Design and Evaluation of Mucoadhesive Buccal Delivery Systems of Felodipine

THESIS

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MURALI MONOHAR PANDEY

Under the Supervision of
Prof. Shrikant Y. Charde

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CERTIFICATE

This is to certify that the thesis entitled **"Design and Evaluation of Mucoadhesive Buccal Delivery Systems of Felodipine"** and submitted by **Murali Monohar Pandey,** ID No. **2008PHXF003P** for award of Ph.D. of the Institute embodies original work done by him under my supervision.

Date: 31-03-2015

Signature of the Supervisor
Prof. Shrikant Y. Charde
Associate Professor & Head
Department of Pharmacy
BITS Pilani, Hyderabad Campus

Dedication

This work is dedicated to the loving memory of
my childhood friend Late Nitya Nand Choubey

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Murali Monohar Pandey

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
<	Less than
=	Equal to
°C	Degree centigrade
µg/l	Microgram per liter
µg/ml	Microgram per milli liter
µl	Micro liter
ACN	Acetonitrile
AFM	Atomic force microscope
ANOVA	Analysis of variance
AR	Agar
ASD	Amorphous solid dispersion
AT	Accelerated temperature (40 ± 2^{o}C/ 75 ± 5 % RH)
$AUC_{(0-\infty)}$	Area under plasma concentration-time curve
AUMC	Area under first moments curve of plasma concentration-time profile
BP	Blood pressure
Ca	Calcium
CH	Chitosan
cm^{-1}	Centimeter inverse
cm^{2}	Centimeter square
C_{max}	Maximum plasma concentration
CMC	Carboxymethyl cellulose
Conc.	Concentration
CP	Carbopol 934 P

CRT	Controlled room temperature (25 ± 2^{o}C/60 ± 5 % RH)
CYP	Cytochrome
DSC	Differential scanning calorimetry
EC	Ethylcellulose
EDTA	Ethylene di amine tetra acetic acid
EG	Eudragit RSPO
et al.	Co-workers
F	Calculated or tabulated value of statistical test analysis of variance
FDA	Food and drug administration
FDP	Felodipine
F_r	Relative bioavailability
FT-IR	Fourier transform infrared
g	Gram
GI	Gastro-intestinal
GM	Guar gum
GRAS	Generally recognized as safe
h	Hour
h^{-1}	Hour inverse
HCl	Hydrochloric acid
HEC	Hydroxyethyl cellulose
HPC	Hydroxypropyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methyl cellulose
HQC	Higher quality control sample
i.m.	Intramuscular
i.v.	Intravenous
ICH	International conference on harmonization
IR	Infrared
J/g	Joules per gram
K	Release rate constant
kD	kiloDalton
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
LLOQ	Lower limit of quantification
LLOQC	Lower limit of quantification quality control
Log % RTD	Log percentage remaining to be degraded
Log P	Log of oil water partition coefficient
LQC	Lower quality control
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
MW	Molecular weight
N	Newton
NC	Nanocrystal
ng/ml	Nanogram per milli liter
NIR	Near infra red
nm	Nanometer
NMR	Nuclear magnetic resonance
PC	Polycarbophil
PXRD	Powder x-ray diffraction
QC	Quality control
R^2	Regression coefficient
RH	Relative humidity
RPM	Revolutions per minute
RT	Retention time
SD	Solid dispersion

sec	Seconds
SEM	Scanning electron microscope
SLS	Sodium lauryl sulphate
SSF	Simulated salivary fluid
STDEV	Standard deviation
$t_{1/2}$	Half life
$t_{50\%}$	Time taken for 50% of drug release from formulations
$t_{60\%}$	Time taken for 60% of drug release from formulations
TCP	Tri calcium phosphate
TEM	Transmission electron microscope
T_{max}	Time to reach maximum concentration
US	United states
USP	United States Pharmacopoeia
UV	Ultraviolet
v/v	Volume by volume
VIS	Visible
w/v	Weight by volume
σ	Standard deviation of *y* intercept of regression equation

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Abstract

The objective of the present work was to design and evaluate mucoadhesive buccal drug delivery systems of felodipine (FDP). FDP, a 1,4-dihydropyridine derivative, is a vasoselective calcium antagonist widely used in treatment of angina pectoris and hypertension. The drug exists as crystalline powder and is very slightly soluble in water. Orally administered FDP has poor bioavailability due to extensive first pass metabolism and is erratically absorbed.

In the present research work, modified release buccal tablets of FDP were designed using various mucoadhesive polymers and process excipients in an effort to increase bioavailability. Prior to the formulation of tablets, solubility of FDP was enhanced by preparing solid dispersions and nanocrystals of the drug. The effect of solubility enhancement on in vitro release and bioavailability of FDP was observed by preparing buccal tablets using pure drug, solid dispersions and nanocrystals. Analytical methods were developed and validated for estimation of drug in variety of samples like bulk, formulations, stability, in vitro and in vivo. Adequate preformulation studies were carried out using instruments like DSC and FT-IR to understand the physicochemical nature and stability of drug in presence of different excipients under variety of conditions. This in turn helped in selection of appropriate excipients.

Mucoadhesive buccal tablets of FDP with 5 mg loading were prepared by direct compression method. Formulations were designed using varying proportions of various mucoadhesive and rate controlling polymers like hydroxyethyl cellulose (HEC), ethylcellulose (EC), hydroxypropyl methyl cellulose (HPMC), chitosan (CH), guar gum (GM), agar (AR), polycarbophil (PC), carbopol (CP) and eudragit (EG). The designed buccal tablets were evaluated for the physical characteristics such as drug content, weight variation, friability, thickness and surface pH. In vitro drug release studies were performed using in housed modified dissolution assembly and in vitro mucoadhesion studies were performed using texture analyzer. Effect of polymer type, polymer proportion and process excipients on drug release and mucoadhesive performance was studied for the designed modified release mucoadhesive buccal tablets. Effect of designed solid dispersions and nanocrystals on in vitro drug release behavior was assessed by preparing buccal tablets with SD and nanocrystals.

Furthermore, in vivo bioavailability studies were performed for the designed mucoadhesive buccal tablets using rabbit model and pharmacokinetic parameters were obtained. Effect of solubility enhancement on bioavailability of the drug was also assessed. This data was compared with availability of drug after oral dosing.

Results indicated that all the developed and validated methods were accurate and precise for estimation of FDP in variety of samples. Preformulation studies indicated that polymorphic Form I of FDP was used for the study. The dissociation constant (pK_a) value determined for FDP was found to be 5.07. Drug was found to be compatible with all the process excipients used during the study.

The developed buccal tablets showed good physical characteristics with acceptable variation. Drug release and mucoadhesive behavior of the designed formulations was found to be dependent upon polymer type, polymer proportion, hydrophilicity or lipophilicity of polymer, polymer combination and type and amount of process excipients used.

The prepared solid dispersions (SDs) and nanocrystals (NCs) showed significant enhancement in dissolution of the drug. The drug was found to be present in amorphous form in the prepared SDs and NCs. The dissolution performance of SDs was found to be dependent on drug-polymer ratio. The particle size of FDP in the solid dispersions reduced with increase in the proportion of polymer used. The particle size of FDP was reduced below 50 nm by method used for nanocrystal preparation. The nanocrystals obtained were of amorphous nature which resulted in substantial enhancement in dissolution of the drug.

Modified release buccal tablets of FDP using pure drug and SDs were formulated using various proportions of HEC, EG, AR and PC. The drug release rate from delivery systems decreased with increasing levels of polymers in formulations. Mucoadhesive strength of formulations increased with increasing proportion of polymers used in the buccal tablets. The drug release rate from all mucoadhesive tablets containing SDs was found to be higher in comparison to those containing pure FDP except the tablets prepared using PC as rate controlling and mucoadhesive polymer. Reduction in the drug release from buccal tablets made with PC might have been caused by the hydrogen bond formation between functional groups present in PC and soluplus® used in preparation of SDs. Buccal tablets containing pure drug and

FDP nanocrystals were prepared using various ratios of HPMC and EC, alone and in combinations. EC tablets containing pure drug showed significant retardation of drug release in comparison to those of HPMC. The mucoadhesive strength of tablets prepared using HPMC was observed to be superior than the ones prepared using EC. The tablets containing NCs showed faster in vitro release as compared with those made with pure FDP. The effect of water soluble (lactose) and water insoluble diluents (tricalcium phosphate) was investigated by preparing buccal tablets using chitosan as mucoadhesive polymer. In this study, buccal tablets prepared with tricalcium phosphate (TCP) showed substantial retardation in drug release in comparison to the ones prepared with lactose. Optimum drug release was observed with the tablets containing combination of lactose and TCP. Modified release buccal formulations using CP and GM were prepared with pure FDP only. The drug release rate from delivery systems decreased with increasing levels of CP and GM and mucoadhesive strength of formulations increased with increasing proportion of the polymers in the buccal tablets.

The release data of all the designed formulations fitted best in the first order kinetic model. The drug release mechanism was found to be non-fickian anomalous type for the designed mucoadhesive buccal tablets.

In vivo studies performed for the selected formulations in rabbits demonstrated significant increase in bioavailability in comparison to that of immediate release oral tablets. The optimized mucoadhesive buccal tablets can be used as an alternative to improve the bioavailability of FDP.

It can be concluded that the designed formulations have potential to overcome the disadvantage of poor and erratic bioavailability associated with presently marketed oral tablet preparations. The process and method executed for design of buccal tablets was relatively simple and can easily be adopted in conventional manufacturing units on a commercial scale. However, further studies need to be carried out to assess designed delivery systems in humans for acceptability and clinical performance.

Chapter 1

Introduction, Literature Survey and Objectives

1.1 Introduction

The need for research in drug delivery systems extends beyond ways to administer new pharmaceutical entities. The safety and efficacy of current treatments may be improved if their delivery rates, biodegradation and site specific targeting can be predicted, monitored and controlled. The oral route of drug delivery is the most preferred route for systemic drug administration by physicians and is highly patient compliant. Oral route offers distinct well-established advantages over other drug delivery routes. However, peroral administration of certain drugs has disadvantages such as unpredictable and erratic absorption, gastrointestinal (GI) intolerance, degradation of drug within GI tract and presystemic metabolism resulting in low bioavailability. Parenteral route is the only well-established route till date that overcomes most of these disadvantages (Shojaei, 1988). However, from both financial and global healthcare perspective, parenteral medications are costly, sometimes lead to serious hazardous effects and are patient incompliant. For this reason, there has been a particular interest in exploring different absorptive mucosae as potential sites for systemic drug delivery. Transmucosal routes of drug delivery such as the mucosal linings of the nasal, rectal, vaginal, ocular, and oral cavity provide significant advantages over peroral administration for systemic drug delivery. Important advantages of transmucosal routes include possible bypass of first pass effect and avoidance of metabolism or degradation of drugs in the harsh environment present in GI tract (Patil and Sawant, 2008).

Drug absorption from nasal mucosa is rapid due to its rich vasculature and high permeability (Asane et al., 2008). The nasal mucosa for systemic drug delivery has been extensively investigated by many research groups (Aungst et al., 1988; Shao and Mitra, 1994; Shoyani and Chien, 1996) and the delivery of drugs through this route has almost reached commercial status for some peptide and protein drugs such as leuprolide, calcitonin etc. (Dal Negra, 1991; Adjei et al., 1992). However, 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 variation in mucus secretion in the nasal mucosa, could significantly affect utility of drug administration through this site (Backett and Triggs, 1967). Although the rectal, vaginal and ocular mucosae all offer certain advantages, poor patient acceptability associated with these sites render them appropriate for local applications rather than systemic drug administration (Shojaei, 1988).

The oral cavity, on the other hand, is highly acceptable by the patients. The mucosa is relatively permeable with rich blood supply, robust and shows short recovery times after stress or damage. Moreover, oral transmucosal drug delivery systems bypass first pass effect and avoid presystemic elimination in the gastrointestinal tract (Patil and Sawant, 2008). These factors make the oral mucosal cavity a very attractive and feasible site for systemic and local drug delivery.

Oral transmucosal drug delivery is mostly accomplished by either sublingual or buccal route. In sublingual delivery, drugs are administered through the mucosal membranes lining the floor of the mouth and in buccal delivery, drugs are transported across mucosal membranes lining the cheeks (Shojaei, 1988). Fig. 1.1 demonstrates various locations of the oral cavity used for drugs administration through sublingual and buccal mucosa (Lam et al., 2013).

The sublingual mucosa is a suitable route for rapid onset of action of drugs due to its properties like good permeability, considerably thin epithelial layer and high blood flow (Lam et al., 2013). It is normally used for acute conditions but this route has some disadvantages (Madhav et al., 2009). Sublingual mucosa lacks expanse of smooth muscles which poses problem in keeping dosage form in contact with the mucosa for longer duration of time and hence is unsuitable for controlled drug delivery. The disturbance due to tongue activity and constant washing by saliva create problem in the retention of the dosage forms rendering sublingual route unfit for the transmucosal controlled drug delivery (Patil et al 2008; Madhav et al., 2009).

The buccal route of drug administration has various advantages over that of sublingual route (Ishida et al., 1982; Nagai and Machida, 1993). Buccal mucosa is 3-5 times thicker making it more resistant to injury and surface area is almost double of sublingual mucosa thereby resulting in sufficient flux for permeation of drugs. Moreover, almost $1/4^{th}$ mucus turnover rate in buccal mucosa renders it suitable for controlled drug delivery (Harris and Robinson 1992; Sohi et al., 2010). It also possess expanse of smooth muscles suitable for adhesive delivery systems, better tolerance to allergens, irritation and damage. These factors play important role in making buccal route a potential route for the sustained delivery of drug molecules which cannot be given orally (Ahmed et al., 2014).

1.2 Anatomy and physiology of buccal mucosa

The outermost part of buccal mucosa is composed of 40-50 layers of non-keratinized stratified squamous epithelial cells. With a surface area of 100 cm^2, it covers one-third of total intraoral surface (Ho and Higuchi, 1971). The turnover time of buccal epithelium is reported to be 5-6 days (Harris and Robinson, 1992). It functions as a protective layer to the underlying tissues from the deleterious substances present in the oral cavity. The epithelium is considerably pervaded by conical papillae of the connective tissue present below it (Kraan et al., 2014). The oral epithelial is connected to lamina propia via basement membrane which in turn is followed by submucosa (Pather et al., 2008) as depicted in Fig. 1.2 (Smart, 2005). Lamina propia is essentially a dense, continuous sheet of connective tissues comprised of collagens and elastic fibers and provides mechanical stability. It also contains blood vessels and nerves fibers. It is noteworthy here that the blood vessels present in lamina propia are connected to internal jugular vein through lingual, facial and retromandibular veins. The direct absorption of drugs into portal veins bypass harsh gastrointestinal environment and first pass effect as well. This is one of the major advantages of buccal over peroral drug delivery (Morales and McConville, 2011). The submucosa is made up of adipose tissue, salivary glands, nerve fibers, blood and lymph vessels.

1.2.1 Biochemistry of buccal mucosa

The buccal epithelium possess considerable amount of extracellular substances. The extracellular matrix performs important role in providing mechanical strength, intercellular adhesion and permeability barrier (Harris and Robinson, 1992; Merkle and Wolany, 1993). The matrix appears like a hydrated polysaccharide gel ingrained with fibrous proteins. The polysaccharides present here are hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate and keratan sulfate (Alberts et al., 1989). The fibrous proteins consist of collagens, elastins and fibronectins. These macromolecules associate to each other through various covalent and non covalent bonds in order to form gel like structure. The buccal epithelium also contains various lipids but relatively little is known about it. A difference in type and distribution of the lipids exists within the oral mucosa. Buccal epithelium primarily contains polar lipids like phospholipids, cholesterol sulfate and glycosylceramide (Squier et al., 1986).

The oral epithelia is ubiquitously covered with a salivary layer and constantly bathed by 0.5-2 liters of saliva daily. Saliva is a watery substance containing 4-6% inorganic salts and mucus (Merkle and Wolany, 1993). The buccal epithelia is covered with a ground substance called mucus having variable thickness of 40-300 µm. It is mainly composed of mucins and inorganic salts suspended in water. Mucus molecules are capable to combine together to form polymeric or extended three-dimensional network. As per the variation in the three-dimensional arrangement different types of mucus namely G, L, S, P and F are formed (Odeblad, 1994).

Mucin, a family of high molecular weight glycoproteins, is essentially a heavily glycosylated large polypeptide core. The gel like appearance of mucus is due to the extensive glycosylation. The oligosaccharide branching is made up of 8-10 units of five different monosaccharides namely L-fucose, D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and sialic acid (Jimmenez-Castellanos et al., 1993). The terminal ends the oligosaccharides side chains contain either sialic acid, sulfonic acid and L-fucose (Gandhi and Robinson, 1994). Mucin is composed of 70-80% sugars, 12-25% protein and up to 5% ester sulphate (Sudhakar et al., 2006). The three fourth of polypeptide backbone is heavily glycosylated and render gel like structure to the mucus. The remaining one-fourth sugar free polypeptide region interacts with adjacent mucin molecules via covalent disulfide bond (Scawen and Allen, 1977; Mantle and Allen, 1981) facilitated by widely available charged amino acids at the site (Silberberg and Meyer, 1982).

1.2.2 Permeability barrier of buccal mucosa

Permeability of buccal mucosa is 4-4000 times better to that of skin (Galey et al., 1976). The outermost mucus layer, due to presence of plenty of charged molecules, is considered as the first permeability barrier for drug administered through buccal route (Merkle and Wolany, 1993). But, the major permeation barrier to the drugs is extended by the one-quarter to one-third of the outermost epithelial layer. It was evidenced by the permeation studies conducted with markers like horseradish peroxidase and lanthanum nitrate. Upon application to the outer layer of the epithelial cells, the marker molecules were found to pass through only 1-2 cells layers. When applied subepithelially, the marker molecules were observed to reach within connective tissues and the intercellular spaces of the epithelium (Squier, 1973;

Squier and Rooney, 1976). Interestingly, the permeation profile remains same in case of keratinized and nonkeratinized epithelial cells (Squier and Hall, 1984).

Membrane coating granules (MCGs) present in between the epithelial cell layers are considered as major contributor of the barrier characteristics of the epithelial layer. They are spherical or oval shaped organelles having 100-300 nm diameter (Matoltsy, 1976; Squier, 1977; Hayward, 1979). The MCGs of keratinized epithelium contains polar lipids such as glycolipids and phospholipids, glycoprotein and several enzymes. These enzymes convert the polar lipids to nonpolar ones which act as the permeability barrier (Silverman and Kearns, 1970; Lavker, 1976). This was evidenced by reduction in the barrier functions after treating buccal mucosa with chloroform or methanol (Squier et al., 1991). In order to determine the chemical nature of these lipids, various regions of porcine oral cavity were separated and the lipids present in each region were extracted and analyzed by thin-layer chromatography (Squier et al., 1986; 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. The non-keratinized buccal and sublingual mucosae showed higher proportion of polar phospholipids, cholesterol esters and minimal amounts of ceramides. Histological staining suggested that polar lipids were localized in intercellular spaces of the non-keratinized oral epithelium (Squier et al., 1986). 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 defferences in permeability observed between these mucosae (Squier et al., 1991).

Three types of intercellular junctions, namely desmosomes, gap junctions and tight junctions exist between the neighboring epithelial cells. These junctions play a crucial role in the maintenance of rigidity and transport of materials. Desmosomes present in the buccal epithelial cells provides 25-30 nm size space between the cells which easily allows movement of drug molecules. In contrast to it, gap junction presents a space of only 2-5 nm size which exercises considerable barrier for the permeation of molecules (Muller and Schroeder, 1980). Furthermore, tight junctions leaves no space in between the cells but these are mostly absent in buccal epithelium (Chen and Squier, 1984).

Finally, proteolytic activity of the enzymes present in buccal oral cavity also serves as a barrier to the permeation of the molecules. The oral mucosa contains various enzymes like aminopeptidases, carboxypeptidases and endopeptidases (Garren and Repta, 1988; Lee, 1988). These enzymes offer a barrier by degrading the drug molecules right in the oral cavity.

1.2.3 Drug transport pathways across buccal mucosa

Dug transport across buccal mucosa occurs mainly through the following pathways (Hao and Heng, 2003)

- Passive diffusion
- Carrier mediated transport
- Endocytosis

Passive diffusion of the drug molecules through buccal membrane happens by two possible route, the transcellular and the paracellular routes. The transcellular route is preferred by lipophilic drugs and the paracellular route by hydrophilic drugs (Rathbone and Tucker, 1993). In fact, simultaneously both routes remain engaged, but the route which extends ease of permeation is primarily used (Hao and Heng, 2003).

Carrier mediated transport is a way of the permeation of substances across the plasma membrane with help of a carrier protein. Lipophilic drugs may be transported across the aqueous cytoplasm with help of many carriers and/or lipoproteins (O'Driscoll, 2002). Various hydrophilic molecules such as sugars, vitamin C, nicotinic acid, nicotinamide, mono carboxylic acids, salicylic acid etc. have been reported to permeate through buccal mucosa by the carrier mediated transport (Manning and Evered, 1976; Sadoogh-Abasian and Evered, 1979; Evered et al., 1980; Utoguchi et al., 1997; Utoguchi et al., 1999). But, still there is no report of such type of transport for lipophilic drug across buccal mucosa.

Although very less but there are some examples of drug transport by endocytosis process. In this process the drug molecules are internalized and transported to the site. It is thought that the acidic stimulation of the salivary glands along with the vasodilatation causes the absorption of drug into the blood vessels (Chen et al., 2002).

1.3 Formulation design

Following factors are required to be contemplated for the effective transmucosal drug delivery via buccal route:

1.3.1 Physiological factors

In order to design a suitable formulation for buccal delivery physiological factors like thickness of mucosal membrane, area available for application of the delivery system, characteristic structure and turn over time of mucosal surface, extent of saliva secretion must be considered. Buccal mucosa with a total surface area of 100 cm^2 can accommodate a delivery system having maximum area of 1-3 cm^2 and per day dose of 25 mg (Gandhi and Robinson, 1994; Robinson and Yang, 1999; Alur et al., 2001). The duration of application of a buccal formulation should not exceed 4-6 h as food intake or water uptake may demand removal of the delivery system (Alur et al., 2001). Increased saliva secretion due to psychological effect of the presence of foreign body (in form of drug) in buccal pouch and involuntary swallowing of the saliva may affect subsequent bioavailability of the drug. To get rid of this problem unidirectional release systems with impermeable backing layer are preferred (Weathercell et al., 1994). Quick turnover rate (3-8 days) of buccal mucosa also affects absorption of drugs by regularly altering the permeability properties (Veuillez et al., 2001).

1.3.2 Pathological factors

The mucus characteristics and level of secretion are affected in some diseased conditions and may result in the change of drug permeation and mucoadhesive properties of delivery systems (Khanvilkar et al., 2001). Certain diseases cause an increase or decrease and even complete loss of the epithelial layer. In these conditions, the permeability profile of the buccal membrane gets changed (Squier and Wertz, 1996). This complicates the application of mucoadhesive drug delivery system designed for controlled release formulations. It has been observed that irradiation treatment of cancer causes considerable reduction in the saliva secretion. Some drugs like miconazole have been reported with decreased antifungal activity in such conditions (Bouckaert et al., 1996). So, the pathological conditions of the buccal cavity requires suffice attention before designing mucoadhesive delivery systems.

1.3.3 Pharmacological factors

The site of action such as local or systemic of the drug plays an important role in design of delivery system. The quantity of drug permeated through the mucous membrane is the primary concern for systemic action whereas the residence time and local concentration are the primary ones for local action (Hao and Hang, 2003).

1.3.4 Pharmaceutical factors

The physicochemical properties of the drugs must be considered for designing a successful buccal drug delivery system. A formulation intended for transmucosal delivery should possess suitable mucoadhesive property. Various mucoadhesive polymers like polycarbophil, chitosan, carbopol, cellulose derivatives etc. have been successfully used in these formulations (Sudhakar et al., 2006). In order to get absorbed, the drug should be having a suitable balance between its hydrophilicity (to get solubilized in saliva) and lipophilicity (to get transported across buccal membrane). A number of solubility enhancement techniques for poorly soluble drugs have been reported. These techniques are classified as drug solubilization using surfactant system, alteration of apparent solubility through complexation, drug derivatization and solubilization by solid state manipulation such as in solid dispersions (Yalkowsky, 1981). After solubilization into saliva the drug must easily get permeated across the buccal membrane. Several molecules known as permeation enhancers e.g. bile salts, fatty acids, cyclodextrins etc. are used to facilitate drug permeation through mucosal membrane (Chattarajee and Walker, 1995). Finally, the formulation should have good organoleptic properties in order to have better acceptability. To achieve this, suitable excipients need to be incorporated in the dosage form. A detailed discussion of solubilizing agents, permeation enhancers and mucoadhesive polymers follows in the next few sections.

1.4 Permeation enhancement

As stated in earlier sections, oral mucosa offers a considerable barrier to the permeation of a variety of drugs. In such cases, permeation enhancement becomes essential to achieve sufficient bioavailability of the drugs. The permeation enhancers exercise their action by one or more of the following mechanisms (Nicolazzo et al., 2005a).

- Increasing the partitioning of the drug into the buccal mucosa

- Increasing the fluidity of buccal membrane by extraction (and not disruption) of intercellular lipids
- Reducing viscosity of the mucus and saliva
- Communicating with the proteins present in buccal epithelium
- Extending residence time of drugs at the mucosal membrane

The suitability of permeation enhancer depends upon physicochemical properties of the drug, nature of vehicle and other excipients used. Sometimes use of enhancers in combination exhibit better result (Nicolazzo et al., 2004a, 2005c; Sudhakar et al., 2006). The permeation enhancers should be chemically and pharmacologically inert, non-toxic, non-irritating and non-sensitizing (Aungst, 1994). In contrast to transdermal permeation enhancers considerably less information is available on buccal permeation enhancers. The correlation between structure and permeation enhancement is not completely known resulting in availability of very few permeation enhancers for buccal drug delivery (Robinson and Yang, 1999; Veuillez et al., 2001). In past decade, research interest focusing on the search of safe and effective enhacers specific to buccal drug delivery has been significantly increased (Nicolazzo et al., 2005b). Table 1.1 presents a list of permeation enhancers reported in the literature.

1.5 Mucoadhesion

Mucoadhesion is of prime importance for drugs intended to be delivered through buccal cavity. It is well reflected in the extensive research conducted on bioadhesive polymers. Bioadhesive polymers are the polymers which can stick to a biological surface. A bioahesive, specifically meant to adhere on mucosal surface is known as mucoadhesive (Peppas and Buri, 1985).

1.5.1 Theories of mucoadhesion

The process of mucoadhesion is not clearly understood. Nevertheless, various theories have been proposed to explain the process (Ahuja et al., 1997).

- Electronic theory
- Adsorption theory
- Wetting theory
- Diffusion theory

- Fracture theory

Electronic theory states that mucoadhesive polymer and mucous layer differs in their electronic properties. This causes electron transfer between both the surfaces which give rise to the force of attraction and inter-diffusion at the interface (Ahuja et al., 1997; Huang et al., 2000).

Adsorption theory suggests that the mucoadhesive polymer gets adsorbed on the mucus membrane by virtue of the formation of covalent and non-covalent bonds. The covalent bond involves formation of disulfide bond between mucus glycoprotein and the polymer. This bond is known to result strongest mucoadhesive force with the buccal mucosa (Guggi et al., 2004). Examples of non-covalent interactions are hydrogen bond, hydophobic bond and van der Walls' force of attractions (Peppas and Buri, 1985; Ahuja et al., 1997; Huang et al., 2000).

Wetting theory suggests appropriate wetting as prerequisite for mucoadhesion. This theory estimates the mucoadhesive force as the capacity of the substance to spread over the mucosal membrane. This theory is mainly applicable to the liquid or semisolid mucoadhesive substances (Van Wachem et al., 1985; Peppas and Buri, 1985; Ahuja et al., 1997; Huang et al., 2000).

Diffusion theory suggests that formation of intermixed network between glycoprotein chains of mucus and the polymer is responsible for the mucoadhesion. Diffusion of polymeric chain into the mucus creates an entangled network between the two. The factors affecting the strength of the intermixing are polymer chain flexibility, surface exposure for contact, similarity in the structure and diffusion coefficient of the mucoadhesive polymer (Peppas and Buri, 1985; Ahuja et al., 1997; Gu et al., 1998; Huang et al., 2000).

Fracture theory links the detachment force of the polymer from the membrane with the adhesion strength. Normally, greater force is required to detach the polymer in case of longer network strands or reduced degree of cross-linking (Peppas and Buri, 1985; Ahuja et al., 1997; Gu et al., 1998; Huang et al., 2000).

1.5.2 Mucoadhesive polymers

Mucoadhesive formulations use polymers as the adhesive agent. The formulation absorbs water available in the buccal cavity and swells to form a viscous sticky gel like substance causing attachment to the mucosal surface. A mucoadhesive

polymer should possess following properties (Longer and Peppas, 1981; Jimmenez-Castellanos et al., 1993).

- It should form strong non covalent bond with mucosal surface.
- It should have minimal effect on the drug release.
- The polymer and its degradation product should not be toxic or irritant.
- It should be stable during shelf life of the drug.
- It should be biodegradable.

1.5.3 Classification of mucodhesive polymers

Mucodhesive polymers reported in the literature can broadly be classified on the basis of their origin such as natural, semi-natural and synthetic mucoadhesive polymers. Naturally occurring mucoadhesive macromolecules are structurally similar to synthetic ones. They are normally linear polymers with high molecular weight containing a substantial number of hydrophobic charged functional groups and form three-dimensional expanded networks (Gu et al., 1998). Agarose, chitosan, gelatin and various gums such as guar and hakea are classified as semi-natural/natural mucoadhesive polymers. Polyacrylic acid based cellulose ester derivatives and poly methacrylate derivatives are examples of synthetic mucoadhesive polymers.

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

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

1.6 Buccal-adhesive dosage forms

Buccal mucoadhesive formulations possess various advantages over conventional oral dosage forms as discussed in earlier sections. The potential of this particular delivery system has catalyzed the research works conducted in the area. As a result of which wide variety of buccal-adhesive dosage forms, both for local and

systemic actions, has achieved substantial place in the literature. The formulations can be categorized as (Sudhakar et al., 2006)

- Solid buccal-adhesive formulations
- Semi-solid buccal-adhesive formulations
- Liquid buccal-adhesive formulations

1.6.1 Solid buccal-adhesive formulations

Formulations such mucoadhesive tablets, patches, films, lozenges, wafers, microparticles fall into this category (Sudhakar et al., 2006).

1.6.1.1 Tablets

Buccal-adhesive tablets are the most extensively used type of formulations studied for the orotransmucoasal drug delivery. These are generally prepared by direct compression method (Charde et al., 2008; Jaipal et al., 2013; Jaipal et al., 2014) but wet granulation method can also be used (Miller et al, 2005). These are placed in buccal pouch and allowed to dissolve or adhere depending upon the excipients used (Ikinci et al., 2004). Buccal tablets are small, flat or oval, having diameter in a range of 5-8 mm (Rathbone et al., 1994). The dry tablets becomes wet on application to the mucosal surface and get attached to it. The buccal tablets can also be prepared in certain physical state like microsphere, before direct compression to exhibit specific properties such as enhanced activity and prolonged drug release (Giunchedi et al., 2002). Buccal-adhesive tablets do not cause much problems in drinking and speaking after proper attachment. Alternate side of mouth can be used to keep successive tablets. This dosage form suffers some drawbacks like inter- and intra-personal variation in absorption and bioavailability because it is difficult to control the drug concentration as the media is constantly diluted by saliva. Unpleasant taste and lack of physical flexibility are the other limitations linked to the dosage form (Madhav et al., 2012). Table 1.3 represents list of some recently reported drugs and polymers used for the preparation of buccal tablets.

1.6.1.2 Buccal patches/films

Buccal patches essentially comprise of an impermeable backing layer, a drug reservoir layer and a bioadhesive surface for mucoadhesion (Miller et al., 2005; Patel

et al., 2011). These delivery systems provide several advantages such as rapid onset of action, sustained drug release and quick reduction in plasma drug concentration after removal (Madhav et al., 2012). In case of local delivery in oral cavity, the films/patches provide a covering layer to the wound and thus help to reduce pain and treat the disease more effectively. For the preparation of these dosage forms two methods namely solvent casting and direct milling are generally used. In solvent casting method, a solution containing drug and excipients is casted on a backing layer surface and the solvent is allowed to dry. In direct milling method, formulation constituents are uniformly mixed and compressed to the required thickness, and patches of desired size and shape are then cut and punched out. Although the solvent casting method is simple but it has certain limitations like long processing time, high cost, issues of uniformity and concerns over solvents used. In order to counter these limitations hot-melt extrusion has been reported (Repka et al., 2002). In hot-melt extrusion, a mixture of drug and other compounds is allowed to melt and pass through a die to produce a homogenous material of various shapes, like tablets, pellets, or films (Miller et al., 2005). Table 1.4 presents some of the works recently reported on buccal patches/films.

1.6.1.3 Lozenges

Mucoadhesive lozenges for the local action within oral cavity have been reported for drugs like cetylpyridinium chloride (Collins and Deasy, 1990), corticosteroids, local anesthetics, antimicrobials, antibiotics and antifungals (Codd and Deasy, 1998; Sudhakar et al., 2006). Both conventional and sustained release lozenges can be prepared as per the need.

1.6.1.4 Wafers

Recently, Mura et al., (2015) reported pectin-based wafers for econazole buccal delivery for the treatment of candidiasis. Kianfar et al., (2013) reported use of mucoadhesive wafers prepared with carrageenan and pluronic acid for both soluble and insoluble drugs. In another work, Bromberg et al. (2001) prepared a novel periodontal drug delivery system that is intended for the treatment of microbial infections associated with periodontitis.

1.6.2 Semi-solid dosage forms

Semi-solid dosage forms intended for buccal delivery are basically of two types viz. gels and ointments. On one hand, they have merits in terms of the ease of dispersion over oral mucosa but they have disadvantages of being linked to the poor retention and dose inaccuracy in comparison to the solid dosage forms. The poor retention problem has been eliminated by formulating the semi-solid dosage forms with entrapped drug molecule that are released by diffusion or erosion (Martin et al., 2003). Generally, these dosage forms have been delivered for the local action in diseases like periodontitis (Jones et al., 2000; Vinholis et al., 2001). Table 1.5 presents list of recently reported drugs prepared as buccal mucoadhesive semi-solid preparations.

1.6.3 Liquid dosage forms

Liquid dosage forms for buccal delivery uses highly viscous liquid to form a layer on the oral cavity in order to protect the underlying layer. Suitable polymers are required to be used to enhance the viscosity of the products which also helps in retention of the dosage form. Dry mouth syndrome has reportedly been treated by application of artificial saliva solutions containing sodium CMC as mucoadhesive agent (Sudhakar et al., 2006).

Aerosol spray has also been reported for the delivery of the drugs into salivary fluid or oral cavity for quick absorption. A buccal insulin spray has been reported to treat type 1 diabetes which produced similar anti-diabetic effect as compared to the subcutaneous injection (Pozzilli et al., 2005).

1.6.4 Commercial status of buccal-adhesive dosage forms

The market proportion of buccal-adhesive drug delivery system is steadily increasing in US and European countries. Table 1.6 lists buccal mucoadhesive dosage forms available in the market commercially (Madhav et al., 2012).

1.7 Evaluation of buccal-adhesive dosage forms

In vitro evaluation of buccal-adhesive dosage forms considerably differ from other dosage forms as the environment in buccal cavity is difficult to simulate. 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), viscosity (for buccal semi-solids), in vitro drug release, permeability, mucoadhesive strength, residence time and acceptability are generally carried out for buccal dosage forms.

1.7.1 Mucoadhesion measurement

A number of methods reported for quantitative mucoadhesion measurement are essentially based upon measuring the force required to break the adhesive bond between the membrane and the dosage form. A mucoadhesive force above 4.5 dyne/cm^2 is needed by a buccal tablet to remain attached in the buccal membrane for at least 4 h (Chio and Kim, 2000). Depending upon the direction of force applied to separate the delivery system from membrane (Fig. 1.3) peel, shear and tensile force required for detachment are measured (Sudhakar et al., 2006).

1.7.1.1 Determination of peel strength

Peel strength of a buccal formulation is the energy required to separate the dosage form from the model mucosal membrane in the direction shown in Fig. 1.3 (Sudhakar et al., 2006). Peel strength is also measured for transdermal patches.

1.7.1.2 Determination of shear strength

Shear strength of a buccal formulation is the force required to slide the dosage form with respect to the membrane in a direction parallel to the plane of contact as shown in Fig. 1.3 (Sudhakar et al., 2006). Some researchers have studied the mucoadhesive strength of calcium polycarbophil, sodium CMC, HPMC using homogenized mucus from pig intestine as a model substrate (Lehr et al., 1991). Two glass plates were coated with the homogenized mucus and the dosage form was allowed to hydrate between these two mucus coated plates. The force required to pull these plates apart was used for the measurement of shear strength. Likewise, mucoadhesive strength of carbopol, CMC, HPMC, gelatin, acacia, polyethylene glycol, PVP, pectin, tragacanth and sodium alginate gels has been studied and reported by measuring 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).

1.7.1.3 Determination of tensile strength

Tensile strength is measured as the force required to detach the dosage form from the mucosal membrane when a force is applied in the vertical direction (Fig. 1.3). This is the most widely reported method for the measurement of mucoadhesion. It can be used as a measure of mucoadhesion for dosage forms like solids, semi-solids and liquids (Wong et al., 1999).

Plenty of literature is available wherein texture analyzer has been reported for the measurement of tensile strength. This instrument is known to precisely measure the mucoadhesive force and work of adhesion of a mucoadhesive polymer (Repka et al., 2005; Owens et al., 2005; Jaipal et al., 2013; Jaipal et al., 2014). Some other methods like colloidal gold staining (Park and Park, 1989), direct staining (Koclkisch et al., 2001), modified pan balance method (Ahuja et al., 1997; Desai and Kumar, 2004) have also been reported for the measurement of mucoadhesion.

1.7.2 In vitro release study

In vitro release study is an important evaluation technique to assess the performance of the dosage forms. A proper release study should be an indicator of in vivo performance of the dosage forms (Siewert et al., 2003).

USP allows use of in vitro release test for some sublingual tablets and apparatus 2 with water as dissolution media (US Pharmacopoeia, 2003). But, in vivo dissolution is limited for these tablets due to presence of less amount of saliva in mouth as dissolution media. So, dissolution tests using standard USP apparatus and large volume of liquids might not produce results that simulate the in vivo dissolution of drugs. Therefore, several methods have been reported for the dissolution study of buccal formulations using smaller volume of dissolution media.

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 mucoadhesive tablet was embedded into paraffin wax which was placed on top of a bovine buccal mucosa as membrane.

In vitro release study using USP apparatus 2 has widely been reported in literature (Ceschel et al., 2001; Rambali et al., 2001; Jain et al., 2002). However, in a quest of mimicking the dissolution behaviour in buccal cavity a variety of

modifications have been reported in the literature (Parodi et al., 1996; Cilurzo et al., 2003; Akbari et al., 2004; ElGindy, 2004). Mohammed and Khedr (2003) used an easier method to perform 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 taken at different time intervals for drug content analysis.

Researchers have also used a system comprising of continuous flow-through cell with a dip tube (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 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 then filtered and analyzed. Simulated salivary fluid was used as dissolution media (Davis et al., 1971; Tavss et al., 1984).

1.7.3 Residence time determination

1.7.3.1 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 used is 800 ml isotonic phosphate buffer pH 6.7 maintained at 37 °C. A 3 cm long mucosa is attached on the surface of a glass slab using glue. The dosage form is then hydrated from one surface using isotonic phosphate buffer and allowed to touch the mucous membrane. The glass slab which is vertically fixed to the apparatus is allowed to move up and down. The time taken for complete erosion or detachment of the dosage form from the mocosal surface is noted as residence time (Nafee et al., 2004; Patel et al., 2006).

1.7.3.2 In vivo residence time

All in vivo residence time experiments reported in literature have been conducted on healthy human subjects of 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 (Desai and Kumar, 2004; Nafee et al., 2004; Charde et al., 2008).

1.7.4 Permeation studies

The developed buccal dosage forms as well as drugs should be evaluated to determine feasibility of the route of administration. Also, this study helps in the establishment of safety and efficacy profile of the drug on administration by buccal route. The reported methods have been discussed below.

1.7.4.1 In vitro methods

Majority of the reported in vitro methods for permeation study have used buccal tissue from various animal models like rats (Aungst et al., 1988), rabbits (Dowty et al., 1992; Gandhi and Robinson, 1992), dogs (Nagai, 1985), porcine (Hoogstraate et al., 1996; Mashru et al., 2005) and Franz diffusion cell (Giannola et al., 2005, 2007). For the study, animals are sacrificed just before the start of the experiment. Buccal mucosa along with underlying connective tissue is surgically removed from the oral cavity and then connective tissue is carefully separated from the buccal mucosa. Now, the mucosa is 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 problem in these in vitro models is the viability and integrity of the surgically removed tissue. The tissue is required to be preserved properly as this directly affects the results of the study.

1.7.4.2 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 determined. The methodology involved swirling of a 25 ml sample of the test solution for up to 15 min 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 demerits of this method include salivary dilution of the drug, accidental swallowing of some amount of the sample solution, and inability to localize the drug solution within a specific site of the oral cavity. Some modifications of the test have also been reported (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.

The in vivo permeation study has also been reported to use dog as animal model (Yamahara and Lee, 1993). In this study, a small perfusion chamber was attached to the upper lip of anesthetized dog. The perfusion chamber was attached to the tissue by cyanoacrylate cement. The drug solution was circulated for a predetermined period of time with help of a device and sample fractions were collected from the perfusion chamber in order to determine the quantity of drug remaining in the chamber. Blood samples were taken to determine the amount of drug absorbed across the mucosa.

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

1.8 Objective of the research

Felodipine (FDP), 1,4-dihydropyridine derivative, is a vasoselective calcium antagonist widely used in treatment of angina pectoris and hypertension (Saltiel et al., 1988; Ekelund et al., 1994). The drug exists as crystalline powder and is very slightly soluble (1 in 2000) in water (Saltiel et al., 1988; USP, 34/NF 29). It is also effective against seizures and central ischemic disorders by acting at L-type calcium channels (Murai et al., 2000).

FDP formulations available in the market show erratic absorption due to interaction with food and poor bioavailability (approximately 15%) due to extensive first pass metabolism with high inter- and intra-patient variability. Alternative route of administration like buccal for FDP can help in combating the above mentioned issues and can result in better therapeutic efficacy.

The objective of the present research work was to design buccal mucoadhesive modified release delivery systems of FDP to overcome the challenges associated with presently marketed formulations of FDP. The venous drainage of buccal route is not subjected to hepatic first pass metabolism and hence it was postulated that this may increase bioavailability of the drug. Due to high vascularization of buccal mucosa, the rate of drug absorption is expected to be faster resulting in quicker onset of action. Moreover, retarding release of drug would help in maintaining the target

concentration of drug for a longer period of time. As delivery of drug through this route bypasses GI tract, chances of food drug interaction are minimal.

The present research work was thus broadly targeted at preparation of buccal mucoadhesive modified release drug delivery systems of FDP using various mucoadhesive and rate controlling polymers for increasing the bioavailability. To achieve this broad objective, research work was carried out in following stages:

- Suitable analytical methods were developed and validated for estimation of FDP in bulk, formulations, in vitro release, stability and plasma samples.
- Extensive preformulation studies were carried out to establish solution and solid state stability of FDP and assess compatibility of selected excipients with FDP.
- Solubility enhancement of FDP, a poorly soluble, was carried out by preparing solid dispersions (SD) and nanocrystals (NC).
- Buccal mucoadhesive modified release delivery systems were designed and various process variables and physical characteristics of the formulations were optimized.
- Designed formulations were evaluated for in vitro release and mucoadhesive properties.
- In vivo pharmacokinetic studies were carried out in rabbits to assess bioavailability of designed delivery systems.

Buccal delivery of FDP is challenging due to its poor solubility. Moreover, availability of limited volume of saliva in buccal cavity might result in drug not dissolving sufficiently in the oral cavity for buccal permeation. Considering this, as first step of the development of mucoadhesive buccal formulation, solid dispersions and nanocrystals of FDP were prepared for the improvement of solubility. Following this, experiments involving development and characterization of modified release buccal mucoadhesive tablets containing pure drug, SDs and nanocrystals prepared by matrix embedding technique were conducted. The mucoadhesive tablets were prepared by direct compression method.

