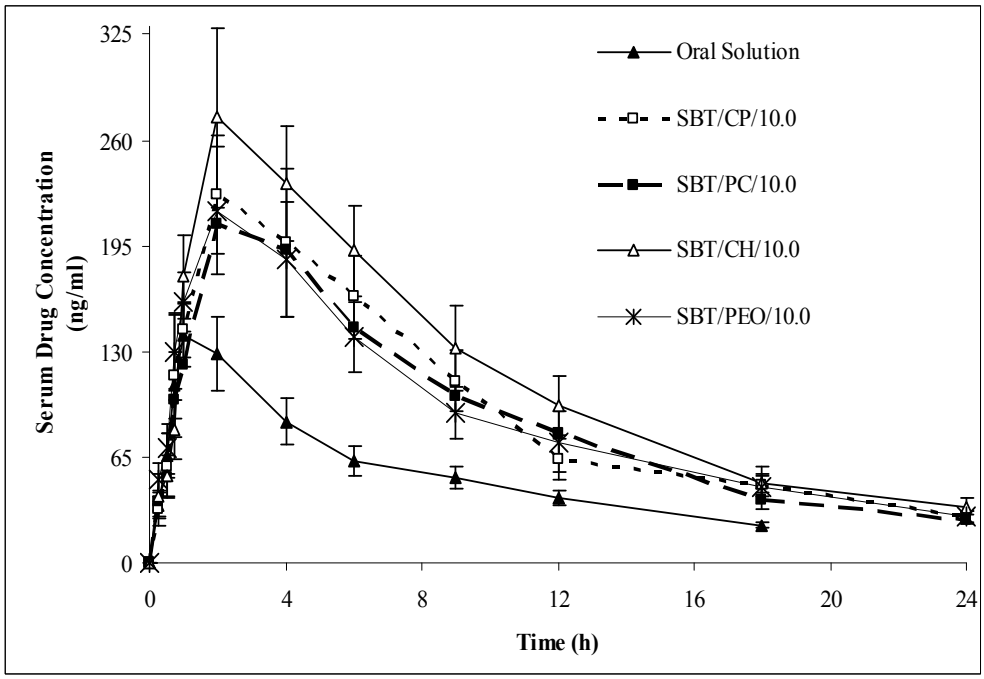

Figure 6.2: In vivo profiles following administration of single dose of LER (10 mg) in rabbits by oral and buccal route

(Each value represents mean of 3 independent determinations with standard deviation)

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Chapter 7
Conclusions

7.1 Conclusions

The need for research in drug delivery systems extends beyond ways to administer new pharmaceutical entities. The safety and efficacy of current drugs can be improved if their delivery rate, biodegradation and site specific targeting can be predicted, monitored and controlled. From a financial and global healthcare perspective, administration of injectable medications is costly and sometime leads to hazardous effects. Hence, inexpensive formulations with better bioavailabilities are needed.

The buccal route has been advocated as possible route for delivery of drugs having poor oral bioavailability because of high first pass metabolism or degradation in the gastrointestinal tract. Buccal adhesive systems offer several advantages in terms of accessibility, administration and withdrawal, retentivity, low enzymatic activity, economy and high patient compliance apart from reduced first pass metabolism of drugs.

Buccal mucoadhesive controlled release tablets of LER designed during this study were found to possess good physical characteristics indicating suitability of direct compression technique adopted for manufacturing quality buccal tablets. The designed formulations were found to be stable for at least two years when stored at CRT indicating that excipients, process and packaging materials adopted were appropriate and compatible with drug. The excipients were rationally selected based upon the extensive preformulation studies.

Drug release from designed tablets was affected by polymer proportion, polymer combination, polymer hydrophilicity/ hydrophobicity and swelling behavior of the polymer. A series of formulations retarding the release of drug from 4-14 h were designed. The release mechanism in almost all the designed formulations was anomalous non Fickian transport. The drug release was rapid initially and tapered off with passage of time indicating that target concentration can be achieved without loading dose. Near zero order release kinetics were obtained in formulations prepared using polymers like PC and CP either alone or in combination with various viscosity grades of HPMC. Mucoadhesive strength of the designed formulations was influenced by polymer concentration, viscosity of gel formed by polymer, flexibility of polymeric chains, polymer hydrophilicity/ hydrophobicity and swelling behavior of the polymer. Results indicated good mucoadhesive property of CP, PC, PEO and CH based formulations.

The designed buccal mucoadhesive tablets were found to be non-irritating and acceptable in human subjects. In vivo human acceptability studies of selected formulations demonstrated that the designed tablets adhered well for 4-6 h without any discomfort to

human subjects. The designed formulations did not interfere with normal functions like eating, drinking and speaking.

Further, in vivo studies of selected formulations in rabbits demonstrated significant increase in bioavailability relative to oral solution of LER due to reduction in the first pass metabolism. Formulation prepared using CH resulted in maximum increase of bioavailability. The designed formulations increased bioavailability 1.78 to 2.28 times relative to oral dose.

For all the analysis work of this project various analytical methods were developed in-house and validated. All the developed methods were found to be sensitive and facilitated accurate and precise estimation of LER in variety of samples. The HPLC methods for estimation of LER in stability and bio samples was found to be highly sensitive and were successfully used for stability and in vivo studies. Lack of interference from excipients/ biomatrix in all these methods indicated specificity of these developed and validated methods.

The study suggested that the designed buccal mucoadhesive tablet formulations are promising for commercialization and may lead to substantial dose reduction and more predictable serum drug concentration profile of LER as compared to conventional marketed preparations. The method used for manufacturing was found to be relatively simple and can easily be adopted in conventional formulation manufacturing units on a commercial scale.

However, further studies of the developed delivery systems need to be carried out in human subjects to establish clinical effectiveness of the designed formulations. The drug to polymer ratio can also be reduced to increase drug loading thereby decreasing the dimensions of the designed tablets without affecting the extension of drug release.

Appendix

List of Publications and Presentations

Publications

1. Charde, S.Y., Mudgal, M., Kumar, L., Saha, R.N., 2008 Development and evaluation of buccoadhesive controlled release tablets of lercanidipine. AAPS PharmSciTech, 9, 182-190.
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