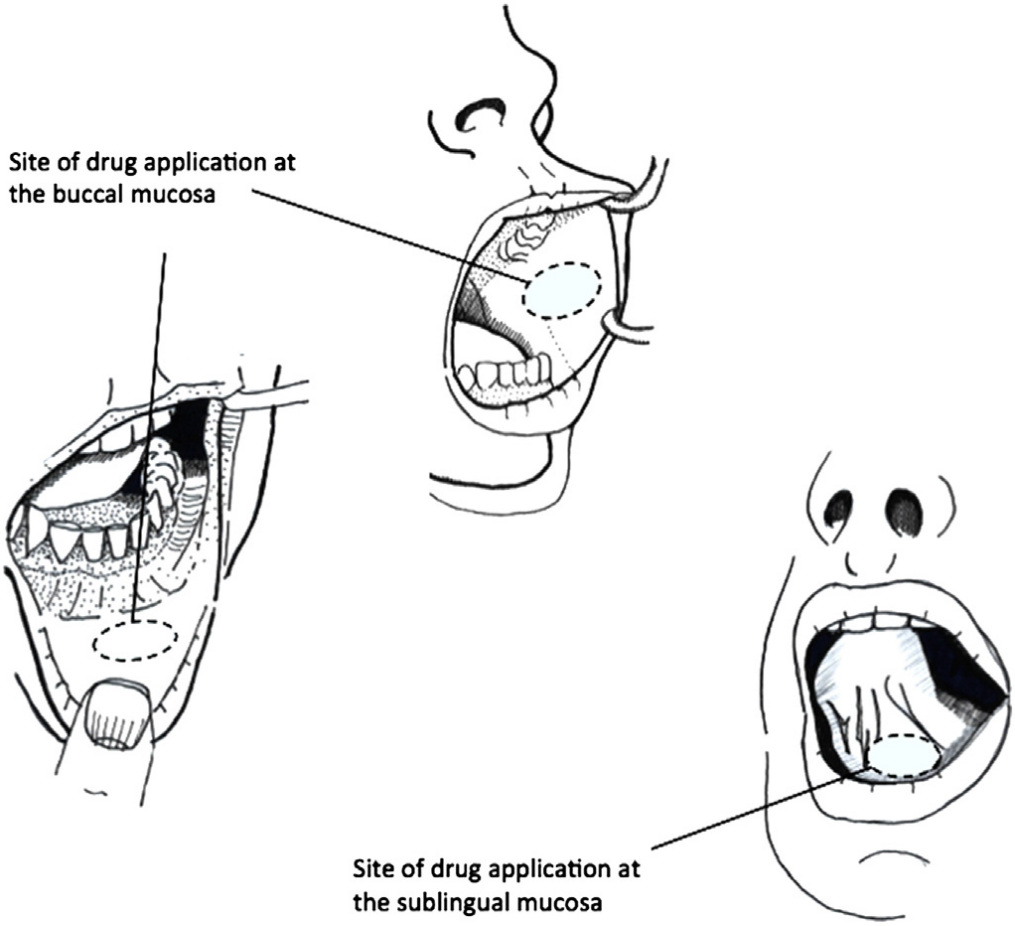

Fig. 1.1. Schematic representation of sites of oral cavity used for the sublingual and buccal delivery of drugs (Lam et al., 2013)

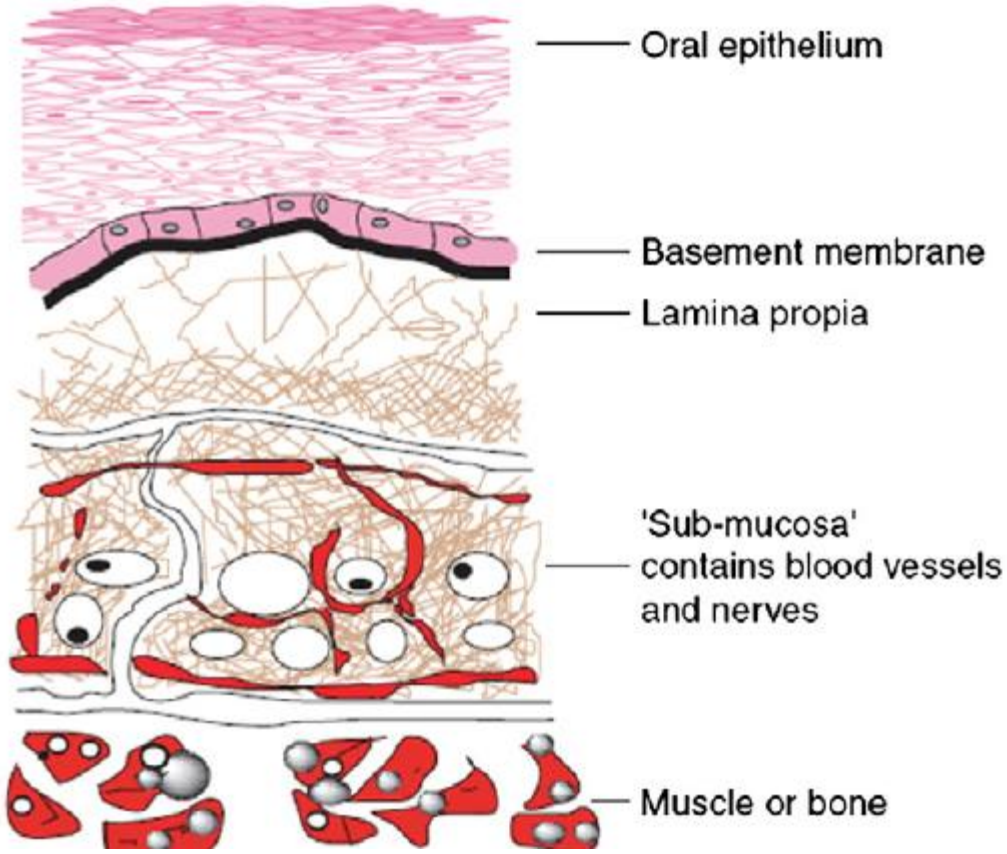

Fig. 1.2. Schematic diagram of oral mucosa (Smart, 2005)

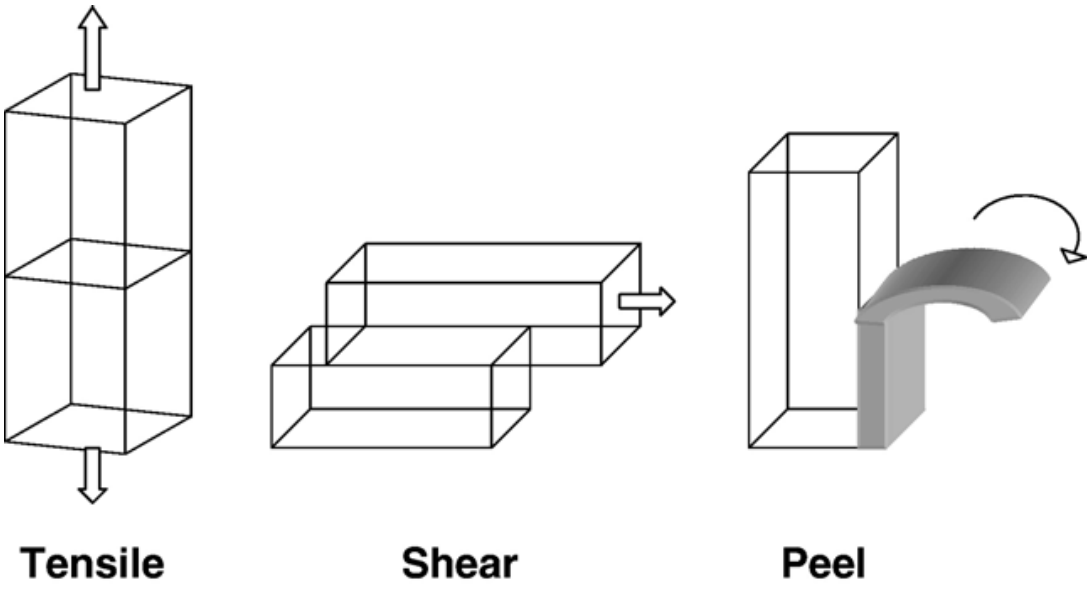

Fig. 1.3. Schematic representation of tensile, shear and peel forces (Sudhakar et al., 2006)

Table 1.1: List of various permeation enhancers used in buccal drug delivery systems

Category	Examples	References
Chelators	EDTA Sodium EDTA Citric acid Sodium salicylate Methoxy salicylates	(Aungst and Rogers, 1988; Coutel-Egros et al., 1992; Wolany et al., 1990; Oh and Ritschel, 1990)
Bile salts	Sodium glycocholate, Sodium deoxycholate, Sodium taurocholate, Sodium glycodeoxycholate, Sodium taurodeoxycholate	(Aungst et al., 1988; Aungst and Rogers, 1989; Nakane et al., 1996; Hoogstraate et al., 1996; Senel et al., 1994; Ishida et al., 1981)
Surfactants	Sodium lauryl sulphate Polysorbate 80 23-lauryl ether Benzalkonium chloride Cetylpyridinium chloride Cetyltrimethyl ammonium bromide	(Rathi et al., 2011; Nicolazzo et al., 2004b, Siegel and Gordon, 1985a; Oh and Ritschel, 1990; Siegel and Gordon, 1985b)
Fatty acids	Oleic acid Capric acid Lauric acid Phosphatidylcholine Lysophosphatidylcholine	(Coutel-Egros et al., 1992; Manganaro and Wertz, 1996; Zhang et al., 1994; Aungst and Rogers, 1989)
Thiolated polymers	Chitosan Trimethyl chitosan Chitosan-4-thiobutylamide	(Cid et al., 2011; Martin et al., 2003; Park and Munday, 2004; Sandri et al, 2004, 2006)
Others	Azone Ethanol Menthol Cyclodextrins Dextran sulphate Sulfoxide Alkyl glycosides	(Challa et al., 2005; Coutel-Egros et al., 1992; Aungst, 1994; Steward et al., 1994; Oh and Ritschel, 1990; Aungst and Rogers, 1989)

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 such as 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, copolymer 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, Polycrbophil, 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 ancohol, Acrylates
	Electrostatic	Chitosan

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

Drug	**Polymer**	**References**
Ibuprofen	Chitosan	Sogias et al., 2012
Ritodrine hydrochloride	Alginate	Onishi et al., 2014
Buspirone	Xanthan Gum, HPMC	Jaipal et al., 2013, 2014
Indomethacin	EC, PEG	Ikeuchi-Takahashi et al., 2013
Curcumin	Anacardium occidentale gum	Gowthamarajan et al., 2012
Amiloride hydrochloride	HPMC, CP, Chitosan, PVP	Reddy et al., 2013
Theophylline	CP, PEO, HPMC	Boyapally et al., 2010
Tizanidine Hydrochloride	HPMC, Sodium CMC	Shanker et al., 2009
Granisetron hydrochloride	HPMC, CP	Ahmed et al., 2014
metoprolol succinate	Badam gum	Mylangam et al., 2014
Timolol maleate	CP, Sodium alginate	Gaikwad et al., 2014
Selegiline hydrochloride	PC	Wasnik et al., 2014
Nicotine	HPMC, Sodium CMC, CP	Bahri-Najafi et al., 2013
Terbutaline sulfate	HPMC, CP	Emami et al., 2013
Atenolol	HPMC, CP	Shirsand et al., 2012
Chlorhexidine	Cordia myxa, HPMC	Moghimipour et al., 2012
Methimazole	Eudragit	De Caro et al., 2012
Fluconazole	CP	Mohamed et al., 2011
Sumatriptan succinate	CP, HPMC	Prasanna et al., 2011
Amitriptyline	HPMC, Sodium CMC	Movassaghian et al., 2011
Nystatin	Xanthan gum	Sakeer et al., 2010.
Miconazole nitrate	HPMC, CP, PVP	Madgulkar et al., 2009
Pravastatin sodium	PVP	Shidhaye et al., 2010
Diltiazem	HPMC, CP	Shayeda et al., 2009
Nimodipine	CP	Hassan et al., 2010

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

Drug	Polymer	References
Tizanidine hydrochloride	Eudragit	Mohamed et al., 2012
Carvedilol	Chitosan	Kaur and Kaur, 2012
Triamcinolone acetonide	PEO	Miro et al, 2013
Triclosan	Pectin	Jug et al., 2012
Ondansetron	HPMC, Eudragit	Kumria et al., 2013
Domperidone	Guar gum, Xanthan gum	Singh et al., 2010
Nicotine	HPMC	Rao et al., 2011
Carbamazepine	HPMC, PVA, PVP	Govinadswamy et al., 2013
Ivabradine hydrochloride	HPMC, CP, PEG	Lodhi et al., 2013
Lidocaine	Chitosan	Xu et al., 2015
Zolmitriptan	Xanthan gum	Shiledar et al., 2014
Ibuprofen	Chitosan	Tang et al., 2014.
Tizanidine hydrochloride	HPMC, PVA	El Mahrouk et al., 2014

Table 1.5: Recently reported drugs and polymers used for developing buccal mucoadhesive semisolid preparations

Drug	Polymer	References
Salbutamol	Xanthan gum	Zeng et al., 2014
Diazepam	Azone®	Meng-Lund et al., 2014
Miconazole nitrate	Cyclodextrins	Rai et al., 2014
Propolis	HPMC, PEG	Augusto et al., 2014
Lidocaine	Thiolated pectin	Hauptstein et al., 2014.
Venlafaxine	Linseed	Nerkar and Gattani, 2013
Triamcinolone acetonide	CP, Poloxamer	Choi et al., 2013
Nystatin	Alginate, Chitosan	Martin et al., 2015

Table 1.6: List of marketed buccal-adhesive dosage forms (Madhav et al., 2012)

Brand Name	**Drugs**	**Uses**	**Manufacturer**	**Dosage Form**
Loramyc	Miconazole lauriad	Oropharyngeal candidiasis	Bioalliance Pharma	Buccal tablet
Lauriad	Acyclovir	Herpes labialis	Bioalliance Pharma	Buccal tablet
Onsolis	Fentanyl citrate	Opioid analgesic	Meda Pharmaceuticals, Inc.	Buccal soluble film
BEMA	Buprenorphine	Opioid analgesic	Biodelivery Sciences International, Inc	Buccal soluble film
Actiq	Fentanyl citrate	Opioid analgesic	Wolters Kluwer Health	Lozenge on a stick
ACT fluoride rinse	Fluoride topical	Anticavity	Cerner Multum, Inc.	Oral solution
Nitrocot	Nitroglycerin	Anti-angina	Thomson Healthcare Products	Sublingual tablet
Saphris	Asenapine maleate	Schizophrenia, bipolar Disorder	Catalent UK Swindon Zydis Ltd.	Sublingual tablet
Gel-kam	Fluoride	Anticavities	Cerner Multum, Inc.	Oral gel

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Chapter 2

Drug Profile: Felodipine

2.1 Felodipine

2.1.1 Chemistry

Felodipine (FDP) is chemically a racemic mixture of 3-ethyl 5-methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4 dihydropyridine-3,5-dicarboxylate with a molecular weight of 384.26. It is a light sensitive crystalline slightly yellowish powder. FDP is chemically synthesized using Hantzsch synthesis. The chemical structure of FDP is shown in Fig. 2.1.

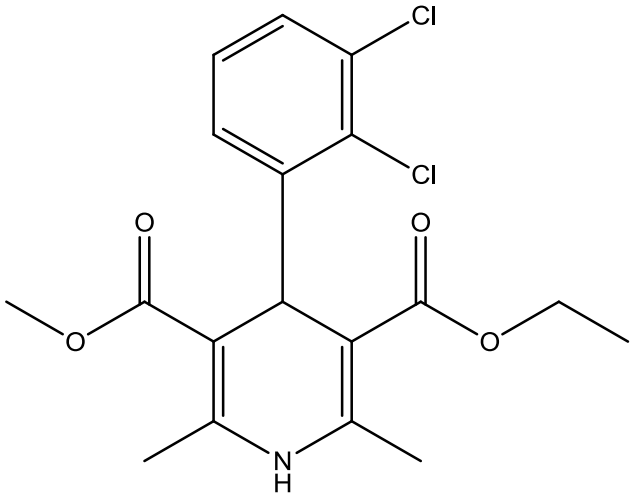

Fig. 2.1. Structure of felodipine

2.1.2 Solubility

FDP is freely soluble in dichloromethane, acetonitrile and ethanol. It is poorly soluble in water with a solubility of 0.5 mg/l (Saltiel et al., 1988). The partition coefficient (Log P) value of felodipine is 4.46 (Desai et al., 2012).

2.1.3 Polymorphism

Different polymorphic forms of a crystalline drug exhibit different physicochemical properties. FDP is known to be present in two polymorphic forms namely From I and Form II. Form I FDP is obtained by recrystallization from methanol, acetonitrile or ethanol. Form II is recrystallized from hexane and methanol mixture (Rollinger and Burger, 2001; Lou et al., 2009). Form I with melting point of around 145 °C, is most stable polymorphic form of FDP and widely used in the marketed formulation. Form II is thermodynamically metastable at room temperature and melts at 136 °C (Lou et al., 2009). Fourier transform infrared (FT-IR) studies

have been reported for confirmation for polymorphic form of FDP. In Form I, N-H vibrational stretch is observed at 3370-3371 cm^{-1} wave number and in Form II, it is positioned at 3336 cm^{-1} (Rollinger and Burger, 2001).

2.1.4 Stereoisomerism

FDP exists in two enantiomeric forms *(S-)* and *(R)*- or as racemate. *(S)*- form is the pharmacologically active enantiomer. Melting point of (S) - and (R)- felodipine are 145.3 and 145.4 °C respectively (Lamm and Simonsen, 1989). In vitro study of dog and rat liver microsomes demonstrated that *(S)*- form was more readily metabolized than *(R)*- enantiomer. However, in case of microsomal preparations of human liver the enzyme catalyzing activity was higher for *(R)*- enantiomer. The intrinsic clearance rate of *(R)*- was observed to be 2 fold higher than the *(S)*- form of the drug (Eriksson et al., 1991). When administered orally to healthy human volunteers, the AUC and C_{max} was found to be 2-fold higher for *(S)*- enantiomer (Soons et al., 1990).

2.1.5 Pharmacodyanamic profile

2.1.5.1 Mechanism of action

FDP, a calcium channel blocker, reversibly competes for dihydropyridine binding sites. FDP acts through voltage-gated L-type calcium channels and inhibits the influx of calcium ions which decreases arterial smooth muscle contractility, mediate negative inotropic cardiac effects and vasodilation (Bostrom et al., 1981).

FDP is a class of drug that antagonizes the effect of calmodulin and inhibits the actin myosin interaction and hence produces vascular smooth muscle relaxation (Hidaka et al., 1979). Calmodulin is required for the activity of myosin light chain kinase which is in turn required for contraction of smooth muscle (Gevers, 1984).

Inhibition of the initial influx of calcium decreases the contractile activity of arterial smooth muscle cells and results in vasodilation. The vasodilatory effects of FDP result in an overall decrease in blood pressure (Saltiel et al., 1988).

In vitro studies showed that FDP had selective action, showing greater effects on vascular smooth muscle than cardiac muscle. Because of its lack of effect on venous smooth muscle and on adrenergic vasomotor control, felodipine does not cause orthostatic hypotension (Saltiel et al., 1988). FDP has been reported to inhibit

sodium and water reabsorption in the collecting duct and hence increases urinary flow rate and sodium excretion thereby decreasing mean arterial pressure in normotensive rats (Dibona and Sawin, 1984).

2.1.5.2 Vascular selectivity

FDP is the first compound discovered which has vascular selectivity factor of 100. This drug is known for the selective inhibition of vascular smooth muscles (Ljung and Nordlander, 1987). It shows negative inotropic actions at concentration 1000 times than the concentration required for action on vascular tissue (Curtis et al., 1985). Animal models were used to evaluate vascular versus cardiac selectivity of felodipine, nifidipine and amlodipine. Dose that reduced heart rate by 25% gives the cardiac potency of a drug. Vascular potency is defined as the dose that reduced mean arterial pressure to the same extent. Vascular versus cardiac chronotropic selectivity was found higher for felodipine than for nifedipine and amlodipine (Norlander et al, 1995). Membranes prepared from human coronary arteries and human heart were used for checking the vascular selectivity based on radioligand binding studies. Nifedipine and amlodipine demonstrated vascular to myocardial binding ratio of 10 whereas felodipine, isradipine, nicardipine, and nitrendipine showed a binding ratio of 100 (Godfraind, 1994).

2.1.5.3 Antihypertensive effect

FDP shows excellent antihypertensive effect due to dilation of arterial vessels (Elmfeldt and Hedner, 1983). It has equal potency compared to minoxidil in the treatment of severe hypertension but have fewer side effects when compared to minoxidil. For the short term treatment of hypertension, it is a better third line drug compared to hydralazine. Greater change in the blood pressure over 6 week was observed in case of felodipine treated patients than that of hydralazine (Muir and Wathen, 1987).

2.1.5.4 Other effects

FDP produces reduction of blood-pressure depending on the dose and also because of elevation of ST segment, it delays the development of signs of ischaemia in ECG (Curtis et al., 1985).

Timmis and Jewitt (1985) studied the effect of FDP in congestive heart failure. When it was given as short term therapy, an increase in cardiac index due to reduction in systemic vascular resistance was observed. Whereas on long term therapy, marked improvement in left ventricular function was observed on the day 2 and it remained the same throughout the therapy. An increase in cardiac output and stroke volume was noted both at rest and at exercise due to vasodilator property of FDP (Timmis and Jewitt, 1985).

FDP produces antianginal effects by virtue of its afterload-reducing action,. When it is used in combination with β-blockers, it reduces the number of episodes of angina and increases exercise capacity (Sheridan et al., 1987). Hence, it is regarded as a suitable candidate for ischemic heart diseases.

2.1.6 Pharmacokinetic profile

2.1.6.1 Absorption

On oral administration, FDP is almost completely absorbed and undergoes extensive first-pass metabolism. Oral systemic bioavailability of felodipine is approximately 15%. Peak plasma concentration of FDP is achieved 2.5 to 5 hours post oral dosing. By increasing the dose of felodipine from 5 mg to 40 mg, the peak plasma concentration and the area under the plasma concentration time curve (AUC) increases linearly indicating linear pharmacokinetics (Edgar et al., 1985).

The peak plasma concentration of felodipine (C_{max}) is significantly increased (1.5 to 2 fold) when it is taken after a high fat or high carbohydrate meal. Because the effects of FDP on blood pressure are related to plasma levels, this increase in C_{max} may cause a clinically significant fall in blood pressure (Todd and Faulds, 1992). Therefore, FDP tablet is usually administered with diet low in carbohydrates and fat content.

The administration of a 5 mg FDP tablet with 200 ml grapefruit juice causes threefold increase in AUC and C_{max} in comparison to that obtained with plain water (Lown et al., 1997).

2.1.6.2 Distribution

FDP is extensively bound (nearly 99%) to plasma proteins. It binds predominantly to albumin. Felodipine has a volume of distribution of approximately 10 l/kg at steady state (Ljung and Nordlander, 1987).

2.1.6.3 Metabolism and Elimination

FDP shows extensive first pass metabolism in the liver and is metabolized by CYP3A4 enzyme of the cytochrome P-450 system. All identified metabolites are inactive. Drug gets completely metabolized and no unchanged drug is found in the urine. FDP has extensive distribution to the extravascular tissues. The elimination half life of FDP has been reported to be 11 to 17 h (Todd and Faulds, 1992).

Elderly people have higher plasma levels of felodipine than the young and middle-aged. Impaired liver function significantly decreases the systemic clearance of drug (Edgar et al., 1985).

The plasma concentration-time curve exhibits triexponential declines in 3 distinct phases after i.v. administration to rats. The mean terminal half-life of felodipine is 8.1±4.4 h. The primary pyridine metabolite of felodipine, dehydrofelodipine, appears very rapidly after i.v administration (Wang et al., 1989).

2.1.7 Dosage and administration

The dose of FDP in hypertension is adjusted individually. Usual starting dose for adults is 5 mg once daily. In elderly patients, a starting dose of 2.5 mg once daily is given. If necessary, the dose can be increased in 2.5 or 5 mg/day increments. The usual maintenance dose is 5 to 10 mg per day. Doses higher than 20 mg daily of FDP are not recommended.

The dose of felodipine is tailored in case of patients with severe impaired hepatic functions. In case of impaired kidney functions, reduction in dose is not required because impaired kidney function does not influence the AUC and the peak plasma concentration of FDP.

2.1.8 Commercially available formulations

AstraZeneca, London, UK was first to launch Plendil ER tablets in dose strengths of 2.5, 5 and 10 mg in market for the treatment of severe hypertension. Subsequently, Sanofi Aventis, Paris, France launched Renedil extended release

tablets, film coated tablets containing felodipine in the strengths of 2.5, 5, and 10 mg. Teva UK Limited markets FDP prolonged release tablets under the brand name FOLPIK® XL in the dose strengths of 2.5, 5, and 10 mg. Actavis UK Ltd. markets it as VASCALPHA 5 mg prolonged release tablets. Felodipine is also available in market in combinations with ramipril (Altace® Plus Felodipine) and enalapril (LEXXEL).

In Aug 2010, Jiangbo Pharmaceuticals, Inc. received approval from the Shandong Food and Drug Administration to start the sales of felodipine sustained release tablets in china.

In Dec 2013, Ranbaxy Laboratories, Inc., Gurgaon, India, a subsidiary of Ranbaxy Laboratories Limited received approval from the US Food and Drug Administration to manufacture and market Felodipine Extended-Release tablets USP, 2.5, 5 and 10 mg which are the generic equivalent of Plendil® Extended-release tablets.

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Chapter 3

Analytical Method Development

3.1 Introduction

Analysis is an important component in formulation development of any drug molecule and characterization of developed formulations. An analytical procedure is developed to test a defined characteristic of the drug substance or drug product against established acceptance criteria for that characteristic. 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 the estimation of drugs in various samples.

3.2 Analytical methods for estimation of felodipine

FDP is widely used in treatment of angina pectoris and hypertension (Saltiel et al., 1988). It is also effective against seizures and central ischemic disorders by acting at L-type calcium channels (Murai et al., 2000). Various analytical techniques have been reported for the determination of FDP in bulk, formulations and biological samples.

Quantitative estimation of FDP in formulations by UV-Vis spectroscopy using pure methanol as solvent system has been reported (Gedil et al., 2004). Recently, spectrophotometric quantification of five dihydropyridine derivatives including FDP in tablets and capsules using vanillin reagent has been reported (El Hamd et al., 2013). Simultaneous determination of FDP along with some drugs like amlodipine, enalapril, metoprolol, ramipril using UV-Visible spectroscopy has also been reported (Basavaiah et al., 2005; Rontogianni et al., 2006; El Yazbi et al., 2008; Desai et al., 2012; Salem and Abdallah, 2007). Estimation of FDP in tablets by liquid chromatography using UV detector has been reported (Basavaiah et al., 2003; Gedil et al., 2004). Stability indicating RP-HPLC method using UV and PDA detector and C18 column has also been reported (Cardoza and Amin, 2002; Annapurna et al., 2013). HPLC methods determining FDP in binary mixtures containing ramipril, enalapril, metoprolol has been developed and validated (Rontogianni et al., 2006; El Yazbi et al., 2008; Desai et al., 2012; Salem and Abdallah, 2012). Simultaneous determination of dihydropyridine derivatives along with felodipine has also been reported (Baranda et al., 2004, 2005a, 2005b). A number of LC-MS/MS method for the determination of FDP in dog and human plasma has been reported (Baranda et al., 2005c; Miglioranca et al., 2005; Yan-yan et al., 2006; Sreedevi et al., 2011a, 2011b). Apart from these, estimation of felodipine by spectrofluorometry and capillary gas

chromatography has also been reported (Ahnoff, 1984; Soons et al., 1990; Nishioka et al., 1991; Sakamato et al., 1993; Dru et al., 1995; El Yazbi et al., 2008).

A comprehensive literature survey suggested that the reported methods were not suitable for the determination of FDP in pharmaceutical tablets and in vitro release samples for the particular research works. In fact, the analytical methods used for the routine analysis should be simple, cost effective and rapid apart from meeting criteria with respect to sensitivity, accuracy and precision. For routine analysis of drug in bulk, formulations and in vitro release samples UV-Visible spectrophotometric methods are of choice. But, the reported spectrophotometric methods were either more suitable for simultaneous determination or having drawback of complex sample preparation (Gedil et al., 2004; El Yazbi et al., 2008; Desai et al., 2012). The HPLC methods reported for the routine analysis were less sensitive (Basavaiah et al., 2003; Gedil et al., 2004) or more suitable for estimation of FDP in mixtures (Rontogianni et al., 2006; Desai et al., 2012; Salem and Abdallah, 2007).

The quantification of FDP in rabbit plasma was planned to test the in vivo performance of the developed formulations. None of the bioanalytical method reported used rabbit serum/plasma as biomatrix. Moreover, almost all methods were found to use LC-MS which is a very sensitive and precise instrument but usually not available in laboratories with relatively modest infrastructure (Baranda et al., 2005c; Miglioranca et al., 2005; Yan-yan et al., 2006). So, a simple and sensitive HPLC method using fluoroscence detector was developed and validated for the determination of FDP in rabbit plasma.

The present work comprises of the development of simple, sensitive, accurate and cost effective UV-Visible spectrophotometric method for the determination of FDP in bulk and formulations. HPLC methods were developed for quantification of FDP in stability and plasma samples. The developed methods were validated according to the standard guidelines (International Conference on Harmonization, 1996; US FDA, 2001; US Pharmacopoeia, 2003). Suitable statistical test were performed in order to validate the analytical methods (Bolton and Bon, 2004). The developed and validated methods were applied for the estimation of FDP in bulk, formulation, in vitro release, stability and plasma samples.

3.3 Materials

FDP was gifted by Ranbaxy Laboratories Limited (New Delhi, India). Methanol (HPLC grade), ortho-phosphoric acid and potassium dihydrogen orthophosphate were purchased from Merck (Mumbai, India). Sodium hydroxide was obtained from Rankem (Faridabad, India). Acetyl chloride and hydrogen peroxide were purchased from SD Fine-Chem Limited (Mumbai, India) and Avra Synthesis Pvt. Limited (Hyderabad, India) respectively. All the other materials used were of analytical grade. Millipore water was used wherever needed. Two commercially available FDP tablet formulations (Plendil® by Astra Zeneca Pharma India Limited; Felogard® by Cipla Limited, India) were purchased from the local market.

3.4 Reagents

Phosphate buffer (pH 6.8): Potassium dihydrogen orthophosphate (6.8 g) and sodium hydroxide (0.896 g) were dissolved in millipore water and volume was made upto 1000 ml.

Ortho phosphoric acid (0.1 M): 85% pure ortho phosphoric acid (6.78 ml) was diluted to 1000 ml using millipore water.

Phosphate buffer 10 mM (pH 3.0): Potassium dihydrogen orthophosphate (1.36 g) was dissolved in millipore water and volume was made up to 1000 ml. The pH of solution was adjusted to 3.0 using 0.1 M ortho phosphoric acid.

Methanolic hydrochloric acid (1.0 M): Acetyl chloride (7.1 ml) was added drop-wise to 92.9 ml of chilled methanol under constant stirring.

Methanolic sodium hydroxide (1.0 M): Sodium hydroxide (4 gm) was dissolved in 5 ml millipore water and volume was made up to 100 ml using methanol.

3.5 Analytical Method 1

Ultraviolet-Visible (UV-Visible) spectrophotometric method for estimation of FDP in bulk and formulations

3.5.1 Instrumentation

Spectrophotometric study was conducted on UV Spectrophotometer (UV-1800 Shimadzu) operated at a wavelength range of 200–400 nm with automatic wavelength correction and a wavelength accuracy of 0.5 nm. A pair of 10 mm matched quartz cells was used for all absorbance measurements. The instrument was

connected with a computer loaded with Spectra -manager software for computational purposes.

3.5.2 Selection of media

Various media were investigated to develop UV-Visible spectrophotometric method for the analysis of FDP in bulk and formulations. The criteria employed for selecting media were stability of the drug, solubility of the drug, sensitivity of the method and cost of solvents in order of priority. Various media were investigated and the media finally selected was methanol- pH 6.8 phosphate buffer (50:50 v/v).

3.5.3 Calibration curve

A stock solution of FDP was prepared by dissolving 10 mg of drug in 100 ml of methanol-pH 6.8 phosphate buffer (50:50 v/v) to get a concentration of 100 µg/ml. The λ_{max} of FDP was determined by scanning a suitable dilution of the stock using spectrophotometer. From this primary stock solution, suitable dilutions were made to obtain concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 µg/ml, and absorbance was measured for all dilutions at the λ_{max} (363 nm) of the drug. For linearity of the proposed method, nine separate calibration sets were prepared and analyzed. Least square regression analysis was carried out for the obtained data and calibration curve 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° C. All the solutions were protected from light by using amber coloured 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 in accordance with the 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 of drug concentration of 20 µg/ml from pure drug stock and with drug solution containing excipients (magnesium stearate, lactose, hydroxyethyl cellulose, soluplus,

polycarbophil, poloxamer, eudragit, chitosan) proposed to be used during the course of current research work. The two spectra were compared for any change in the absorbance pattern of FDP in presence of excipients.

For determining the accuracy of proposed method, different quality control (QC) levels of drug concentrations [lower quality control samples (LQC) = 8 µg/ml, medium quality control samples (MQC) = 28 µg/ml, and higher quality control samples (HQC) = 47 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.] ×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=6) to check the analytical recovery. The percent analytical recovery of the added pure drug was calculated as, % Analytical Recovery = [(Cv - Cu)/Ca] X 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 discussed in accuracy. Inter- and Intra-day variation was studied to determine intermediate precision of proposed method. Different QC samples in triplicates were prepared twice in a day and studied for intra-day variation. The same protocol is repeated 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 quantification (LOQ) of FDP by the proposed method were calculated by using standard deviation (SD) of intercept and the slope of regression equation based upon replicate measurement.

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 methanol by ±2% in the selected media.

3.5.5 Estimation of drug content in commercial tablets

Two commercially available tablets brands of FDP (containing 10 mg of the drug) were randomly selected for the estimation of total drug content per tablet. For each brand, 20 tablets were weighed, finely powdered and mixed. An accurately

weighed aliquot (equivalent to 10 mg of FDP) was transferred to a series of 100 ml volumetric flask (5 in each case) and dissolved in methanol 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 suitably diluted with the selected media to obtain a concentration of 20 µg/ml and the samples were analyzed using proposed method.

3.5.6 Results and discussion

3.5.6.1 Selection of media

Solubility of the FDP was studied in series of solvent like ethanol, methanol, methanol-pH 6.8 phosphate buffer (50:50 v/v) and acetonitrile- pH 6.8 phosphate buffer (50:50 v/v). Absorbance of the drug was found to be stable at least for 72 h in methanol- pH 6.8 phosphate buffer at all wavelength. Finally methanol- pH 6.8 phosphate buffer (50:50 v/v) was selected as media on the basis of sensitivity and stability.

3.5.6.2 Calibration curve

The spectrum of FDP showed a distinct λ_{max} at 363 nm. Fig. 3.1 shows overlaid spectra of FDP and blank. The absorbance at 363 nm was found to be stable for at least 72 h at 25 ± 2° C, indicating stability of the drug in the selected media. Absorbance values for different drug concentrations as given in Table 3.1. At all levels of concentrations the standard deviation was found to be low and the % RSD did not exceed 0.85. The concentrations predicted by using calibration curve equations were nearly matching with the nominal concentrations. The linearity range for FDP was found to be 5–50 µg/ml. The linear regression equation obtained was Absorbance = [0.019 x concentration (µg/ml)] + 0.007; with regression coefficient value of 0.9999. Individual values of slopes and intercepts obtained were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (5.77×10^{-5}) and standard error of intercept (9.00×10^{-4}) indicated high precision of the proposed method. Lower calculated *F*- value [calculated *F*-value (8, 81) of 6.53×10^{-5} and critical *F*- value of 2.05] further confirmed precision of the method.

3.5.6.3 Analytical method validation

Fig. 3.2 shows overlaid spectra of pure FDP and combination of FDP with HEC in 1:1 proportion in the selected media. Estimation of FDP in formulations and comparison of pure drug spectrum with drug spectrum in presence of common excipients used in the formulations confirmed lack of interference at the wavelength used (363 nm) in this method. Absence of interference confirmed the specificity and selectivity of the method.

All three QC levels (LQC, MQC, HQC) showed an accuracy (% bias) ranging from -0.23 to 0.40. The high (nearly 100%) mean percentage recovery values and their low STDEV values (STDEV< 0.9) represented the accuracy of the method (Table 3.2). In the standard addition method, the mean percentage analytical recoveries (± STDEV) for 10, 20 and 30 µg/ml concentration were found to be 100.95 (± 0.95), 99.83 (± 1.25) and 101.25 (± 1.19) respectively. This result further established the validity and reliability of the proposed method. Fig 3.3 shows overlaid spectra of blank, LQC (8 µg/ml), MQC (28 µg/ml) and HQC (47 µg/ml).

In repeatability study, the % RSD ranged from 0.52 to 0.80 (Table 3.2). % RSD values were low for intermediate precision, with intra-day variation not more than 1.81 % and inter-day variation less than 1.85 (Table 3.3). Lower % RSD values indicated the repeatability and intermediate precision of the method.

LOD and LOQ values were found to be 0.47 µg/ml and 1.42 µg/ml respectively. The method was found to be robust as variation of the pH of the selected media by 0.5 units and variation of concentration of methanol by ± 2 % did not affect absorbance significantly.

The results of the estimation of FDP in pharmaceutical formulations by proposed method ranged from 100.24 to 100.36 % of the claimed amount with maximum STDEV of 1.25 (Table 3.4). This indicated absence of interference of excipient matrix in estimation of FDP by the proposed method. The estimated drug content with low values of STDEV further established precision of the method (Table 3.4).

3.6 Analytical method 2

Stability indicating liquid chromatographic method for estimation of FDP in bulk and formulations

3.6.1 Instrumentation and chromatographic conditions

The liquid chromatographic instrument used was of Shimadzu (LC-2010CHT, Kyoto, Japan) equipped with fluorescence detector (RF-20A prominence Flourescence Detector). The stability study was conducted on Shimadzu (LC10ATvp, Kyoto, Japan) equipped with fluorescence detector (RF-10AXL Fluorescence Detector). Shimadzu CLASS-VP, version 5.33 software was used for acquiring and processing the data. Chromatographic separation was achieved on C18 column (BDS Hypersil endcapped; 250 mm x 4.6 mm; 5 µm; Thermo Scientific, Mumbai, India).

Other instrument used in the experiments 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 (Centrifuge 5430 R, Eppendorf, Germany) and deep freeze (Vestfrost, Australia).

3.6.2 Selection of mobile phase

Phosphate buffers of various pH in different combinations of methanol or acetonitrile were tested for the optimization of mobile phase. The mobile phase selected finally comprised of aqueous media (10 mM dihydrogen phosphate buffer of pH 3.0 adjusted with 0.1 M ortho phosphoric acid) and methanol (20:80 v/v). The prepared mobile phase was degassed by sonication for 30 min. The criteria employed for the selection of mobile phase were peak characteristics (retention time and asymmetric factor), sensitivity (height and area), ease of sample preparation, and applicability of the method for current research work.

3.6.3 Calibration curve

Primary stock solution of 100 µg/ml FDP was prepared by dissolving accurately weighed 10 mg of the drug in methanol and making up the volume to 100 ml. A secondary stock solution of 10 µg/ml was prepared by taking appropriate aliquot from the primary stock and diluting it with the mobile phase. Suitable quantity of secondary stock solution of FDP was taken and properly diluted to achieve

standard solutions of 10, 20, 50, 100, 200, 400, 800 and 1000 ng/ml. 50 µl of each solution was injected, and the peak area was recorded using fluorescence detector set at excitation and emission wavelength of 368 and 434 nm respectively. In order to obtain linearity, three separate calibration sets were prepared and analyzed. Least 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. All the solutions were kept in amber colour glass container to protect the solutions from light.

3.6.4 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 estimated as per standard guidelines.

To study selectivity of the method, FDP stock solutions (100 µg/ml) were prepared separately in the optimized mobile phase with and without excipients (magnesium stearate, lactose, hydroxy ethyl cellulose, soluplus, polycarbophil, poloxamer, eudragit and chitosan). All the solutions were diluted suitably with the mobile phase to get a drug concentration of 200 ng/ml and were analyzed. A blank solution containing only excipients was also injected and interference near the drug peak was checked.

In order to determine accuracy of the developed method, various QC levels of the drug concentrations [lower quality control samples (LQC) = 15 ng/ml, medium quality control samples (MQC) = 480 ng/ml and high quality control sample (HQC) = 840 ng/ml] were prepared independently and analyzed (n =6). Accuracy was assessed by calculating percentage RSD and percentage bias (% bias). % bias was calculated as, % bias = [(Predicted conc.- Nominal conc.)/ Nominal conc.] x 100. Different concentrations of pure drug (100, 200 and 400 ng/ml) were added to a known pre-analyzed formulation sample and analyzed using the proposed method (n=6) to check analytical recovery. The percent analytical recovery of the added pure drug was calculated as, % Analytical Recovery = [(Cv - Cu)/Ca] x 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 QC samples of drug concentrations (n=6) as mentioned in accuracy. Intra- and inter-day variation was studied to determine intermediate precision of the proposed method. Various levels of drug concentrations in triplicates were prepared twice in a day and studied for the 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 concentration from the regression equation was taken as precision.

The limit of detection (LOD) and limit of quantification (LOQ) of FDP by the proposed method were calculated using STDEV of intercept and slope of the regression equation based upon replicate measurement.

Robustness of the developed method was determined by varying the pH of the media by ± 0.2 units. Benchtop and stock solution stability of FDP was studied by storing the samples at controlled room temperature of 25 ± 1 °C for a period of 24 h.

3.6.5 Force degradation studies

Forced degradation studies of FDP were carried out to explore stability indicating nature and specificity of the developed method. Therefore, FDP was intentionally degraded by exposing it to acidic and alkaline hydrolytic as well as photolytic and oxidative stress conditions.

3.6.5.1 Hydrolytic treatments

3.6.5.1.a. Acidic degradation

Acid degradation was carried out by refluxing (at 100°C temperature) FDP in 1.0 M methanolic hydrochloric acid (2 mg/ml) for 3 h.

3.6.5.1.b. Basic degradation

This was performed by refluxing (at 100°C temperature) FDP in 1.0 M methanolic sodium hydroxide (2 mg/ml) for 3 h.

To exclude possible degradative effect of the refluxing, drug was also refluxed with methanol (2 mg/ml) for 3 h.

3.6.5.2 Oxidative degradation

Oxidative degradation was performed by adding 0.5 ml FDP solution (1 mg/ml in methanol) to 2 ml of 30% H_2O_2. The resultant mixture was kept at room temperature for 1 h.

3.6.5.3 Photodegradation

For photodegradation study, methanolic solution of the FDP (1 mg/ml) was exposed to UV light at a wavelength of 254 nm, at a distance of 20 cm for a period of 24 h.

After completion of stress treatments, samples were allowed to cool to room temperature (if needed) and appropriately neutralized when required (samples of acid and base degradation studies). To exclude possible degradative effect of the light on FDP, acid, base and oxidative degradation studies were performed in dark. All the degradation samples were suitably diluted using mobile phase before injecting into the HPLC system. The degraded samples were compared with control sample (freshly prepared samples lacking degradation treatment).

3.6.6 Estimation of drug content in commercial tablets

Two commercially available tablets brands of FDP (containing 10 mg of the drug) were randomly selected for the estimation of total drug content per tablet. For each brand, 20 tablets were weighed, finely powdered and mixed. An accurately weighed aliquot (equivalent to 5 mg of FDP) was transferred to a series of 50 ml volumetric flask (5 in each case) and dissolved in methanol 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 suitably diluted with the mobile phase to obtain a secondary stock of concentration of 10 µg/ml. An aliquot of secondary stock was suitably diluted with mobile phase to obtain a concentration 400 ng/ml and the samples were analyzed using the proposed method.

3.6.7 Results and discussion

3.6.7.1 Selection of mobile phase

Mobile phase was optimized by considering the aspects of peak characteristics (retention time and asymmetry factor) and sensitivity (peak height and area). Mobile

phase used in the study comprised of aqueous phase (10 mM potassium dihydrogen ortho-phosphate buffer, pH adjusted to 3.0 with 0.1 M ortho phaosphoric acid) and methanol (20:80 v/v). With the optimized mobile phase retention time of FDP was observed at 6.40 ± 0.13 min with an asymmetric factor of 1.01 ± 0.08 (Fig. 3.4). The retention time of FDP increased to 7.13 min and decreased to 5.61 min with decrease and increase in proportion of methanol by 2% v/v in the mobile phase respectively. However, there was no effect on the peak area, peak height and asymmetry factor. Change in pH of the aqueous phase above 3.0 caused increase in the asymmetry factor of drug peak. Hence, aqueous phase (10 mM potassium dihydrogen phosphate buffer, pH 3.0) and methanol (20:80 v/v) was selected as mobile phase.

3.6.7.2 Calibration curve

Various concentrations and their corresponding area (determined at the excitation and emission wavelength of 368 and 434 nm) are shown in Table 3.5. At all concentrations, the STDEV was low and % RSD did not exceed 1.68. Overlaid chromatograms of blank and 400 ng/ml are shown in Fig. 3.5.

The retention time of FDP was 6.40 ± 0.13 min with an asymmetric factor of 1.01 ± 0.08. Total run time for single injection was 15 min for the proposed method. The predicted concentration were nearly matching and well within the acceptable limit provided by the guidelines. The linear regression equation obtained was, Peak area = [76.30 x concentration (ng/ml)] + 760.85; with regression coefficient of 0.9994.

Individual values of slopes and intercepts obtained were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (3.82×10^{-1}) and standard error of intercept (14.42) indicated high precision of the proposed method. Lower calculated *F*- value [calculated *F*-value (2, 21) of 1.95×10^{-4} and critical *F*- value of 3.46] further confirmed precision of the method.

3.6.7.3 Analytical method validation

Fig. 3.6 shows overlaid chromatogram of pure FDP and combination of FDP with HEC in 1:1 proportion. Estimation of FDP in formulations and comparison of pure drug peak with drug peak in presence of common excipients used in the formulations confirmed lack of interference at the retention time of FDP. The blank

samples of excipients did not show any interference near the drug peak. In presence of excipients, peak characteristics of the drug (retention time, area and asymmetric factor) were not affected. This indicated that there is no 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 - 0.72 to 0.19 (Table 3.6). The high (nearly 100 %) mean % recovery values and their low STDEV values (STDEV<1.8) represented the accuracy of the method. In the standard addition method, the mean percentage analytical recoveries (± STDEV) for 100, 200 and 400 ng/ml concentrations were found to be 99.39 (± 0.82), 100.56 (± 0.57) and 100.56 (± 0.97) respectively. This result further established the validity and reliability of the proposed method.

In repeatability study, the % RSD values ranged from 1.35 to 1.72 (Table 3.6). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 1.88% and inter-day variation not more than 1.36% (Table 3.7). Lower % RSD values indicated the repeatability and intermediate precision of the method.

LOD and LOQ were found to be 1.08 and 3.27 ng/ml respectively. The method was found to be robust as the variation of pH of the selected media by ± 0.2 unit did not have any significant effect on the retention time, peak height, peak area and asymmetric factor. Various concentrations of bench-top FDP solutions and stock solutions of FDP showed % RSD values less than 1.76%, indicating stability of FDP in the stock solutions. These solutions exhibited no change in peak characteristics (retention time, asymmetric factor) at least until 24 h at room temperature. During this period no additional peaks were observed in the chromatograms across all concentrations.

The results of the estimation of FDP in pharmaceutical formulations by the proposed method ranged from 100.18 to 100.32% of the claimed amount with maximum STDEV of 0.53 (Table 3.8). Assay values of the formulations were very close to the label claim. This indicated absence of interference of excipients matrix in estimation of FDP by the proposed method. The estimated drug content with low values of STDEV further established precision of the proposed method.

3.6.7.4 Force degradation studies

The representative chromatogram of pure FDP sample is shown in Fig. 3.7a. The observed HPLC results of force degradation study demonstrated that FDP is susceptible to the hydrolytic (acidic/basic), oxidative and photolytic stress conditions. The percentage degradation was calculated by the formula: % degradation = [(peak area of pure FDP - peak area of treated FDP)/peak area of pure FDP] × 100.

3.6.7.4.a. Hydrolytic treatments

In the chromatogram of acid degradation sample, degradation product peak was observed at retention time (RT) of 5.55 min. Reduction in the peak area of FDP (RT = 6.50 min) was also noticed for acid degradation sample (Fig. 3.7b). A 36.85% degradation of FDP was found after acid hydrolysis at 100°C for 3 h (Table 3.9). The chromatogram of alkaline degradation sample demonstrated significant reduction in peak area. Further, peaks of two degradation products were also observed (Fig. 3.7c). Reduction in peak area and two degradation product peaks in chromatogram suggested that FDP is highly susceptible to alkaline hydrolysis. Moreover, there was 51.45% degradation of FDP observed which demonstrated faster rate of alkaline hydrolysis compared to the acid hydrolysis (Table 3.9). Two degradation products formed during alkaline hydrolysis were eluted at RT of 4.20 and 5.62 min whereas intact FDP was eluted at 6.54 min (Fig. 3.7c).

It is hypothesized that the alkaline degradation process proceeds in two steps (Walash et al., 2014): first step involves partial hydrolysis of the methyl ester resulting in compound I. Second step involves partial hydrolysis of the ethyl ester linkage resulting into formation of compound II (Fig. 3.8). Compound I and II eluted before FDP probably due to more polarity than FDP. Furthermore, compound II (disodium salt) was eluted before compound I (monosodium salt) due to higher polarity. This pathway has been proposed on the basis of previous studies on FDP (Walash et al., 2014) and similar compounds containing dihydropyridine nucleus (Abdine et al., 2001).

Acid hydrolysis followed same pathway as alkaline hydrolysis, but at slower rate (Walash et al., 2014). Therefore, only one peak (probably for compound III) was observed in case of acid degradation sample at RT of 5.55 min (Fig. 3.7b and Table 3.9). Furthermore, degradation of FDP was not observed upon refluxing it with methanol demonstrating drug degradation was solely due to hydrolysis in acidic and

basic conditions excluding the effect of heat.

3.6.7.4.b. Oxidative degradation

FDP was also found to be susceptible to oxidative degradation by hydrogen peroxide (30%) resulting in reduction of FDP peak area by 26.40% when exposed for 1 h (Table 3.9). Furthermore, chromatogram for oxidative degradation sample did not show any peak for the degradation product (Fig. 3.7d). This may be because of oxidation of the dihydropyridine ring by hydrogen peroxide into corresponding pyridine ring (compound IV) with a loss of fluorescence (Fig. 3.8) (Walash et al., 2014). In order to confirm the hypothesis, samples were analyzed using HPLC coupled with UV detector at 238 nm. Observed chromatogram with UV detector demonstrated degradation product peak at RT of 5.17 min and intact FDP peak at 6.30 min (Fig. 3.9a). This study was conducted on different HPLC instrument Shimadzu (LC-2010CHT, Kyoto, Japan) coupled with UV detector therefore little shift in FDP peak was observed.

3.6.7.4.c. Photolytic degradation

In case of photolytic degradation sample, peak of degradation product was not observed in the chromatogram, however a significant reduction in peak area of FDP was seen (Fig. 3.7e). There was 47.90% degradation of FDP observed after exposure to UV light for 24 h (Table 3.9). Upon UV exposure, dihydropyridine ring of FDP might have undergone oxidation by atmospheric oxygen to the corresponding pyridine ring (compound IV) with a consequent loss of fluorescence (Fig. 3.8) (Walash et al., 2014). Degradation product formed due to photolytic oxidation process was also evaluated by analyzing samples using HPLC coupled with UV detector. Observed chromatogram is represented in Fig. 3.9b. Similar to oxidative degradation product peak, photolytic degradation product peak was also observed at same RT (5.14 min) demonstrating photolytic degradation process also follows similar degradation pathway (Fig. 3.8). The degradation product peak was well separated from the intact FDP peak (Fig. 3.9b) demonstrating stability indicating property of the method.

3.7. Analytical Method 3

RP-HPLC method for estimation of felodipine in rabbit plasma

3.7.1. Instrumentation and chromatographic conditions

The liquid chromatographic instrument used was of Shimadzu (LC-2010CHT, Kyoto, Japan) equipped with fluorescence detector (RF-20A prominence Flourescence Detector). Chromatographic separation was achieved on C18 column (BDS Hypersil endcapped; 250 mm x 4.6 mm; 5 µm; Thermo Scientific, Mumbai, India).

Other instrument used in the experiments 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 (Centrifuge 5430 R, Eppendorf, Germany) and deep freeze (Vestfrost, Australia).

3.7.2. Blood collection and isolation of plasma

New Zealand white rabbits weighing 2.0-2.5 kg weight were used for the blood collection. Blood was collected in 2.0 ml centrifuge tubes containing 100 µl of EDTA solution (1.0 mg/ml) from the marginal ear vein of the animal and with the permission of Institutional Animal Ethics Committee (Protocol approval number :IAEC/RES/16/04). The collected blood was kept at room temperature for 30 min and centrifuged at 4000 rpm for 20 min. The clear supernatant plasma layer was subsequently collected.

3.7.3 Selection of mobile phase

Phosphate buffers of various pH in different combinations with methanol or acetonitrile were tested for the optimization of mobile phase. The mobile phase selected for the chromatography was comprised of aqueous media (10 mM dihydrogen phosphate buffer of pH 3.0 adjusted with 0.1 M ortho phosphoric acid) and methanol (20:80 v/v). The prepared mobile phase was degassed by sonication for 30 min. The criteria employed for the selection of mobile phase were peak characteristics (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 the in vivo studies in rabbits.

3.7.4 Calibration curve

Primary stock solution of 100 µg/ml FDP was prepared by dissolving accurately weighed 10 mg of the drug in methanol and making up the volume to 100 ml. A secondary stock solution of 10 µg/ml was prepared by taking aliquot from the primary stock and diluting with selected mobile phase. Suitable quantity of secondary stock solution of FDP was spiked into rabbit plasma to achieve standard solutions of 10, 20, 50, 100, 200, 400, 800 and 1000 ng/ml.

In order to prepare plasma standards, a simple and one-step protein precipitation method was employed. 1.5 ml of acetonitrile was added to 500 µl of spiked plasma samples and vortex mixed for 1 min. The samples were allowed to stand for 10 min on bench top condition for thorough precipitation of proteins. The samples were then centrifuged at 13000 rpm at 4°C for 20 min. Clear supernatant of the centrifuged sample was taken in clean and dry 2 ml centrifuge tube and evaporated to dryness using vacuum concentrator [vacuum concentrator-Maxi Dry Lyo 230v (Heto-holten, Denmark)]. The dried residue was reconstituted in 500 µl of the selected mobile phase, vortex mixed for 5 min and centrifuged at 13000 rpm 4°C for 10 min. The sample were then transferred to clean and dry auto-sampler vials. In order to establish linearity of the method eight sets of plasma standard were prepared and analyzed. 50 µl of the standard solutions were injected in to column and the peak area at the retention time of FDP was recorded. Least square regression analysis was exercised for the obtained calibration data. An analysis of variance test (one-way) was performed with respect to the peak area observed for each concentration during the replicate measurement of the plasma standards.

3.7.5 Analytical method validation

The developed method was validated for selectivity, linearity, range, precision, accuracy, sensitivity and stability in plasma samples.

3.7.5.1 Selectivity

The test for selectivity was performed using six different lot of blank plasma batches processed by the same method and analyzed to determine the extent of interference caused by endogenous substances at retention time (RT) of drug. These samples were compared with those containing FDP, at lower limit of quantification (LLOQ). The rational of this comparison is to ensure the quality of the results in the

analysis. The area of interfering peak, if any, at retention time of drug should be ≤ 20 % of the mean area of analyte peak at LLOQ.

3.7.5.2 Precision and accuracy

Accuracy and precision of the proposed method was determine by analyzing QC standards prepared at LOQQC (10 ng/ml), LQC (15 ng/ml), MQC (480 ng/ml) and HQC (840 ng/ml) levels. Each QC standards was processed and analyzed in six replicates. The samples were analyzed at three validation batches, one batch on a particular day and remaining two on another day. Concentration of FDP in QC standard was calculated from the regression equation. Accuracy was expressed as % bias and precision was determined as intra- and inter- batch variation and expressed as % RSD.

3.7.5.3 Recovery

Recovery of FDP from plasma samples was assessed (n=6) at LQC (15 ng/ml), MQC (480 ng/ml) and HQC (840 ng/ml) respectively. The extraction efficiency was determined by comparing the areas obtained from the processed plasma samples to those of corresponding concentration of analytical samples injected directly in the HPLC system.

3.7.5.4 Sensitivity

Sensitivity of the method was obtained by determining the lowest concentration of FDP which could be estimated with acceptable accuracy and precision (% RSD ≤ 20). This lower limit of quantification (LLOQ) was taken as a concentration of 10 ng/ml. Six replicates of LLOQ were prepared and analyzed by the proposed method on three different occasions. Concentration of FDP was calculated from the regression equation and parameters such as % bias and % RSD were determined.

3.7.5.5 Stability studies

The stability of drug is governed by various factors such as chemical properties of the drug, biological matrix and storage conditions. Stability of the drug in stock solution and plasma was assessed. All the experimental conditions which the drug actually encountered during the sample analysis were simulated during the

method validation. Long term stability at -20 °C (for 90 days), freeze- thaw stability (for 3 cycles), bench-top stability at room temperature (for10 hours), stock solution stability (for 15 days) were assessed.

In order to evaluate long term stability, aliquots of QC samples were first frozen at -20 °C for 90 days, then thawed and analysed against fresh samples. The difference between the starting concentration and the concentration after 90 days shows whether the drug in plasma remains stable under this condition for the stated period of time. Evaluation of freeze-thaw stability involved estimation of analytes after three freeze-thaw cycles. Bench-top stability for 10 hours of the spiked samples was checked. The stock solution stability of the drug was evaluated at refrigerated condition for 15 days by comparing the response of the samples prepared from stored stock solutions to that of samples prepared using fresh stock solution. All stability studies were carried out using six replicates of LQC and HQC samples and the results were compared with freshly spiked CC standards and fresh QC samples.

3.7.6 Results and discussion

3.7.6.1 Selection of mobile phase

Mobile phase was optimized by considering the aspects of peak characteristics (retention time and asymmetric factor), sensitivity (peak height and area) and separation of peak from proteins present in the plasma. Mobile phase used in the study comprised of aqueous phase (10 mM potaassium dihydrogen ortho-phosphate buffer, pH adjusted to 3.0 with 0.1 M ortho phaosphoric acid) and methanol (20:80 v/v). With the optimized mobile phase retention time of FDP was observed at 6.80 ± 0.19 min with an asymmetric factor of 1.070 ± 0.099. The retention time of FDP increased to 7.43 min and decreased to 5.96 min with decrease and increase in proportion of methanol by 2% v/v in the mobile phase respectively. However, there was no effect on the peak area, peak height and asymmetric factor. Change in pH of the aqueous phase above 3.0 caused increase in the asymmetric factor drug peak. Hence, aqueous phase (10 mM potassium dihydrogen phosphate buffer, pH 3.0) and methanol 20:80 v/v was selected as mobile phase.

3.7.6.2 Linearity and range

The linearity regression analysis indicated good linearity between average peak area and plasma drug concentration over the range of 10 - 1000 ng/ml with weighted regression equation-

Peak area = 69.84 x concentration (ng/ml) + 672.3 (weighted $1/x^2$)

The regression coefficient (R^2) value of the calibration curve was found to be 0.9990. Table 3.10 shows various calibration curve concentrations and their corresponding areas. The STDEV of the area was found to be acceptable and % RSD did not exceed 13.92. Overlaid chromatogram of entire calibration curve range and QC samples are shown in Fig. 3.10 and Fig. 3.11 respectively. Retention time of FDP was found to be 6.80 ± 0.19 min (Fig. 3.10) in the selected mobile phase. The obtained peak was of good resolution with asymmetry factor of 1.070 ± 0.099. Total run time for single injection was 15 min.

One way ANOVA was performed for peak area obtained at individual concentration and lower calculated *F*- value [calculated *F*-value (2, 21) of $4.34x10^{-3}$ and critical *F*- value of 3.46] further confirmed precision of the method.. Therefore, there was no significant difference between the measured calibration curve standards. Good weighted linear relationship existed between average peak area and the plasma concentration of felodipine.

3.7.6.3 Analytical method validation

3.7.6.3.a. Selectivity

Chromatogram of blank plasma (n=6) revealed that there was no peak present at the retention time of felodipine (Fig. 3.12). The absence of response in the blank biological matrix confirmed the selectivity of the method from the endogenous substances. Furthermore, chromatogram of test samples from in vivo study demonstrated no interference from the metabolite or degradation product near retention time of the drug (Fig. 3.13). Thus the proposed method was found to be selective in determination of FDP from the spiked as well as test samples.

3.7.6.3.b. Precision and Accuracy

The obtained results confirmed the accuracy of the proposed method as the % bias ranged from -0.07 to 10.52. The method was found to be precise with % RSD of 2.49

to 11.38 (intra-batch) and 2.76 to 9.62 (inter-batch). Results of precision and accuracy study were in acceptable limits which indicated that the method was accurate and precise (Table 3.11).

3.7.6.3.c. Recovery study

The proposed method showed high and consistent recovery of felodipine from rabbit plasma. Mean absolute recovery in plasma was found to be in range of 92.60 - 95.57% over the calibration range (Table 3.12). As the recovery obtained was nearly 100%, internal standard was not used during the course of analysis.

3.7.6.3.d. Sensitivity

Six replicate injections of LLOQ (10 ng/ml) indicated % RSD and % bias of less than 12% and 11% respectively. Hence, it can be concluded that the method is sensitive with high signal to noise ratio at excitation and emission wavelength of 368 nm and 434 nm respectively.

3.7.6.3.e. Stability studies

Results obtained for bench-top stability at two QC levels (LQC and HQC) demonstrated that FDP was stable in rabbit plasma under the bench top conditions. The drug did not show significant change (% RSD) in response up to 10 hours as compared with the response obtained from fresh standards. Similarly, in long term stability study, FDP was found to be stable in rabbit plasma at -20 °C at both the QC levels as there was no significant difference between response of the standard at zero time and at the end of 90 days. The deviation observed was within the acceptable limit (% RSD < 15). Insignificant degradation was observed in the QC standards up to 3 freeze thaw cycles. Percentage deviation calculated for all stability studies were within the acceptable limit of ± 15% at LQC and HQC levels exhibiting good stability of the of FDP under the various conditions of the study. Table 3.13 shows results of the stability studies. Summary of the results is also presented in Table 3.14.

3.8 Conclusions

The proposed methods were found to be simple, precise, accurate and suitable for the quantification of FDP in bulk, in vitro release samples, formulations and rabbit plasma samples. The UV-Visible method was cost effective and produced prompt

results. The UV-Visible method was successfully applied for the estimation of drug content in the in vitro release samples.

The developed liquid chromatographic method for the determination of FDP in bulk and formulations was sensitive as compared to the earlier reported methods using same instruments. The method was also observed to be specific as evidenced by the non-interference of regularly used excipients for the formulations. In forced degradation study, peaks of degradation products were not interfering with FDP peak demonstrating selectivity and stability indicating capability of developed and validated HPLC method. Peaks of all the degradation products were found to be well separated from the FDP peak. Finally, the developed HPLC bioanalytical method was also specific, sensitive and successfully employed in the determination of FDP in plasma samples obtained from the pharmacokinetic study of the optimized formulation in rabbits.

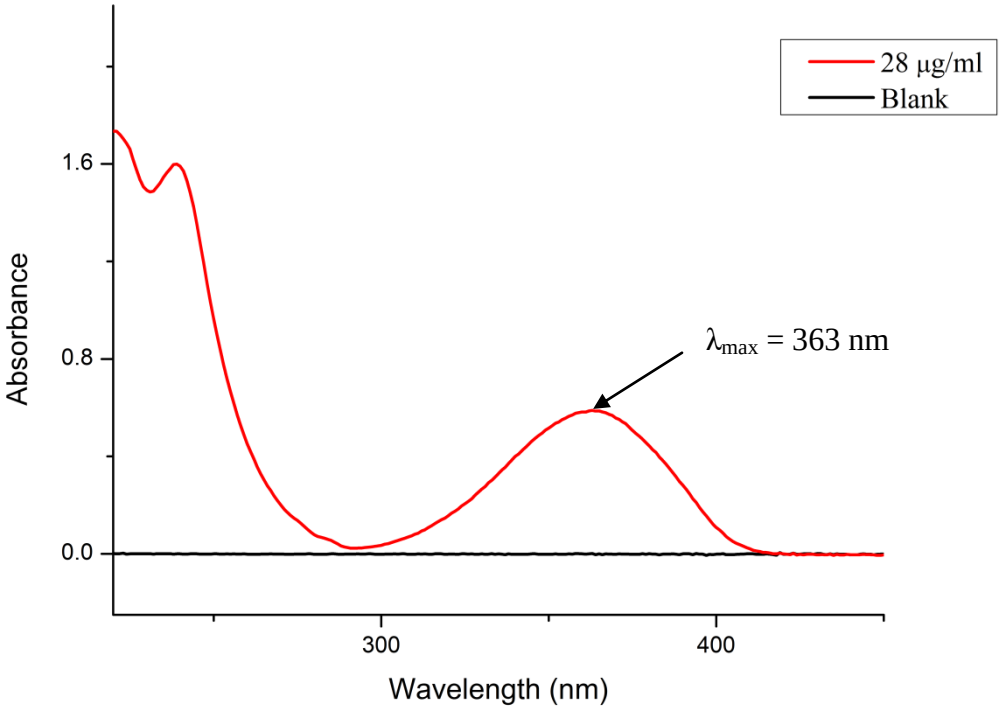

Fig. 3.1. Overlaid UV-Visible absorption spectra of FDP (28 µg/ml) and blank for analytical method 1

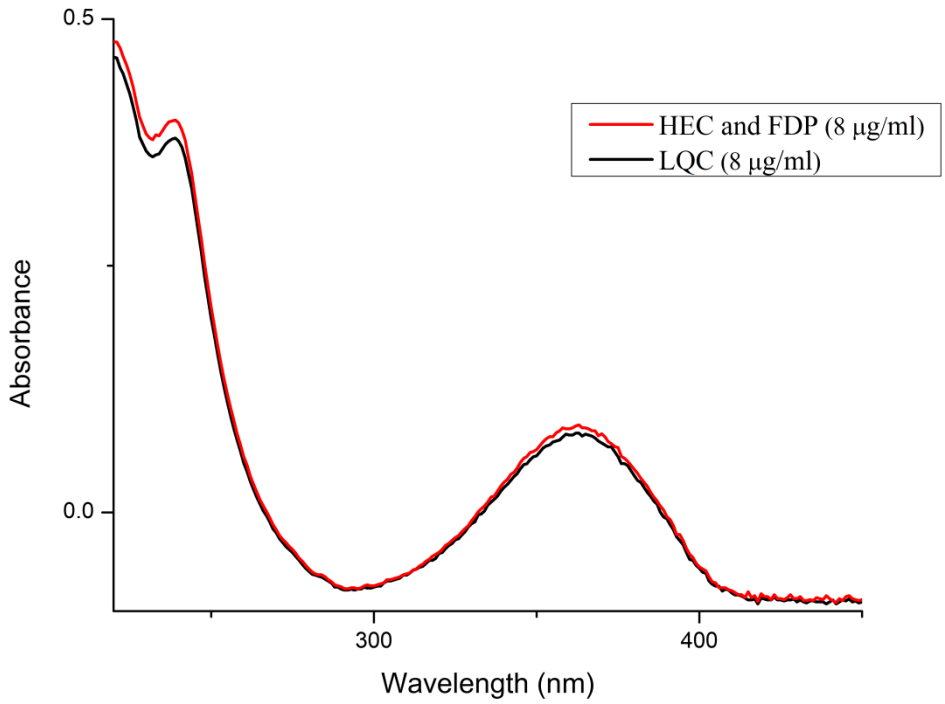

Fig. 3.2. Overlaid spectra of pure FDP solution and solution containing FDP and HEC in 1:1 ratio obtained using analytical method 1

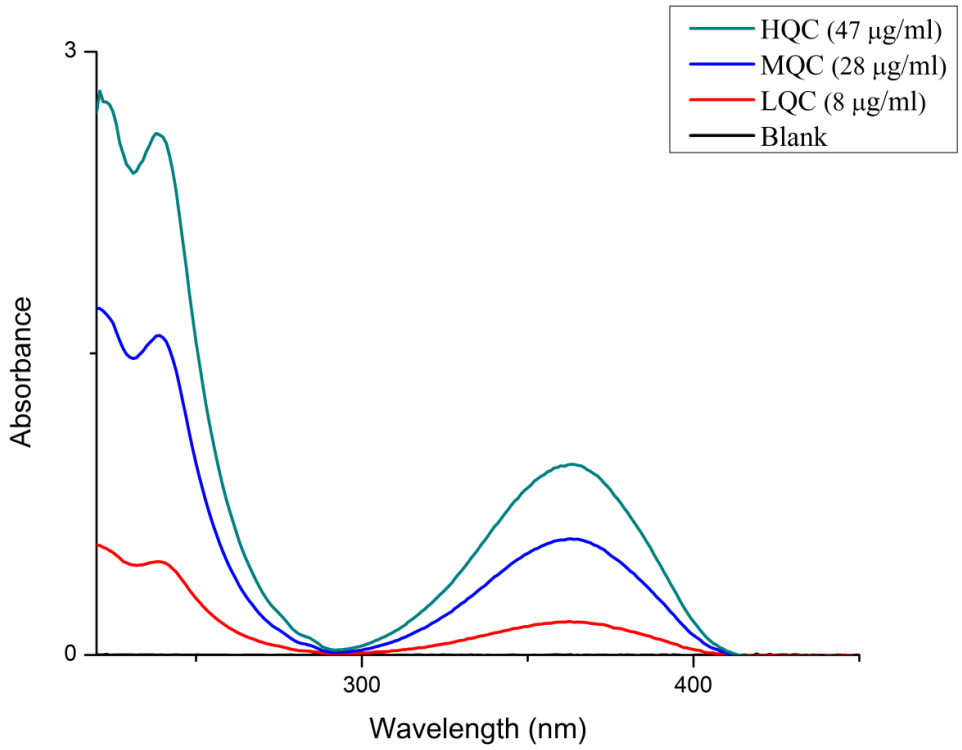

Fig. 3.3. Overlaid spectra of blank, LQC (8 µg/ml), MQC (28 µg/ml) and HQC (47 µg/ml)

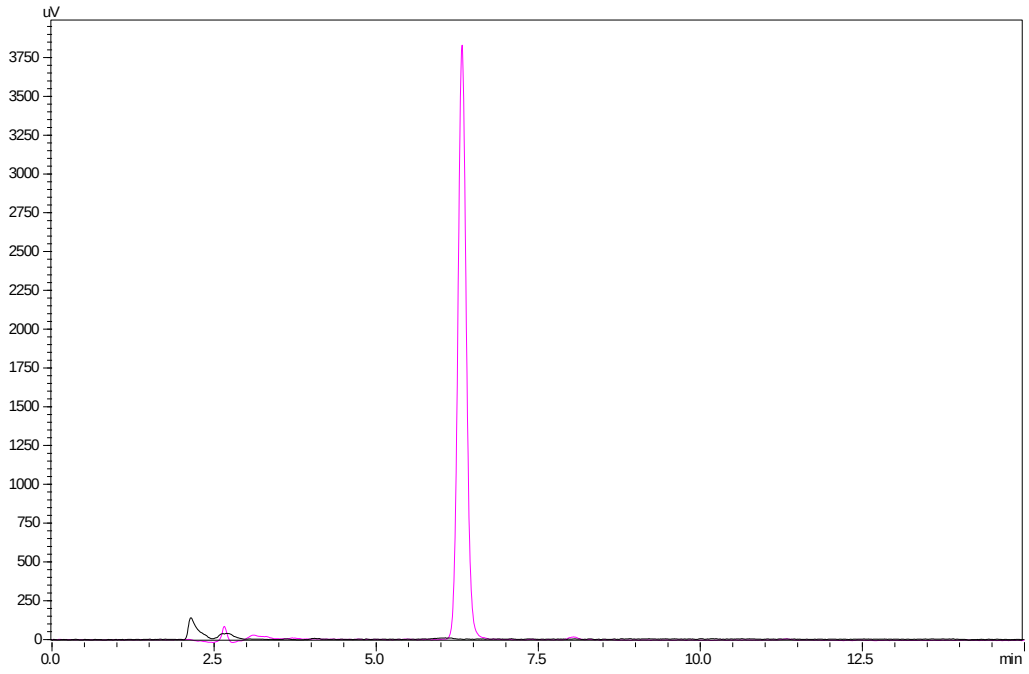

Fig. 3.4. Representative chromatogram of pure FDP (400 ng/ml)

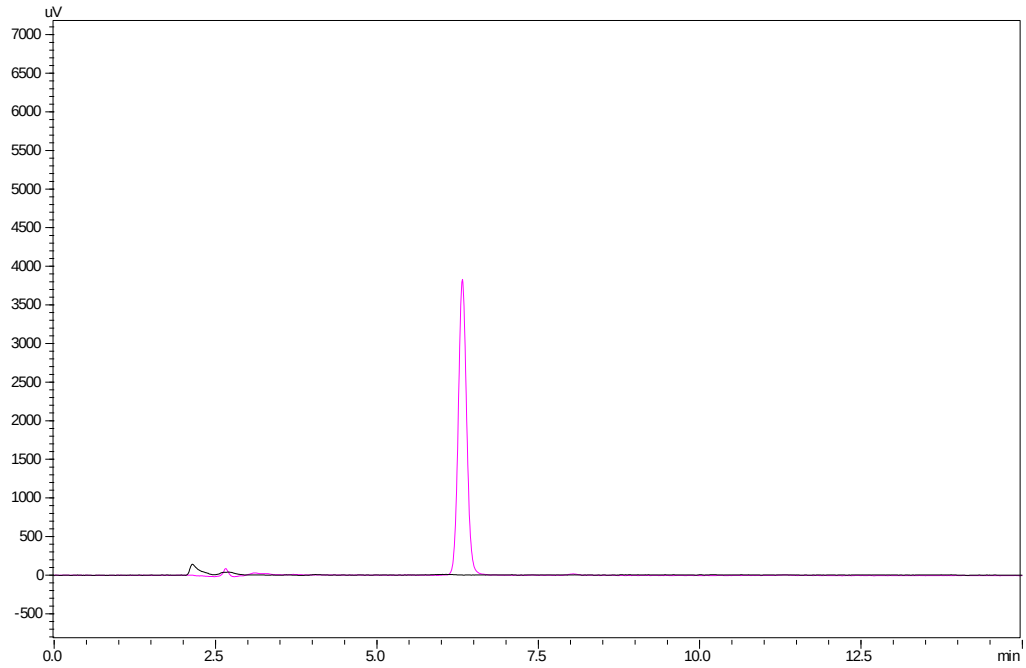

Fig. 3.5. Overlaid chromatogram of blank (mobile phase) and pure FDP (400 ng/ml)

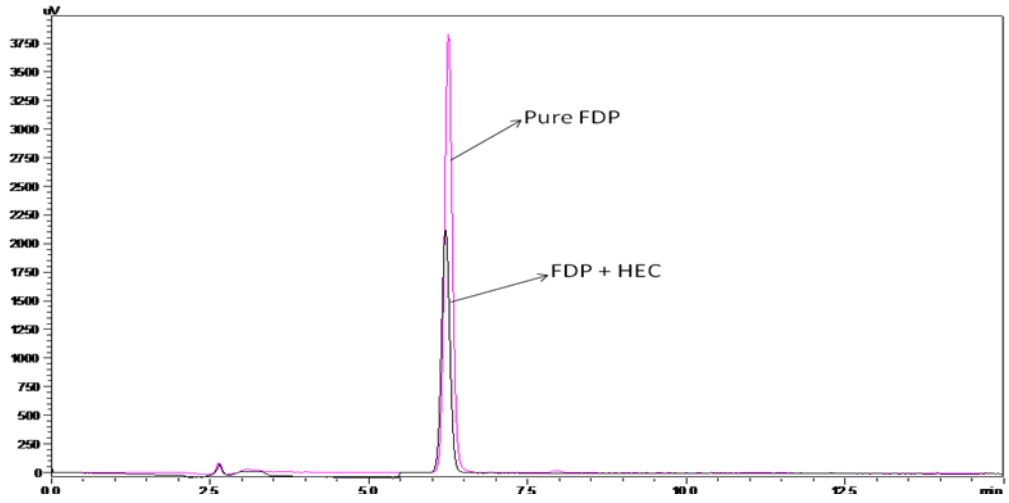

Fig. 3.6. Overlaid chromatogram of pure FDP (400 ng/ml) and combination of FDP (200 ng/ml) with HEC in 1:1 proportion

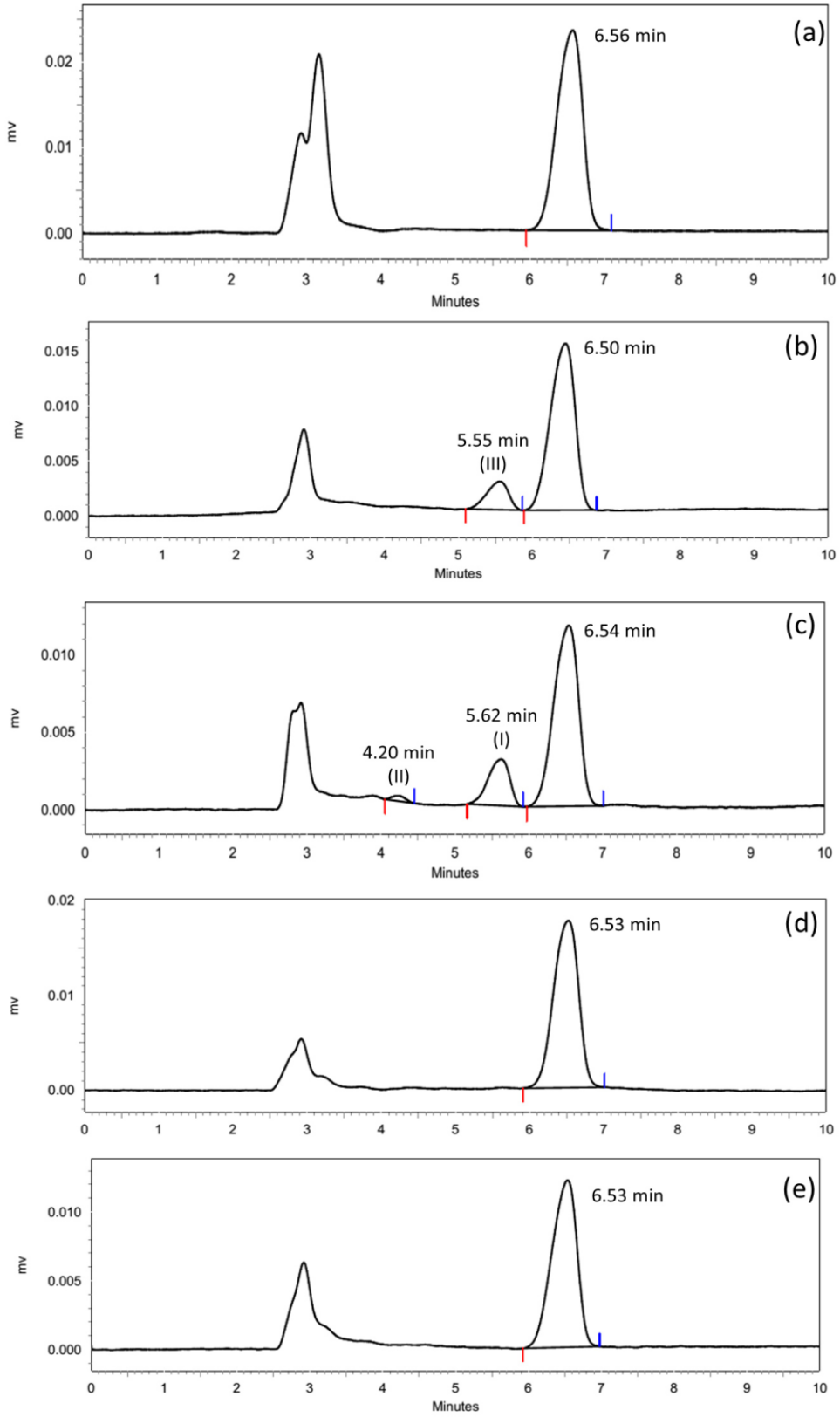

Fig. 3.7. Representative chromatograms of samples of (a) pure FDP (b) acid degradation (c) base degradation (d) oxidative and (e) photolytic degradation

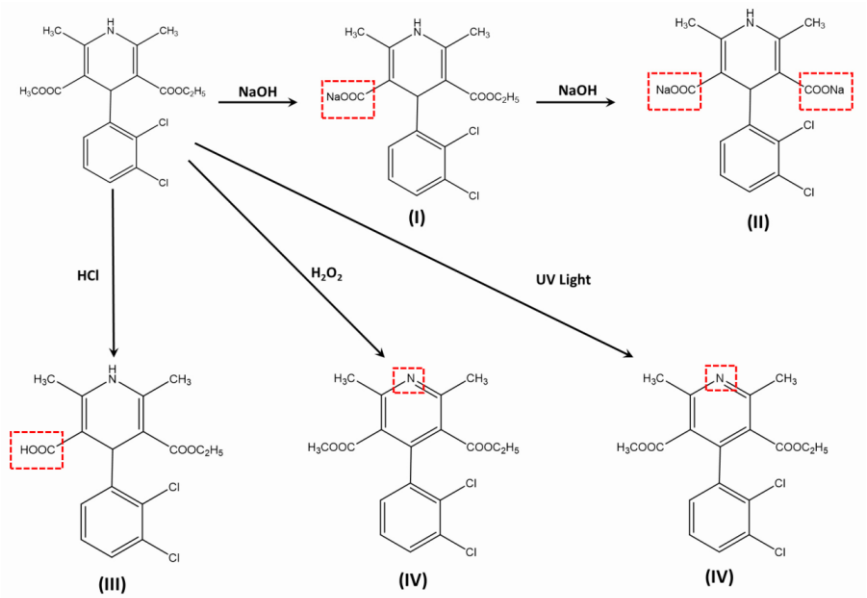

Fig. 3.8. Illustration of probable degradation pathways of FDP under different stress conditions

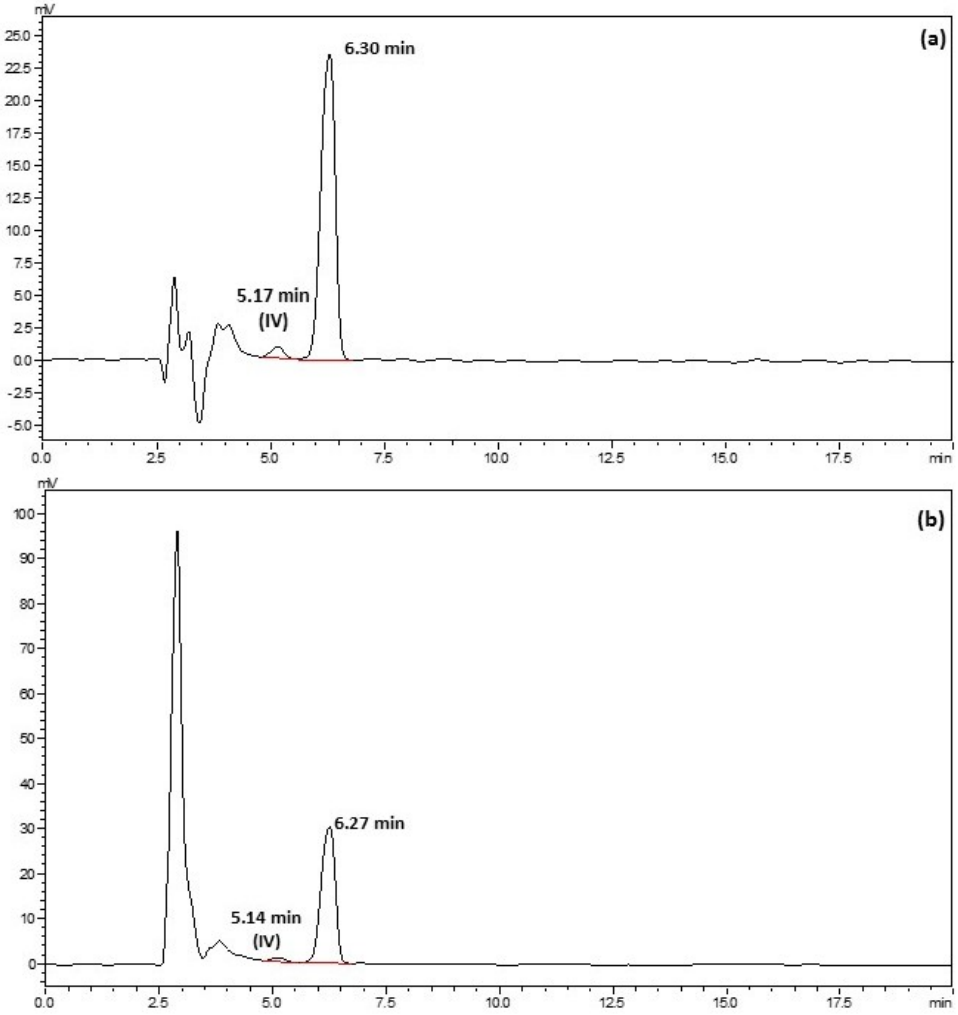

Fig. 3.9. Representative chromatograms acquired using UV detector for samples of (a) oxidative degradation and (b) photolytic degradation

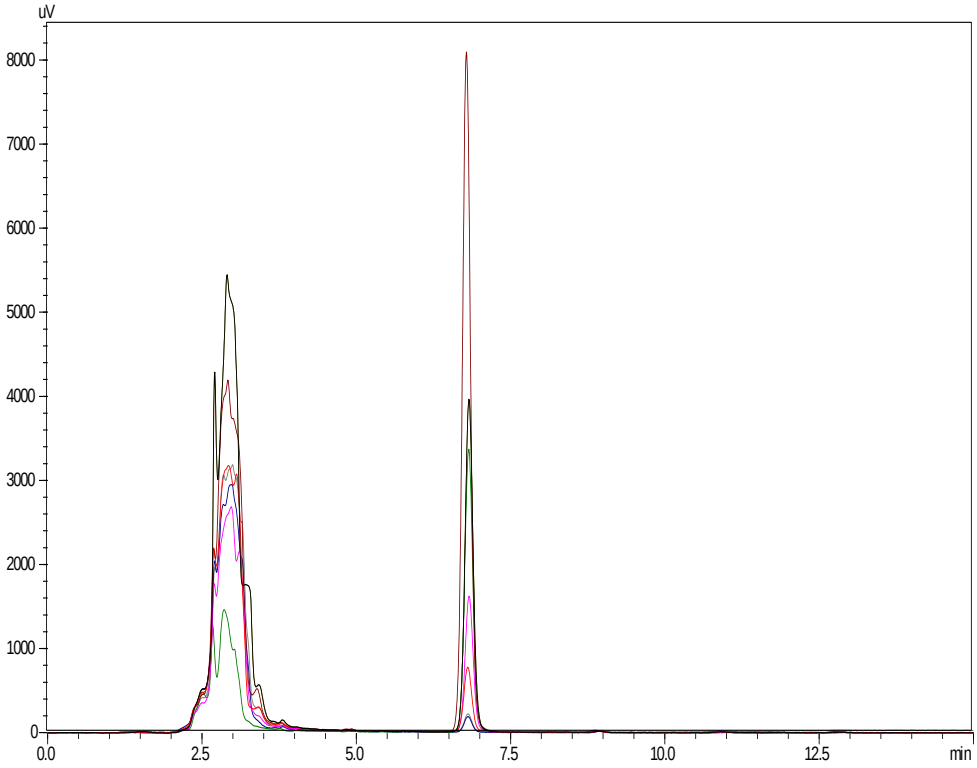

Fig. 3.10. Overlaid chromatogram of all calibration curve concentrations for analytical method 3

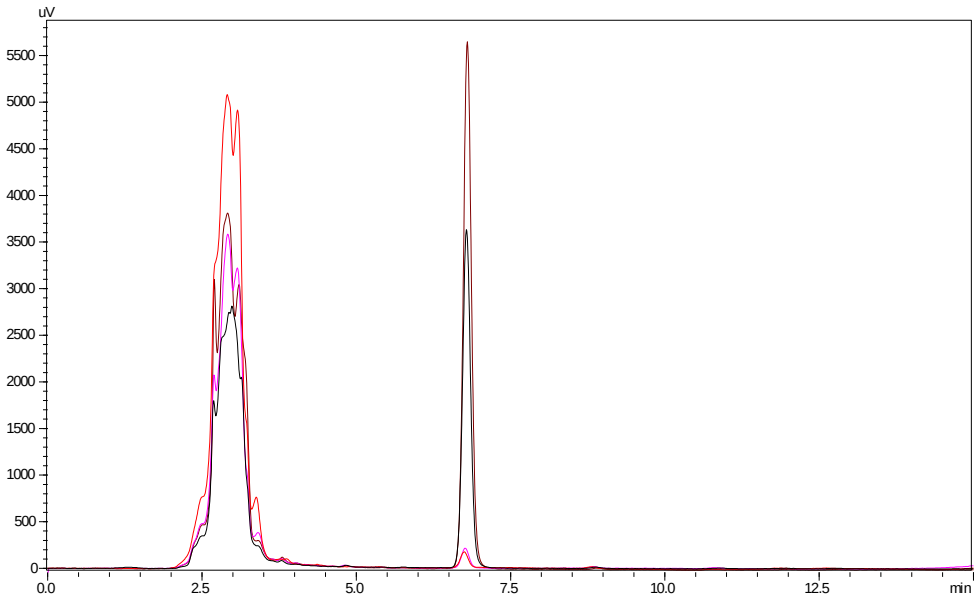

Fig. 3.11. Chromatogram of LLOQ, LQC, MQC, HQC (10, 15, 480, 840 ng/ml)

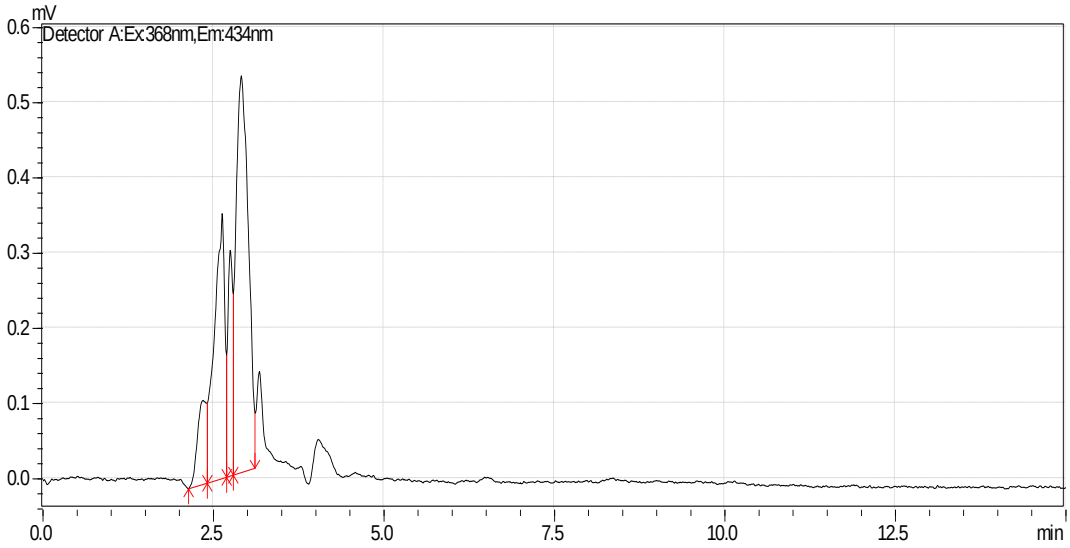

Fig. 3.12. Representative chromatogram of blank plasma

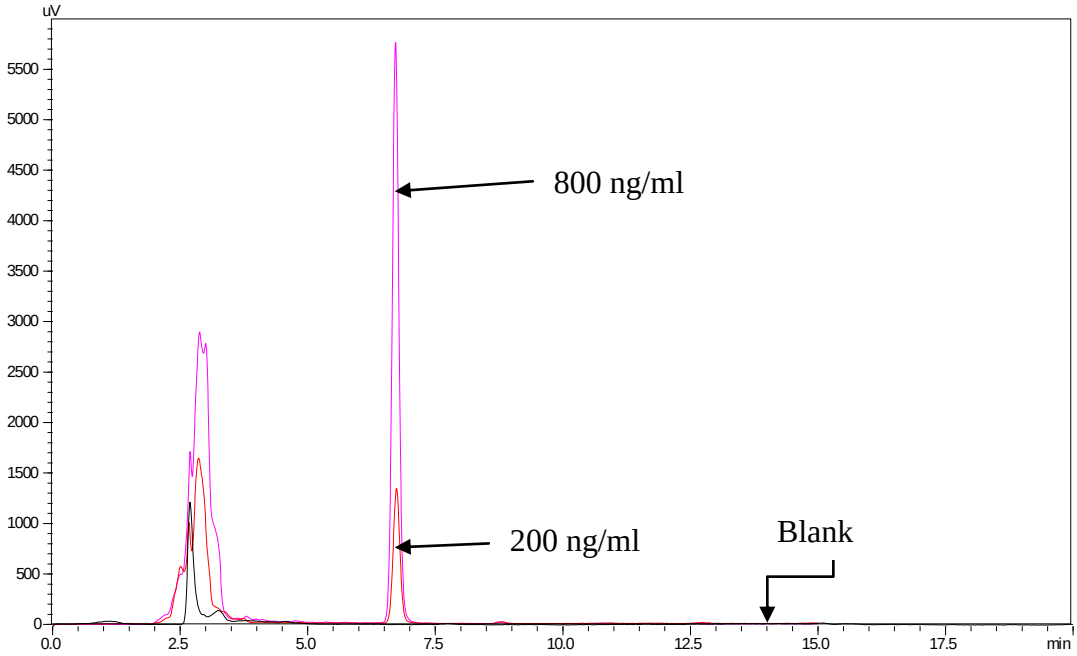

Fig. 3.13. Overlaid chromatograms of blank plasma, plasma standard (800 ng/ml) and in vivo test sample (200 ng/ml)

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

Conc. (µg/ml)	Mean Absorbance[a] (± STDEV)	% RSD[b]	Predicted Conc.[c] (µg/ml)
5	0.102 ± 0.001	0.77	4.99
10	0.198 ± 0.001	0.62	10.05
15	0.292 ± 0.002	0.63	15.01
20	0.388 ± 0.002	0.56	20.05
25	0.482 ± 0.004	0.78	24.99
30	0.575 ± 0.005	0.85	29.92
35	0.680 ± 0.005	0.69	35.42
40	0.768 ± 0.004	0.57	40.06
45	0.863 ± 0.007	0.75	45.05
50	0.958 ± 0.005	0.53	50.04

[a] Each value is mean of nine 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] (± STDEV)	% Bias[c]
	Range	Mean[b] (± STDEV)	% RSD		
LQC	7.95 - 8.10	8.03 ± 0.06	0.80	100.40 ± 0.80	0.40
MQC	27.73 - 28.17	27.94 ± 0.17	0.55	99.77 ± 0.55	-0.23
HQC	46.68 - 47.36	46.95 ± 0.24	0.52	99.89 ± 0.52	-0.11

[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	0.90	1.58	1.68	1.82
	0.87	1.64	1.21	
MQC	0.82	0.80	0.56	1.03
	0.30	1.29	0.33	
HQC	0.29	0.48	1.80	1.58
	0.55	1.81	1.74	

Table 3.4: Determination of FDP in marketed products using analytical method 1

Commercial Products	Mean Amount Found[a] (mg) (± STDEV)	% Assay[a] (± STDEV)
Plendil Tablets (10 mg)	10.02 ± 0.13	100.24 ± 1.25
Felogard Tablets (10 mg)	10.04 ± 0.11	100.36 ± 1.10

[a] Each value is the mean of five independent determinations.

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

Conc (ng/ml)	Mean Peak Area[a] (± STDEV)	%RSD[b]	Predicted Conc.[c] (ng/ml)
10	1531.67 ± 12.43	0.81	10.11
20	2097.67 ± 35.30	1.68	17.52
50	4267.33 ± 51.60	1.21	45.96
100	8003.33 ± 107.39	1.34	94.93
200	15831.00 ± 258.63	1.63	197.54
400	33013.33 ± 424.30	1.29	422.76
800	61448.00 ± 806.06	1.31	795.48
1000	76739.67 ± 404.17	0.53	995.92

[a] Each value is the mean of three 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] (ng/ml)			Mean % Recovery[b] (± STDEV)	% Bias[c]
	Range	Mean[b] (± STDEV)	% RSD		
LQC	14.68 - 15.17	14.89 ± 0.20	1.35	99.28 ± 1.35	-0.72
MQC	469.59 - 487.32	479.76 ± 8.25	1.72	99.95 ± 1.72	-0.05
HQC	829.47 - 858.19	841.63 ± 11.47	1.36	100.19 ± 1.37	0.19

[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	1.09 0.35	1.63 1.26	1.57 1.64	1.30
MQC	1.88 1.83	1.25 1.54	0.76 1.44	1.35
HQC	1.79 1.19	0.98 0.76	0.72 0.44	0.93

Table 3.8: Determination of FDP in marketed products using analytical method 2

Commercial Products	Mean Amount Found[a] (mg) (± STDEV)	% Assay[a] (± STDEV)
Plendil Tablets (10 mg)	10.03 ± 0.05	100.32 ± 0.53
Felogard Tablets (10 mg)	10.02 ± 0.05	100.18 ± 0.47

[a] Each value is the mean of five independent determinations.

Table 3.9: Summary of forced degradation studies

Treatment	Exposure (h)	FDP undegraded (%)	RT of degradation products (min)
Acid hydrolysis (1M methanolic HCl)	3	63.15	5.55 (III)[a]
Base hydrolysis (1M methanolic NaOH)	3	48.55	4.20 (II)[a], 5.62 (I)[a]
Oxidation (H_2O_2, 30%)	1	73.60	-
UV light (λ= 254 nm)	24	52.10	-

[a]number in parenthesis indicates probable degradation product observed in HPLC chromatograms

Table 3.10: Calibration curve data of plasma standard of FDP by analytical method 3

Conc (ng/ml)	Mean peak area[a]	STDEV	% RSD
10	1415.17	117.63	8.31
20	1930.39	128.06	6.64
50	3926.13	546.54	13.92
100	7443.39	903.04	12.13
200	14881.26	1421.27	9.55
400	30701.88	3241.50	10.56
800	56532.29	4526.63	8.01
1000	73535.83	4221.74	5.74

[a]Each value represents the average of three independent determinations

Table 3.11: Intra and inter-batch accuracy and precision of analytical method 3

Level	Repeatability (n=6)			Intermediate Precision (n=18)		
	Mean (ng/ml)	%RSD	% Bias	Mean (ng/ml)	% RSD	% Bias
LOQQC	11.05	11.38	10.52	10.99	9.62	9.93
LQC	14.92	8.38	-0.52	15.49	8.11	3.30
MQC	479.66	4.02	-0.07	482.67	3.69	0.56
HQC	847.99	2.49	0.95	845.13	2.76	0.61

Table 3.12: Recovery study of analytical method 3

Quality Control Sample	% Recovery[a] (n=6)	
	Mean ± STDEV	% RSD
LQC	92.60 ± 2.61	3.15
MQC	91.48 ± 3.47	2.49
HQC	95.57 ± 3.98	2.86

[a] %Recovery = [(Peak area of plasma standard/Peak area of analytical standard)*100]

Table 3.13: Stability study of FDP in rabbit plasma

Storage period and storage conditions	Nominal Conc. (ng/ml)	Mean Conc.[a] (ng/ml)	% RSD	% Recovery
Stock solution~ 15 days, refrigerated temp.	15	14.65	8.30	97.63
	840	851.07	1.68	101.32
Bench top ~ 10 hours, room temp.	15	15.74	9.31	104.94
	840	858.60	2.10	102.21
Freeze thaw cycle (3 cycles),	15	16.81	5.31	112.09
	840	860.02	1.00	102.38
Long term stability,	15	16.38	11.77	109.22
(- 20 °C ~90 days)	840	859.92	2.03	102.37

[a]Each value represents the average of six independent determinations

Table 3.14: Summary of validation parameters of analytical method 3

Parameter	**Value**
Calibration range	10 - 1000 ng/ml
Linearity (Coefficient)	$R^2 = 0.9990$
Regression equation	peak area = 69.84 x concentration (ng/ml) + 672.3
Lower limit of quantification (LLOQ)	10 ng/ml
Absolute recovery/Recovery efficiency	89.603 % - 92.574 %
Accuracy (% Bias)	- 0.071 - 10.517 (Intraday) 0.556 - 9.928 (Interday)
Precision (%RSD)	2.489 - 11.375 (Intraday) 2.755 - 9.623 (Interday)
Selectivity	Selective

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Chapter 4

Preformulation Studies

4.1 Introduction

Preformulation study is the foundation of formulation development of any candidate drug. It provides complete information of pharmaceutically significant physicochemical properties of the selected drug. The objective of preformulation study is to select appropriate polymorphic form of the drug, analyze its physicochemical properties and present a comprehensive knowledge of its stability under various conditions that are useful for the development of an optimum dosage form. This information is necessary to minimize difficulties in formulation development, reduce formulation development cost and reduce time required to successfully launch the drug into market (Ravin and Radebaugh, 1990; Chen et al., 2006; Niazi, 2007). Preformulation study is regarded as the interface between new drug discovery and the further formulation development process. Knowledge obtained from this study help to decide rational and effective roadmap to design a proper dosage form for maximum availability of the drug. Preformulation studies include determination of solubility, stability, dissociation constant, partition coefficient and particle size of the drug molecule. Comprehensive knowledge of the stability of drug in pure form and in physical mixture with proposed excipients under various conditions of temperature, light and humidity is essential for the selection of compatible excipients for design of formulation.

Certain physicochemical parameters like solubility and partition coefficient have already been reported in the literature and hence were not determined during the current research work. Aqueous solubility of FDP has been reported to be 0.5 mg/l (Saltiel et al., 1988) in literature while the Log P value of 4.46 has been reported (van der Lee et al., 2001).

During the course of this research work, pH and photo stability of FDP was determined. The dissociation constant value (pK_a) of FDP was also determined. Solid state stability of the drug at controlled room temp (CRT) and accelerated condition (AT) was assessed. Moreover, compatibility study of FDP with various excipients proposed to be used during course of current research was carried out.

4.2 Experimental

4.2.1 Materials

FDP pure samples and chitosan (CH) were gifted by Ranbaxy Laboratories Limited (New Delhi, India). Potassium dihydrogen orthophosphate, methanol (HPLC grade) and ortho-phosphoric acid (85% pure) was purchased from Merck (Mumbai, India). Hydrochloric acid and sodium hydroxide was obtained from Fisher Scientific (Mumbai, India) and Rankem (Faridabad, India) respectively. Sodium chloride, lactose and magnesium stearate were purchased from SD Fine-Chem Limited (Mumbai, India). Soluplus and poloxamer 407 were obtained as gift sample from BASF, India. Hydroxyethyl cellulose (HEC) and guar gum (GM) were supplied as gift sample by Signet Chemical Corporation Pvt. Ltd., India. Eudragit RSPO (EG), and tri-calcium phosphate (TCP) were purchased from Sigma Aldrich Chemicals, USA. Polycarbophil (PC) and carbopol 934P (CP) were obtained as gift samples from Noveon Inc., USA and Cadila Pharmaceutical Ltd., India respectively. Ethylcellulose (EC) and hydroxypropyl methylcellulose (HPMC) were obtained as gift samples for Colorcon, India. Agar (AR) was purchased from Himedia Labs., India. Millipore water was used throughout the study.

4.2.2 Instrument/Equipment

Dissociation constant (pK_a) determination was conducted on UV-Vis Spectrophotometer (UV-1800 Shimadzu) operated at a wavelength range of 200-400 nm. Validation study of the estimated pK_a value was performed using Jasco V-570 UV/VIS/NIR spectrophotometer. Phase solubility and stability study was conducted on orbital shaker incubator (Macroscientific Works Pvt. Ltd, Delhi). The pH of the buffer solution was measured using digital pH meter (Eutech® pH meter). Thermal analysis was performed using differential scanning calorimeter (Shimadzu, Japan; model: DSC-60; integrator: TA-60 WS thermal analyzer; integrating software: TA-60; principle: heat flux type; temperature range: -150-600 °C; heat flow range: ± 40 mW; temperature program rate: 0-99 °C per min; atmosphere: inert nitrogen at 30 ml/min). A five digit analytical balance (Metter Toledo, Switzerland) was used for all weighing purpose. Compatibility

studies were mainly conducted on differential scanning calorimeter (DSC). For some of the compatibility studies, Fourier Transform-Infrared (FT-IR) Spectrophotometer (Perkin-Elmer Spectrum 400-FT-IR/FT-FIR) was used. Analytical instruments mentioned in chapter 3 were used for all sample analysis.

4.3 Methods

Analytical method 1 mentioned in chapter 3 was used for the pK_a determination, and analytical method 2 was used for stability analysis.

4.3.1 Identification and characterization of polymorphic form

Identification of drug was carried out by comparing DSC thermogram and infra red (IR) spectra of the drug with that of reported values.

Different polymorphic forms of a crystalline drug exhibit different physicochemical properties. FDP has been reported to be present in two polymorphic forms namely From I and Form II. Form I FDP is obtained by recrystallization from methanol, acetonitrile or ethanol. Form II can be recrystallized from hexane and methanol mixture (Rollinger and Burger, 2001; Lou et al., 2009). The Form I with melting point of around 145 °C, is most stable polymorphic form of FDP and widely used in the marketed formulation. Form II is thermodynamically metastable at room temperature and melts at 136 °C (Lou et al., 2009). In Form I, N-H vibrational stretch is observed at 3370 cm^{-1} wavenumber and in Form II it is positioned at 3336 cm^{-1} (Rollinger and Burger, 2001).

FT-IR and DSC studies of FDP were carried out in order to determine the polymorphic form of the drug. For FT-IR study, pellet of FDP was prepared by mixing the drug with appropriate quantity of potassium bromide. Pellet was prepared using Perkin Elmer hydraulic press by applying 7-10 N force. The IR spectra was obtained in % transmission mode in the spectral region 450-4000 cm^{-1}. Eight scans were taken at a resolution of 4 cm^{-1} for each sample. The data were processed using Spectrum v5.3.1 software provided along with the instrument by Perkin Elmer. For DSC study, approximately 2-4 mg of sample was weighed accurately and heated at a rate of 10°C/min from 25°C to 400°C in hermetically sealed aluminium pans. Nitrogen was used as purge gas at a flow rate of 30 ml/min.

4.3.2 Determination of dissociation constant (pK_a)

The procedure developed by Albert and Serjeant (1962) was used for the exact determination of the pK_a of felodipine. In order to exercise this method, rough estimate of the pK_a of the compound in question is essential. There was no information available anywhere regarding pK_a value of the drug to the best of our knowledge. Hence, a preliminary study for the approximate determination of pK_a of felodipine by inflection method was carried out (Cox and Nelson, 2008). For this purpose, the absorbance of drug solutions were plotted against their corresponding pH. The inflection point in this curve was considered as approximate value of felodipine pK_a.

Furthermore, to establish that the molecule has only one pK_a value, absorbance diagram which is essentially a plot of absorbance of the drug at one wavelength versus that of another wavelength at various pH was analyzed. For a system governed by one equilibrium, absorbance diagram produces a linear curve without any change in its course (Blanco et al., 2005). To achieve this, absorbance of felodipine, in various buffers of pH ranging from 1.3 to 12.0, at 364 nm was plotted against absorbance of same solutions at 381 nm wavelength. The absorbance values are given in Table 4.1 and the absorbance diagram is presented in Fig. 4.1.

Using the approximate pK_a value determined by the inflection method, method reportedly Albert and Serjeant was carried out to ascertain the exact pK_a value for felodipine. A series of seven buffer solutions of pH 4.3, 4.5, 4.8, 5.0, 5.2, 5.5 and 5.7 were made. The buffer solutions were prepared by using appropriate amount of 10 mM Potassium dihydrogen orthophosphate (KH_2PO_4) and 0.1 M ortho phosphoric acid (H_3PO_4). Appropriate quantity of sodium chloride (NaCl) was added in order to maintain the ionic strength of all solutions to 0.02. Primary stock solution (2 mg/ml) of felodipine was prepared in HPLC grade methanol as it is poorly water soluble (0.5 mg/l) (Saltiel et al., 1988). Working solutions of 20 µg/ml strength were prepared in respective buffers using primary stock solutions and all the samples were analyzed in triplicate. UV-spectrum of all the sample solutions were taken and the absorbance at 364 nm wavelength was recorded. The difference in the optical densities of the ion and the molecule was considerable at this wave length. Likewise, absorbance of ionic and neutral molecule was estimated using 0.01M hydrochloric acid (HCl) and 0.01 M sodium

hydroxide (NaOH) respectively. The absorption values at corresponding pH are shown in Table 4.2. UV-spectrum of FDP at various pH is given in Fig. 4.2. All the solutions were kept in glass containers at 25°C and protected from light.

4.3.2.1 Calculation

pK_a of felodipine, a weakly basic drug, was determined by using the following Eq. (4.1)

$$pK_a = pH + \log \frac{d_M - d}{d - d_I} \quad (4.1)$$

Where, d_M is the absorbance of unionized molecule, d is the absorbance of respective buffers tested and d_I is the absorbance of ionized molecule and pH is the value recorded on pH meter. In order to avoid errors in averaging and meet the proper degree of precision, the individual pK_a values were first converted to their antilogarithm and the average of these antilogarithms were calculated. The logarithm of this average was reported as the average pK_a value.

To validate the results, the experiment was repeated using another working solution of 10 µg/ml concentration. Furthermore, the experiment was conducted by a different member of the team and also on an another spectrophotometer. Table 4.3 gives the validation results.

4.3.3 Stability study

4.3.3.1 Solution state stability

The solution state stability of FDP was carried out in disparate buffered solutions of different pH (pH 1.2, 2.0, 3.0, 4.0, 5.0, 6.0, 6.8, 7.0, 8.0, 9.0). A stock solution (1 mg/ml) of FDP was prepared in methanol. 625 µl of this stock solution was added to buffered solution 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 ± 1 °C in screw capped containers. The entire experiment was carried out in triplicates. Samples were protected from light during the study. Samples were withdrawn at different time points, suitably diluted and were analyzed by analytical method 2 of chapter 3.

In order to determine photostability, 25 µg/ml solution of FDP was prepared in pH 6.8 buffer as mentioned above. The samples were exposed to natural sunlight. All the experiments were conducted in triplicates. Samples were withdrawn at different time points and were analyzed using analytical method 2 of chapter 3.

4.3.3.2 Solid state stability

Solid state stability of FDP and compatibility with the excipients proposed to be used in the development of buccal mucoadhesive tablets was assessed. Excipients used for the study were AR, CP, CH, EC, EG, HEC, HPMC, GM, TCP, lactose, magnesium stearate, poloxamer 407 and soluplus.

DSC was used to study solid state stability and compatibility of FDP with various excipients proposed to be used for the formulation of buccal mucoadhesive dosage forms. Mixed samples of drug and excipients were analysed by analytical method 2 of chapter 3 for content uniformity. The experiment was conducted for pure FDP, pure excipients and physical mixture of the drug and various excipients (1:1 ratio). Around 2-4 mg of sample (pure FDP, pure excipients and physical mixture of the drug and individual excipients) was taken and sealed in standard aluminium pan with lid and DSC thermogram was recorded as discussed in section 4.3.1. All the samples were stored at CRT (25 ± 2 °C and 60 ± 5% RH) away from light for 12 months and study was repeated.

FT-IR study was also carried out for pure FDP, individual excipients and combination of FDP with excipients (mixed in 1:1 ratio) as mentioned earlier in section 4.3.1. All the samples were stored at CRT (25 ± 2 °C and 60 ± 5% RH) away from light for 12 months and study was repeated.

Different excipients and FDP (#60) were physically mixed in 1:10 ratio. The physical mixtures were prepared carefully by geometrical mixing and analyzed for content uniformity with help of analytical method 2 of chapter 3. The prepared mixture were taken in vials and kept at different temperature conditions like controlled room temperature (CRT: 25 ± 2 °C and 60 ± 5% RH) and at accelerated condition (AT: 40 ± 2^{o}C and 75 ± 5% RH). At predetermined time intervals, samples (in triplicates) were taken and analyzed for drug content by analytical method 2 of chapter 3 after suitable dilution.

4.4 Results and discussion

4.4.1 Identification and characterization of polymorphic form

DSC and FT-IR studies were conducted for the identification and confirmation of polymorphic form of drug used during the current research work. Fig. 4.3 presents DSC thermogram of FDP with a distinct endothermic peak of drug at 146.9 °C. Literature review revealed presence of two polymorphic forms of crystalline FDP of which From I shows melting range of 145-149 °C (Lou et al., 2009). This provides evidence that Form I of FDP was used in the study. The FT-IR spectra of the drug is displayed in Fig 4.4. The IR peak at 3370 cm^{-1} corresponding to the N-H group further confirmed that Form I was used in the study.

4.4.2 Determination of dissociation constant (pK_a)

The inflection point in the plot of absorbance of drug solution versus its pH was found at pH 5.1 which was considered as approximate value of felodipine pK_a.

Fig. 4.1 exhibits a typical linear curve of absorbance diagram. The absorbance data of felodipine at 364 nm and 381 nm are given in Table 4.1. The linear nature of the absorbance diagram as discussed in the previous section, extends suffice evidence of the presence of one equilibrium in the ionization system of the felodipine (Blanco et al., 2005).

Fig. 4.2 shows the variation in the UV-spectrum of felodipine in buffer solutions of various pH. It is clear from the figure that the drug exhibits pH dependent UV-absorption. The pK_a value of the compound was calculated using Eq. (4.1) for the set of seven buffers containing 20 µg/ml drug and pH ranging from 4.3 to 5.7. The average of the seven pK_a value was calculated as per the method discussed earlier and found to be 5.0667. Table 4.2 gives the absorbance at respective pH, corresponding pK_a values and average of the pK_a value.

The pK_a determination of drug was repeated for the same buffer solutions containing 10 µg/ml of drug. The ruggedness of the study was assessed as described earlier. The results of the validation study are presented in the Table 4.3.

Finally, the pK_a of felodipine can reported to be 5.07 as calculated from the experiments. It was observed that all the values falls within a spread of ± 0.05 which proves the preciseness of results.

4.4.3 Stability study

4.4.3.1 Solution state stability

FDP was found to be stable over the pH range of 1.2 to 9.0 for at least 2.5 days. The profile of log % RTD versus time at various pH was linear indicating first order degradation kinetics (Fig. 4.5). Table 4.4 presents first order degradation kinetic parameters of the drug at various pH. Degradation rate constant (K_{deg}) values obtained were ranging from 12.44 x 10^{-3} (pH 6.8) to 37.31 x 10^{-3} day^{-1} (pH 1.2) and $t_{90\%}$ values obtained were ranging from 2.81 to 8.44 days.

On the other hand, in photostability study, FDP was found to be extremely sensitive to light. First order degradation rate constant obtained from slope of the log % RTD versus time profiles of drug (Fig. 4.6) was used to determine $t_{90\%}$ value. The linear nature of the plot with R^2 close to one indicated first order degradation kinetics. In this study K_{deg} and $t_{90\%}$ values were obtained to be 17.96 x 10^2 h^{-1} and 0.58 h respectively.

4.4.3.2 Solid state stability

DSC study was carried out for pure FDP, individual excipients and mixture of FDP with various excipients (1:1 ratio). The content uniformity of all the samples was found to be in range of 98.57 to 101.46 % with maximum STDEV of 1.05.

DSC thermogram of FDP showed a distinct melting point at 146.9 °C (Fig. 4.3) with an enthalpy value of - 57.71 J/g (Table 4.5). Fig 4.7 to Fig 4.14 present DSC thermograms of pure FDP, individual excipients and physical mixtures of FDP with different excipients used in the study. Melting thermogram of FDP was found to be undisturbed in all the cases. In few cases, minor change in peak shape was noticed which could be due to the process of mixing that causes decrease in the purity of the components (Verma and Garg, 2004).

DSC profile of lactose exhibits three endothermic peaks at 146.5 °C, 225.3 °C and 249.5 °C (Fig 4.7). Peak at 146 °C can be attributed to the loss of bound water (Araujo et al., 2003). Remaining two peaks correspond to the melting of the two forms namely α and β form of lactose at the respective temperatures (Choi et al., 1949). This confirms presence of both the forms of lactose in the sample. In physical mixture, the DSC thermogram of drug was retained at same temperature with almost similar enthalpy value indicating compatibility.

The DSC thermogram of magnesium stearate demonstrated an endothermic peak at 110 °C. Both the endothermic peaks of the drug and magnesium stearate was retained in the DSC study of physical mixture (Fig 4.8). Hence, it was concluded that the drug was compatible with magnesium stearate.

In DSC thermogram of HEC, EG, CP, EC, HPMC, CH and PC in pure form, no sign of melting endotherm was observed (Fig. 4.9 - 4.14). The DSC profile of the physical mixtures demonstrated retention of FDP melting endotherm at 146 °C without any additional endothermic peaks. The enthalpy value obtained were close to that obtained from in pure form of the drug. This strongly suggests the complete compatibility of these excipients with the drug.

The summary of the DSC study with enthalpy value for FDP with peak onset and end set for all the endothermic peak is given in Table 4.5.

Table 4.6 lists wavelength attribution of FT-IR spectra of FDP. The characteristics peaks of FDP were retained in all the mixtures studied indicating absence of chemical interactions between drug and the excipients. Similar results were obtained when the study was repeated with the samples stored at CRT for 12 months.

Physical mixtures of FDP prepared in 1:10 ratio with various excipients showed good content uniformity between 98.15 to 102.35 % with maximum STDEV of 1.57. Table 4.7 gives the first order degradation kinetics of drug alone and combination of drug with various excipients. At controlled room temperature (CRT: 25 ± 2 °C and 60 ± 5% RH) and accelerated conditions (AT: 40 ± 2 °C and 75 ± 5% RH), the log % RTD versus time profiles were linear indicating first order degradation kinetics. The degradation rate constant (K_{deg}) for pure drug was found to be 20.50 x 10^{-4} and 47.21 x 10^{-4} $month^{-1}$ at

CRT and AT conditions respectively. The $t_{90\%}$ of the drug at CRT and AT were 51.23 and 22.24 months respectively.

At CRT condition, the K_{deg} values of all the physical mixtures were ranging from 18.42 x 10^{-4} to 35.70 x 10^{-4} $month^{-1}$. The highest degradation rate was observed with magnesium stearate and lowest degradation was observed with agar. At this storage conditions, $t_{90\%}$ values were ranging from 29.41 to 56.99 months. FDP alone and in combination with various excipients was found to be stable for nearly 30 months at this condition.

Upon storage at AT conditions, the K_{deg} values for all the drug excipient mixtures were ranging from 43.99 x 10^{-4} to 92.58 x 10^{-4} $month^{-1}$. The highest degradation rate constant was again observed with magnesium stearate and lowest degradation rate constant was observed with soluplus. At this storage condition $t_{90\%}$ ranged between 11.34 to 23.87 months. FDP alone and in combination of various excipients was stable for at least 10 months at this condition.

4.5 Conclusions

In the present study, Form I of polymorphic form of FDP was used as confirmed by DSC and FT-IR data obtained from the experimental results. The experimental value of pK_a was found to be 5.07. FDP was found to be compatible and stable with all the proposed excipients in solid state stability studies. FDP was found stable over a pH range of 1.2 to 9.0 at 25 °C and in presence of sun light the drug followed first order degradation kinetics, indicating that FDP need to be protected from light. The results obtained from the above preformulation studies were helpful in the design and development of buccal mucoadhesive formulations.

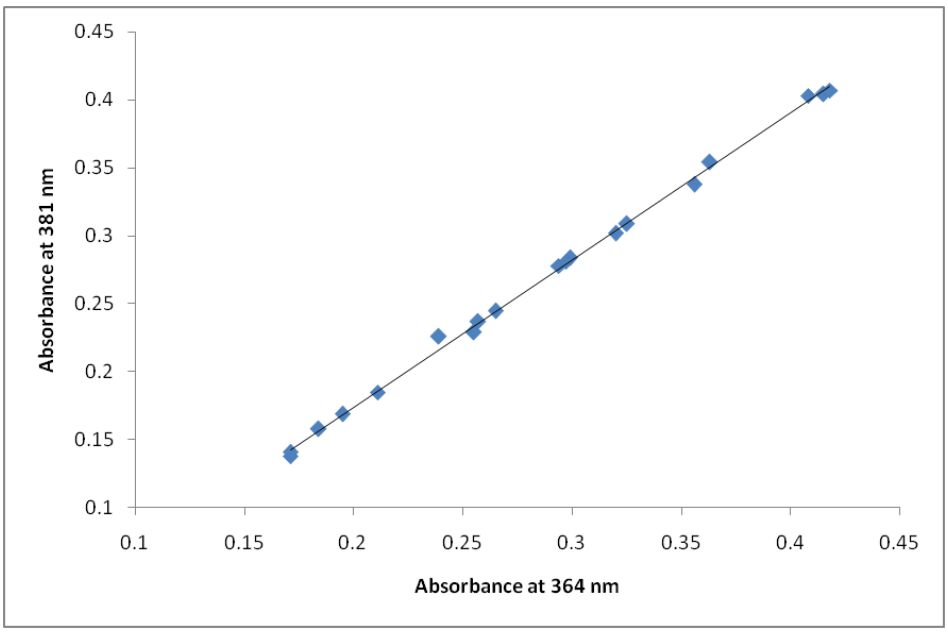

Fig. 4.1. Absorbance diagram of felodipine in buffer solutions of pH 1.3-12.0

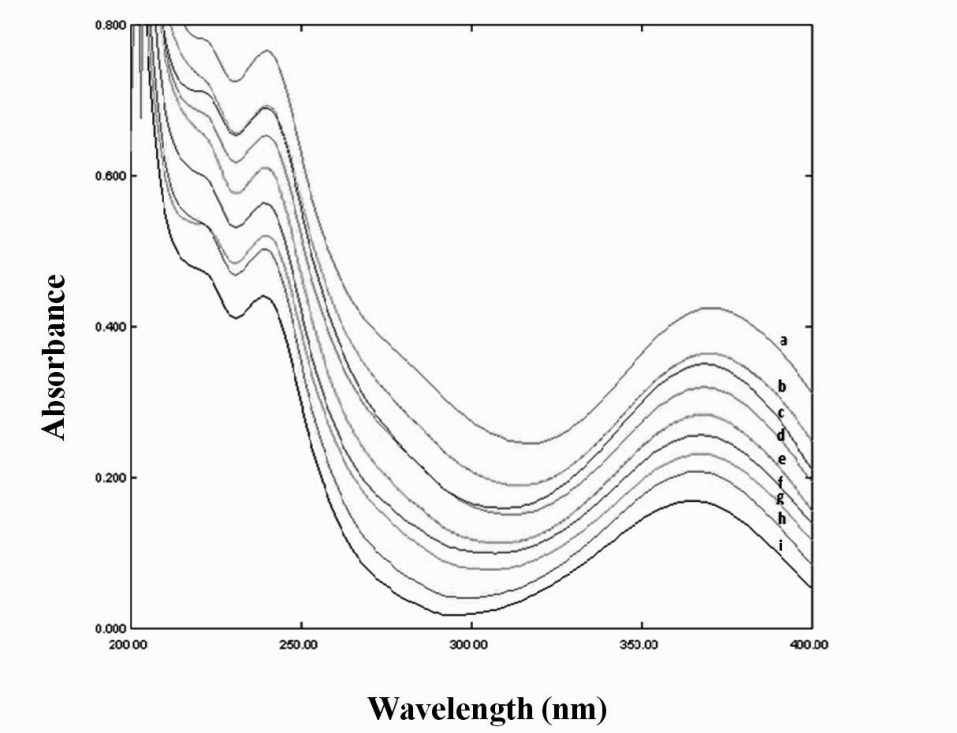

Fig. 4.2. UV-spectrum of felodipine in different pH : (a) 0.01 N NaOH; (b) pH 5.7; (c) pH 5.5; (d) pH 5.2; (e) pH 5.0; (f) pH 4.8; (g) pH 4.5; (h) pH 4.3; (i) 0.01 N HCl

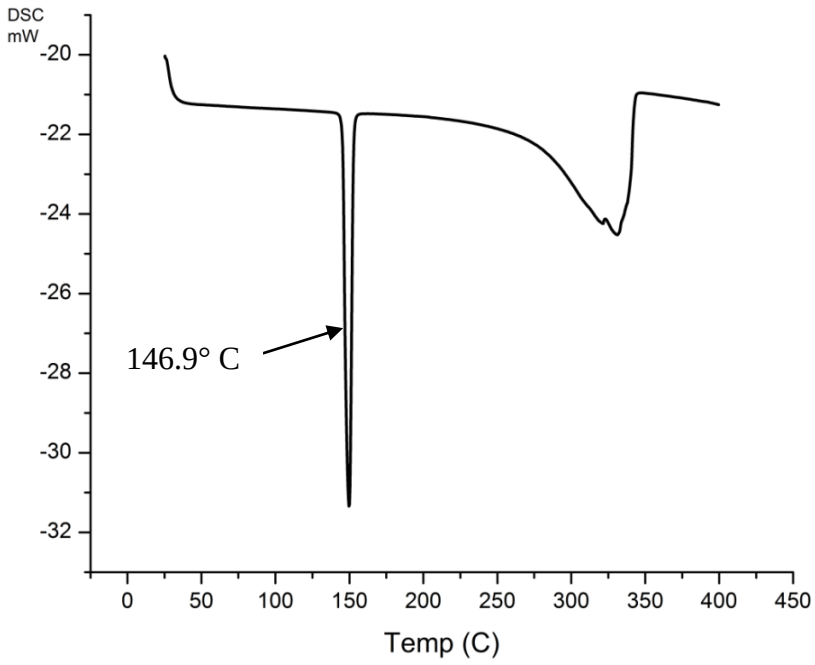

Fig. 4.3. DSC thermogram of pure FDP

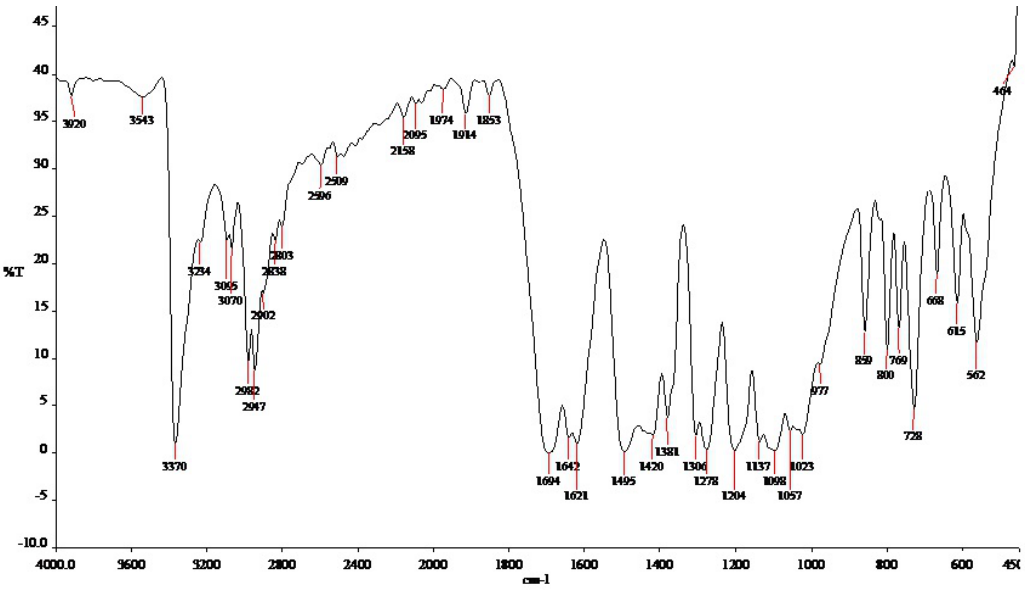

Fig. 4.4. FT-IR spectra of pure FDP

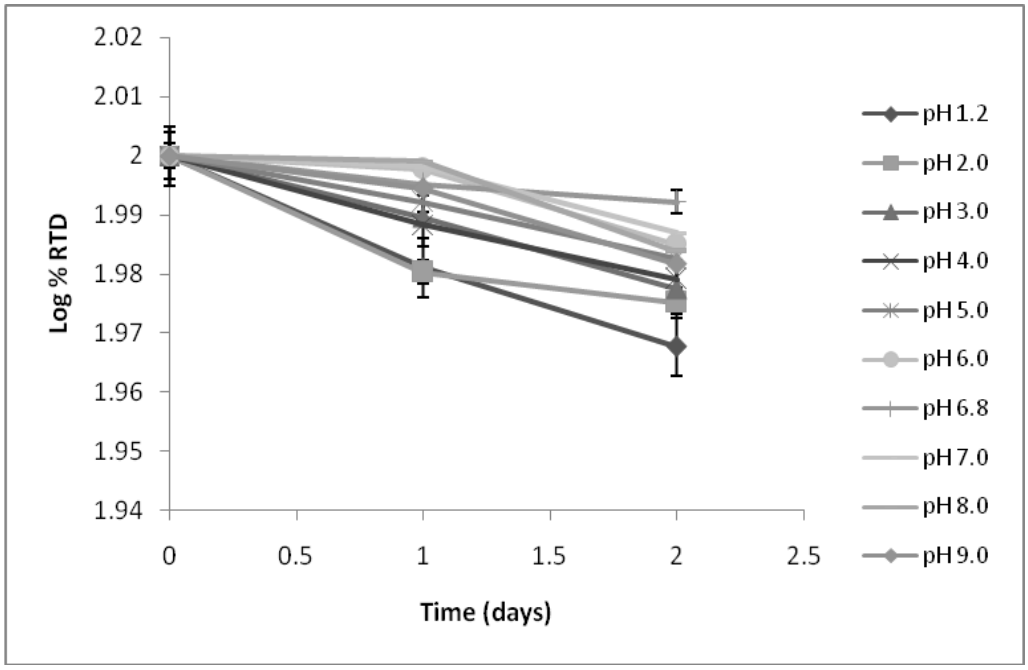

Fig. 4.5 Solution state stability of FDP in various buffer solutions

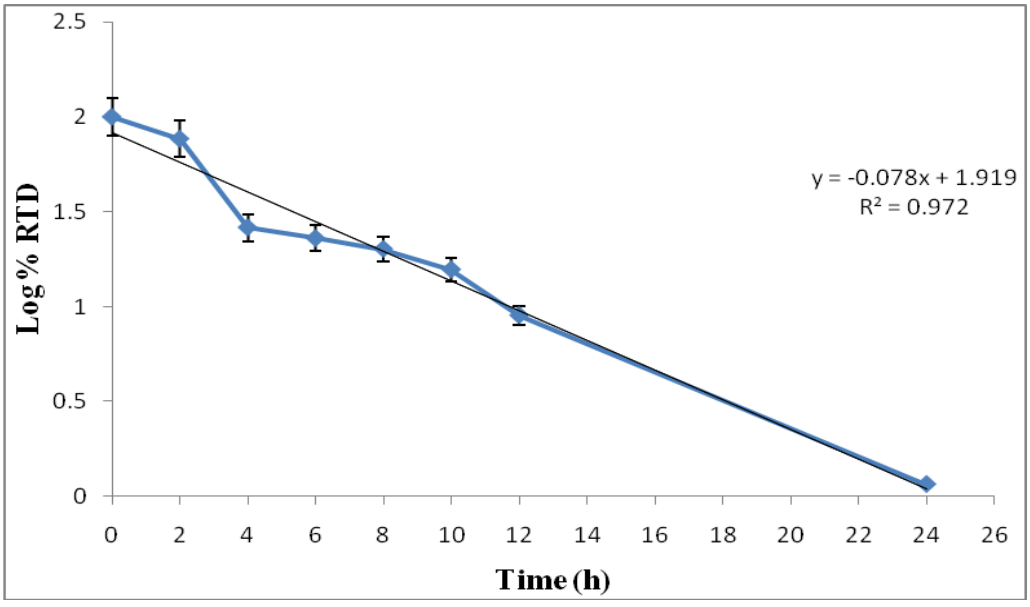

Fig. 4.6. Photostability profile of FDP in phosphate buffer (pH 6.8)

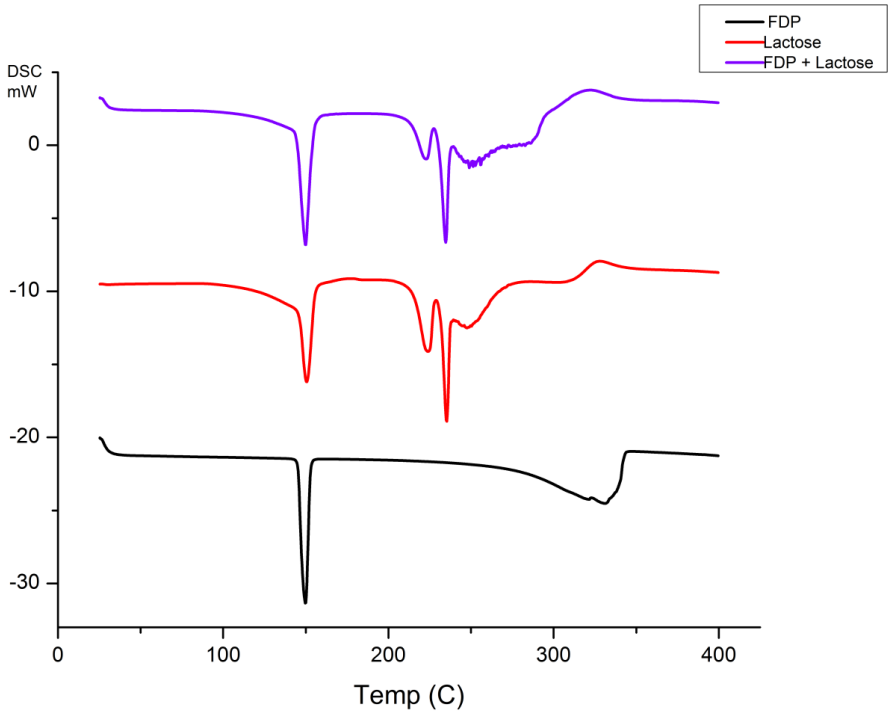

Fig. 4.7. DSC thermogram of pure FDP, lactose and 1:1 physical mixture

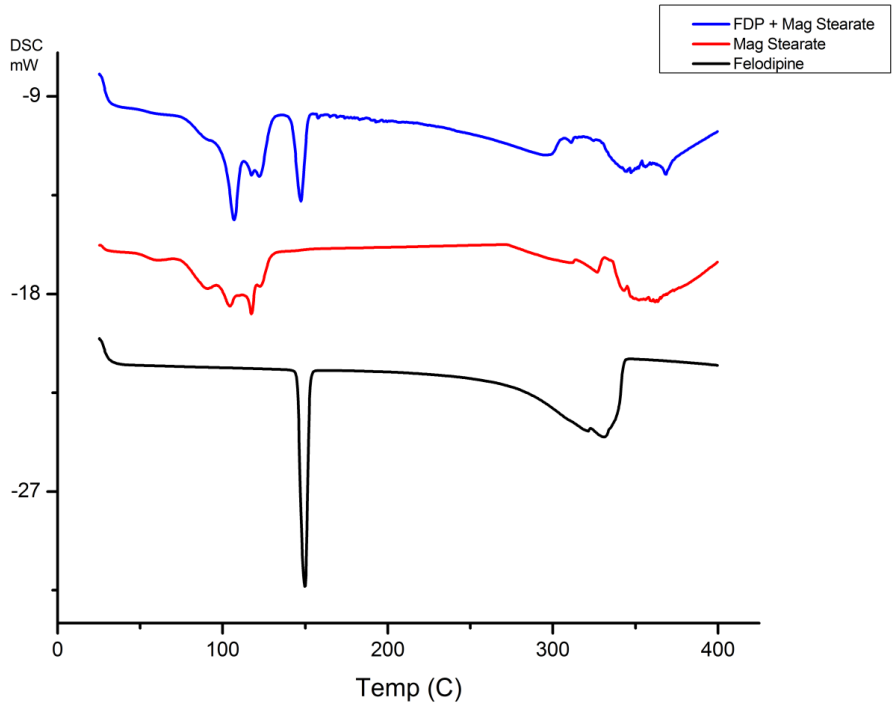

Fig. 4.8. DSC thermogram of pure FDP, magnesium stearate and 1:1 physical mixture

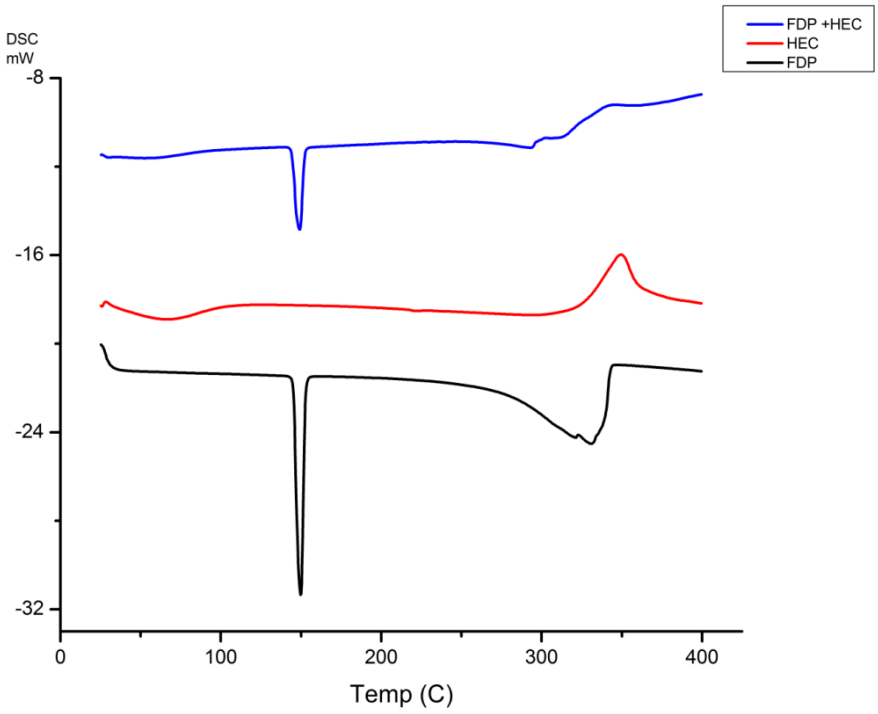

Fig. 4.9. DSC thermogram of pure FDP, HEC and 1:1 physical mixture

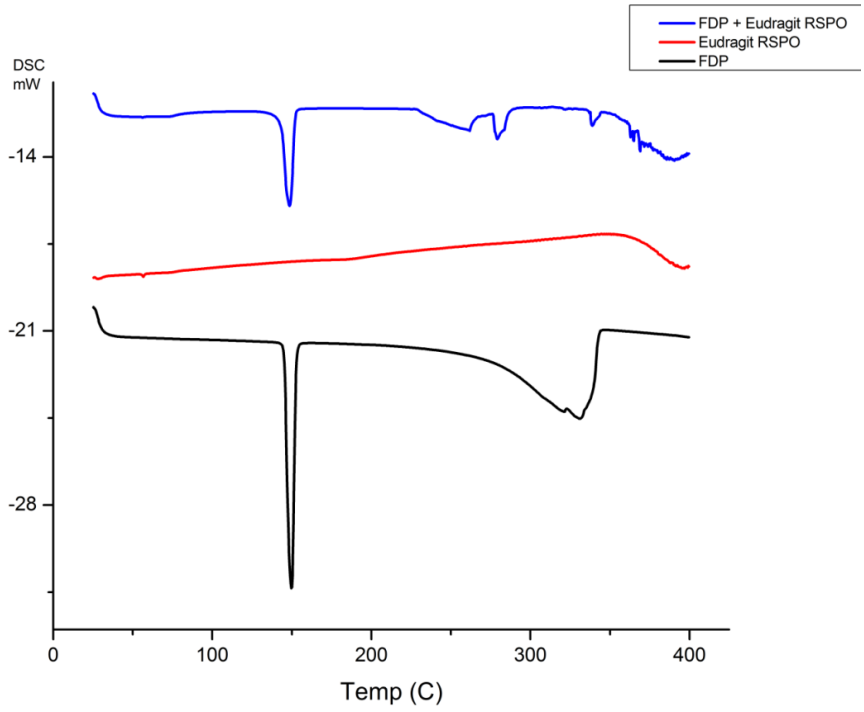

Fig. 4.10. DSC thermogram of pure FDP, EG and 1:1 physical mixture

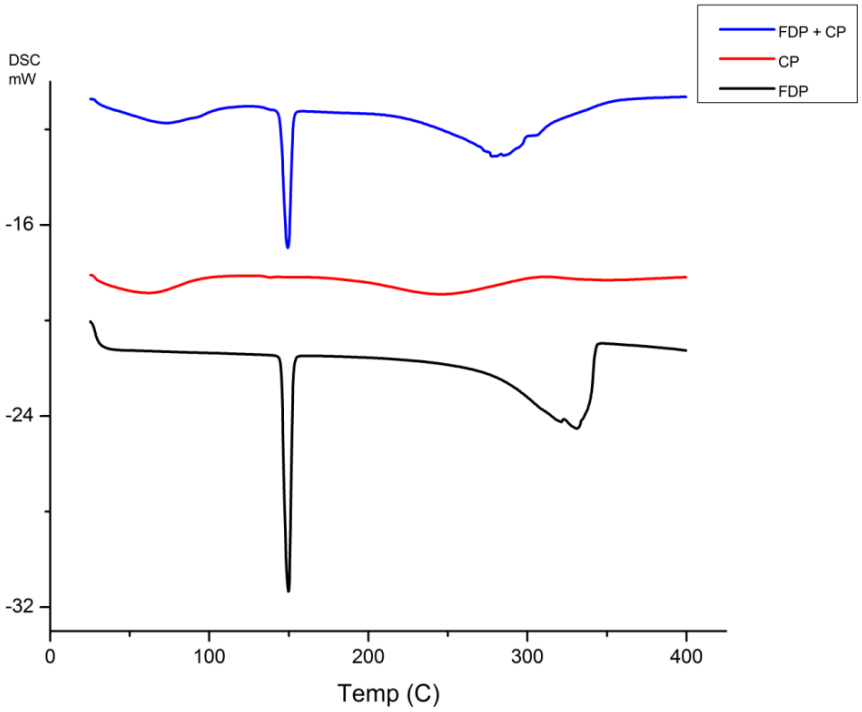

Fig. 4.11. DSC thermogram of pure FDP, CP and 1:1 physical mixture

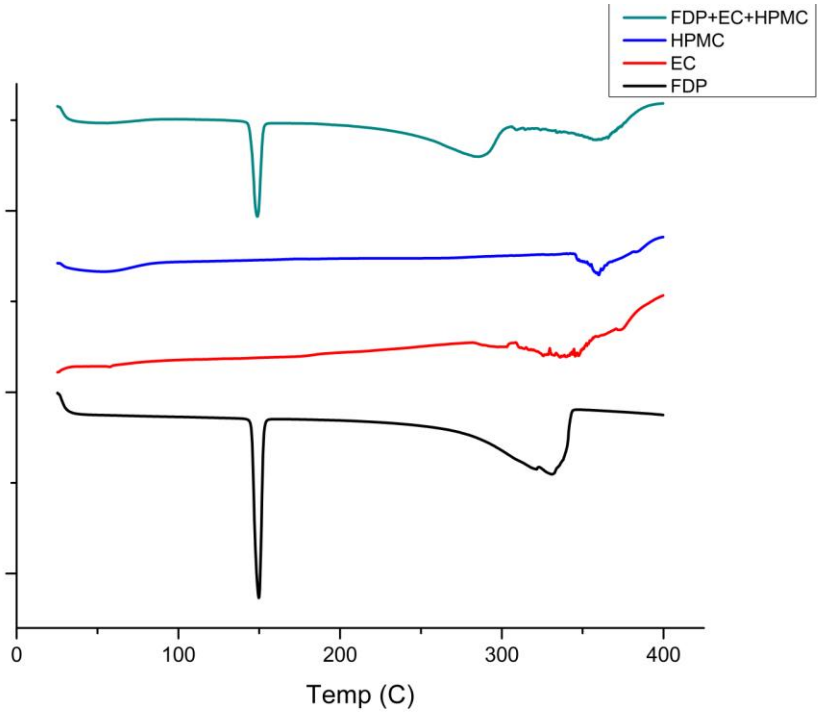

Fig. 4.12. DSC thermogram of pure FDP, EC, HPMC and physical mixture of all three in equal proportion

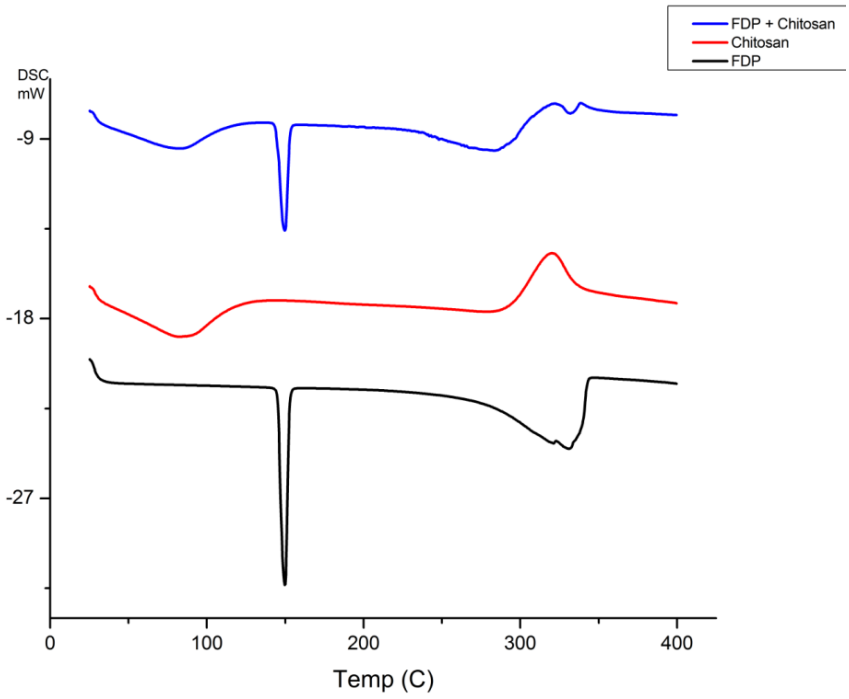

Fig. 4.13. DSC thermogram of pure FDP, CH and 1:1 physical mixture

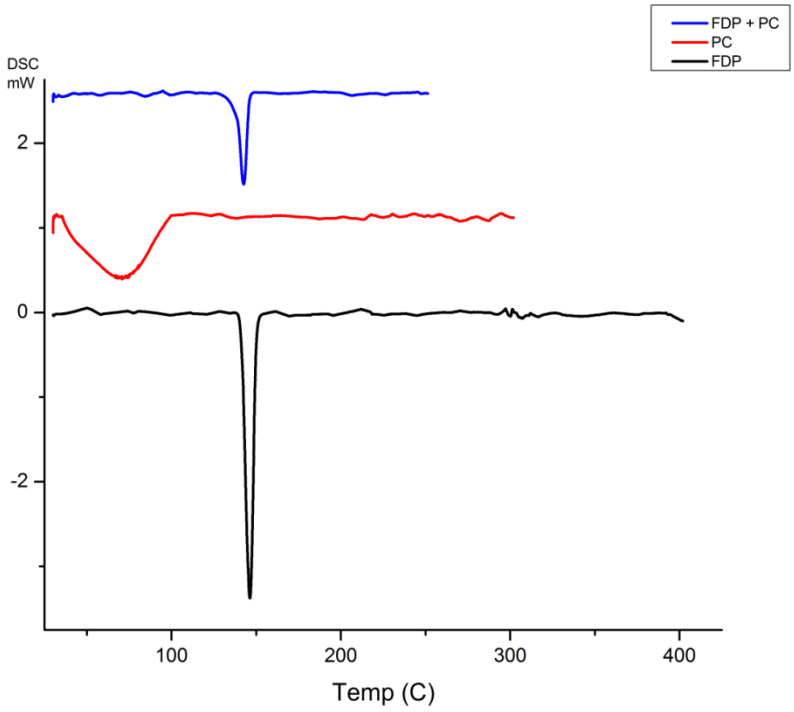

Fig. 4.14. DSC thermogram of pure FDP, PC and 1:1 physical mixture

Table 4.1: Absorbance value of felodipine at 364 and 381 nm in buffer solutions of pH 1.3-12.0

pH	Absorbance data	
	364 nm	**381 nm**
1.3	0.239	0.226
1.5	0.184	0.158
2.0	0.171	0.138
2.5	0.195	0.169
3.0	0.171	0.141
3.5	0.32	0.302
4.0	0.211	0.185
4.8	0.255	0.229
5.5	0.356	0.338
6.0	0.265	0.245
6.5	0.363	0.354
7.0	0.299	0.284
7.5	0.294	0.278
8.0	0.257	0.237
8.5	0.297	0.281
9.0	0.408	0.403
10.0	0.325	0.309
11.0	0.415	0.404
12.0	0.418	0.407

Table 4.2: Determination of pK_a using absorbance values at respective pH.

pH	**Absorbance (*d*)**	**pK_a = pH + log (d_M[a]-*d*/*d*-d_I[b])**	**Mean pK_a= Log (Avg of antilog)**
4.3	0.2070	5.0445	
4.5	0.2200	5.0891	
4.8	0.2550	5.0777	
5.0	0.2810	5.0875	5.0667 ± 0.0461
5.2	0.3160	5.0413	
5.5	0.3560	5.0206	
5.7	0.3680	5.1001	

[a]Absorbance of unionized molecule = d_M = 0.418 (in 0.01 N NaOH)
[b]Absorbance of ionized molecule = d_I = 0.169 (in 0.01 N HCl)

Table 4.3: Validation of ruggedness of the pK_a value

Instrument 1			**Instrument 2**
Person 1, day 1, drug strength=20 µg/ml	Person 1, day 2, drug strength=20 µg/ml	Person 1, day 3, drug strength=10 µg/ml	Person 2, day 4, drug strength=20 µg/ml
5.0667±0.0461	5.0659±0.0397	5.0681±0.0521	5.0693±0.0492

Table 4.4: First order degradation kinetics of FDP in buffered media of varying pH at 25 ± 2 °C

pH	Degradation Rate Constant $K_{deg} \times 10^{-3}$ (Day^{-1})	$t_{90\%}$ (Days)
1.2	37.31	2.81
2.0	28.56	3.68
3.0	26.02	4.03
4.0	23.95	4.38
5.0	20.27	5.18
6.0	17.27	6.08
6.8	12.44	8.44
7.0	14.97	7.01
8.0	18.65	5.63
9.0	21.19	4.96

Table 4.5: Thermal properties of drug and excipients alone and in combination

Sample	Peak onset (ºC)	Peak (ºC)	Peak End set (ºC)	Heat (J/g)
FDP	141.57	146.91	151.57	-57.71
Lactose	139.41	146.52	153.37	-53.96
	219.32	225.31	233.28	-87.25
	243.48	249.53	257.36	-93.26
FDP + Lactose	141.28	146.37	152.37	-54.38
Magnesium stearate	103.68	110.59	118.25	-16.38
FDP + Magnesium stearate	143.49	147.13	151.28	-52.16
HEC	---	---	---	---
FDP + HEC	142.53	147.65	153.57	-59.62
EG	---	---	---	---
FDP + EG	142.53	146.91	152.38	-56.72
CP	---	---	---	---
FDP + CP	142.48	147.32	152.59	-58.29
EC	---	---	---	---
HPMC	---	---	---	---
FDP + EC + HPMC	143.73	148.52	154.26	-58.37
CH	---	---	---	---
FDP + CH	142.76	147.37	153.73	-57.39
PC	---	---	---	---
FDP + PC	143.26	148.13	154.26	-58.38

Table 4.6: Wavelength attribution of IR spectra of FDP

Wavelength (cm^{-1})	**Attribution**
3370	N-H
2947	Aliphatic C-H
1694	C=O
728	C-Cl

Table 4.7: First order degradation kinetics of incompatibility study of FDP with various excipients

FDP + Excipient (1:10)	**CRT(25 ± 2°C/60 ± 5 % RH)**			**AT (40 ± 2°C/75 ± 5 % RH)**		
	$K_{deg} \times 10^{-4}$ ($month^{-1}$)	**$t_{90\%}$ (month)**	**R^2**	**$K_{deg} \times 10^{-4}$ ($month^{-1}$)**	**$t_{90\%}$ (month)**	**R^2**
FDP	20.50	51.23	0.9778	47.21	22.24	0.9913
FDP + HEC	22.34	47.00	0.8990	51.36	20.45	0.9711
FDP+ AR	18.42	56.99	0.7648	50.44	20.82	0.9024
FDP + EG	19.58	53.64	0.8302	72.31	14.52	0.9862
FDP + TCP	25.56	41.07	0.9660	60.57	17.34	0.9949
FDP + Lactose	21.42	49.02	0.9337	66.10	15.89	0.9847
FDP + Magnesium stearate	35.70	29.41	0.9976	92.58	11.34	0.9998
FDP + CH	25.56	41.07	0.9228	62.87	16.70	0.9772
FDP + GM	21.65	48.50	0.9960	54.35	19.32	0.9437
FDP + EC	27.87	37.58	0.8087	49.51	21.21	0.9485
FDP + HPMC	21.88	47.99	0.7082	75.77	13.86	0.9666
FDP + Soluplus®	21.88	47.99	0.7983	43.99	23.87	0.9538
FDP + Poloxamer 407	21.65	48.50	0.7854	51.36	20.45	0.9902

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Chapter 5

Formulation Development and In Vitro Characterization

5.1 Introduction

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

In an endeavor to achieve sufficient and predictable bioavailability, various researchers have developed controlled release buccal mucoadhesive drug delivery systems (Shanker et al., 2009; Bahri-Najafi et al., 2013; Govinadswamy et al., 2013; Onishi et al., 2014). Buccal drug delivery systems are designed to remain in contact with buccal mucosa for desired time interval and release drug at a predetermined rate to obtain steady plasma drug concentration with higher bioavailability thereby reducing total dose and dosing frequency (Rossi et al., 2003; Sudhakar et al., 2006; Mylangam et al., 2014). Several mucoadhesive polymers reported in literature for the development of buccal drug delivery systems have been comprehensively reviewed in chapter 1 and the list of the polymers with their respective class is presented in Table 1.2 of chapter 1. However, the availability of considerably less amount of aqueous media in form of salivary fluid hampers the rate and extent of dissolution of drugs thereby posing a serious hurdle in the achievement of desired bioavailability. This problem becomes more intense in the case of poorly water soluble drugs. So, it becomes necessary to first, enhance the aqueous solubility of those drugs and then deliver it as buccal mucoadhesive dosage form. Recently, many researchers have been working on this line (Kohda et al., 1997; Cappello et al., 2006; Llabot et al., 2007; Miro et al., 2009; Jug et al., 2010; Sakeer et al., 2010;).

In this chapter, firstly, studies on the solubility enhancement of poorly soluble drug FDP, achieved by solid dispersion (SD) and nanocrystal (NC) technology have been presented. Following this, experiments involving development and characterization of modified release buccal mucoadhesive tablets containing pure drug, SDs and NCs prepared by matrix embedding technique have been presented. The mucoadhesive tablets were prepared by direct compression method. Effect of polymer type, polymer proportion and form of the drug (pure, SD or NC) on in vitro release profile and mucoadhesion were studied. The quality of the tablets was tested by various quality control tests.

Soluplus® and Poloxamer 407 were used to prepare SD and NC of drug for improvement of solubility. Various polymers used for design of buccal mucoadhesive tablets of pure FDP or SD/NC of FDP were agar (AR), eudragit RSPO (EG), polycabophil (PC), carbopol 934P (CP), guar gum (GM), hydroxypropyl methylcellulose (HPMC), ethylcellulose (EC), hydroxyethyl cellulose (HEC), chitosan (CH). While some of these polymers were discussed briefly in section 1.5.3 of chapter 1, further information on the excipients/polymers used during current research work is presented in subsequent sections.

PC, CP and EG used in this research endeavor are polyacrylic acid polymers. PC is an acrylic acid polymer, cross linked with divinyl glycol. It is not soluble in water but at neutral pH it has a high swelling capacity and the volume can increase by 100 folds (Ludwig, 2005). It has been reported as an excellent mucoadhesive in buccal, intestinal, nasal, vaginal and rectal mucoadhesive dosage forms (Kissel and Werner, 1998; Bruschi and Freitas, 2005; Madhav et al., 2009; De Araujo Pereira and Bruschi 2012). Buccal tablets containing PC have been reported to demonstrate high mucoadhesion for considerably long time. This is due to the presence of large number of carboxylic acid groups in its polymeric network which attach to the mucosal surface by forming extensive hydrogen bonding. It is non-toxic, non-irritating, non-sensitizing and listed as GRAS (generally recognized as safe) by USFDA (Wei and Xin-guo, 2002). CP is also an acrylic acid polymer but it is crosslinked by allyl sucrose. It is rich with carboxylic acid groups. On dry basis, it contains 56-68% carboxylic acid groups. It is considered as water soluble but does not get dissolve in water and only swells. It is listed in USFDA Inactive Ingredient Guide for tablets, oral suspensions, ophthalmic, rectal, vaginal and transdermal preparations. CP has been widely explored by pharmaceutical industry due to its high viscosity at low concentration and less toxicity (Taylor and Bagley, 1975). Eudragit polymers are derived from esters of acrylic acid and methacrylic acid. These polymers are available in wide range of physical forms and physicochemical properties of the polymers are determined by attached functional groups. Eudragit L, S, FS and E polymers with acidic or alkaline groups enable pH-dependent release of the active ingredient. Eudragit RL and RS polymers with alkaline and eudragit NE polymers with neutral groups enable controlled release of the active ingredient by pH-independent

swelling. Eudragit RSPO (EG) was used in the present research work. EG is a hydrophobic acrylic acid polymer used as matrix forming substance. It is a copolymer of acrylic and methacrylic acid esters having 5% of functional quaternary ammonium groups. The ammonium groups are present as salts and give rise to pH-independent permeability of the polymer. It is water insoluble and possess low swelling index. It is widely used in sustained release formulations. It has recently been reported to be used as mucoadhesive agent in buccal tablets and patches (De Caro et al., 2012; Mohamed et al., 2012). It is listed in USFDA Inactive Ingredient Guide for oral capsules and tablets.

Cellulose derivatives such as HEC, EC and HPMC were used as mucoadhesive polymer in some of the formulations. HEC is a partially substituted poly(hydroxyethyl) ether of cellulose. It is prepared by treating alkali cellulose with ethylene oxide (Li et al., 2014). It is a non ionic water soluble polymer. It presents wide range of viscous solutions upon solubilization in water. It is one of the extensively utilized polymer due to its ability to get bonded to a number of functional group with help of its easily accessible hydroxyl groups (Li et al., 2014). It is used as thickening agent in ophthalmic and topical preparations. It has widely been reported as mucoadhesive polymer in various formulation such as ocular, buccal, sublingual and vaginal (Ludwig, 2005; Miller et al., 2005; Valenta, 2005). It is listed in USFDA Inactive Ingredient Guide for ophthalmic preparations, oral syrups, tablets, otic and topical preparations. EC is available in various grades which differ in their molecular weight. The EC grade used in this study was Ethocel Standard 7 Premium. It is practically insoluble in water. The viscosity of a 5% solution of Ethocel standard 7 Premium in an 80:20 solvent mixture of toluene:ethanol is reported to be 6.6 cP. It is widely used in controlled release oral and topical formulations. HPMC is a partly *O*-methylated and *O*-(2-hydroxypropylated) cellulose. It contains 22 and 18% of methoxy and hydroxypropoxy group respectively as calculated on dry basis. It forms a viscous colloidal solution in cold water. The HPMC grade used in this study was Methocel K4M. HPMC has widely been reported to be used as a mucoadhesive polymer for transmucosal administration of various drug by buccal, nasal, rectal and vaginal routes (Miller et al., 2005; Valenta, 2005; Koffi et al., 2006; Gabal et al., 2014). Both EC and HPMC are GRAS listed ingredient and included in USFDA Inactive

Ingredient database used in manufacturing of variety of dosage forms available commercially.

CH, a cationic biodegradable and biocompatible biopolymer, has been most extensively investigated for bioadhesive buccal formulations (Ayensu et al., 2012; Giovino et al., 2012; Sander et al., 2013). CH is a cationic polysaccharide, produced by the deacetylation of chitin, the most abundant polysaccharide in the world, next to cellulose (Elsabee and Abdou, 2013; Geisberger et al., 2013). It is a copolymer of N-acetyl-D-glucosamine and D-glucosamine units and available in various grades based upon the degree of deacetylation. CH is available in two grades of low and high molecular weight. The molecular weight of lower one ranged from 20 to 190 kD with less than 75% degree of deacetylation. The higher ones molecular weight ranged from 190 to 375 kD with more than 75% degree of deacetylation. In this study, CH having lower molecular weight was used. Reports suggest that bioadhesion mechanism of CH is by ionic interactions between primary amino functional groups and the sialic acid and sulphonic acid substructures of mucus (Rossi et al. 2003; Hassan et al. 2010). CH can be tailored by adding various functional groups, using this modification it can be customized to suit various formulation requirements (Geisberger et al. 2013).

AR and GM both are polysaccharide in nature. AR is hydrophilic, colloidal polysaccharide complex extracted from the agarocytes of algae of the Rhodophyceae. It is soluble in boiling water but insoluble in cold water. It is extensively used in food applications as a stabilizing agent. It has been reported in literature to be used as sustained release agent in oral tablets, gels and microspheres (Sakr et al., 1995; Bhardwaj et al., 2000). In pharmaceutical suspensions, it is used as a suspending agent (Kahela et al., 1978). GM is a high-molecular weight hydrocolloidal polysaccharide, composed of galactan and mannan units combined through glycoside linkages, which may be described chemically as a galactomannan. In hot and cold water, it gets disperse and swells quickly to form a highly viscous, thixotropic sol. It is used as a binder and disintegrant in solid dosage forms. It is also used as a release controlling, suspending, thickening and stabilizing agent in oral and topical products. It is GRAS listed ingredient and included in USFDA Inactive Ingredient database.

Solulpus®, an amphiphilic polymeric solubilizer, was used to enhance the aqueous solubility of FDP by solid dispersion and nanocrystal techniques. Soluplus® is designed and developed by BASF especially for applications in solid solutions (Hardung et al., 2010). It is a graft copolymer made up of 13% polyethylene glycol, 57% vinyl caprolactam and 30% vinyl acetate. Essentially, it has a polyethylene glycol backbone containing one or two side chains of vinyl acetate randomly copolymerized with vinyl caprolactam. This chemical structure confers soluplus® with properties like low hygroscopicity, low glass transition temperature, capacity to form stable formulations, excellent solubilization efficacy, ability to inhibit crystal nucleation etc. In addition to these properties, poor absorption of soluplus® following oral administration makes it a potential candidate to be used as a polymeric solubilizer in amorphous solid dispersions of poorly water soluble drugs (Reintjes, 2011). These physico-chemical properties of soluplus® actuated us to use it in the formulation of solid dispersions for the dissolution enhancement of the poorly water soluble drug.

Poloxamer 407, a polyol, was used to improve solubility of FDP by nanocrystal technology. Poloxamers are copolymers of ethylene oxide and propylene oxide. The polyoxyethylene part is hydrophilic while the polyoxypropylene part is hydrophobic in nature. It is freely soluble in water and widely used as tablet lubricant, solubilizing and emulsifying agents. Poloxamer 407, containing 70% w/w polyoxyethylene units, has been listed in USFDA Inactive Ingredient database for i.v. injections, inhalations, ophthalmic formulations, solutions, suspensions, oral powdres, syrups and topical preparations (Albertinia et al., 2010). It has been reported to be non-irritating and non-sensitizing to skin and shows good tolerance in topical, rectal and ocular preparations (Dumortier et al., 2006). Poloxamer 407 has also been reported to be safe for drug delivery into mice lungs (Desigaux et al., 2005).

5.2 Materials and reagents

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

5.3 Equipment

An eight station tablet compression machine (Rimek Mini Press, India) equipped with 4 mm punches was used for the preparation of tablets. A five digit analytical balance (Mettler Toledo, TA 215D, India) was used for all weighing purposes. Mucoadhesion study of the designed formulations was performed using texture analyzer (Stable Micro Systems TA-XT Plus, UK). In vitro release studies were carried out using in-house modified USP Type I Dissolution Apparatus (Electrolab-Tablet Dissolution Tester, Mumbai, India). Hardness and friability of the tablets was determined on electrolab digital hardness tester (EBT-2PRL, Electrolab, Mumbai, India) and USP Friability Test Apparatus (Campbell, India) respectively. A digital pH meter (Eutech® Instruments, Singapore) was used for the pH measurement. Water uptake study was conducted using humidity chamber (MAC Instruments, India). Analytical instruments mentioned in chapter 3 were used for all sample analysis.

5.4 Methods

5.4.1 Dissolution enhancement of FDP by solid dispersion technique

5.4.1.1 Preparation of solid dispersions and physical mixtures

Solid dispersions (SDs) with varying ratio of drug and soluplus® were prepared by solvent evaporation method. SDs with drug : soluplus® ratio (w/w) of 1:2, 1:4, 1:6 and 1:10 were prepared. For this, required amount of felodipine and soluplus® were dissolved in appropriate quantity of ethanol and stirred with a glass rod to form a clear solution. The solutions were then poured in petriplates and kept in an oven maintained at 37±1°C for complete drying. The dried mass was scratched with help of a spatula and passed through sieve number 60. The final selection of the ratio of the drug and polymer for this work was based upon preliminary experiments conducted. Physical mixtures (PMs) of same ratio were prepared by geometric mixing of the suitable amount of felodipine and soluplus®. The mixture was then powdered using a mortar and pestle and sifted through mesh (#60). Solid dispersions and physical mixtures thus prepared were stored in a desiccator at room temperature away from light until use.

5.4.1.2 Content uniformity

Sample of prepared SD (#60) equivalent to 10 mg of felodipine was taken in 100 ml of solvent system (methanol and phosphate buffer pH 3.0; 4:1 v/v). The content uniformity was determined using in house developed and validated HPLC analytical method 2 discussed in chapter 3. All the samples were appropriately diluted and analyzed in triplicate. Finally, average drug content was calculated.

5.4.1.3 Solubility measurements and determination of thermodynamic parameters

The procedure developed by Higuchi and Connors was employed for the determination of solubility of felodipine in presence of polymer (Higuchi and Connors, 1965). In order to exercise this method, excess amount of the drug was taken in 10 ml of disparate aqueous solutions containing 1, 2, 4, 6 % w/v of the polymer placed in cotton plugged conical flasks. The conical flasks were kept on rotation (100 revolutions/min) in an orbital shaker incubator maintained at 25 ± 0.5°C or 45 ± 0.5°C. After 48 hours, aliquots were taken and filtered through 0.22 µm Millipore filter. In order to avoid precipitation of the drug, all the materials were kept at the same temperature as that of the solutions during filtration. All samples were analyzed in triplicates after proper dilution using HPLC analytical method 2 as discussed in chapter 3. Similarly, saturation solubility of felodipine was also estimated in water. Amber colored glass containers were used in the experiment.

Thermodynamic parameters like heat of solution (ΔH in J/mol) and change in Gibbs free energy (ΔG in J/mol) were used to understand the effect of soluplus® on solubility of felodipine (Table 5.1). Heat of solution was measured using Eq. (5.1)-

$$\log S_S = \frac{-\Delta H}{2.303RT} + C \qquad (5.1)$$

Gibbs free energy at 25°C and 45°C was determined using Eq. (5.2)-

$$\Delta G = -2.303\, RT \log \frac{S_S}{S_W} \qquad (5.2)$$

Where, S_s is the saturation solubility of felodipine at temperature T in degree Kelvin, R is universal gas constant (8.3143 $J.mol^{-1}K^{-1}$), C is a constant and S_W is aqueous saturation solubility of the drug.

5.4.1.4 Dissolution studies

In house modified USP Type I dissolution apparatus as reported earlier (Charde et al., 2008) was used for assessing rate and extent of drug from dissolution of solid dispersions and physical mixtures and this was compared with rate and extent of pure drug dissolution. Crystals of the pure drug were sifted through mesh (#60) before use to maintain particle size uniformity in the experiment. Samples equivalent to 10 mg FDP were taken in 100 ml dissolution media maintained at 37 ± 1 °C. The baskets were rotated at a speed of 25 rpm. Sodium phosphate buffer (pH 6.8) containing 1% sodium lauryl sulphate was used as dissolution media. Samples (1 ml) were withdrawn at 5, 10, 15, 30 and 60 min and replenished with the equal volume of fresh media. All collected samples were filtered through 0.22 µm membrane filter. The samples were then analyzed for drug content using analytical method 2 of chapter 3. All readings were taken in triplicates and cumulative percentage drug dissolved at various time points was calculated.

5.4.1.4.1. Model independent approach

In order to facilitate the interpretation and comparison of the dissolution profiles of pure drug, PMs and SDs, model-independent approach was used.

(a) Dissolution efficiency (DE): DE is defined as the area under the dissolution curve up to a particular time (t), expressed as a percentage of the area of the rectangle outlined by 100% dissolution within the same time (Khan, 1975). DE value at 5, 15 and 60 minutes was determined using Eq. (5.3)-

$$DE = \frac{\int_0^t y\,dt}{y_{100}\,t} \times 100\,\% \qquad (5.3)$$

(b) Dissimilarity (f1) and similarity (f2) factors: Dissimilarity (*f1*) and similarity (f2) factors were used to statistically compare the dissolution pattern of the samples (Moore

and Flanner, 1996). *f1* is defined as the measure of percent error between two dissolution profiles. *f2* is a logarithmic transformation of the sum-squared error of the difference between the percentage dissolved of test and reference product over all time points. These values were calculated using Eqs. (5.4) and (5.5)-

$$f_1 = \frac{\sum_{j=1}^{n} \left| R_j - T_j \right|}{\sum_{j=1}^{n} R_j} \times 100 \tag{5.4}$$

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{j=1}^{n} \left| R_j - T_j \right|^2 \right]^{-0.5} \times 100 \right\} \tag{5.5}$$

Where, n is the number of sampling time points in the study, Rj and Tj are the percent drug dissolved of the reference and test at a time point j respectively.

The *f1* factor assumes value of 0 in case of identical dissolution profiles of the test and the reference and increases with the increase in the dissimilarity. A *f2* value of 100 indicates the identical dissolution profile of the test and reference. Higher *f2* value, in the range of 50 and 100 indicates similar dissolution profile and values of *f2* lesser than 50 signify dissimilarity.

(c) Mean dissolution time (MDT) and mean dissolution rate (MDR): Mean (MDT), the arithmetic mean of dissolution profile, was calculated using Eq. (5.6)-

$$MDT = \frac{\sum_{j=1}^{n} t_{mid} \, \Delta M}{\sum_{j=1}^{n} \Delta M} \tag{5.6}$$

Where, j is the dissolution sample number, n is the number of sampling time points, t_{mid} is the time at the midpoint between t_j and t_{j-1} and ΔM is the amount of drug dissolved between t_j and t_{j-1}.

Similarly, mean dissolution rate (MDR) was calculated using Eq. (5.7)-

$$MDR = \frac{\sum_{j=1}^{n} \Delta M / t_{mid}}{n} \tag{5.7}$$

5.4.1.5 Powder X-ray diffraction (PXRD)

PXRD studies were carried out to decipher crystalline or amorphous nature of prepared SDs. The study was conducted using a desktop X-ray diffractometer (Rigaku Miniflex II) operating at 30 kV and 15 mA. The X-ray measurements were performed at a scanning rate of 3°/min over a 2θ range of 10° to 60° using Cu Kα radiation (λ= 1.5418A˚) as the X-ray source. The temperature of the samples was maintained at 20 ± 0.5 °C throughout the study.

5.4.1.6 Differential scanning calorimetry (DSC)

Thermal behaviour of the samples was studied using differential scanning calorimeter (DSC 60, Shimadzu, Japan equipped with TA-60 WS thermal analyzer). Approximately 5 mg of sample was weighed accurately and heated at a rate of 10°C/min from 25°C to 400°C in hermetically sealed aluminium pans. Nitrogen was used as purge gas at a flow rate of 30 ml/min.

5.4.1.7 Fourier transform-infrared spectroscopy (FT-IR)

FT-IR spectra were acquired using Perkin-Elmer Spectrum 400 (FT-IR/FT-FIR) spectrometer. Pellets of all the samples were prepared by mixing samples with appropriate quantity of potassium bromide. Pellets were prepared using Perkin Elmer hydraulic press by applying 7-10 N force. The IR spectra were obtained in % transmission mode in the spectral region 450-4000 cm^{-1}. Eight scans were taken at a resolution of 4 cm^{-1} for each sample. The data were processed using Spectrum v5.3.1 software provided along with the instrument by Perkin Elmer.

5.4.1.8 Scanning electron microscopy (SEM)

The scanning electron micrographs of SD 1:10, felodipine and soluplus® were recorded on S-3,400 N scanning electron microscope (Hitachi, Tokyo, Japan). For this, the sample was fixed on a double-sided carbon adhesive tape previously adhered to a metallic stub. In order to achieve conducting surface, the samples were gold coated using Hitachi Ion Sputter for 30 sec at 15 mA and then studied under the electron microscope.

5.4.1.9 Transmission electron microscopy (TEM)

For transmission electron microscopy (TEM), the samples suspended in water were loaded on carbon coated 300 mesh copper grid (diameter 3 mm). The samples were examined at 0.02 nm resolution under H 7500 transmission electron microscope (Hitachi, Tokyo, Japan) operating at 120 kV. The electron micrographs were processed using Amtv542 software.

5.4.1.10 Atomic force microscopy (AFM)

Samples suspended in water were taken on silicon wafers with help of a micropipette and allowed to dry in air. The AFM study was performed in non-contact mode on Park systems XE-70 atomic force microscope (Park systems, Korea). The cantilever of the instrument was working with the force constant of 42 N/m. The images were processed with XEI software.

5.4.1.11 ^{1}H NMR spectroscopy

^{1}H NMR experiments of all SDs, pure FDP and soluplus® were conducted on a Bruker Advance II 400 NMR spectrometer. The instrument was equipped with TopSpin 1.3 software. Deuteronized chloroform $CDCl_3$ was used as solvent.

5.4.1.12 Particle size analysis during dissolution

The particle size of the solid dispersions during dissolution was analyzed at 25°C using Zetasizer Nano ZS NanoSeries (Malvern Instrument Ltd, Malvern, UK) working on the principle of dynamic light scattering. The data was analyzed with Zetasizer software version 6.01. He-Ne laser at 633 nm was used as the light source.

5.4.2 Dissolution enhancement of FDP by nanocrystal (NC) Technique

5.4.2.1 Preparation of nanocrystals and physical mixtures

FDP nanocrystals with varying ratio of the drug and poloxamer 407 were prepared by precipitation-ultrasonication method. In practice, this method of nanocrystal preparation involves two steps- first, anti-solvent precipitation and second,

ultrasonication. NCs with FDP to poloxamer 407 ratio of 1:6, 1:8 and 1:10 (w/w) were prepared and named as NCP 1:6, NCP 1:8 and NCP 1:10.

For precipitation, suitable amount of poloxamer 407 was added in 60 ml of millipore water taken in 100 ml capacity beaker and was allowed to cool below 4°C. FDP (2 ml of 20 mg/ml) solution in ethyl acetate, all at a time, was added to the above solution under stirring at 1000 rpm. The temperature of the beaker was maintained below 4°C by keeping in an ice bath during the mixing process. In ultrasonication step, the formed particles during anti-solvent precipitation were treated with an ultrasonic probe (Biosonik IV-Model Bio IV, Bronwill VMR Scientific, San Fransisco, California) of 2 cm tip diameter for 1 min. The probe sonicator was operated at 220 volts, 2.25 amp and 50-60 Hz. With the help of an ice bath, the temperature of the liquid undergoing ultrasonication was kept constant.

The prepared nanosuspension was transferred into a round bottom flask and ethyl acetate were removed using rotary evaporator (Rotavapour® R-215, Buchi, Switzerland) for 4 hours at 25°C and 100 mBar of pressure. The nanosuspensions were dried by lyophilisation (Maxi Dry Lyo 230v, Heto-holten, Denmark) to form solid nanocrystals.

In order to discern the effect of surfactants on particle size, nanocrystals of same ratios i.e. 1:6, 1:8 and 1:10 (w/w of drug and surfactants) were prepared with sodium lauryl sulphate and tween 80. Similarly, effect of organic solvent were assessed by preparing nanocrystals using acetone.

After observing the excellent performance of soluplus® on dissolution enhancement front via solid dispersion, it was decided to prepare nanocrystals using soluplus® as a stabilizer for nanocrystals. Thus, NCs with FDP to soluplus® ratio (w/w) of 1:2, 1:4 and 1:6 were also prepared and termed as NCS 1:2, NCS 1:4 and NCS 1:6 respectively. In this case ethanol was used as organic solvent.

5.4.2.2 Particle size and zeta potential analysis

The particle size and zeta potential of all freshly prepared nanocrystals was determined at 25°C by dynamic light scattering on Zetasizer Nano ZS NanoSeries (Malvern Instrument Ltd, Malvern, UK). The data was analysed with Zetasizer software version 6.01. He-Ne laser at 633 nm was used as the light source. All analysis were

performed in triplicates and mean particle size (z-average, d-nm) and polydispersity index (PDI) was determined.

5.4.2.3 Content uniformity

Sample of prepared NCs equivalent to 10 mg of FDP was taken in 100 ml of solvent system (methanol and phosphate buffer pH 3.0; 4:1 v/v). The content uniformity was determined using in house developed and validated analytical method 2 of chapter 3. All the samples were appropriately diluted and analyzed in triplicate. Finally, average drug content was calculated.

5.4.2.4 In vitro dissolution study

In vitro dissolution study of FDP, PMs and NCs were performed by dialysis membrane method. Samples of pure drug, PMs and NCs (equivalent to 10 mg) were suspended in 3 ml of sodium phosphate buffer pH 6.8. and taken in 3 cm long regenerated cellulose membrane (Spectrum Laboratories Inc, USA) with molecular weight cutoff of 12-14 kDa. The whole assembly was placed in a beaker containing 100 ml sodium phosphate buffer pH 6.8 containing 1% SLS. The solution was stirred at 37 ± 1 °C on a magnetic stirrer. Samples (1 ml) were withdrawn at 5, 10, 15, 30 and 60 min and replenished with the equal volume of fresh media. The samples were collected and analyzed for drug content using analytical method 2 of chapter 3. All readings were taken in triplicates and cumulative percentage drug dissolved at various time points was calculated.

Model independent dissolution parameters such as dissolution efficiency, dissimilarity (*f1*) and similarity (*f2*) factor were used for comparing dissolution profile of FDP, PMs and NCs.

PXRD, DSC, SEM, TEM, ^{1}H NMR studies were carried out adopting the procedure used for SDs as mentioned earlier.

5.4.3 Preparation and evaluation of buccal mucoadhesive modified release tablets

5.4.3.1 Preparation of buccal mucoadhesive modified release tablets

Modified release mucoadhesive buccal tablets were prepared by direct compression method using various polymers such as HEC, EG, AR, GM, PC, CP, EC and HPMC. To study the effect of combination of polymers on in vitro drug release and mucoadhesion, formulations were designed using combination of EC and HPMC. Formulations were also designed using cationic natural polymer chitosan (CH). To study the effect of diluents on drug release from CH matrices, formulations were prepared using lactose or tricalcium phosphate (TCP).

Effect of designed solid dispersions and nanocrystals on in vitro drug release behavior was assessed by preparing buccal tablets with SD (SD 1:10) and nanocrystals (NCP 1:10). Considering this objective and limited availability of SD and NC, only representative polymers were used for preparation of tablets containing SD and NC. Modified release buccal tablets containing FDP solid dispersions were prepared using representative polymers HEC, EG, AR and PC. Modified release buccal tablets containing FDP nanocrystals were prepared using EC and combination of EC and HPMC.

Each formulated tablet contained 5 mg of FDP. Drug/SD/NC (60#), polymer (60#) and other excipients (60#) were carefully mixed using geometrical technique and compressed using 4 mm round flat faced bevel edge (FFBE) punches. Lactose and magnesium stearate were used as diluent and lubricant respectively. Three batches of tablets for each formulation were prepared to check for batch reproducibility.

5.4.3.2 Effect of various formulation parameters

Buccal tablets were formulated using varying ratio of polymers like HEC, EG, AR, GM, CH, EC, HPMC, PC and CP to investigate the effect of type and quantity of polymer on in vitro release profile, release mechanism and in vitro mucoadhesion. Effect of the nature of polymers on in vitro release and mucoadhesion was also studied by selecting non ionic (HEC, EC, HPMC), cationic (CH) and anionic (CP, PC) polymers. To

observe the impact of combination of polymers, formulations were prepared using various combinations of HPMC and EC.

Generally, water soluble diluents easily get dissolved in water and thereby improves wetting and in vitro release of the drugs from polymer matrix. On the other hand, water insoluble diluents are poorly wettable and present a barrier between the drug and water molecules resulting in slow release of the drugs from polymer matrix. To study this phenomenon, chitosan buccal tablets with lactose (water soluble) or TCP (water insoluble) as diluents were designed.

5.4.3.3 Evaluation of buccal mucoadhesive modified release tablets

(a) Physical characteristics

In order to estimate weight variation of each batch, weight of 20 tablets was taken. Vernier caliper was used to determine the thickness and diameter of the tablets. For friability test, 20 tablets were subjected to falling shocks in friabilator for 4 min at 25 rpm. Percentage friability was determined using initial and final weights of 20 tablets taken for testing.

(b) Assay

For the determination of drug content, 20 tablets were weighed and finely powdered. An aliquot of this powder equivalent to 10 mg of FDP was weighed and dissolved in methanol-pH 6.8 phosphate buffer (50:50 v/v). The solution was appropriately diluted and analyzed using analytical method 1 of chapter 3.

(c) Hardness

Hardness of the designed buccal tablets was determined using electrolab digital hardness tester. For this, 3 tablets from each batch were taken and force employed to break the tablets were measured in Kg. Mean hardness with STDEV of all the buccal tablets were reported.

(d) Surface pH

Buccal tablets with acidic or alkaline pH may cause irritation in oral cavity so, neutral surface pH is desired (Botternberg et al., 1991). The buccal tablets were first allowed to swell in contact with 5 ml of millipore water (pH 7.0) for 2 h in petriplates. The surface pH of the tablets was determined using digital pH meter and allowing it to equilibrate for 1 min.

(e) In vitro drug release study

In house modified USP Type I dissolution apparatus as mentioned in earlier section was used for assessing in vitro release of FDP from prepared mucoadhesive modified release buccal tablets (Charde et al., 2008). The buccal tablets were taken in 100 ml dissolution media maintained at 37 ± 1 °C. The baskets were rotated at a speed of 25 rpm. Sodium phosphate buffer (pH 6.8) containing 2% sodium lauryl sulphate was used as dissolution media. Samples (10 ml) were withdrawn at 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6 h and replenished with the equal volume of fresh media. All collected samples were filtered through 0.22 µm membrane filter. The samples were then analyzed for % CDR using analytical method 2 of chapter 3. All readings were taken in triplicates and in vitro percentage cumulative drug released at various time points was calculated.

In vitro release study data was treated using various mathematical models to assess impact of polymer and excipients on drug release mechanism and kinetics (Gurny et al. 1982; Korsmeyer et al. 1983a; Korsmeyer et al. 1983b; Ritger and Peppas 1987; Peppas and Sahlin, 1996). Extent and rate of drug release rate from embedded matrices depends on swelling behavior of the polymer, shape of the matrices, diffusion and erosion properties of the polymer and dissolution characteristics of the drug. Drug solubility, type and quantity of diluents and the nature of polymer affects the mechanism of the drug release. Drug release data obtained from the release studies was modeled using Higuchi’s, Korsmeyer-Peppas, first order and zero order equations in order to predict the drug release mechanism and kinetics from designed buccal discs of various polymers (Korsmeyer et al. 1983b).

(f) Mucoadhesion studies

In vitro mucoadhesion studies of manufactured buccal tablets were performed using texture analyzer (Stable Micro Systems TA-XT Plus, UK). Freshly excised porcine buccal mucosa was obtained from the local slaughter house and stored frozen in a simulated salivary solution and thawed to room temperature just before the study. The tissue was placed in simulated salivary fluid (SSF) and stored at -20 °C till further usage. The components of SSF were sodium chloride (0.8% w/v), potassium phosphate monobasic (0.019% w/v) and sodium phosphate dibasic (0.238% w/v).

The thawed mucosal membrane was fixed at the base of instrument using a teflon hollow disc and screws in temperature controlled bath containing simulated salivary fluid. Designed buccal tablet was attached to the base of the texture analyzer's movable probe (SMSP/10) using a double sided adhesive tape. The probe was lowered at 0.5 mm/sec till the buccal tablet was in contact with mucosal membrane. Upon contact of tablet with mucosal membrane a contact force of 0.01 N was applied for 300 sec and then the probe was dragged in opposite direction. The force required to detach the buccal tablet from the mucosal surface was recorded.

(g) Water uptake studies

Water uptake of the designed buccal tablets was performed on 2% aqueous agar plates maintained at 37 °C and 40% RH in a humidity chamber. The percentage water uptake was calculated at different time points using Eq. (5.8). All the readings were recorded in triplicate for each time point.

$$Percentage\ water\ uptake = \frac{\left(Final\ weight - Initial\ \ weight\right)}{Initial\ weight} \times 100 \qquad (5.8)$$

(h) Stability studies

Representative formulation from each lot 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 room temperature (CRT: 25 ± 2^{o}C/

60 ± 5% RH) and accelerated condition (AT: 40 ± 2°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 and 12 months for CRT). 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 analytical method 2 of chapter 3. 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 were calculated at different storage conditions for all the formulations.

5.5 Results and discussions

5.5.1. Dissolution enhancement of FDP by solid dispersion technique

5.5.1.1 Content uniformity

The prepared SDs appeared colourless and translucent on observation after complete drying in the petriplates. The complete loss of yellowish shade of FDP even in SD 1:2 suggested excellent miscibility and effective formation of the dispersion system. Percentage drug content in all the SDs was found to be acceptable with a range of 99.5 to 101.4% w/w.

5.5.1.2 Solubility measurements and determination of thermodynamic parameters

Fig. 5.1 exhibits phase solubility diagram of felodipine with respect to various soluplus® concentrations at 25° and 45°C. It was observed that the increase in the soluplus® concentration causes an increase in the aqueous solubility of felodipine. As per Higuchi and Connors the phase solubility diagram was of A_L type (Higuchi and Connors, 1965). The apparent stability constants, calculated using slope of phase diagram were found to be 249.6 mg/ml and 156.8 mg/ml at 25° C and 45° C respectively. This indicated presence of some type of interactions between drug and polymer.

In an effort to further explore the role of polymer on solubility enhancement, thermodynamic parameters ΔH (J/mol) and ΔG (J/mol), listed in Table 5.1, were

calculated with help of Eqs. (5.1) and (5.2). At all concentrations of polymer, ΔG was negative which reveals that the solubilization of drug was spontaneous in presence of the polymer. Furthermore, the spontaneity increases with increase in the concentration of polymer as evidenced by subsequent decrease in free energy of the process. Also, a gradual decrease in ΔH value with respect to the increase in polymer concentration may be attributed to the role of polymer in rendering the solubilization process more favorable.

5.5.1.3 Dissolution studies

Dissolution profiles of FDP, PMs and SDs are shown in Fig. 5.2. Pure FDP showed very poor dissolution as less than 19% drug was dissolved in 1 hour. On the other hand, an increase in the rate and extent of dissolution was observed with the increase in the quantity of soluplus® in all PMs and SDs. Furthermore, a significant enhancement of dissolution was observed with solid dispersions in comparison to the corresponding physical mixtures. Solid dispersion with 1:10 drug/polymer ratio showed fastest dissolution with more than 90% cumulative release in 30 min.

Model-independent approach was used to comprehensively compare and explore the dissolution behaviour of all the formulations. The cumulative percentage of drug dissolved after 5 min (Q5), 15 min (Q15) and 60 min (Q60), dissolution efficiency at 5 min (DE5), 15 min (DE15) and 60 min (DE60), mean dissolution time (MDT), mean dissolution rate (MDR), dissimilarity (*f1*) and similarity (*f2*) factors were calculated and the results are presented in Table 5.2. For pure drug, Q5, Q15 and Q60 were found to be 5.32%, 10.39%, 18.36% whereas corresponding values for the SD 1:10 were 27.13%, 66.63% and 101.46% respectively. This indicates outstanding dissolution enhancement of FDP when formulated as solid dispersion using soluplus®. SD with lowest drug to polymer ratio (SD 1:2) resulted in dissolution of 23.85% of drug in first five minutes which was greater than the drug dissolution from the pure drug at the end of one hour (18.36%). In first 15 minutes, SD 1:2 showed nearly 45% dissolution and solid dispersions of ratios 1:4 onwards exhibited more than 50% dissolution in the same period. Dissolution efficiencies calculated at 5, 15 and 60 minutes were highest in case of SD 1:10 and were found to be 5.10, 6.27 and 6.19 times greater than that of the pure drug

respectively. Mean dissolution time was found to be minimum and mean dissolution rate was found to be maximum in the case of SD 1:10. So, dissolution of drug from all the SDs increased with increasing proportion of soluplus® with respect to drug.

Furthermore, dissimilarity (*f1*) and similarity (*f2*) factors were also used to compare the dissolution profile of the drug from prepared SDs, PMs and pure drug. The *f1* values for SDs were found to be in range of 76.63- 83.72 (>>15) providing sufficient evidence of the substantial difference in the dissolution profile of pure drug and the SDs. This is well supported by the lower *f2* values of SDs in range of 12.70-22.77 (<50). This confirms the existence of substantial disparity in the dissolution nature of felodipine in its pure form and from prepared SDs.

5.5.1.4 Powder X-ray diffraction (PXRD)

Fig. 5.3 demonstrates the comparison of X-ray diffraction patterns of SDs, PMs, soluplus® and felodipine. The crystalline nature of felodipine was revealed by the exhibition of an array of sharp and distinct peaks notably at 2θ diffraction angles of 10.32°, 11°, 16.32°, 16.64°, 20.6°, 23.38°, 24.6°, 25.5° and 26.6°. X-ray patterns showed complete disappearance of the crystalline peaks in solid dispersion of all ratios indicating that felodipine crystals were thoroughly transformed to amorphous forms. Moreover, the characteristic peaks of the drug were present in all the physical mixtures but with lower intensity.

5.5.1.5 Differential scanning calorimetry (DSC)

DSC studies of the pure drug and solid dispersions were performed and the thermograms are shown in Fig. 5.4. The thermal analysis of pure drug revealed an endothermic peak at 146.7°C corresponding to its melting point, indicating crystalline nature of the drug. The melting peak of the drug was completely lost in case of all solid dispersions which connotes the conversion of crystalline drug into amorphous form. Thus, the finding of DSC experiment was observed to be in accordance with that of PXRD study.

5.5.1.6 Fourier transform-infrared spectroscopy (FT-IR)

FT-IR spectroscopy is a potential and widely used tool for examining intermolecular interactions such as hydrogen bonding between drug and polymer in solid dispersions (Weuts et al., 2005). In pure crystalline felodipine, the NH group of one molecule is hydrogen bonded to carbonyl group of another molecule. The stretching vibrations of both NH and carbonyl groups are very sensitive to their degree of involvement in hydrogen bonding (Tang et al., 2002). Hence, in order to examine the interactions between the drug and soluplus®, special focus on both NH and carbonyl region of IR spectra is required. IR spectra of solid dispersions in NH and carbonyl region has been shown separately in Fig. 5.5. The IR of pure drug showed stretching vibrations at 3370 (Fig. 5.5a) and 1694 cm^{-1} (Fig. 5.5b) corresponding to the hydrogen bonded NH and carbonyl groups respectively.

FT-IR spectra of all solid dispersions showed considerable shift in the NH stretch towards lower wave number and the degree of shift increased with the increase in the percentage of soluplus®. As a result, NH stretching vibrations are seen at 3294, 3271, 3254 and 3228 cm^{-1} in solid dispersions of ratio 1:2, 1:4, 1:6 and 1:10 respectively (Fig. 5a). It is established elsewhere that the downward shift of N-H bond is caused by lengthening of the bond due its involvement in hydrogen bonding and also more the downward shift stronger is the hydrogen bond formed (Tang et al., 2002). So, we can conclude that in solid dispersions, NH group of felodipine is involved in stronger hydrogen bonding with acceptor groups of soluplus® relative to that existed in the pure drug. Furthermore, within the solid dispersions, the hydrogen bonding has become stronger in the solid dispersions containing higher quantity of the polymer as evidenced by downward shift of NH peak by 76, 99, 116 and 142 cm^{-1} in SDs 1:2, 1:4, 1:6 and 1:10 respectively. A synchronous shift in carbonyl group from 1694 to higher wave number in the range of 1697-1700 cm^{-1} in all solid dispersions was observed (Fig. 5.5b). The stretching vibration of carbonyl group in this region is attributed to the non-hydrogen bonded carbonyl group (Konno and Taylor, 2006). This upward shift indicates that the carbonyl group is now free and not involved in hydrogen bonding in the solid dispersions (Lin-Vien et al., 1991). The concomitant event of NH group of felodipine getting involved in stronger hydrogen bonding relative to that of in pure drug and transformation

of carbonyl group from hydrogen bonded to non-hydrogen bonded form indicates disruption of intermolecular hydrogen bonding between felodipine molecules which is vital for the 3D crystal lattice conformation of felodipine. This phenomenon could have resulted in conversion of crystalline felodipine to amorphous form in the solid dispersions.

5.5.1.7 Scanning electron microscopy (SEM)

SEM images of pure felodipine, pure soluplus® and SD 1:10 are displayed in Fig. 5.6. On scanning electron micrographs, felodipine appeared as cuboid shaped crystals with particle size ranging from 10-200 µm (Fig. 5.6a). Soluplus® was visualized as irregular surfaced spherical particles with a diameter of approximately 300 µm under the microscope (Fig. 5.6b).

The prepared solid dispersion appeared as soft and thin flakes. SEM micrograph at a resolution of 10 µm did not reveal any sign of drug particles with one µm or larger size. This indicates a drastic decrease in drug particle size in the solid dispersion (Fig. 5.6c). Moreover, a complete loss of the individual morphological characteristics of drug and the polymer could easily be visualized in the SEM image of the solid dispersion confirming effective formation of the SD system. This observation led to conclusion that the solid dispersions was of complete amorphous nature containing drug particles at sub-micron level. These results were found to be in complete agreement with the PXRD and DSC results.

5.5.1.8 Transmission electron microscopy (TEM)

Transmission electron microscopy is considered as the state of the art method for characterization of amorphous solid dispersion containing very small particles especially at nano scale. TEM images of pure felodipine, SD 1:6 and SD 1:10 are shown in Fig. 5.7. In all images, drug particles can be visualized as black spots. Majority of the particles in pure drug sample were found to be of micrometer size (Fig. 5.7a). The numbers of particles less than one micron were very few, smallest being of 700 nm. A tremendous decrease in the particle size was observed in solid dispersions containing higher proportions of soluplus®. Particle of approximately 30 nm could be seen in SD 1:6 (Fig.

5.7b). Solid dispersions containing 1:10 ratio of drug and polymer comprised of particles of size lesser than 10 nm (Fig. 5.7c). Furthermore, a closer look reveals that in fact the drug particles are of around 5 nm size only, surrounded by 3-4 nm thick layer of soluplus®. This drastic reduction of drug particle size in solid dispersions could be ascribed to the capacity of the polymer to be involved in very strong hydrogen bonding with the drug molecule replacing the intermolecular hydrogen bonding between the drug molecules. Moreover, it is reported in literature that greatest increase in the saturation solubility could be achieved by formulating amorphous particles of less than 50 nm (Junghanns and Muller, 2008). Hence, particle size reduction to the level of 5-7 nm in SD 1:10 resulted in maximum enhancement in the rate and extent of dissolution.

5.5.1.9 Atomic force microscopy (AFM)

AFM non-contact mode images of felodipine and SD 1:10 are presented in Fig. 5.8. Crystalline drug particles of several micron size were clearly visible in the pure drug sample (Fig. 5.8a). The atomic force micrograph of solid dispersion (Fig. 5.8b) revealed presence of nano-sized particles with uniform distribution. Good miscibility of drug and soluplus® indicated effective formation of the solid dispersion. The solid dispersion micrograph also showed existence of several pores of around 5 nm size. The AFM results were found to be in absolute unison with the findings of SEM experiment.

5.5.1.10 ^{1}H NMR spectroscopy

^{1}H NMR spectroscopy was used to probe the electronic interactions between felodipine and soluplus®. In this study $CDCl_3$ was used as solvent as both drug and polymer were soluble in it. Moreover, $CDCl_3$ being an aprotic solvent does not interfere with the proton spectra of NH group of felodipine and hence negates any difference in the position of the proton spectra of NH in solution and solid state (Karavas et al., 2006).

Fig. 5.9 shows the comparative NMR spectra of pure drug, soluplus® and all solid dispersions recorded in $CDCl_3$. As discussed earlier, intermolecular hydrogen bonding among felodipine molecules via secondary amino-group and carbonyl groups is crucial for the maintenance of its crystal lattice (Tang et al., 2002). The position of the proton of the secondary amino-group in NMR spectrum could be used as a measure of the extent of

involvement of NH group in hydrogen bonding. In pure drug spectrum, signal at 5.72 ppm corresponding to the proton of NH group can be seen. This peak underwent gradual upfield shift in all solid dispersions and consequently was recorded at 6.26, 6.24, 6.52 and 7.16 ppm for SDs 1:2, 1:4, 1:6 and 1:10 respectively. Thus, an increase in extent of the shift was observed with increase in the quantity of the polymer used indicating better protection and lesser ability for movement of the NH group proton (Karavas et al., 2007). This suggests that the intensity of hydrogen bonding in felodipine-soluplus® is greater than felodipine-felodipine intermolecular hydrogen bonding.

The NMR results support results of FT-IR spectroscopy and both the studies strongly complement each other.

5.5.1.11 Particle size analysis during dissolution

In situ particle size analysis of pure polymer and SD 1:10 during dissolution was conducted for further understanding of the drug dissolution process. The samples were collected at predetermined time of 10, 30, 45 and 60 min of the dissolution process and analyzed on zetasizer. Results in terms of particle size distribution by volume are displayed in Fig. 5.10. Dissolution of soluplus® demonstrated presence of very small particles with average diameter of 4-5 nm within first 10 min (Fig 5.10a). A gradual shift of particle mean diameter towards lower range was observed throughout the dissolution process. At the end of one hour, mean particle size of about 2.3 nm was observed. Dissolution of SD 1:10 showed similar trend with average particles of 5.19 nm at 10 min and 3.11 nm at 60 min of the dissolution process (Fig. 5.10b). This phenomenon emphasize the substantial solubilization characteristics of the amphiphilic polymer, soluplus®.

5.5.2 Dissolution enhancement of FDP by nanocrystal (NC) technique

5.5.2.1 Particle size and zeta potential analysis

The results of particle size and zeta potential analysis of nanocrystals are given in Table 5.3. Nanocrystals were successfully prepared with all three surfactants namely poloxamer 407, SLS and tween 80 and both organic solvents (ethyl acetate and acetone),

used in the process. Furthermore, poloxamer 407 was found to be the most suitable surfactant to yield nanocrystals with smallest particle size. FDP nanocrystals of 1:10 ratio (drug: poloxamer 407) prepared with ethyl acetate produced very small drug particles of approximately 30 nm (z-average) mean particle size as represented in Fig. 5.11. The particle size distribution of the nanocrystals (NCP 1:10) was narrow with mean PDI of 0.305. The formulated nanocrystals exhibited good stability against inter-particle aggregation as characterized by absolute mean zeta potential of approximately 31. The mean particle size and size distribution were found to increase with the decrease in the quantity of poloxamer in nanocrystals. NCP 1: 8 and NCP 1: 6 produced particles with z-average of approximately 161 (PDI = 0.456) and 280 (PDI = 0.876) nm respectively (Table 5.3). Lowering amount of the surfactant resulted in increased chances of aggregation of nanocrystals as indicated by the observed zeta potential values. Nanocrystals formulated with tween 80 in 1:10 ratio of drug and surfactant exhibited considerably larger particles (z-average = 146.9 nm) as compared to that with poloxamer at same ratio. Again, a consequent increase in the particle size was observed with the decrease in the quantity of tween 80. SLS as a stabilizer was found to stand at last position in terms of particle size among the three surfactants. The mean particle size obtained were 310, 426.9 and 460.3 nm with 1:10, 1:8 and 1:6 ratio of the drug and SLS respectively. The performance of poloxamer 407 as a best stabilizer in this case could be attributed to its merit in the ability of reduction in interfacial tension and consequently free energy of the system. Instead, the system would achieve it by reducing the surface area either by dissolving nascent nuclei or by agglomerating small particles. It seems that poloxamer 407 and tween 80 being non-ionic surfactants interacts in a better way with respect to the SLS (an ionic surfactant), with FDP molecules which is hydrophobic in nature. Both, poloxamer 407 and tween 80 prevented intermolecular agglomeration of FDP molecules by demonstrating steric repulsion. Moreover, molecular weights of poloxamer 407 and tween 80 are 9840–14600 and 1310 respectively. So, due to higher molecular weight poloxamer 407 exhibited greater steric repulsion than tween 80 and resulted in the formation of smaller nanocrystals.

Nanocrystals prepared using acetone as organic solvent at the same ratio for all the surfactants, produced particles with larger size and broader size distribution as

compared to those formulated using ethyl acetate. The rationale of this phenomenon lies in the difference of boiling points of the two solvents. Acetone (B.P. = 56 °C) being more volatile than ethyl acetate (B.P. = 77.1 °C) rapidly gets evaporated during sonication causing quick supersaturation of FDP in aqueous media. This results in the formation of comparatively larger nanocrystals.

Nanocrystals of various ratio were also formulated using soluplus® and ethanol as stabilizer and organic solvent respectively. The results of tests performed on designed nanocrystals are given in Table 5.4. Nanocrystals of ratio 1:6 produced particles of 36.8 nm size as demonstrated in Fig. 5.12. The mean PDI of the nanocrystals (1:6) was found to be 0.212 indicating a narrow size distribution. The mean absolute zeta potential value of 35.7 suggested lesser tendency of agglomeration of the prepared nanocrystals. NCS 1:4 and NCS 1:6 yielded particles with z-average of 90.59 nm and 143.16 nm respectively suggesting increase in particle size with the decrease in the amount of the surfactant. The decrement in the quantity of soluplus® also resulted in decrease in the tendency of the nanocrystals against aggregation.

5.5.2.2 Content uniformity

Percentage drug content in FDP nanocrystals prepared with poloxamer and soluplus® was found to be acceptable with a range of 98.7% to 102.5% and 99.3% to 101.6% w/w respectively.

5.5.2.3 In vitro dissolution study

In vitro dissolution profiles of FDP, PMs and nanocrytals prepared with poloxamer 407 and ethyl acetate as organic solvent are presented in Fig. 5.13. FDP exhibited very poor dissolution as less than 19% drug was dissolved in one hour. An increase in the rate and extent of dissolution was observed with the increase in the quantity of poloxamer in all PMs and NCs. Furthermore, a significant enhancement of dissolution was observed in all nanocrystals in comparison to the corresponding physical mixtures. NCP 1:10 showed fastest dissolution with approximately 90% cumulative release in 15 minutes. Model independent parameters were used to compare the dissolution behaviour of pure drug, NCs and the corresponding physical mixtures. The

cumulative percentage of drug dissolved after 15 min (Q15), 30 min (Q30) and 60 min (Q60), dissolution efficiency at 15 min (DE15), 30 min (DE30) and 60 min (DE60), dissimilarity (*f1*) and similarity (*f2*) factors were calculated and the summary of the results is presented in Table 5.5. For pure drug, Q15, Q30 and Q60 were found to be 10.07%, 11.62% and 18.36% whereas corresponding values for NCP 1:10 were 89.07%, 91.91% and 94.56% respectively. This indicated substantial dissolution enhancement of FDP when formulated as nanocrystals with poloxamer 407. The *f1* values for NCs were found to be in range of 82.18-87.38 suggesting considerable difference in the dissolution behaviour of FDP and NCs. This is well supported by lower *f2* values of NCs in range of 8.02-16.85.

Fig. 5.14 presents dissolution profiles of FDP nanocrystals prepared with soluplus®. The model independent dissolution parameters are shown in Table 5.6. NCS 1:6 exhibited Q15, Q30 and Q60 values of 59.24%, 71.48% and 92.6%. This gives sufficient evidence of considerable dissolution enhancement of the drug. The NCs demonstrated high *f1* values ranging from 74.9 to 82.65 and low *f2* values in range of 15.44-25.77. This establishes substantial difference in the dissolution pattern of NCs and the drug. So, the dissolution enhancement of FDP was achieved successfully by preparing its nanocrystals.

5.5.2.4 Powder X-ray diffraction (PXRD)

Fig. 5.15 shows PXRD pattern of FDP, poloxamer 407, PMs and NCs. FDP being crystalline exhibited an array of sharp and distinct peaks. Poloxamer 407 displayed two peaks at 19.18 and 23.24°. These peaks were present in all PMs but with lower intensity. In all NCs, FDP peaks were found to be absent and characteristic peaks of poloxamer 407 were present. This could be attributed to the conversion of FDP to amorphous form.

PXRD pattern of NCs prepared with soluplus®, PMs, FDP and soluplus® has been shown in Fig. 5.16. Drug peaks completely disappeared in all nanocrystals but were present in all PMs. This provides evidence of formation of amorphous nanocrystal.

5.5.2.5 Differential scanning calorimetry (DSC)

DSC studies of the FDP and poloxamer nanocrystals were performed and the thermograms are shown in Fig. 5.17. The thermal analysis of pure drug revealed an endothermic peak at 146.7°C corresponding to its melting point, indicating crystalline nature of the drug. Pure poloxamer being crystalline molecule, exhibited melting endotherm at 61°C. It was interesting to see that in all nanocrystals, FDP melting peak disappeared and poloxamer melting peak was invariably present. This observation confirms the formation of amorphous FDP nanocrystals.

The DSC results of nanocrystals prepared with soluplus® are shown in Fig. 5.18. The melting peak of the drug was found to be completely lost in case of all nanocrystals. This suggests conversion of crystalline drug into amorphous form in the prepared nanocrystals.

5.5.2.6 Scanning electron microscopy (SEM)

SEM images of pure FDP, pure poloxamer 407 and NCP 1:10 are displayed in Fig. 5.19. On scanning electron micrographs, FDP appeared as cuboid shaped crystals with particle size ranging from 10-200 µm (Fig. 5.19a). Poloxamer 407 was visualized as smooth surfaced spherical particles with a diameter of approximately 15 µm under the microscope (Fig. 5.19b). A complete loss of the individual morphological characteristics of the drug and the surfactant was observed in the prepared nanocrystals. SEM micrograph at a resolution of 5 µm did not reveal any sign of drug particles with 1 µm or of bigger size. This indicates a drastic decrease in drug particle size in the nanocrystlas (Fig. 5.19c).

Fig. 5.20 presents SEM images of pure drug, soluplus® and NCS 1:10. Soluplus® was visualized as irregular surfaced spherical particles with a diameter of approximately 300 µm under the microscope (Fig. 5.20b). The prepared nanocrystal appeared as soft and thin flakes. SEM micrograph of NCS 1:10 did not reveal any sign of drug particles. This indicated a drastic decrease in drug particle size in the nanocrystal (Fig. 5.20c). Moreover, a complete loss of the individual morphological characteristics of drug and the polymer could easily be visualized.

This observation led to the conclusion that the nanocrystals of amorphous nature were effectively prepared.

5.5.2.7 Transmission electron microscopy (TEM)

Transmission electron microscopy is considered as the state of the art method for characterization of amorphous system containing very small particles especially in nano range. Fig. 5.21 gives TEM micrographs of pure FDP, NCS 1:10 and NCP 1:10. In all images, drug particles can be visualized as black spots. Majority of the particles in pure drug sample were found to be of micrometer size (Fig. 5.21a). The numbers of particles less than one micron were very few, smallest being of 600 nm. The size of FDP particles was found to be in a range of 25-35 nm and 29-39 nm in the nanocrystals prepared with poloxamer and soluplus® respectively. These results are in complete agreement with that of particle size analysis conducted by zetasizer.

5.5.2.8 ^{1}H NMR spectroscopy

^{1}H NMR spectroscopy was used to study the electronic interactions between felodipine and the stabilizers. In this study $CDCl_3$ was used as solvent due to solubility of drug, poloxamer 407 and soluplus® in it. Fig. 5.22 and Fig. 5.23 presents comparison of ^{1}H NMR spectra of FDP-poloxamer and FDP-soluplus® nanocrystals respectively.

In pure drug spectrum, signal at 5.72 ppm corresponding to the proton of NH group can be seen. In poloxamer nanocrystals, this peak underwent gradual upfield shift and consequently was recorded at 5.81, 5.94 and 6.35 ppm for NCP 1:6, 1:8, and 1:10 respectively (Fig. 5.22). In case of soluplus-nanocrystals, it was positioned at 5.73, 5.79 and 6.50 ppm for NCS 1:2, 1:4 and 1:6 respectively (Fig. 5.23). Thus, an increase in extent of the shift was observed with increase in the quantity of the polymer used indicating better protection and lesser ability for movement of the NH group proton (Karavas et al., 2007). This suggests that the intensity of hydrogen bonding in felodipine-stabilizer is greater than felodipine-felodipine intermolecular hydrogen bonding. This incorporation of new pattern of hydrogen bonding with stabilizers replacing intermolecular hydrogen bonding in FDP might have caused the disruption of 3-D lattice structure of crystalline felodipine and eventual formation of amorphous nanocrystals.

5.5.3 Formulation of modified release buccal tablets

5.5.3.1 Evaluation of buccal mucoadhesive modified release tablets

Composition of all the batches of buccal mucoadhseive modified release tablets prepared during the course of research work is presented in Table 5.7a to 5.7c.

The developed buccal tablets containing FDP in pure, SD and NC forms were found to possess good physical properties as shown in the results tabulated in Table 5.8a to 5.8c. Weight variation of all the formulations was within permissible limits (±10% of the tablet weight) as per USP. Maximum value of weight variation was observed to 1.33 mg (Table 5.8a). A friability value of less than 1% was observed for all the designed tablets which indicated suitability of the method employed in the formulation of tablets.

The prepared tablets exhibited maximum thickness of 3.17 mm with maximum STDEV of 0.08 (Table 5.8a). Formulations containing CH and AR were found to be thicker when compared to tablets prepared with other mucoadhesive polymers. This may be due to the lower bulk density of CH and AR. Assay results reveal the variation in drug content of the designed formulation. Drug content was found to be in a range of 98% to 103% of the theoretical value with a maximum STDEV of 2.27 (Table 5.8b). This provided further evidence of the reliability and reproducibility of the manufacturing process.

The designed tablets were found to possess good hardness. The hardness values of the manufactured tablets were found to be in a range of 4.06 to 6.61 Kg. Buccal tablets prepared with AR and CH were observed to be less hard in comparison to the tablets of same weight prepared with other polymers using same compressive force. The surface pH of the designed buccal tablets was within a range of 6.51 to 7.26 (data not shown). Surface pH value of tablets close to neutral pH is customary for better patient compliance.

5.5.3.2 In vitro release studies

An ideal in vitro release study should provide suitable information regarding in vivo performance of the dosage forms. The in vitro release study was conducted in 100

ml of the phosphate buffer (pH 6.8). As FDP is practically insoluble in water, 2% SLS was added to the buffer to maintain sink condition.

Present study was aimed at the development of buccal tablets with modified release for 4-6 h in order to maintain steady plasma concentration for longer time. Also, release of 10-20% of drug within first hour would help in the attainment of minimum effective concentration quickly and avoid the use of loading dose in the formulation. Control of drug release for more than 6 h was not targeted just to avoid patient incompliance.

The duration of drug release was found to be governed by proportion of polymer used in the formulation as suggested by in vitro release studies. Increase in the amount of polymer causes decrement in the diffusion of drug by increasing viscosity of the gel layer and eventually extending the diffusion path length. The investigation of release mechanism with help of Korsmeyer-Peppas model suggested presence of anomalous non-Fickian transport for all the designed buccal tablets. A combination of polymer erosion, polymer swelling and diffusion plays a role in release of drug from matrices. An initial higher release rate could be attributed to the time taken by the polymer to swell properly. After complete polymer swelling, the drug release rate decreases with time and becomes dependent upon polymer erosion and effective molecular diffusional area (Hosny, 1993).

The cumulative percentage drug release (%CDR) of the buccal tablets prepared with various polymers was determined and the comparative profiles are presented in Fig. 5.24 to Fig. 5.31. All the tablets showed faster drug release initially (minimum 10-15% drug release in first 30 min) which might be sufficient to elicit target concentration thereby precluding use of loading dose in the formulations. The result of drug release of all formulations was fitted to zero order and first order release kinetics. Table 5.9a to 5.9c gives release rate constants (K) and regression coefficients (R^2) of zero and first order kinetics. In order to predict the release mechanism the release data were also fitted to Higuchi and Korsmeyer-Peppas model. The results are presented in Table 5.9a to 5.9c. The 'n' values obtained from Korsmeyer-Peppas model ranged between 0.45 to 0.89 indicating diffusion, polymer relaxation and erosion as predominant mechanism of drug release (Korsmeyer et al., 1983b).

Fig. 5.24 demonstrates comparative plot of %CDR versus time for matrix embedded modified release buccal mucoadhesive tablets prepared using pure FDP, SD and varying proportions of HEC. Tablets containing pure FDP and HEC ratio of 1:2 and 1:4 exhibited 73% and 51% release at the end of 6 h respectively. In contrast to it, tablets prepared using SD containing equivalent amount of FDP and same ratio of HEC (1:2 and 1:4) showed 100% drug release in approximately 4.5 and 5.5 h respectively. The release data for all the formulations prepared using HEC fitted best in first order kinetic model as evidenced by the regression coefficient (R^2) values (Table 5.9a). The 'n' values determined from Korsmeyer-Peppas equation for all tablets ranged from 0.683 to 0.740 suggesting that the release mechanism was anomalous non-Fickian transport. Tablet prepared with SD 1:10 containing 1:4 ratio of equivalent drug and HEC resulted in preferable drug release profile.

Fig. 5.25 presents comparative release profile of buccal mucoadhesive tablets prepared with pure FDP, SD and varying proportions of EG. At the end of 6 h, tablets made with 1:2 and 1:3 ratio of the pure drug and EG showed 65% and 53% cumulative drug release respectively. On contrary to it, tablets formulated with SD and same ratio of EG demonstrated 100% release in approximately 4 and 5 h respectively. All the tablets made with EG showed first order release irrespective of the form of drug. The 'n' values for all the tablets ranged from 0.653 to 0.752 indicating anomalous non-Fickian transport (Table 5.9a). Tablet made with SD containing 1:3 ratio of equivalent drug and EG resulted in preferable drug release profile.

Agar (AR) was also used as mucoadhesive and rate controlling polymer in the buccal tablets. Buccal tablets containing drug/SD and AR in 1:3 and 1:4 ratio were prepared. The percentage release of 88% and 68% drug release was observed from tablets containing 1:3 and 1:4 ratio of pure drug and AR respectively over a period of 6 h (Fig. 5.26). Buccal tablets prepared with SD and same ratio of AR showed 100% drug release in 3 and 4 h respectively. All the tablets made with AR showed first order release irrespective of the form of drug. The 'n' values for all the tablets ranged from 0.508 to 0.762 indicating anomalous non-Fickian transport (Table 5.9a). Tablet made with SD containing 1:4 ratio of equivalent drug and AR resulted in desirable drug release profile.

PC, an anionic mucoadhesive polymer, has been widely reported as mucoadhesive polymer over last two decades (Garbnar et al., 2003). The in vitro release study of buccal tablets made with PC presented a surprising result. Fig 5.27 presents comparative release profile of buccal mucoadhesive tablets containing pure FDP, SD with varying proportions of PC. At the end of 6 h, tablets made with 1:1 and 1:2 ratio of the pure drug and PC showed 97% and 75% cumulative drug release respectively. Tablets made with SD and same ratio of PC showed 53% and 21% drug release which was just opposite to the release behavior seen with other polymers. This result gives an indication to some sort of interactions between the acrylic acid polymer and soluplus®. The carboxylic acid and hydroxyl groups present in PC might have interacted with hydroxyl groups of soluplus® through hydrogen bonding. However, all the tablets demonstrated first order release kinetics. The 'n' values ranged from 0.682 to 0.864 suggesting anomalous non-Fickian transport (Table 5.9b). In this study, tablet made of pure drug and PC in ratio of 1:1 was found to give desirable release profile.

Fig. 5.28 shows release profile of buccal tablets made with CP and pure FDP. In this preparation, drug to polymer ratio used were 1:1 and 1:2. 100% drug release was observed over 4 and 6 h time respectively. All the tablets made with CP showed first order release irrespective of the form of drug. The 'n' values for the buccal tablets were found to be 0.603 and 0.692 which indicates anomalous non-Fickian transport (Table 5.9b). Tablet made with 1:2 ratio of drug : CP was found to result in desirable drug release profile.

Fig. 5.29 demonstrates comparative plot of %CDR versus time for matrix embedded modified release buccal mucoadhesive tablets prepared using pure FDP with varying proportions of CH. In this study, effect of water soluble and water insoluble diluent in form of lactose and tricalcium phosphate respectively was investigated. Tablets containing 1:2 and 1:4 ratio of drug and polymer with lactose as diluent showed 100% release in approximately 1.5 and 3 h respectively. Drug to CH ratio was 1:1 and 1:2 in buccal tablets prepared with TCP which exhibited only 50% and 28% drug release at the end of 6 h. So, combination of lactose and TCP (1:1) was tried and tablets with 1:1 and 1:2 ratio of FDP:CH were prepared. In this case, almost 100% drug release was observed in 3 and 5 h in case of tablets containing 1:1 and 1:2 ratio of drug to polymer

respectively. All the tablets showed first order release as indicated by R^2. The buccal tablet containing mixture (1:1) of lactose and TCP and 1:2 ratio of drug and CH resulted in desirable drug release profile.

Fig. 5.30 shows release profile of buccal tablets made with GM and pure FDP. In this preparation, drug to polymer ratio used were 1:1 and 1:2. Tablets prepared using drug:polymer ratio of 1:1 showed 100% drug release in 4 h and tablets with drug polymer ratio of 1:2 showed 78 % release at the end of 6 h. All the tablets made with GM showed first order release kinetics. The 'n' values for the buccal tablets were found to be 0.647 and 0.792 which indicates anomalous non-Fickian transport (Table 5.9b). Tablet of 1:1 ratio was found to give desirable drug release profile.

Buccal tablets were also prepared using FDP nanocrystals and its release profile was compared with those made with pure drug. The release profile is shown in Fig. 5.31. In this study, buccal tablets containing pure drug were made with varying proportion of EC and HPMC alone. Further, tablets were also designed using combination of EC and HPMC in varying proportions. Buccal tablets with 1:2 and 1:4 ratio of drug to HPMC resulted in 90% drug release in 4 and 5 h respectively. Buccal tablets with 1:2 and 1:4 ratio of drug to EC resulted in 73% and 39% drug release at the end of 6 h respectively.

Further, EC and HPMC were also used in combination for making buccal tablets. The ratio used were 1:2:2 and 1:3:1 of pure FDP:EC:HPMC. At the end of 6 h, 92% and 68% drug release was seen in case of tablets with 1:2:2 and 1:3:1 ratio of pure FDP:EC:HPMC respectively. In place of pure drug, nanocrystals of equivalent amount of drug were used and buccal tablets of 1:3:1 ratio of NC:EC:HPMC were made. In this case almost 100% drug release was achieved in 5 h. Buccal tablets using FDP nanocrystals with EC as polymer were also prepared in NC:EC ratio of 1:2 and 1:4. Almost 100% drug release was obtained within 5 h and 68% drug dissolution was observed at the end of 6 h for tablets with NC:EC ratio of 1:2 and 1:4 respectively. All the tablets followed first order drug release as suggested by R^2 values. The 'n' values of the in vitro release study ranged from 0.621 to 0.814 suggesting that the release mechanism was anomalous non-Fickian transport. The results are tabulated in Table 5.9c. Buccal tablets prepared with 1:2 ratio of pure drug:HPMC, 1:2 ratio of NC:EC and 1:3:1 ratio of NC:EC:HPMC resulted in desired release profile.

5.5.3.3 Mucoadhesion studies

In vitro mucoadhesion studies of the designed tablets were conducted with porcine buccal mucosa on texture analyzer. The force required for detachment of the buccal tablets from the mucosal membrane was recorded and taken as measure of the mucoadhesive strength. Various polymeric networks containing polar functional groups such as -COOH, -OH, -NH_2 etc. have been reported to possess mucoadhesive properties (Smart, 2005). In order to prepare mucoadhesive buccal tablets for FDP a variety of swellable polymers were used. These polar groups present in the polymers interacts with long glycoprotein chain of mucin. The polymeric network absorbs water from local environment and form gel like structure and get entangled with glycoprotein chain of mucin to form crosslinked bonds causing mucoadhesion.

Table 5.8a to 5.8c exhibits the mucoadhesive strength of each formulations. The mucoadhesive strength was found to be directly proportional to the amount of polymer used in the buccal tablets. At lower concentration, the presence of less number of penetrating polymeric chains per unit volume of the mucus membrane results weaker interaction (Peppas and Buri, 1985). In the present study, hydrophilic polymers such as HEC, HPMC, CP, PC, CH and water insoluble polymers like EG, EC, AR and GM was used.

The hydrophilic polymers possess many polar groups like -COOH, -OH etc. These polar groups attract water and form hydrogels which helps in adhesion to the biological surfaces. For example, PC and CP contains large number of carboxylic acid group in their molecular network. They remains tightly coiled in anhydrous environment. Upon hydration, they undergo considerable swelling resulting in uncoiling of polymeric chains. In this state they get entangled with the glycoprotein networks of mucin. More are the number of available groups to form H-bond more is the mucoadhesive strength achieved (Zhaolu et al., 2013). The increase in bioadhesion with the increase in quantity of PC and CP (Table 5.8b) provides evidence to this theory. The buccal tablets containing pure FDP to PC ratio of 1:1 and 1:2 showed a mucoadhesive strength of 0.75 and 1.54 N respectively. Tablets made with SD exhibited increased mucoadhesive strength due to presence of more hydrophilic group -OH in soluplus®. Tablet made with 1:1 and 1:2 proportions of pure FDP and CP demonstrated a mucoadhesive strength of 0.57 and 0.96

N respectively. CH is a copolymer composed of N-acetyl glucosamine and D-glucosamine units. It is a polycationic polymer containing one -NH_2 and two -OH groups. CH undergoes considerable swelling upon hydration and can interact with mucin networks through various linkage including amide, ester bonding and or ionic linkages (Dash et al., 2011). Formulations containing CH exhibited excellent mucoadhesive behaviour. Tablet containing 1:2 and 1:4 ratio of drug and CH were found to possess mucoadhesive strength of 0.74 and 1.35 N respectively. The use of TCP instead of lactose resulted in decrease in the mucoadhesive strength. Tablet containing 1:2 ratio of drug to CH demonstrated mucoadhesion of 0.67 N. This decreased mucoadhesion is due to cross linking of CH chains by calcium which results in decrease in the number of CH chains available for linkage with mucin chains.

Except EG, all water insoluble polymers easily swell upon hydration and interact with mucus membrane though various interactions. EG posses low swelling index but can interact with mucin chain with help of the -COOH groups present in it. Buccal tablets made with pure FDP and EG of ratio 1:2 and 1:3 showed mucoadhesion of 0.46 and 0.63 N respectively. The buccal tablets made with SD exhibited better mucoadhesion in comparison to those made with pure drug due to hydrophilic nature of the soluplus®.

5.5.3.4 Water uptake studies

Water uptake studies for all the designed buccal tablets were performed. Drug release is determined by swelling behaviour (due to water uptake) of polymer, drug dissolution, drug diffusion and matrix erosion. All these events get affected by the interaction between polymer matrix, water and the drug. Water is required to penetrate the polymer matrix resulting in polymer swelling and drug dissolution before the drug can diffuse out of the matrix. Water penetration causes transformation of glassy polymer into a rubbery mass enhancing the mobility of polymeric chains (Jamzad et al., 2005). This enhanced mobility is responsible for the transport of water and dissolved drug (Nazzal et al., 2007). Furthermore, more is the mobility, higher will be the flexibility of polymeric networks leading to better interaction with mucin resulting in greater mucoadhesion (Miller et al., 2005).

The results of water uptake studies are shown in Fig. 5.32 to 5.39. Formulations prepared with PC showed higher water uptake capability in comparison to other formulations. Formulation MBP/PC/2, containing 10 mg of PC, demonstrated a total of 270.94% water uptake. The percentage of water uptake was found to be proportional to the amount of polymer used. Formulation MBS/PC/4, containing same amount of polymer and equivalent amount of FDP in form of solid dispersion showed better swelling than MBP/PC/2. This can be attributed to the presence of hydrophilic compound, soluplus® in it. Formulations made with HPMC, CH, CP also showed considerably good water uptake behaviour. Formulations MBP/CH/1 and MBT/CH/4, prepared with same amount of polymer but different diluent (i.e lactose and TCP) showed water uptake capability of approximately 240 % and 210 % respectively at end of 6 h. MBP/CH/1 showed 200% water uptake in 4 h and MBT/CH/4 showed same extent of water uptake in nearly 6 h. The nature of the diluent in terms of hydrophilic or hydrophobic created the difference. TCP being hydrophobic diluent resulted in slower rate of water uptake in comparison to lactose, a hydrophilic diluent.

In all the formulations, the water uptake capacity was found to be directly proportion to the amount of polymer present in it. Initially, enhanced water uptake was observed in case of water soluble excipients however, total water uptake was found to be nearly similar at the end of 6 h and was completely dependent on polymer concentration.

5.5.3.5 Stability studies

Results of stability studies carried out using designed formulations at different condition of temperature and humidity like controlled room temperature (CRT: 25 ± 2°C/ 60 ± 5 % RH) and at accelerated condition (AT: 40 ± 2^0C/ 75 ± 5 % RH) are shown in Table 5.10.

At accelerated condition, the maximum degradation rate of the drug was found to be 131.04 x 10^{-4} $month^{-1}$ with predicted $t_{90\%}$ value of 8.01 months for formulations prepared using AR. The minimum degradation rate constant of 78.99 x 10^{-4} $month^{-1}$ was observed for formulation prepared using EG with a predicted $t_{90\%}$ value of 13.29 months. For SD 1:10, the degradation rate constant observed was 47.44 x 10^{-4} $month^{-1}$ with a predicted $t_{90\%}$ value of 22.13 months. At AT conditions, nanocrystals prepared with both

soluplus and poloxamer 407 demonstrated almost similar stability profile with the $t_{90\%}$ values of 16.89 and 17.47 months respectively. All the formulations were stable for the entire study duration (6 months) with no apparent change in physical characteristics, in vitro release and mucoadhesion behaviour.

In the formulations stored at CRT, the maximum degradation rate for the drug was found to be 68.86 x 10^{-4} $month^{-1}$ with predicted $t_{90\%}$ value of 15.25 months for formulations prepared using AR. The minimum degradation rate constant observed was 33.39 x 10^{-4} $month^{-1}$ with a predicted $t_{90\%}$ value of 31.44 months for buccal tablets prepared with HEC. The values were almost comparable to degradation rate constant (20.50 x 10^{-4} $month^{-1}$) and $t_{90\%}$ (51.23 months) value of pure drug obtained during preformulation studies (section 4.4.3.1 of chapter 4). For SD 1:10, the degradation rate constant observed was 22.57 x 10^{-4} $month^{-1}$ with a predicted $t_{90\%}$ value of 46.52 months. Nanocrystals prepared with soluplus (NCS 1:6) showed better stability in comparison to that prepared with poloxamer 407 (NCP 1:10). For NCS 1:6, the degradation rate constant observed was 23.49 x 10^{-4} $month^{-1}$ with a predicted $t_{90\%}$ value of 44.70 months. All the formulations were stable for the entire duration (12 months) with no apparent change in physical characteristics and in vitro release and mucoadhesive behaviour.

5.6 Conclusions

Amorphous solid dispersions (ASDs) and nanocrystals with excellent enhancement of the rate and extent of dissolution of FDP were prepared. In SD, the dissolution performance was dependent on the drug-polymer ratio. Soluplus®, by virtue of its capacity to interfere with the intermolecular interactions among felodipine molecules and establishing new pattern of hydrogen bonding, successfully produced ASDs. The particle size of the FDP in the dispersions reduced with increasing proportions of soluplus®. Nanocrystals of size approximately 30 nm and 37 nm were prepared using ethyl acetate-poloxamer 407 and soluplus®-ethanol systems respectively. The nanocrystals obtained were of complete amorphous nature which resulted in effective dissolution enhancement of the drug.

The developed mucoadhesive modified release buccal tablets were found to possess good physical properties suggesting suitability of the direct compression method

used for the preparation of tablets. Weight variation and content uniformity of all the formulations was found to be within permissible limits. Acceptable values of tablet hardness, friability, thickness and low inter batch variations further confirmed the suitability of the process used in manufacturing.

The release of FDP from the matrix embedded buccal mucoadhesive modified release tablets was influenced by nature and amount of polymer, existence of drug in pure or SD or NC form. In the present study, a number of buccal tablets capable of sustaining FDP release from 4-6 h were prepared. All the formulations exhibited first order release kinetics. The release mechanism of all the tablets was anomalous non-Fickian transport.

Moreover, the mucoadhesive strength of the prepared tablets was found to be considerably good. The mucoadhesive strength was affected by quantity, hydrophilicity, hydrophobicity and swelling behaviour of the polymer used.

Buccal tablets exhibiting drug release for 4-6 h and good biadhesion value were selected for further in vivo studies. MBP/HEC/2, MBS/HEC/4, MBP/PC/2, MBS/PC/4, MBP/EM/6, MBN/EM/7 were finally selected for further in vivo pharmacokinetic studies and comparision of pharmacokinetic parameters against immediate release oral tablet of FDP.

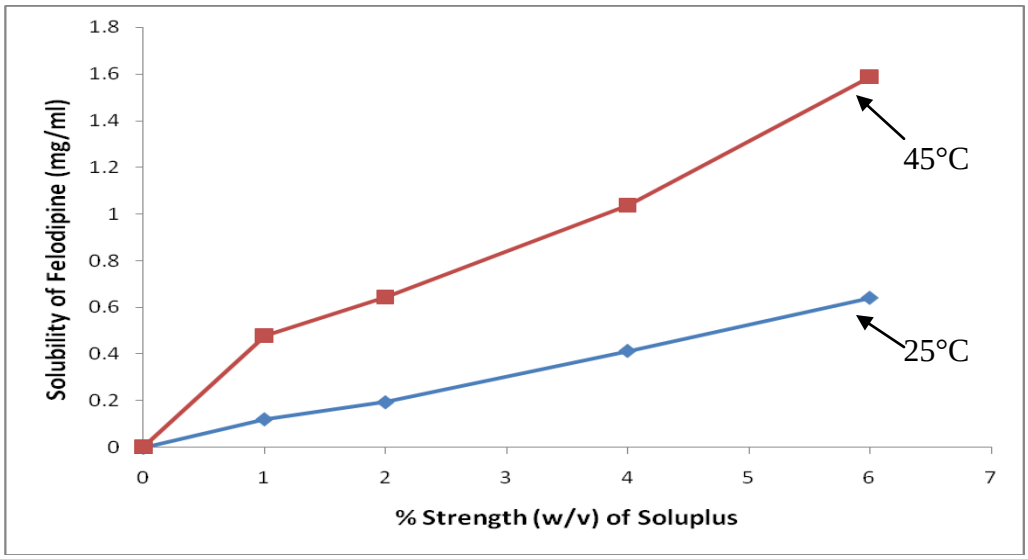


Fig. 5.1. Phase solubility diagram of felodipine in water-soluplus® mixtures at 25° and 45°C

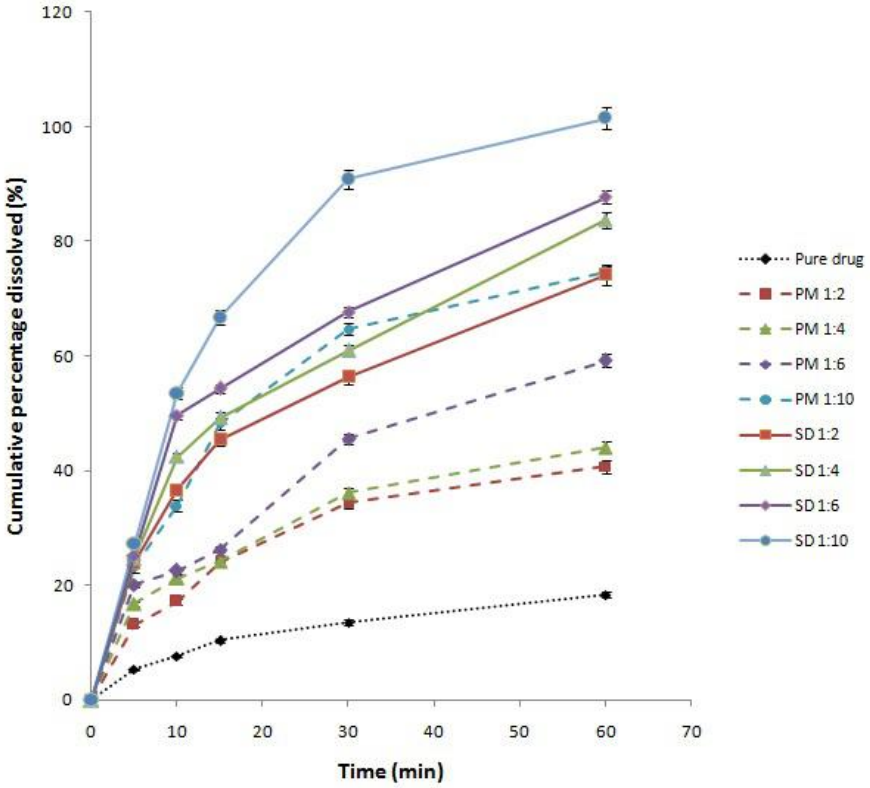


Fig. 5.2. Dissolution profiles of pure drug (felodipine), physical mixtures and solid dispersions

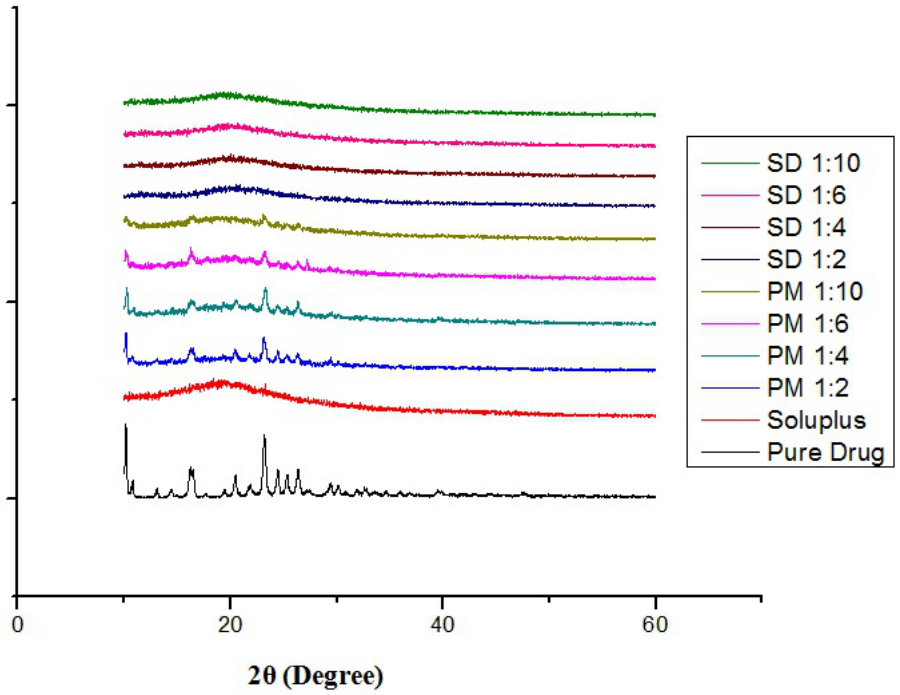


Fig. 5.3. Comparison of PXRD pattern of pure drug, soluplus®, physical mixtures and solid dispersions

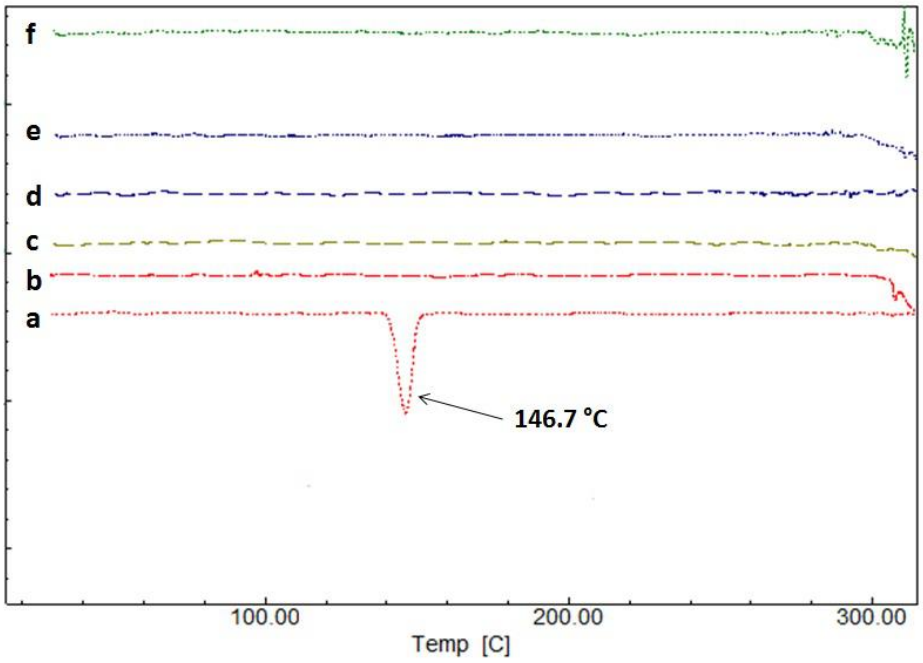


Fig. 5.4. DSC thermograms of (a) pure felodipine; (b) soluplus®; (c) SD 1:2; (d) SD 1:4; (e) SD 1:6 and (f) SD 1:10

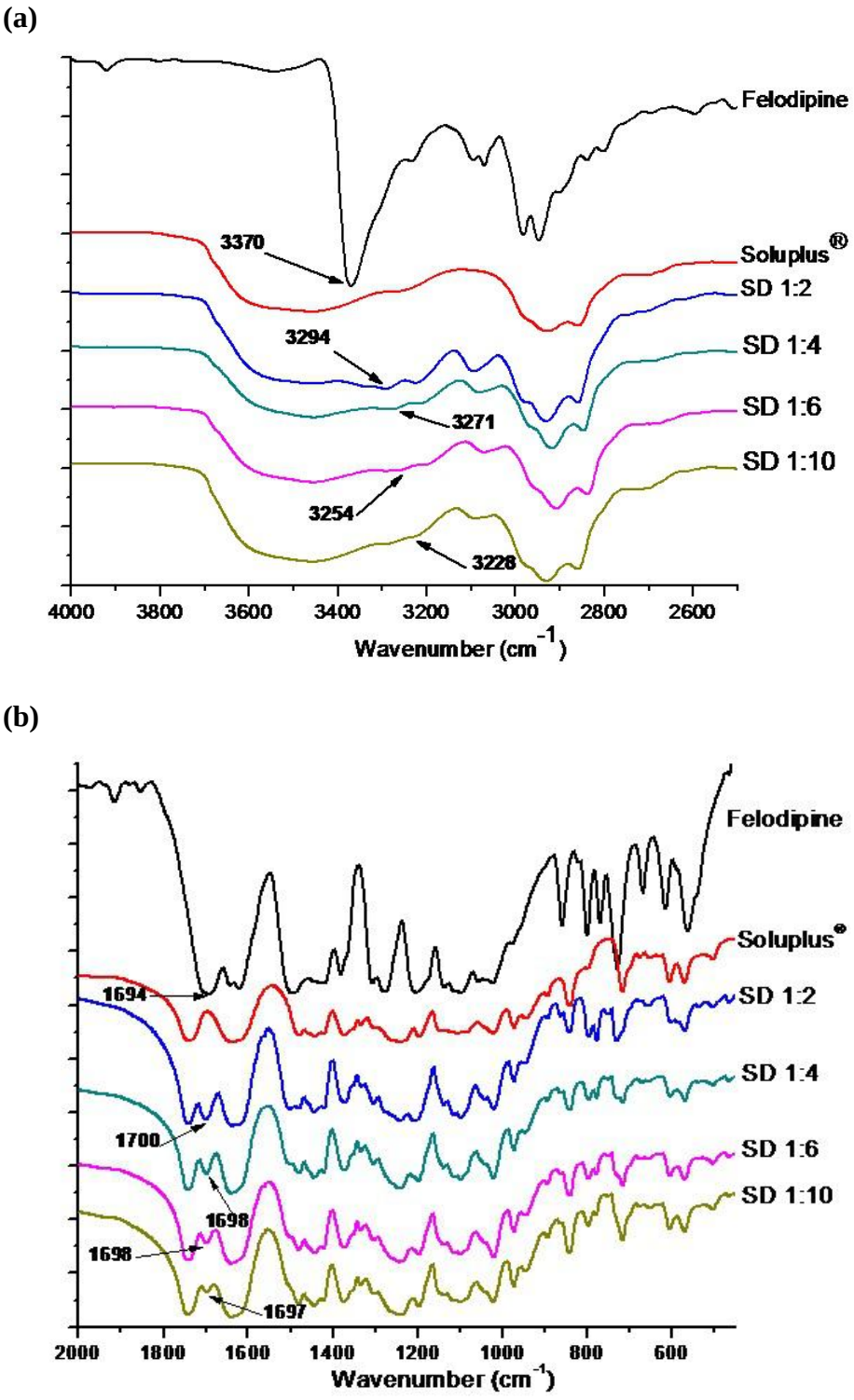


Fig. 5.5. FT-IR spectra of felodipine-soluplus® solid dispersion systems (a) NH stretching region (2500-4000 cm^{-1}) and (b) carbonyl stretching region (400-2000 cm^{-1})

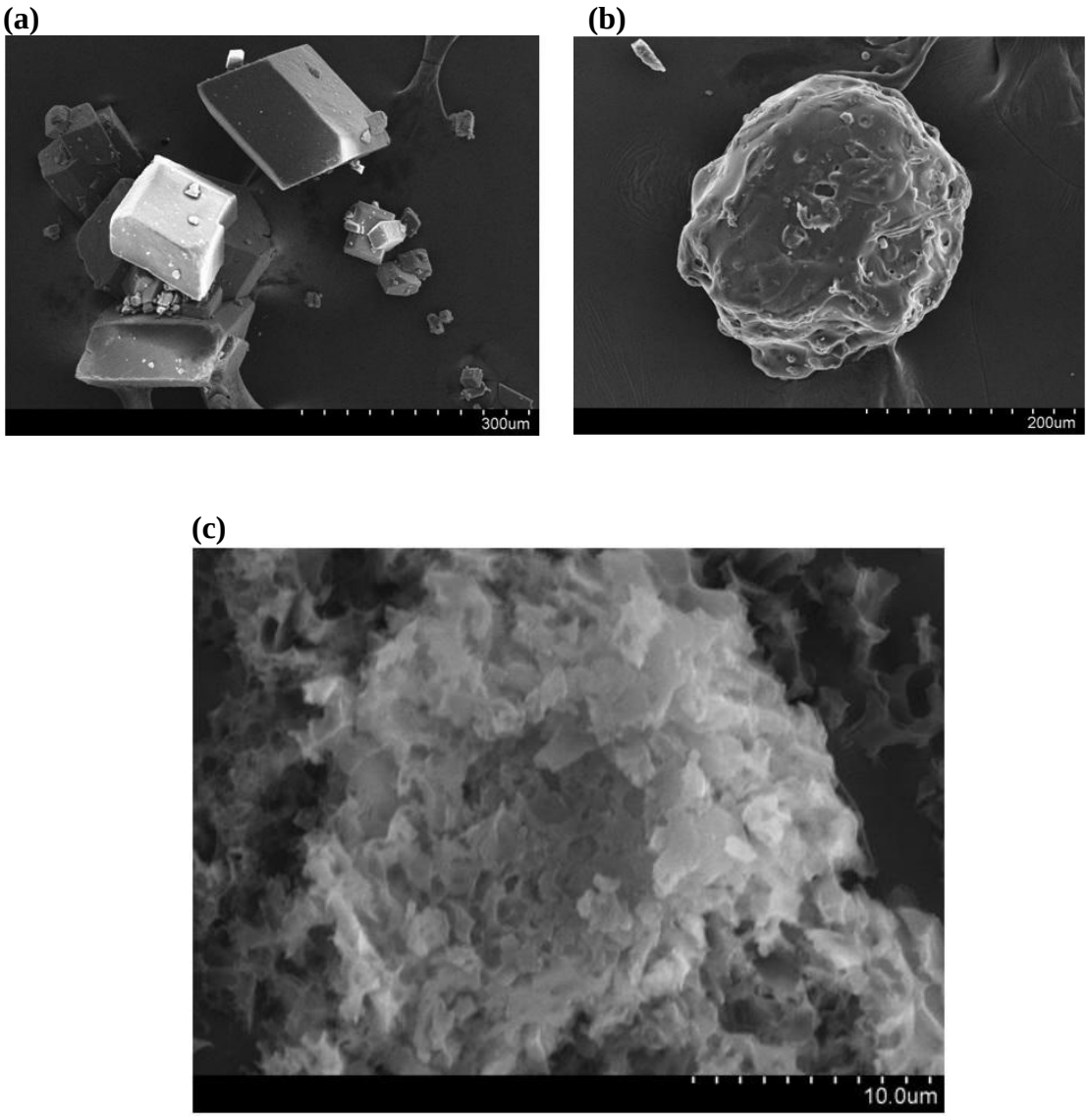


Fig. 5.6. Scanning electron micrographs of (a) pure drug (b) pure soluplus® (c) SD 1:10

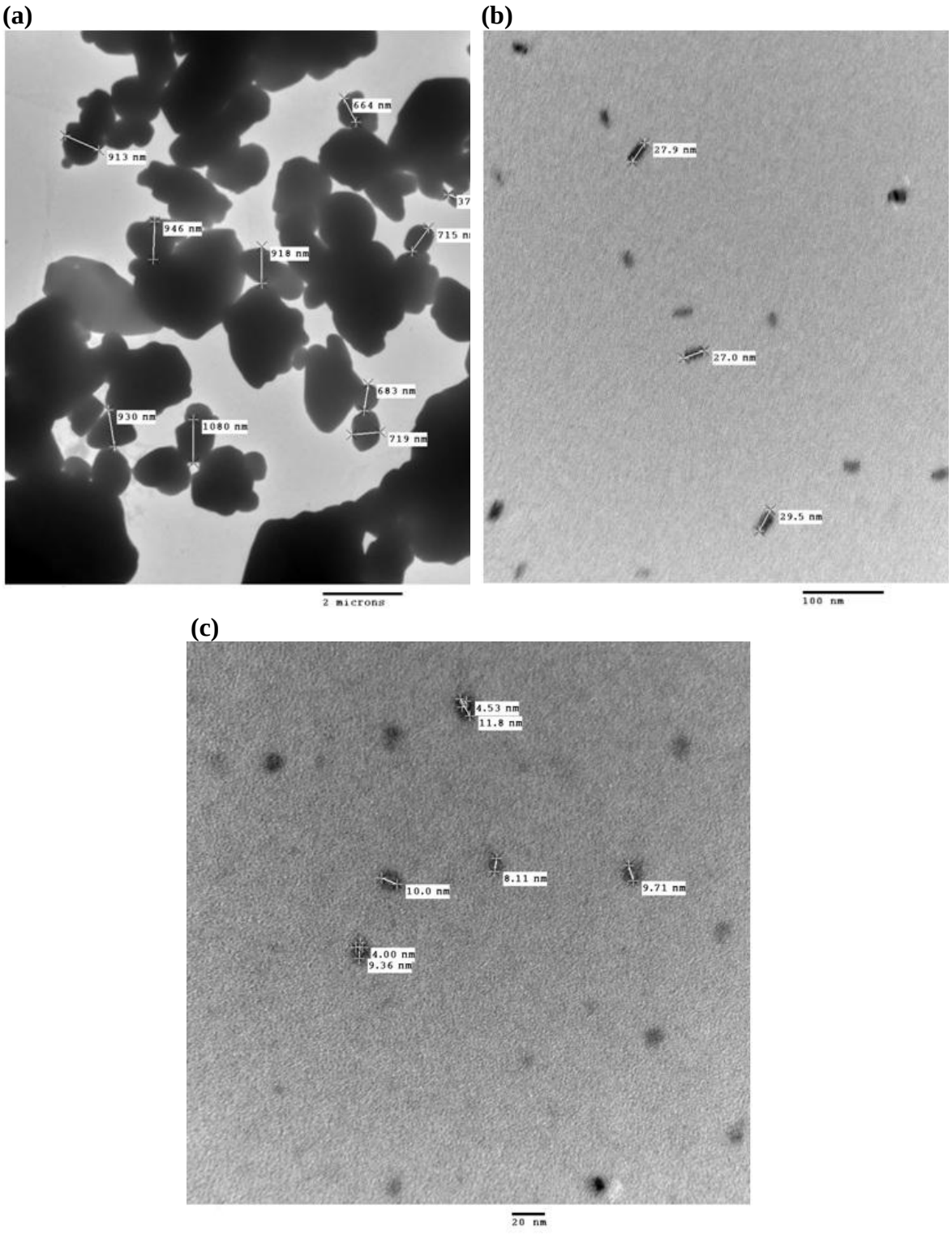
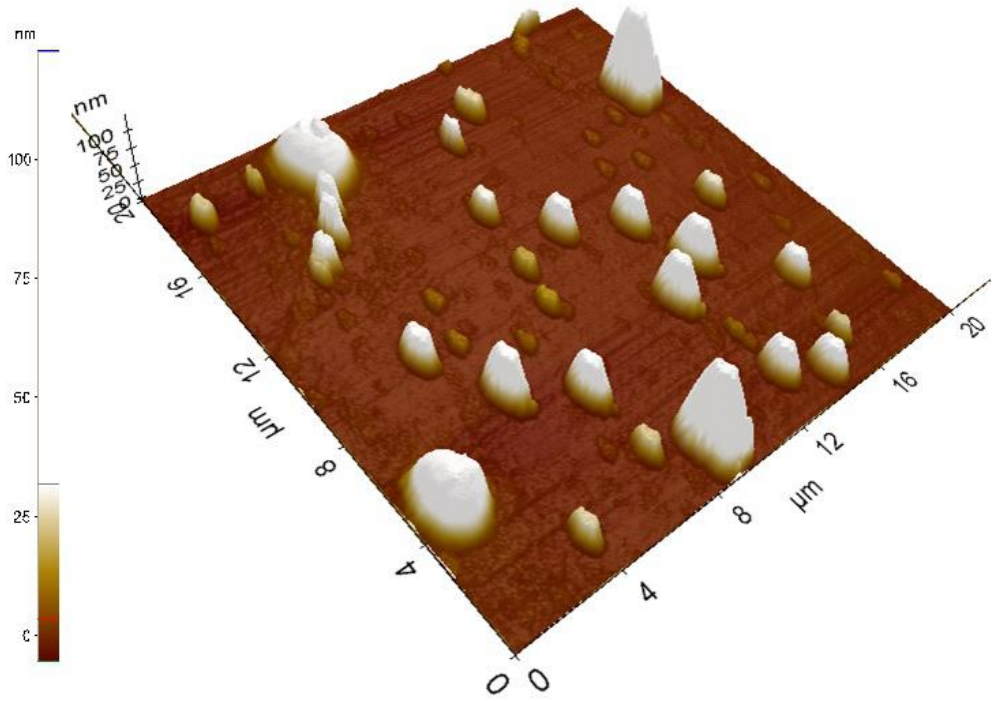


Fig. 5.7.Transmission electron micrographs of (a) pure drug (b) SD 1:6 (c) SD 1:10

(a)



(b)

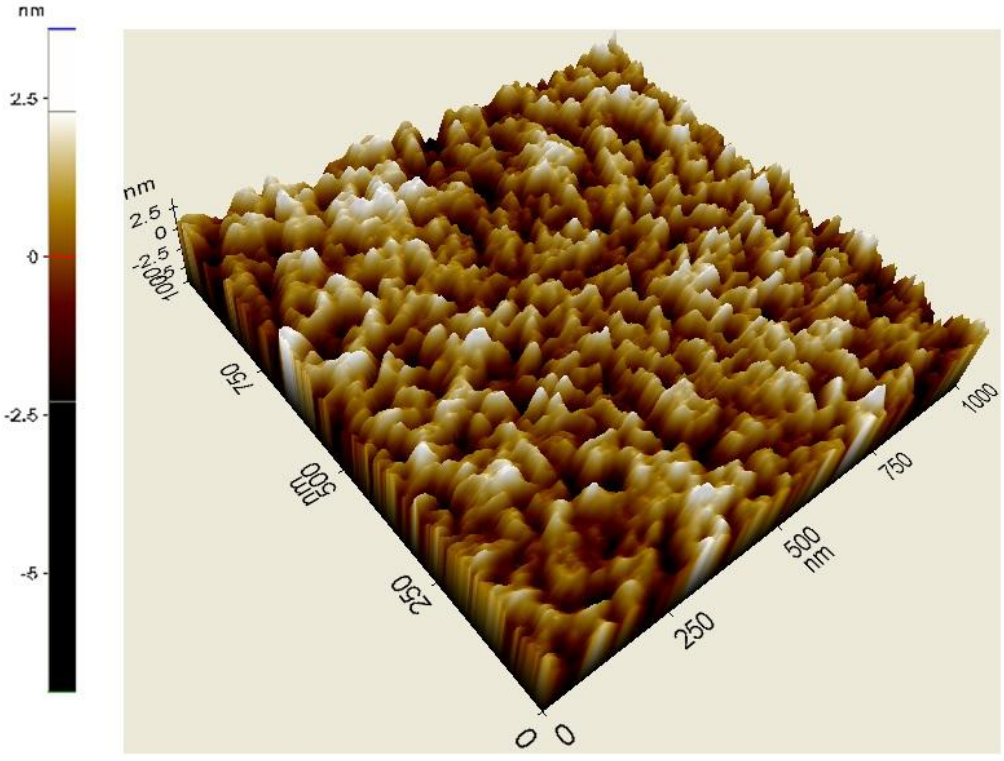


Fig. 5.8. Atomic force micrographs of (a) pure drug (b) SD 1:10

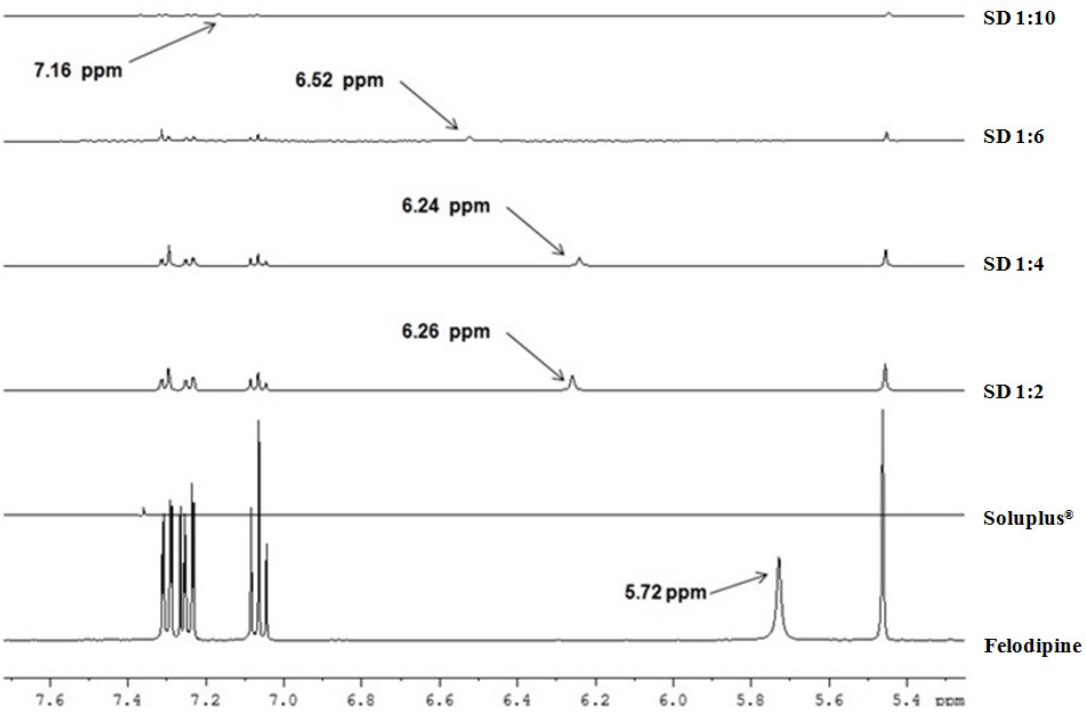
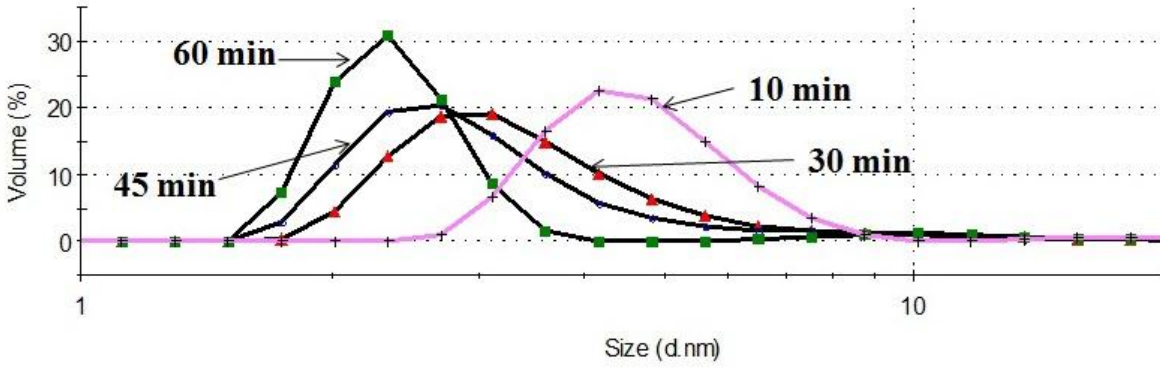


Fig. 5.9. Comparison of ^{1}H NMR spectra of felodipine-soluplus® solid dispersion systems

(a)



(b)

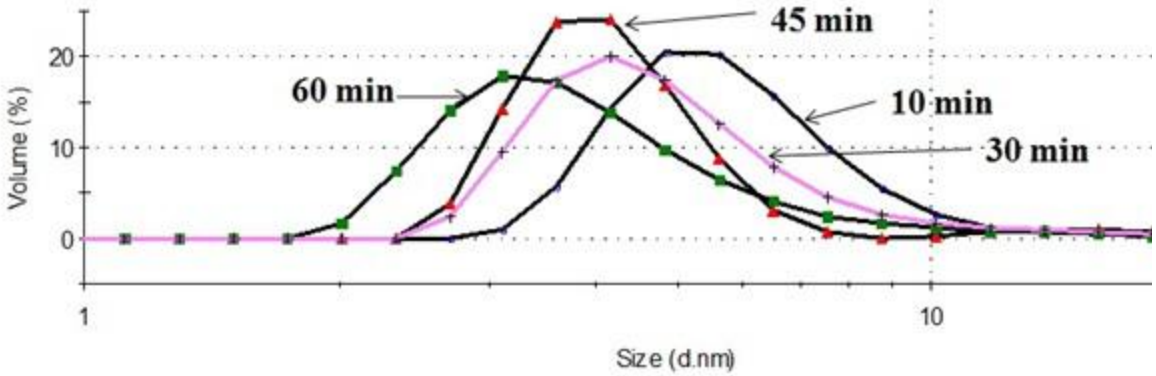


Fig. 5.10. Particle size distribution (by volume) at various time points of dissolution: (a) pure soluplus® (b) SD 1:10

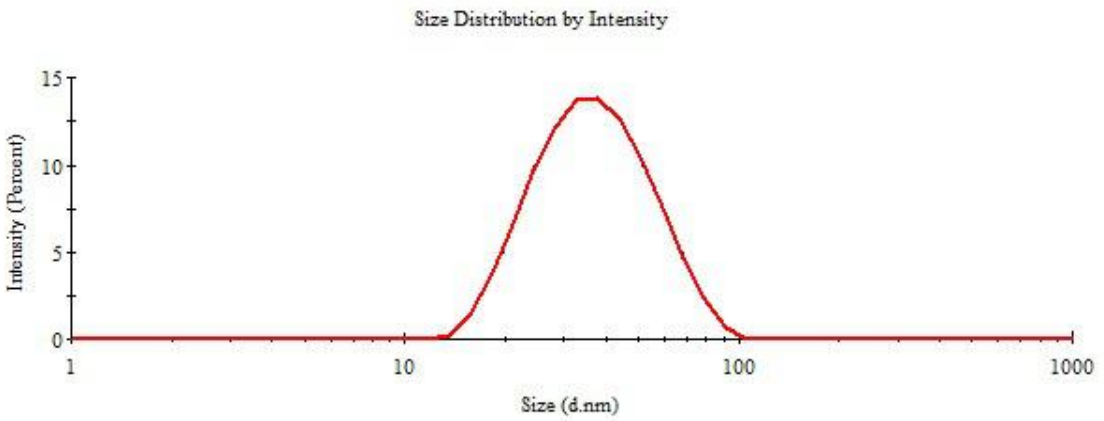


Fig. 5.11. Particle size distribution (by intensity) of NCP 1:10

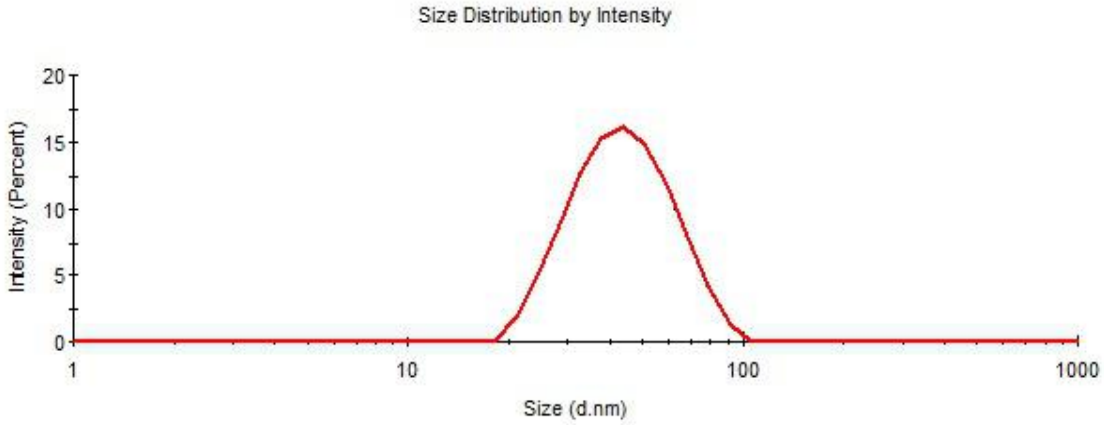


Fig. 5.12. Particle size distribution (by intensity) of NCS 1:6

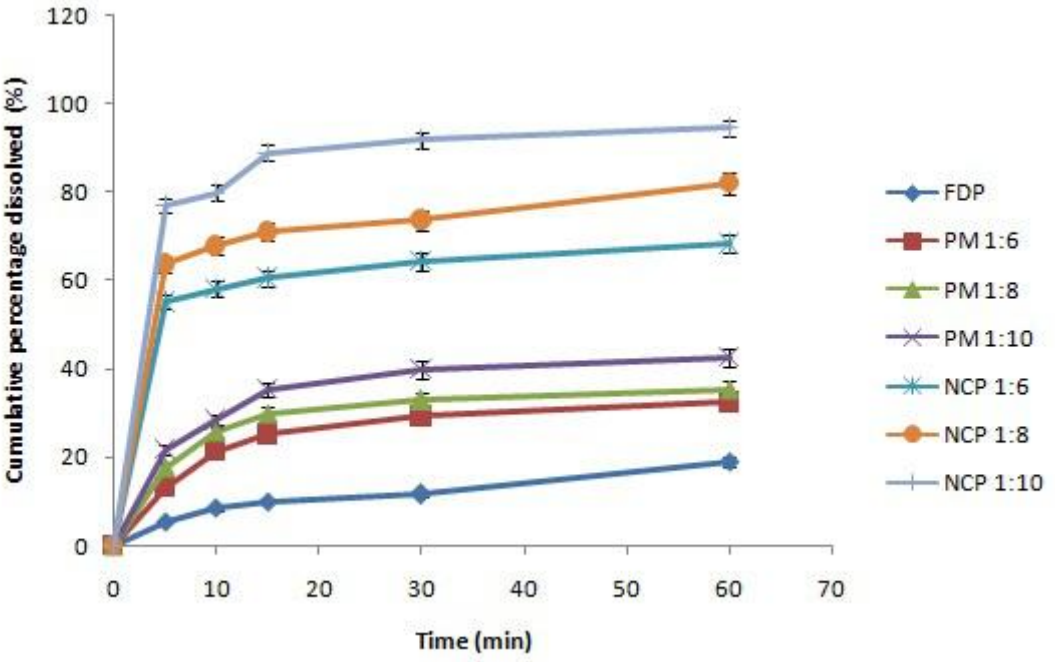


Fig. 5.13. Dissolution profile of pure drug (FDP), physical mixtures and poloxamer 407 nanocrystals

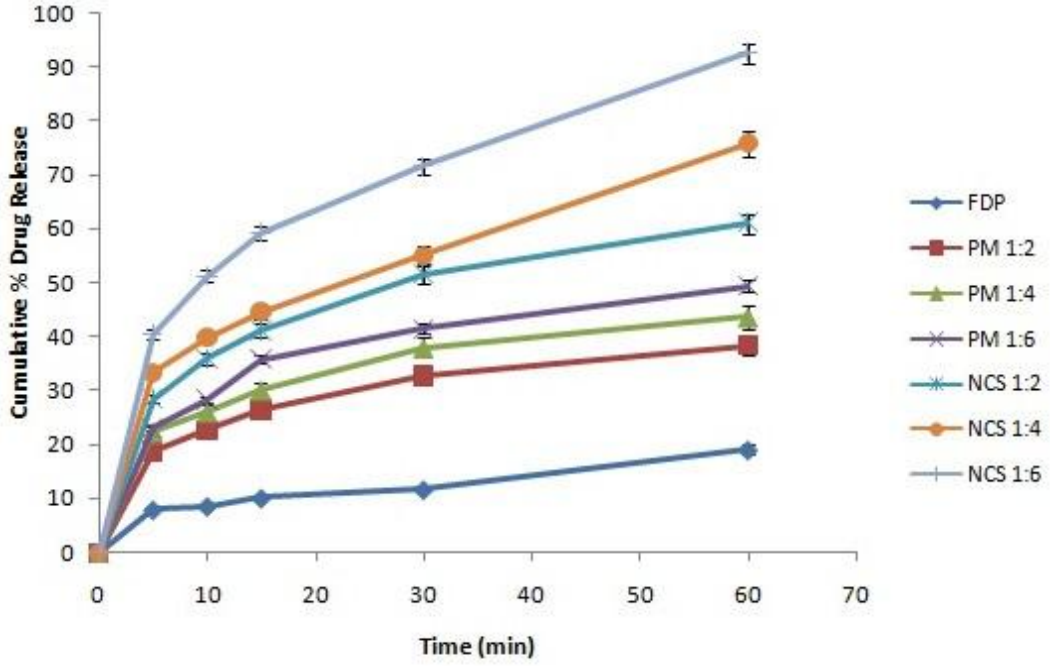


Fig. 5.14. Dissolution profiles of pure drug (FDP), physical mixtures and soluplus® nanocrystals

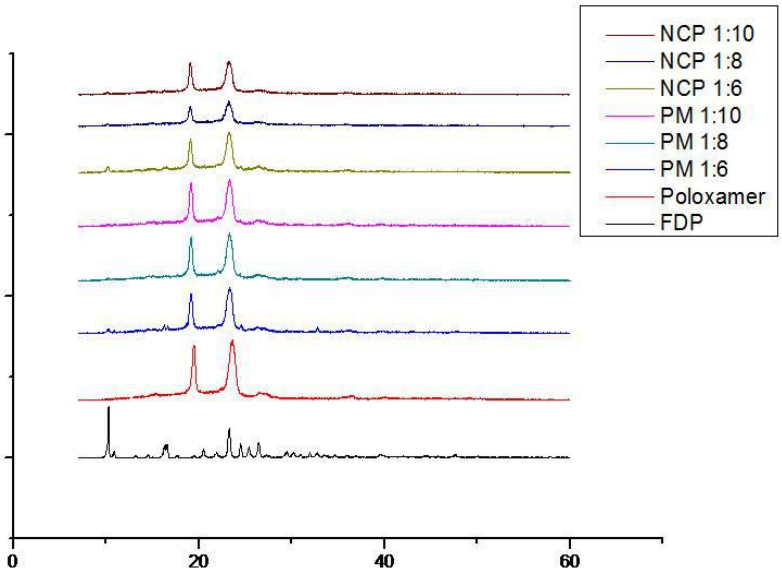


Fig. 5.15. Comparison of PXRD pattern of pure drug, poloxamer 407, physical mixtures and nanocrystals

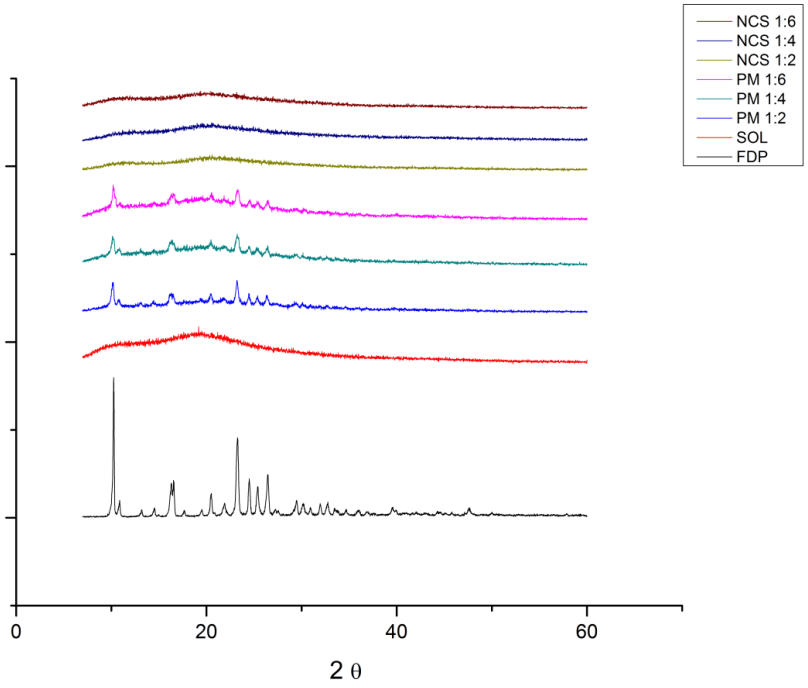


Fig. 5.16. Comparison of PXRD pattern of pure drug, soluplus®, physical mixtures and nanocrystals

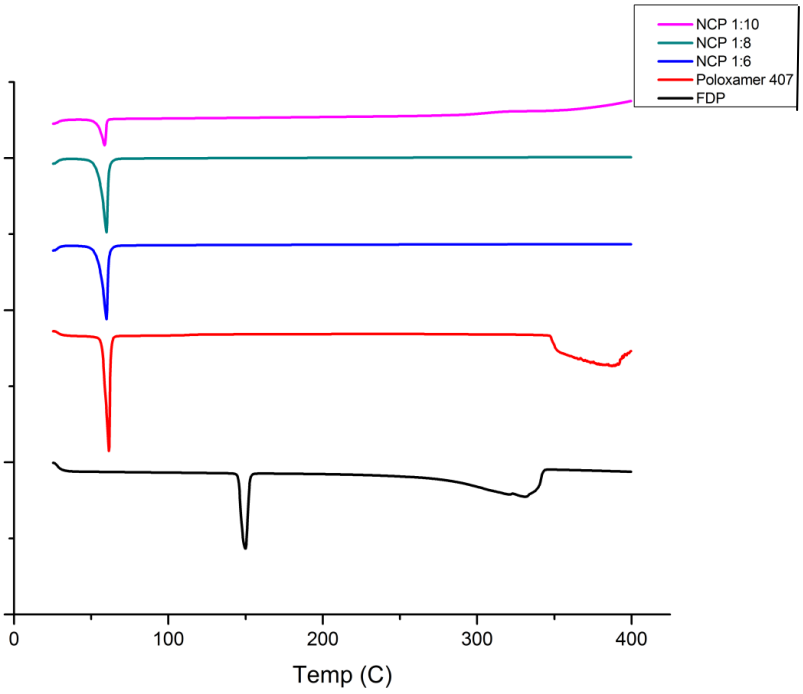


Fig. 5.17. DSC thermograms of FDP nanocrystals prepared using poloxamer 407

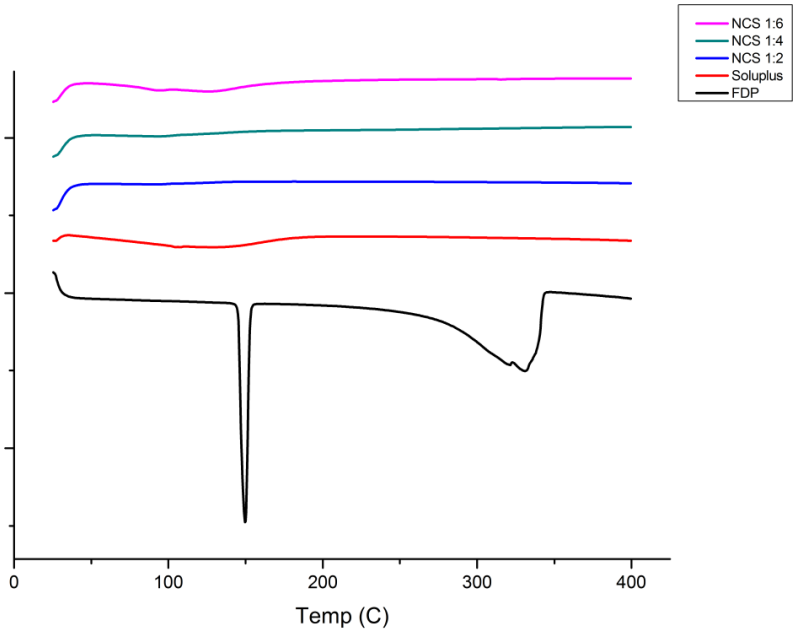


Fig. 5.18. DSC thermograms of FDP nanocrystals prepared using soluplus®

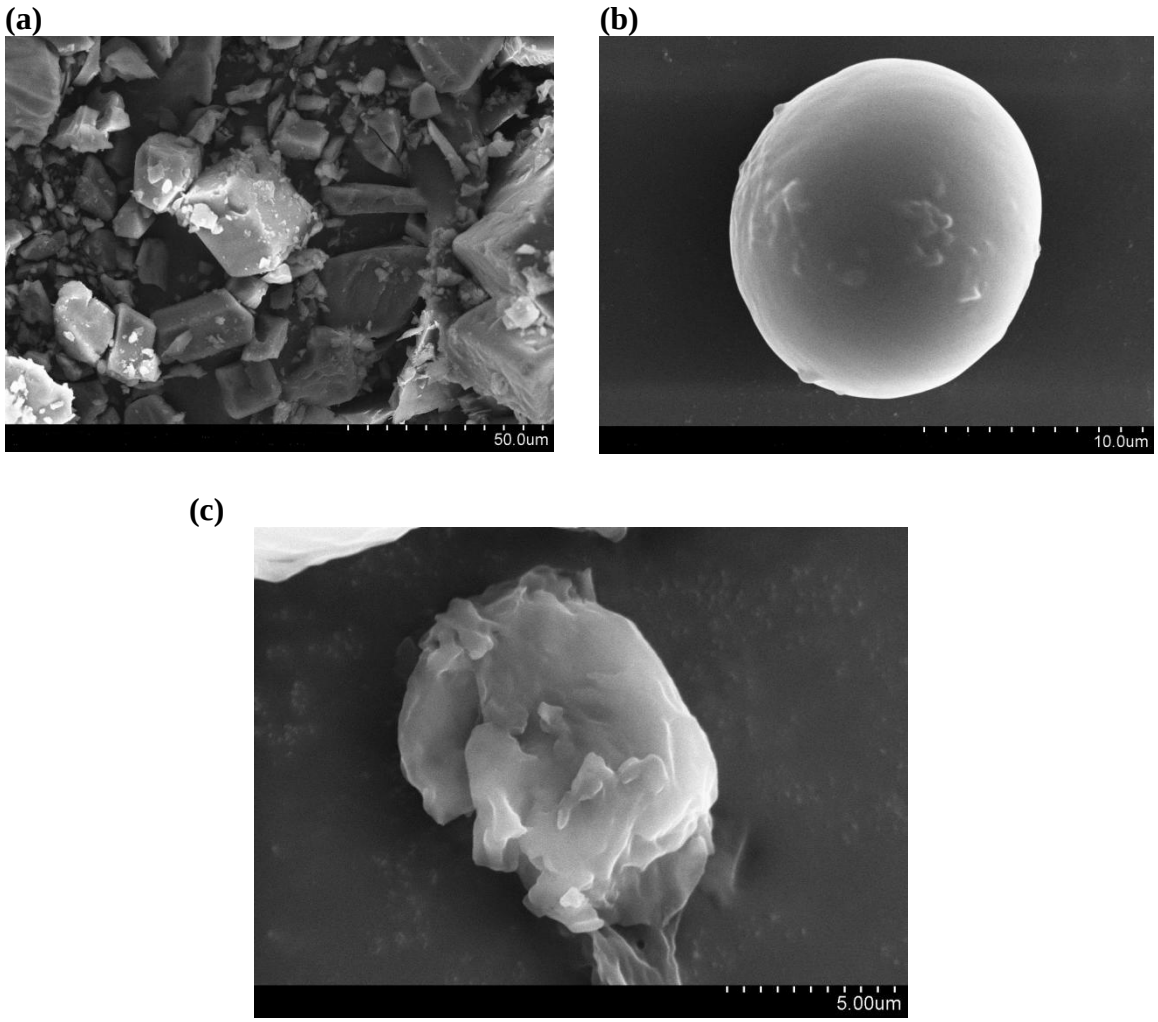


Fig. 5.19. Scanning electron micrographs of (a) pure drug (b) pure poloxamer 407 (c) NCP 1:10

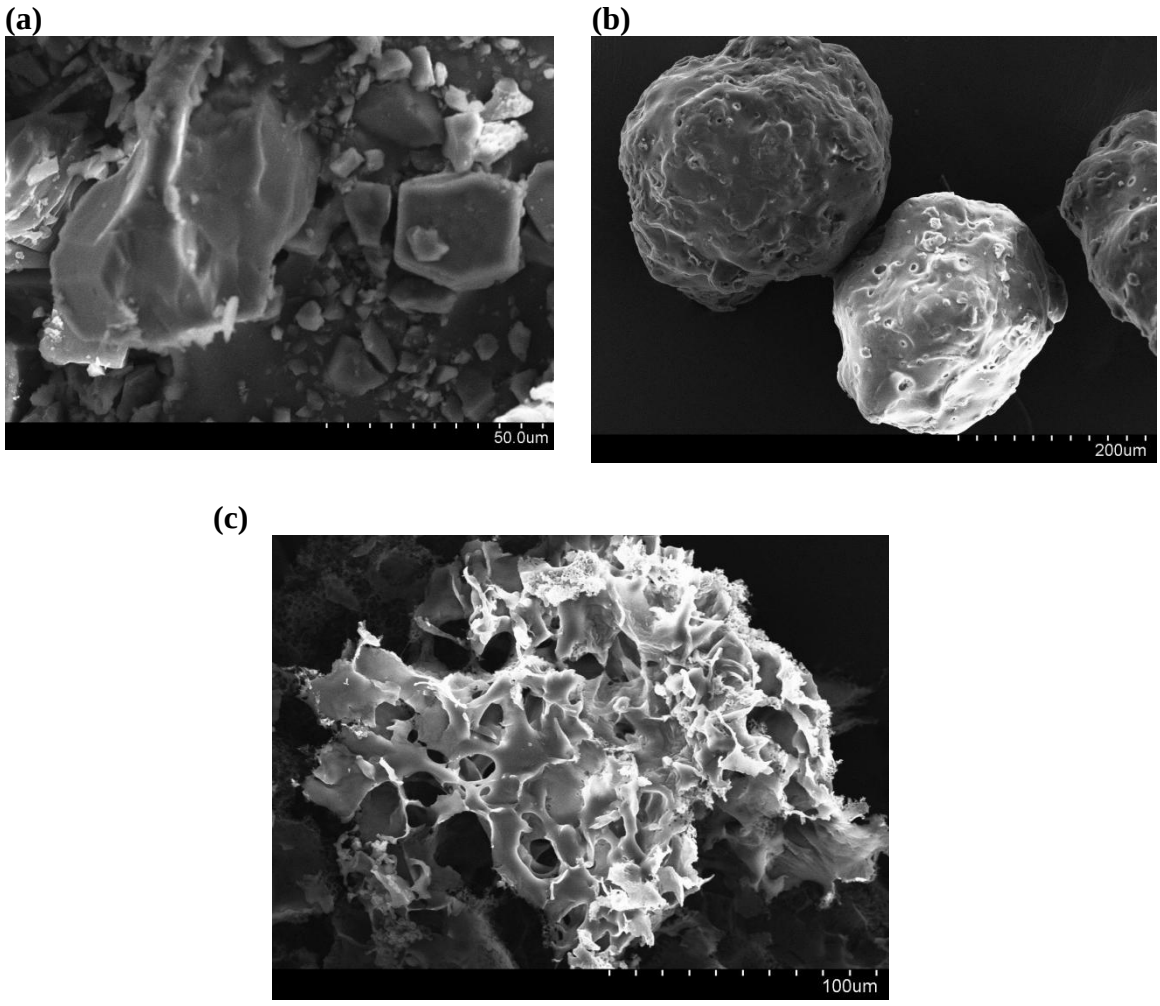


Fig. 5.20. Scanning electron micrographs of (a) pure drug (b) pure soluplus® (c) NCS 1:10

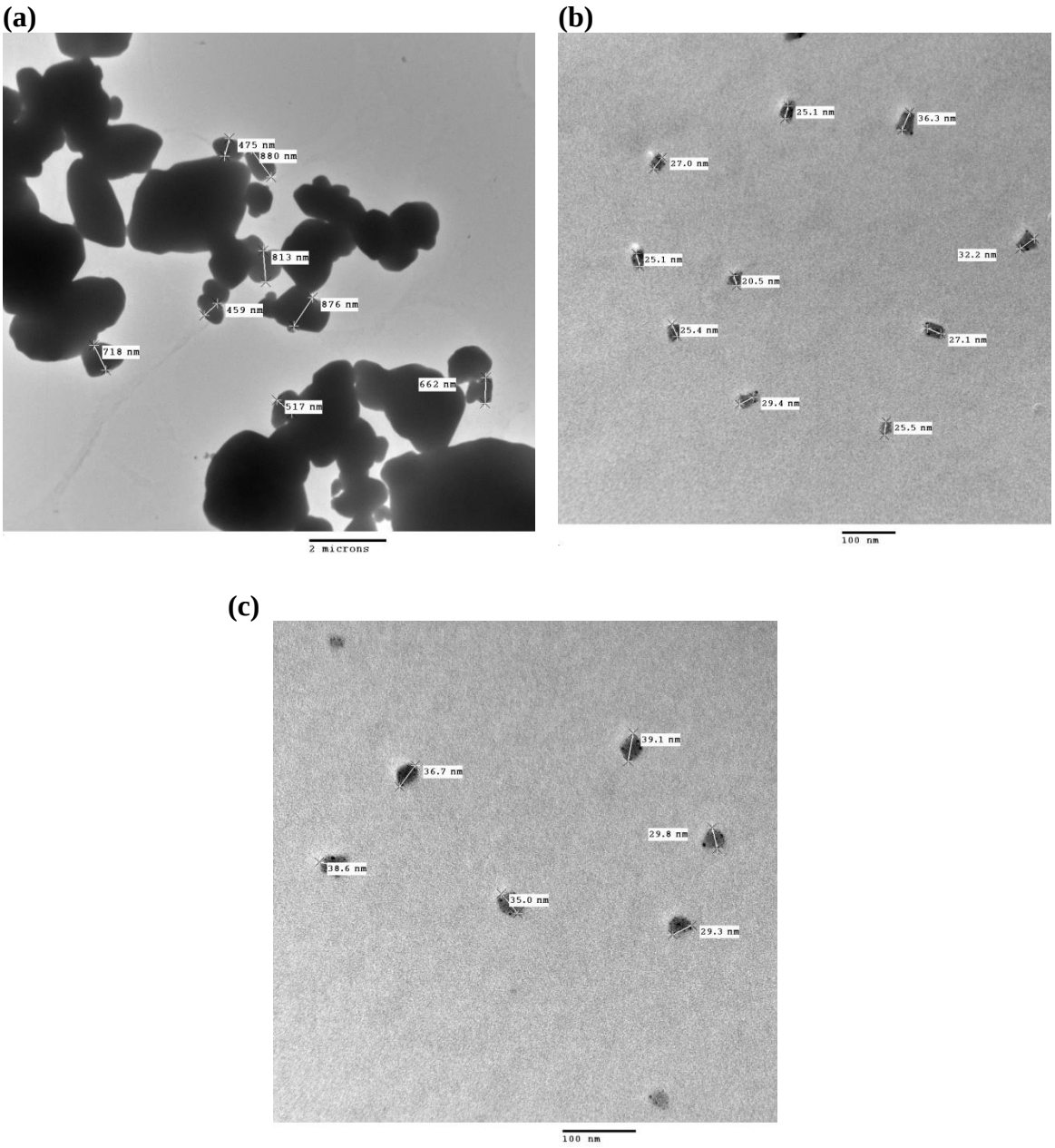


Fig. 5.21. Transmission electron micrographs of (a) pure drug (b) NCP 1:10 and (c) NCS 1:10

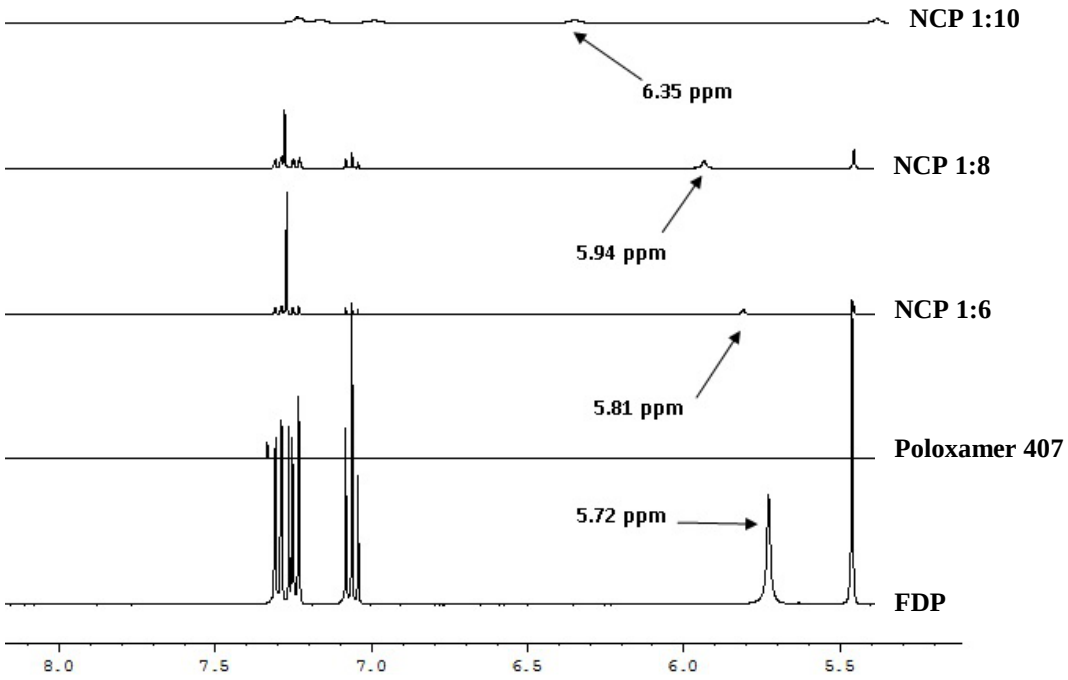


Fig. 5.22. Comparison of ^{1}H NMR spectra of felodipine-poloxamer 407 nanocrystals

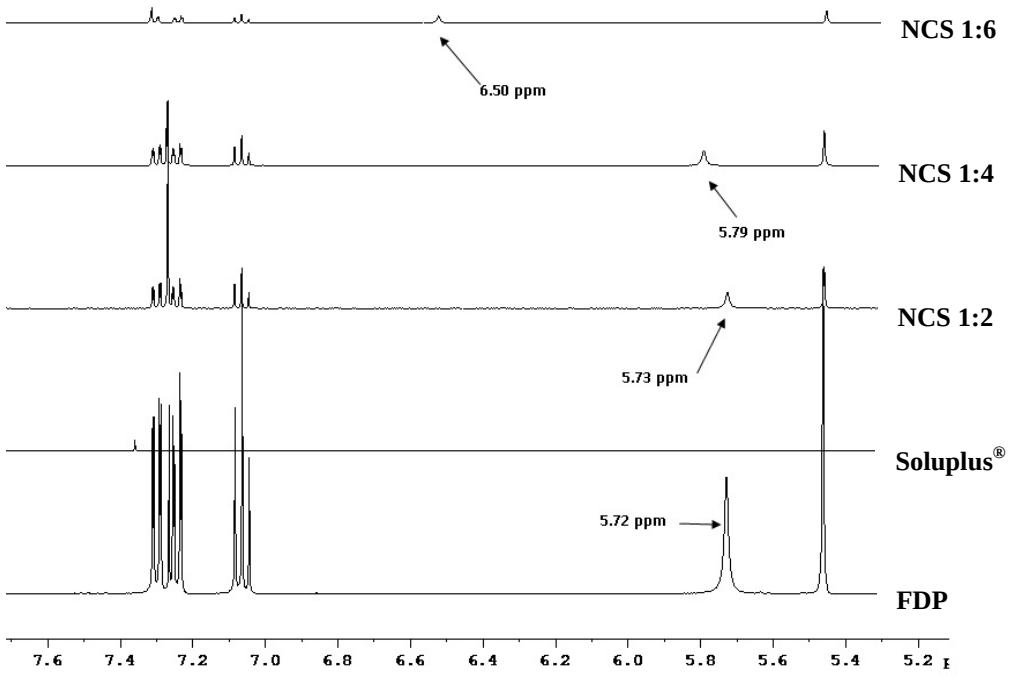


Fig. 5.23. Comparison of ^{1}H NMR spectra of felodipine-soluplus® nanocrystals

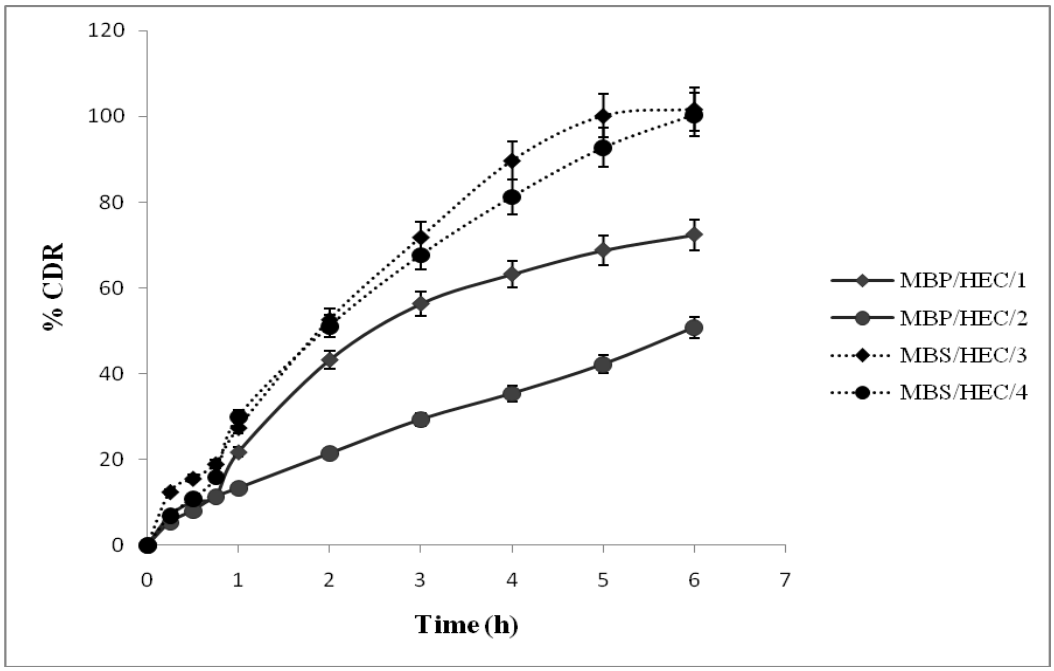


Fig. 5.24. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of HEC

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

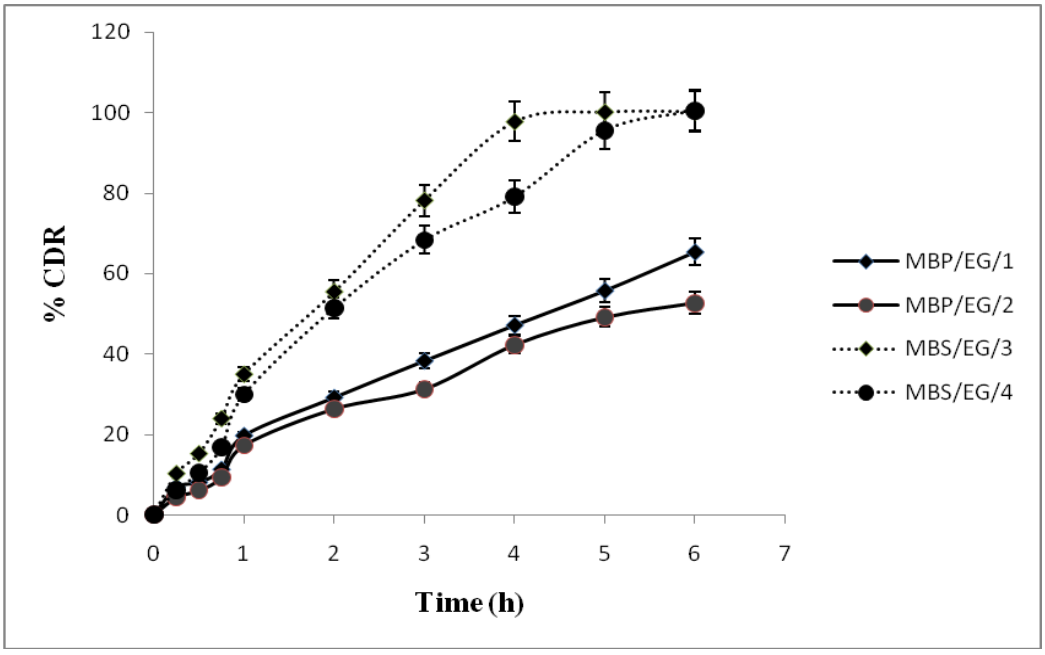


Fig. 5.25. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of EG

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

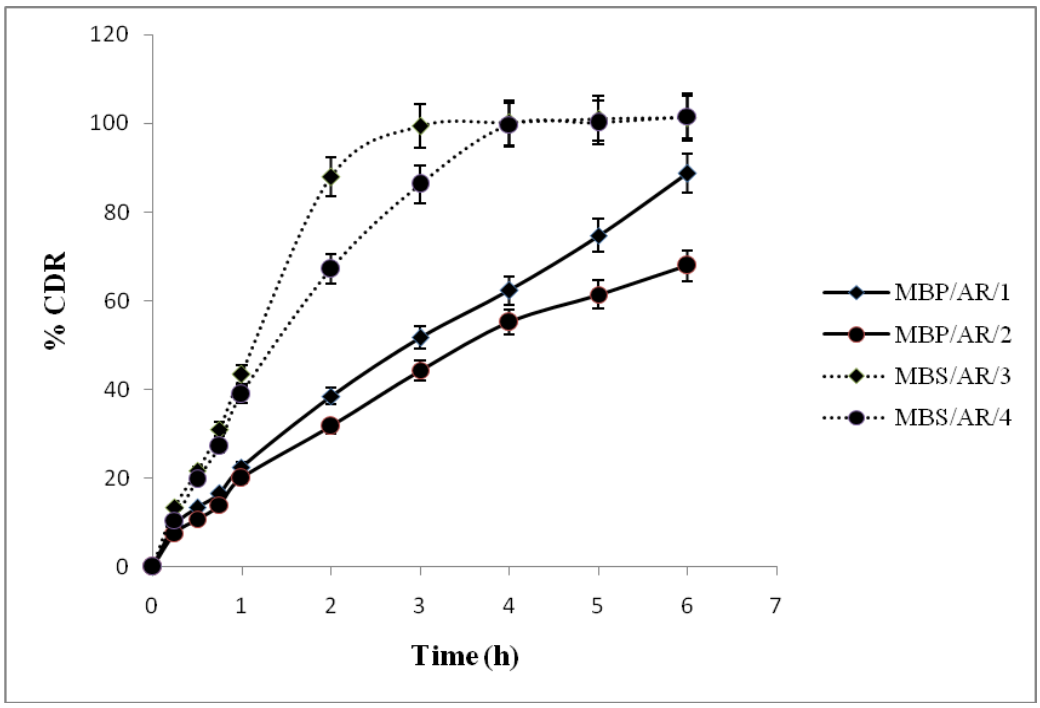


Fig. 5.26. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of AR

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

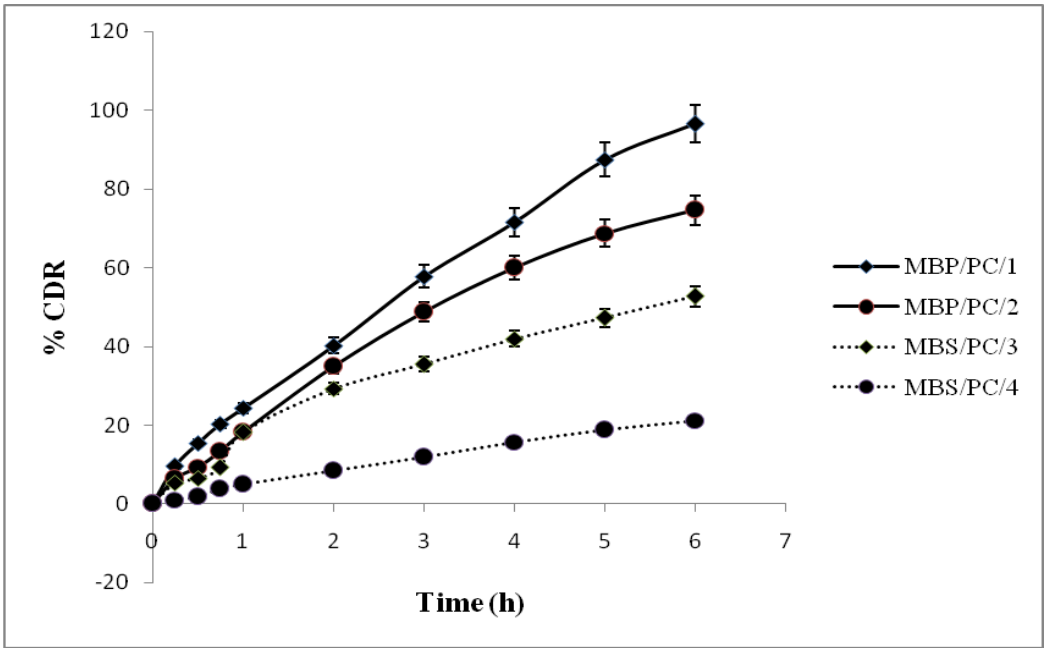


Fig. 5.27. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of PC

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

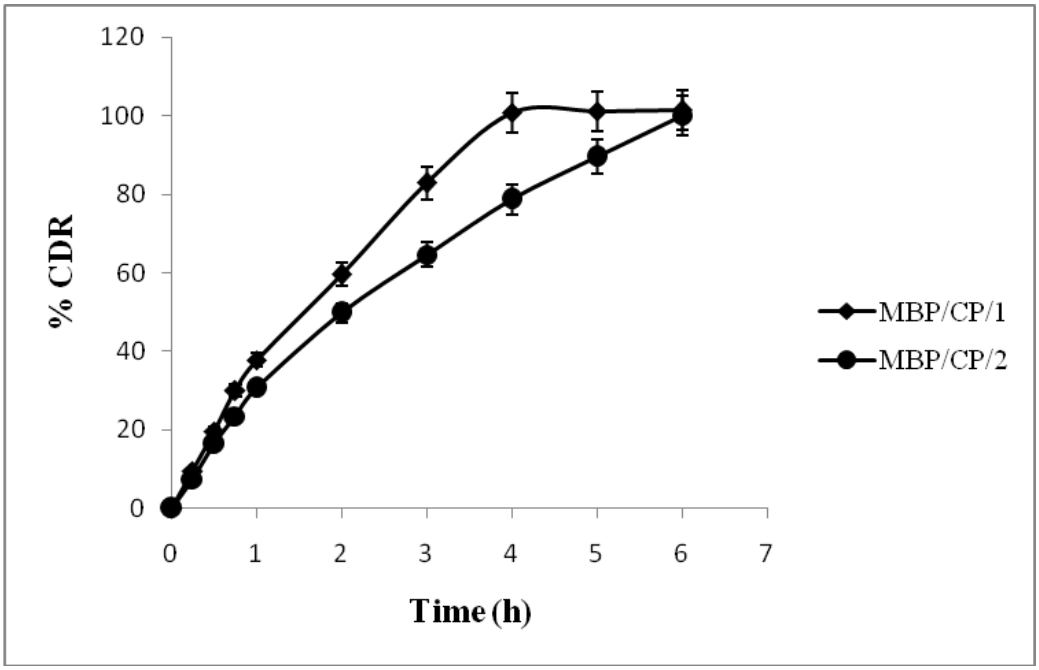


Fig. 5.28. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of CP

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

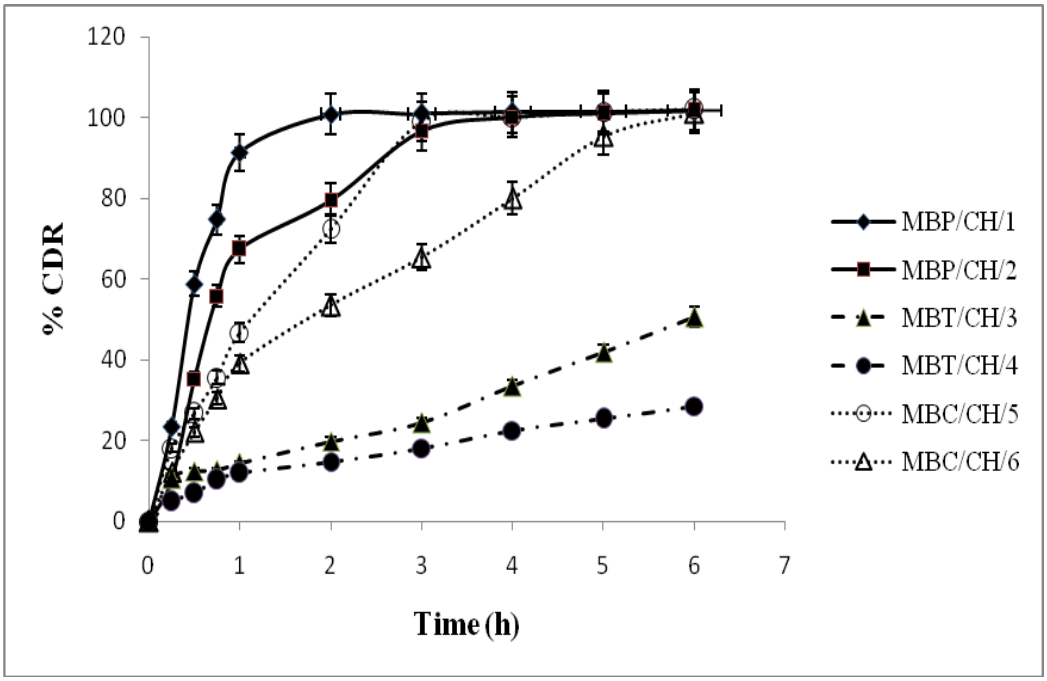


Fig. 5.29. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of CH

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

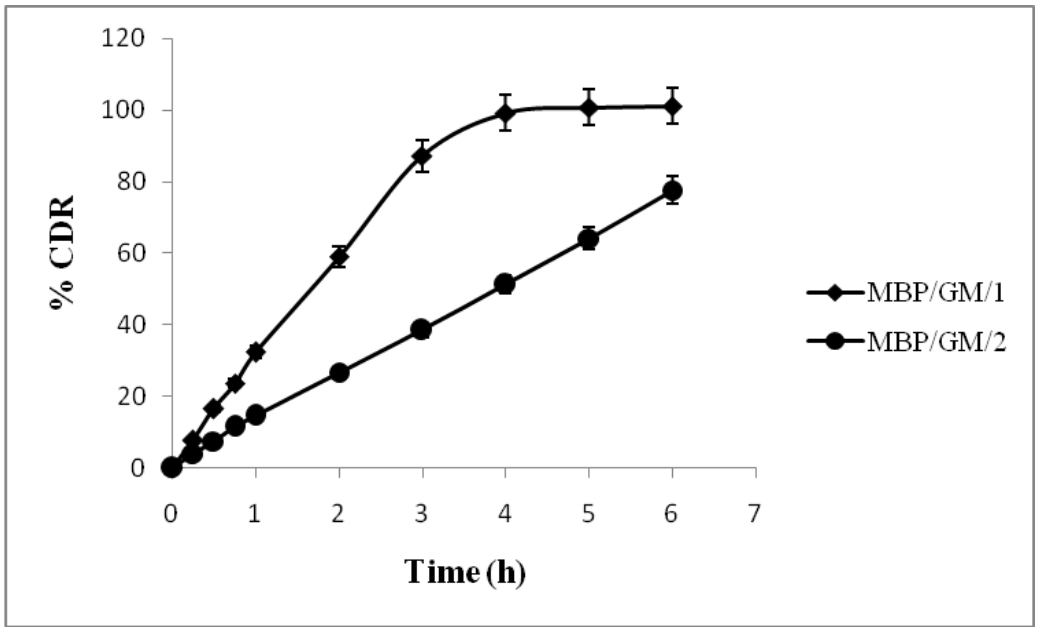


Fig. 5.30. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of GM

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

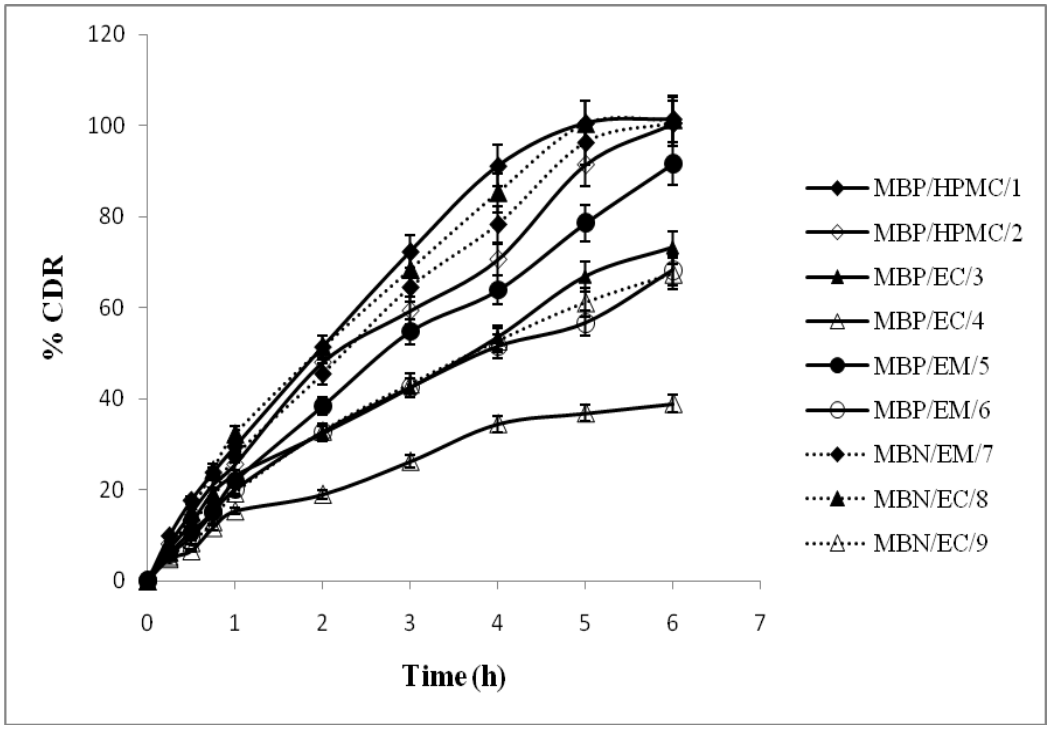


Fig. 5.31. Comparative in vitro release profile of FDP from tablet formulations prepared using varying quantity of EC and HPMC alone and in combination

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

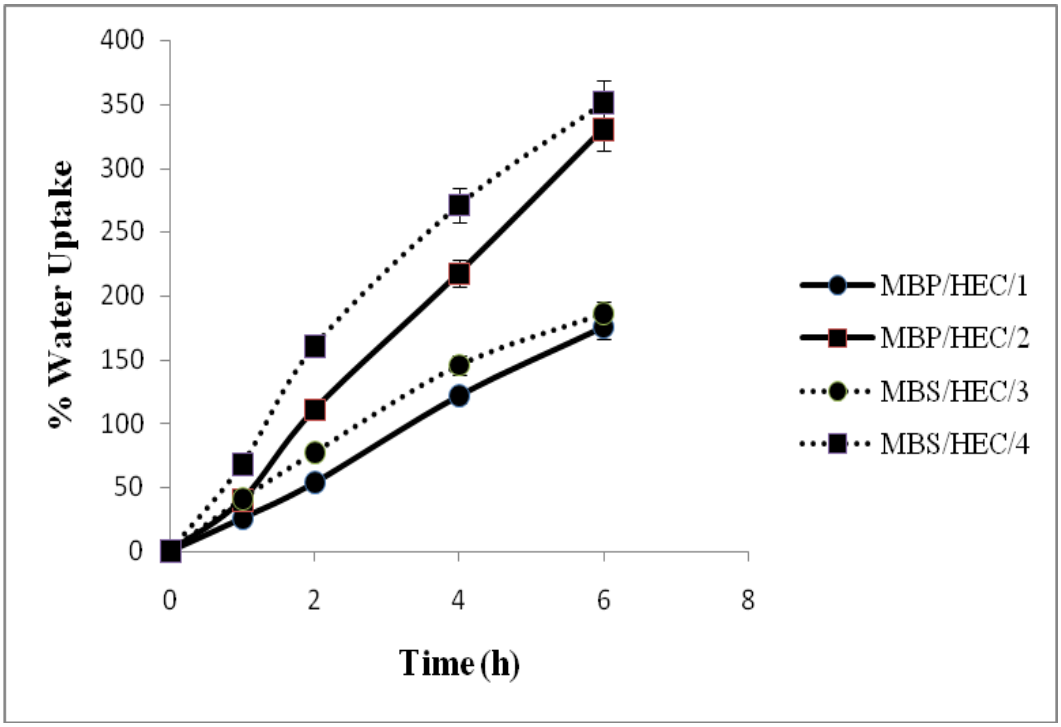


Fig. 5.32. In vitro water uptake studies of FDP buccal tablets prepared with HEC

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

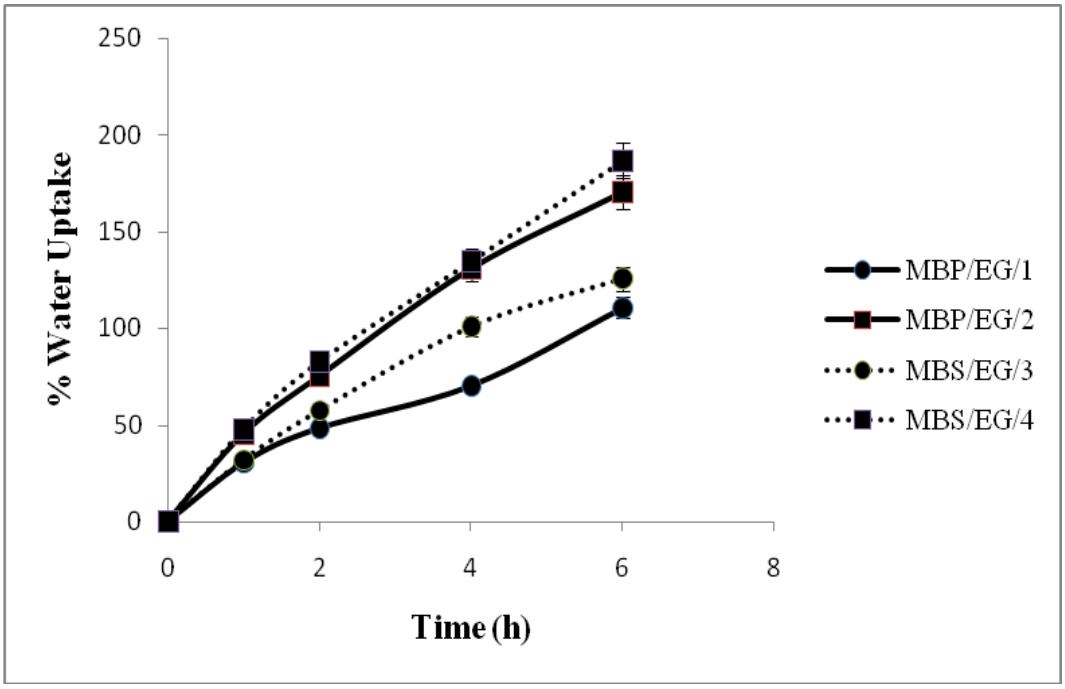


Fig. 5.33. In vitro water uptake studies of FDP buccal tablets prepared with EG

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

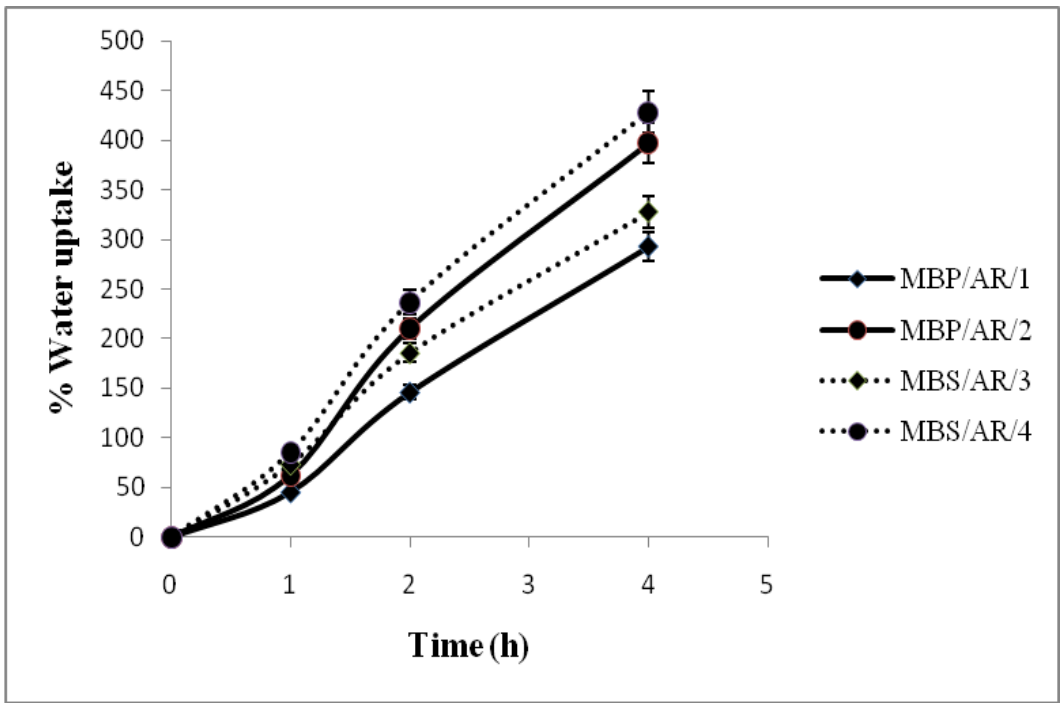


Fig. 5.34. In vitro water uptake studies of FDP buccal tablets prepared with AR

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

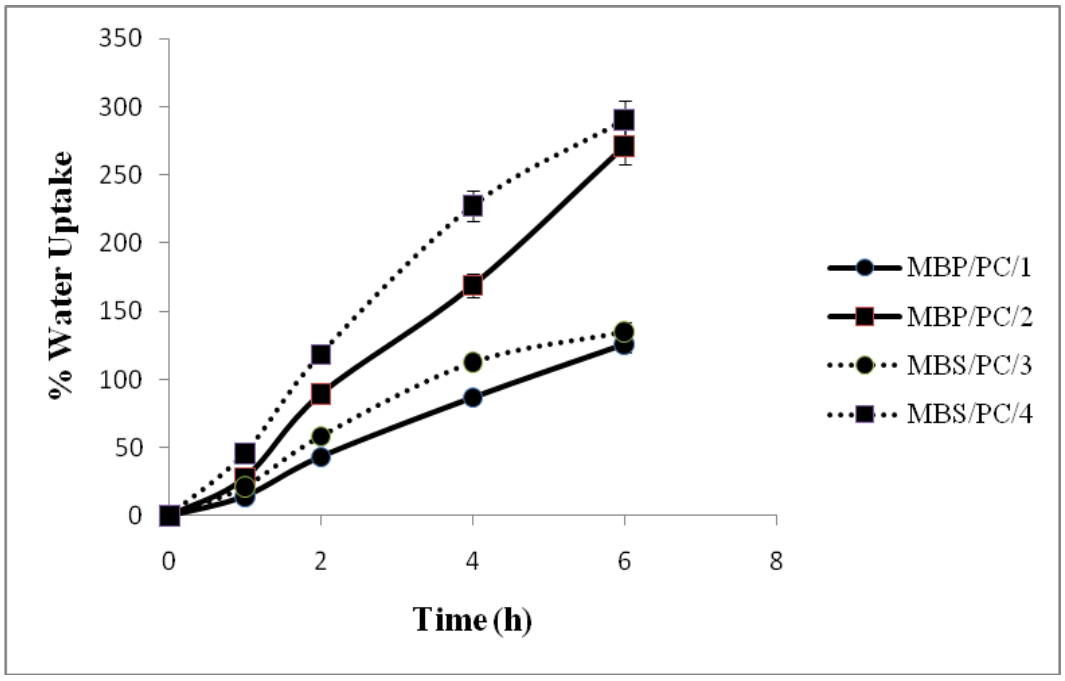


Fig. 5.35. In vitro water uptake studies of FDP buccal tablets prepared with PC

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

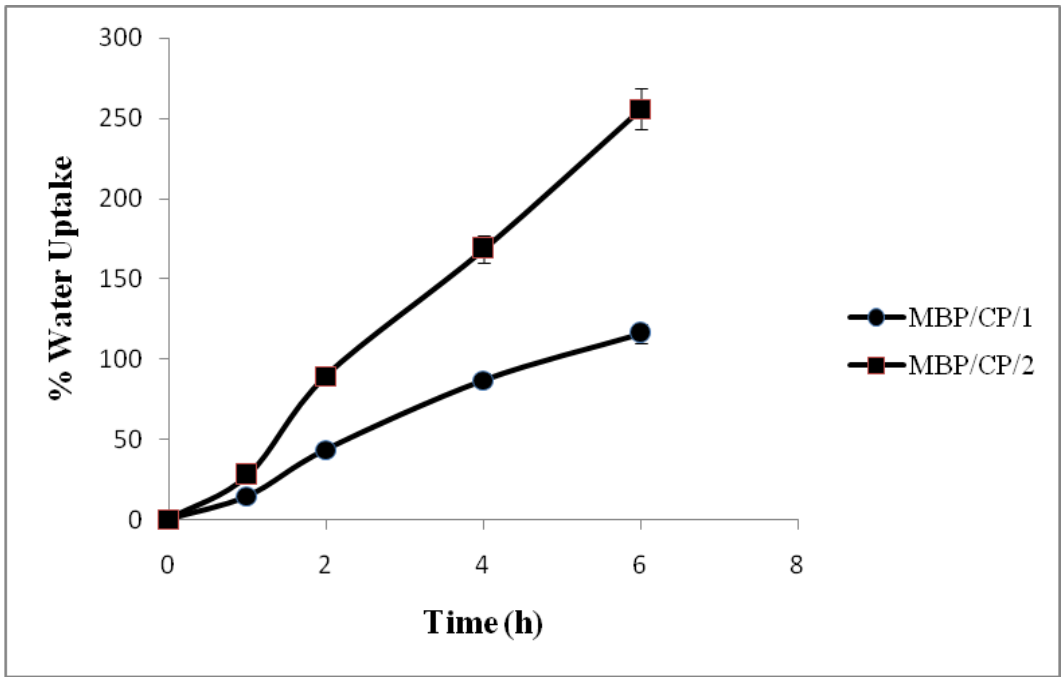


Fig. 5.36. In vitro water uptake studies of FDP buccal tablets prepared with CP

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

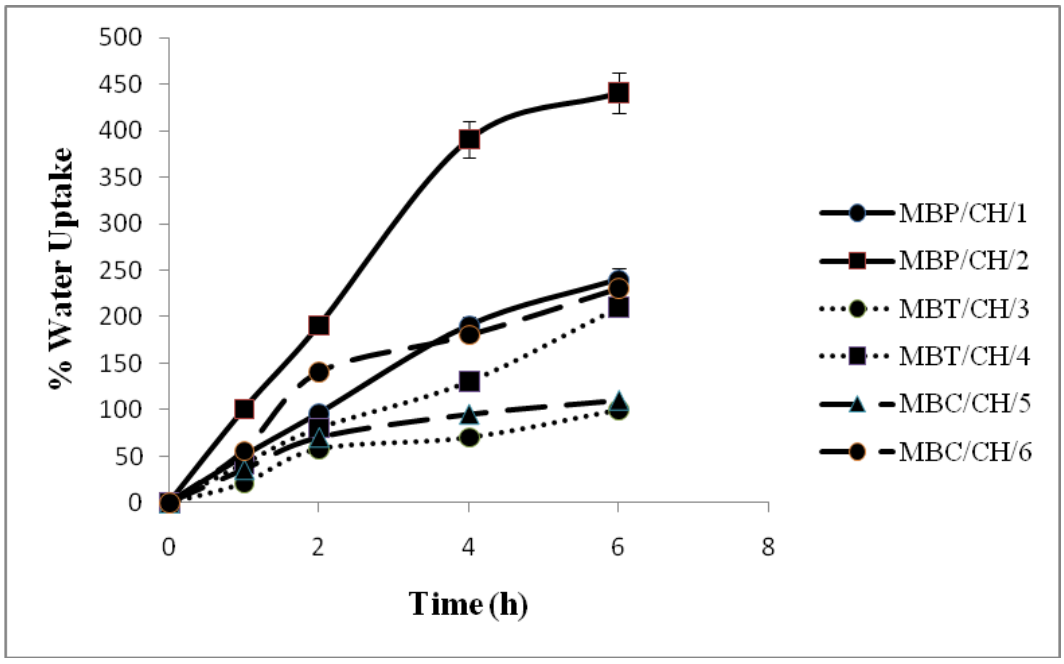


Fig. 5.37. In vitro water uptake studies of FDP buccal tablets prepared with CH

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

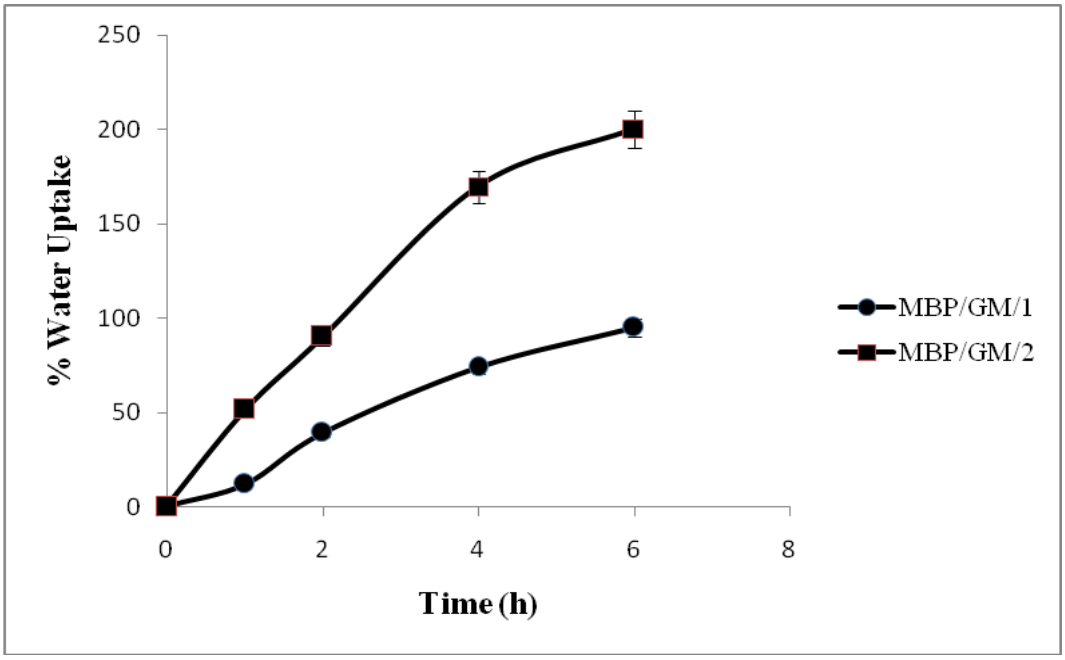


Fig. 5.38. In vitro water uptake studies of FDP buccal tablets prepared with GM

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

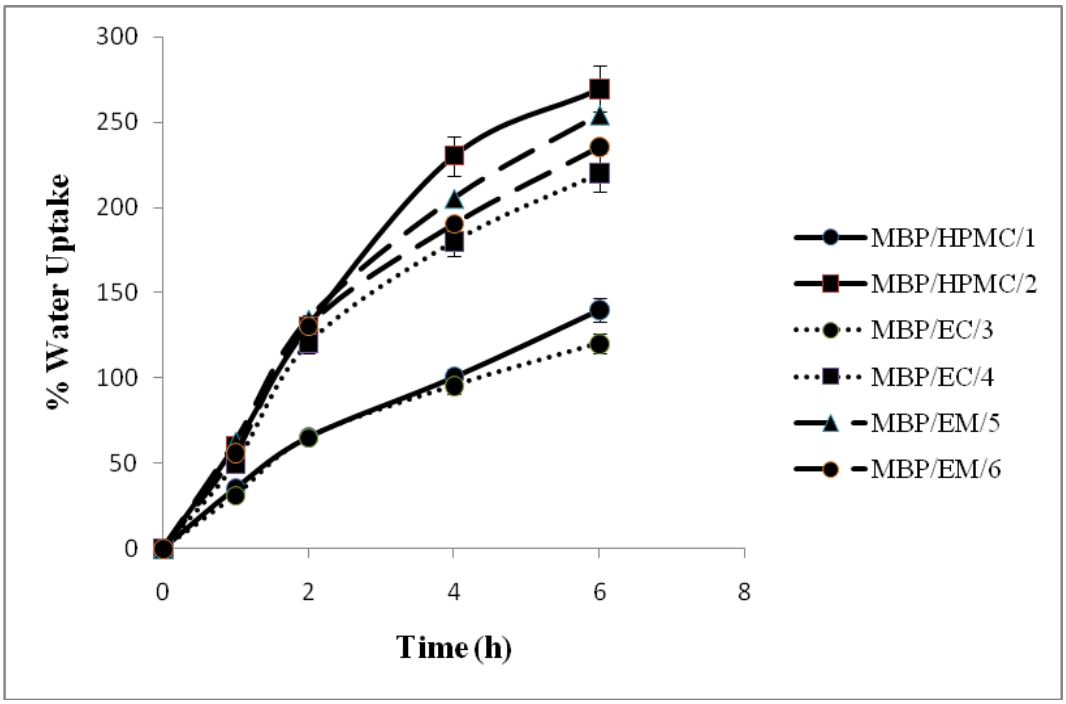


Fig. 5.39. In vitro water uptake studies of FDP buccal tablets prepared with EC and HPMC alone and in combination

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

Table 5.1: Thermodynamic parameters for solubilization of felodipine in aqueous solutions of soluplus®

Soluplus® (% w/v)	ΔH (KJ/mol)	ΔG (KJ/mol)	
		25 °C	45 °C
0	58.51	-	-
1	53.65	-13.79	-14.39
2	46.99	-14.94	-15.17
4	36.10	-16.81	-16.43
6	35.73	-17.89	-17.56

Table 5.2: Model independent dissolution parameters of pure drug and solid dispersions

Formulation	Dissolution parameter									
	Q5	**Q15**	**Q60**	**DE5**	**DE15**	**DE60**	**MDT**	**MDR**	**f_1**	**f_2**
Pure Drug	5.32	10.39	18.36	2.66	6.06	12.48	19.22	0.04	–	–
PM 1:2	13.13	23.98	40.69	6.57	14.09	29.57	16.40	0.10	57.25	41.83
PM 1:4	16.78	24.17	43.98	8.39	16.68	31.76	16.67	0.12	61.16	38.82
PM 1:6	20.07	26.23	59.18	10.04	18.55	39.79	19.66	0.14	68.14	31.22
PM1:10	23.13	48.17	74.53	11.57	27.03	55.65	15.20	0.18	77.38	21.60
SD 1:2	23.85	45.47	74.13	11.93	27.76	52.30	17.67	0.18	76.63	22.77
SD 1:4	24.68	49.26	83.75	12.34	30.57	57.58	18.75	0.20	78.82	19.88
SD 1:6	25.19	54.28	87.74	12.60	33.99	62.60	17.19	0.21	80.57	17.62
SD 1:10	27.13	66.63	101.46	13.57	37.98	77.26	14.31	0.24	83.72	12.70

Table 5.3: Particle size, size distribution and zeta potential of the nanocrystals

Solvent	**Surfactant**	**Drug: Surfactant ratio**	**Mean particle size[a] ± STDEV (z-average, nm)**	**Polydispersity index[a] (PDI) ± STDEV**	**Zeta potential[a] ± STDEV (mV)**
Ethyl acetate	Poloxamer 407	1:6	280.3 ± 35.6	0.876 ± 0.113	-9.76 ± 1.52
		1:8	160.9 ± 20.6	0.456 ± 0.223	-15.5 ± 0.6
		1:10	30.19 ± 5.13	0.305 ± 0.131	- 31.3 ± 0.6
	SLS	1:6	470.3 ± 21.3	0.471 ± 0.213	-9.43 ± 2.61
		1:8	426.9 ± 15.6	0.565 ± 0.067	-23.7 ± 0.5
		1:10	310.3 ± 20.5	0.362 ± 0.023	-28.8 ± 0.7
	Tween 80	1:6	340.5 ± 26.3	0.374 ± 0.237	-4.07 ± 2.15
		1:8	285.6 ± 15.7	0.350 ± 0.176	-2.11 ± 1.69
		1:10	146.9 ± 23.6	0.698 ± 0.218	-4.87 ± 5.36
Acetone	Poloxamer 407	1:6	332.5 ± 33.5	0.853 ± 0.133	-6.89 ± 5.38
		1:8	248.0 ± 30.6	0.764 ± 0.219	-10.6 ± 5.9
		1:10	154.5 ± 21.2	0.653 ± 0.232	-13.6 ± 2.7
	SLS	1:6	685.4 ± 40.5	0.769 ± 0.196	-3.58 ± 2.54
		1:8	540.5 ± 35.3	0.715 ± 0.212	-6.98 ± 3.50
		1:10	458.8 ± 21.9	0.672 ± 0.198	-15.4 ± 4.6
	Tween 80	1:6	427.4 ± 34.3	0.813 ± 0.172	-13.6 ± 5.3
		1:8	357.1 ± 31.5	0.715 ± 0.267	-21.6 ± 7.6
		1:10	269.0 ± 19.7	0.614 ± 0.153	-27.5 ± 8.7

[a] Each value is mean of three different determinations

Table 5.4: Particle size, size distribution and zeta potential of nanocrystals prepared with soluplus®

Solvent	Surfactant	Drug: Surfactant	Mean particle size[a] ± STDEV (z-average, nm)	Polydispersity index[a] (PDI) ± STDEV	Zeta potential[a] ± STDEV (mV)
Ethanol	Soluplus®	1:2	143.6 ± 20.5	0.557 ± 0.213	-11.8 ± 3.6
		1:4	90.9 ± 13.7	0.418 ± 0.149	-19.5 ± 5.9
		1:6	36.80 ± 7.26	0.212 ± 0.183	- 35.7 ± 4.6

[a] Each value is mean of three different determinations

Table 5.5: Model independent dissolution parameters of FDP nanocrystals prepared using poloxamer 407

Formulation	Dissolution parameters							
	Q15	Q30	QE60	DE15	DE30	DE60	f1	f2
FDP	10.07	11.62	18.36	7.16	8.58	11.94	–	–
PM 1:6	25.21	29.25	32.48	15.67	21.45	26.16	54.98	45.01
PM 1:8	29.97	32.9	35.41	19.56	25.50	29.83	61.53	39.49
PM 1:10	35.35	39.94	42.59	22.58	30.11	35.69	67.48	33.82
NCP 1:6	60.61	64.33	68.32	47.88	55.18	60.75	82.18	16.85
NCP 1:8	71.05	73.8	82.05	55.71	64.07	70.99	84.76	12.78
NCP 1:10	89.07	91.91	94.56	67.23	78.86	86.05	87.38	8.02

Table 5.6: Model independent dissolution parameters of FDP nanocrystals prepared using soluplus®

Formulation	Dissolution parameters							
	Q15	**Q30**	**QE 60**	**DE15**	**DE30**	**DE60**	**f1**	**f2**
FDP	10.07	11.62	18.99	6.33	8.58	11.94	–	–
PM 1:2	26.49	32.78	38.34	18.25	23.95	29.75	60.7386	40.2106
PM 1:4	30.06	37.91	43.62	21.13	27.56	34.16	65.8495	35.4306
PM 1:6	35.86	41.47	49.37	23.14	30.90	38.16	69.3473	31.8506
NCS 1:2	41.04	51.42	60.95	28.27	37.25	46.72	74.9109	25.7735
NCS 1:4	44.65	54.97	75.72	31.83	40.82	53.08	78.0199	21.7973
NCS 1:6	59.24	71.48	92.63	40.43	52.88	67.46	82.6542	15.4435

Table 5.7a: Composition of designed buccal mucoadhesive modified release tablets made using HEC/EG/AR

Formulation code	Formulation composition (mg/tablet)							Theoretical tablet weight (mg)
	FDP	SD	Lactose	HEC	EG	AR	Magnesium stearate	
MBP/HEC/1	5	-	58	10	-	-	2	75
MBP/HEC/2	5		58	20	-	-	2	85
MBS/HEC/3	-	55	23	10	-	-	2	90
MBS/HEC/4		55	23	20	-	-	2	100
MBP/EG/1	5	-	58	-	10	-	2	75
MBP/EG/2	5	-	58	-	15	-	2	80
MBS/EG/3	-	55	23	-	10	-	2	90
MBS/EG/4	-	55	23	-	15	-	2	95
MBP/AR/1	5	-	58	-	-	15	2	80
MBP/AR/2	5	-	58	-	-	20	2	85
MBS/AR/3	-	55	23	-	-	15	2	95
MBS/AR/4	-	55	23	-	-	20	2	100

Table 5.7.b: Composition of designed buccal mucoadhesive modified release tablets made using PC/CP/CH/GM

Formulation code	Formulation composition (mg/tablet)								Theoretical tablet weight (mg)
	FDP	SD	Lactose	PC	CP	CH	TCP	Magnesium stearate	
MBP/PC/1	5	-	58	5	-	-	-	2	70
MBP/PC/2	5		58	10	-	-	-	2	75
MBS/PC/3	-	55	23	5	-	-	-	2	85
MBS/PC/4		55	23	10	-	-	-	2	90
MBP/CP/1	5	-	58	-	5	-	-	2	70
MBP/CP/2	5	-	58	-	10	-	-	2	75
MBP/CH/1	5	-	58	-	-	10	-	2	75
MBP/CH/2	5	-	58	-	-	20	-	2	85
MBT/CH/3	5	-	-	-	-	5	58	2	70
MBT/CH/4	5	-	-	-	-	10	58	2	75
MBC/CH/5	5		29			5	29	2	70
MBC/CH/6	5		29			10	29	2	75
MBP/GM/1	5	-	58	-	-	5	-	2	70
MBP/GM/2	5	-	58	-	-	10	-	2	75

Table 5.7c: Composition of designed buccal mucoadhesive modified release tablets made using HPMC/EC/EM

Formulation code	Formulation composition (mg/tablet)								Theoretical tablet weight (mg)
	FDP	NC	Lactose	EC	HPMC	GM	TCP	Magnesium stearate	
MBP/HPMC/1	5	-	58	-	10	-	-	2	75
MBP/HPMC/2	5		58	-	20	-	-	2	85
MBP/EC/3	5		58	10	-	-	-	2	75
MBP/EC/4	5		58	20	-	-	-	2	85
MBP/EM/5	5	-	58	10	10	-	-	2	85
MBP/EM/6	5	-	58	15	5	-	-	2	85
MBN/EM/7	-	55	23	15	5	-	-	2	100
MBN/EC/8	-	55	23	10	-	-	-	2	90
MBN/EC/9		55	23	20	-	-	-	2	100

Table 5.8a: Results of quality control tests carried out on designed buccal mucoadhesive tablets made using HEC/EG/AR

Formulation code	Mean weight[a] (mg) ± STDEV	Friability[a] (% w/w)	Mean thickness[b] (mm) ± STDEV	Mean assay[b] ± STDEV (%)	Hardness[b] (Kg) ± STDEV	Mean bioadhesion[b] (N) ± STDEV
MBP/HEC/1	76.13 ± 0.76	0.79	2.41 ± 0.03	101.83 ± 2.26	4.26 ± 1.39	0.68 ± 0.47
MBP/HEC/2	84.42 ± 0.18	0.87	2.70 ± 0.01	99.28 ± 1.03	4.78 ± 1.63	1.11 ± 0.26
MBS/HEC/3	91.39 ± 1.20	0.65	2.91 ± 0.05	100.57 ± 1.59	5.21 ± 1.28	0.70 ± 0.27
MBS/HEC/4	99.05 ± 1.33	0.72	3.15 ± 0.06	102.38 ± 1.62	5.05 ± 1.49	1.44 ± 0.19
MBP/EG/1	74.37 ± 0.73	0.53	2.36 ± 0.02	99.19 ± 0.98	5.19 ± 1.48	0.46 ± 0.26
MBP/EG/2	81.58 ± 0.27	0.42	2.48 ± 0.07	101.27 ± 1.73	5.59 ± 1.85	0.63 ± 0.35
MBS/EG/3	89.32 ± 1.10	0.62	2.61 ± 0.03	100.28 ± 0.99	6.13 ± 1.74	0.57 ± 0.63
MBS/EG/4	96.29 ± 1.17	0.48	2.83 ± 0.05	99.29 ± 1.49	6.61 ± 1.26	0.81 ± 0.22
MBP/AR/1	81.37 ± 0.69	0.75	2.56 ± 0.03	99.28 ± 1.83	4.68 ± 1.47	0.31 ± 0.20
MBP/AR/2	84.51 ± 1.01	0.87	2.74 ± 0.08	98.53 ± 1.68	4.06 ± 1.58	0.42 ± 0.32
MBS/AR/3	96.39 ± 1.15	0.69	2.94 ± 0.02	100.82 ± 1.32	5.16 ± 1.92	0.40 ± 0.26
MBS/AR/4	101.07 ± 1.25	0.71	3.17 ± 0.07	101.83 ± 1.20	4.99 ± 1.58	0.52 ± 0.37

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

Table 5.8b: Results of quality control tests carried out on designed buccal mucoadhesive tablets made using PC/CP/CH/GM

Formulation code	Mean weight[a] (mg) ± STDEV	Friability[a] (% w/w)	Mean thickness[b] (mm) ± STDEV	Mean assay[b] ± STDEV (%)	Hardness[b] (Kg) ± STDEV	Mean bioadhesion[b] (N) ± STDEV
MBP/PC/1	71.59 ± 0.52	0.45	2.29 ± 0.04	101.31 ± 1.49	5.93 ± 1.84	0.75 ± 0.21
MBP/PC/2	74.52 ± 0.84	0.37	2.41 ± 0.06	100.79 ± 1.23	6.28 ± 1.58	1.54 ± 0.30
MBS/PC/3	86.19 ± 1.10	0.40	2.68 ± 0.08	99.22 ± 1.69	6.18 ± 1.46	0.82 ± 0.18
MBS/PC/4	91.93 ± 1.27	0.26	2.74 ± 0.03	101.19 ± 1.38	6.45 ± 1.53	1.73 ± 0.20
MBP/CP/1	71.59 ± 0.59	0.37	2.48 ± 0.02	100.21 ± 1.05	5.82 ± 1.58	0.57 ± 0.29
MBP/CP/2	77.05 ± 0.58	0.31	2.74 ± 0.06	99.88 ± 1.27	6.31 ± 1.42	0.96 ± 0.26
MBP/CH/1	74.39 ± 0.69	0.73	2.78 ± 0.06	101.64 ± 1.59	4.50 ± 1.92	0.74 ± 0.20
MBP/CH/2	86.13 ± 1.13	0.84	2.91 ± 0.03	98.15 ± 2.27	4.18 ± 1.74	1.35 ± 0.27
MBT/CH/3	69.38 ± 0.28	0.53	2.57 ± 0.08	100.28 ± 1.84	5.14 ± 0.96	0.42 ± 0.16
MBT/CH/4	76.27 ± 0.62	0.64	2.69 ± 0.03	99.94 ± 1.72	6.38 ± 1.38	0.67 ± 0.18
MBC/CH/5	70.19 ± 0.59	0.48	2.53 ± 0.07	101.91 ± 1.43	4.79 ± 1.67	0.52 ± 0.24
MBC/CH/6	76.38 ± 0.38	0.57	2.69 ± 0.05	100.38 ± 1.32	5.82 ± 1.58	0.84 ± 0.16
MBP/GM/1	71.48 ± 0.25	0.42	2.42 ± 0.05	101.73 ± 1.95	5.92 ± 1.73	0.43 ± 0.19
MBP/GM/2	75.98 ± 0.82	0.35	2.69 ± 0.07	99.85 ± 1.68	6.47 ± 1.59	0.73 ± 0.32

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

Table 5.8c: Results of quality control tests carried out on designed buccal mucoadhesive tablets made using HPMC/EC/EM

Formulation code	**Mean weight[a] (mg) ± STDEV**	**Friability[a] (% w/w)**	**Mean thickness[b] (mm) ± STDEV**	**Mean assay[b] ± STDEV (%)**	**Hardness[b] (Kg) ± STDEV**	**Mean bioadhesion[b] (N) ± STDEV**
MBP/HPMC/1	76.41 ± 0.84	0.67	2.49 ± 0.05	101.59 ± 1.75	4.68 ± 1.48	0.57 ± 0.27
MBP/HPMC/2	84.20 ± 0.99	0.71	2.81 ± 0.04	99.36 ± 1.58	4.79 ± 1.74	0.98 ± 0.19
MBP/EC/3	75.95 ± 0.83	0.48	2.38 ± 0.03	98.29 ± 1.79	5.39 ± 1.63	0.42 ± 0.14
MBP/EC/4	86.31 ± 0.58	0.34	2.52 ± 0.08	100.32 ± 0.96	6.13 ± 1.49	0.85 ± 0.15
MBP/EM/5	84.59 ± 1.19	0.52	2.78 ± 0.07	102.81 ± 1.80	4.89 ± 1.68	0.75 ± 0.25
MBP/EM/6	85.88 ± 1.02	0.69	2.64 ± 0.05	100.78 ± 1.69	5.53 ± 1.58	0.82 ± 0.17
MBN/EM/7	89.29 ± 1.14	0.49	2.86 ± 0.06	98.29 ± 1.75	5.31 ± 1.43	0.80 ± 0.13
MBN/EC/8	96.49 ± 1.23	0.57	2.99 ± 0.03	100.53 ± 1.84	4.90 ± 1.53	0.40 ± 0.27
MBN/EC/9	91.59 ± 1.30	0.66	2.89 ± 0.06	99.29 ±1.37	5.38 ± 1.48	0.83 ± 0.30

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

Table 5.9a: Data of drug release kinetics study of designed buccal mucoadhesive tablets made using HEC/EG/AR

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	R^2	k_0 (mg%/h)	R^2	k_1 (h^{-1})	R^2	k_H ($h^{-0.5}$)	R^2	k_{KP} (h^{-n})	n-Value
MBP/HEC/1	0.882	14.404	0.984	0.245	0.915	29.255	0.961	23.117	0.683
MBP/HEC/2	0.954	8.870	0.984	0.116	0.931	17.866	0.997	13.075	0.740
MBS/HEC/3	0.920	20.057	0.959	0.433	0.923	40.546	0.980	30.793	0.713
MBS/HEC/4	0.926	19.061	0.969	0.395	0.919	38.477	0.979	28.850	0.723
MBP/EG/1	0.956	11.617	0.990	0.168	0.924	23.362	0.995	16.833	0.752
MBP/EG/2	0.942	9.875	0.985	0.134	0.919	19.886	0.987	14.566	0.740
MBS/EG/3	0.860	20.706	0.964	0.488	0.929	42.255	0.964	34.753	0.653
MBS/EG/4	0.942	19.114	0.967	0.389	0.912	38.437	0.984	27.814	0.749
MBP/AR/1	0.963	15.575	0.982	0.266	0.924	31.285	0.998	22.248	0.762
MBP/AR/2	0.934	12.732	0.996	0.196	0.939	25.749	0.995	19.659	0.709
MBS/AR/3	0.568	22.503	0.952	0.713	0.882	47.233	0.882	46.736	0.508
MBS/AR/4	0.771	21.489	0.974	0.569	0.937	44.371	0.951	39.801	0.586

Table 5.9b: Data of drug release kinetics study of designed buccal mucoadhesive tablets made using PC/CP/CH/GM

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	R^2	k_0 (mg%/h)	R^2	k_1 (h^{-1})	R^2	k_H ($h^{-0.5}$)	R^2	k_{KP} (h^{-n})	n-Value
MBP/PC/1	0.965	17.488	0.969	0.324	0.922	35.107	0.998	24.803	0.766
MBP/PC/2	0.958	13.952	0.997	0.224	0.914	27.990	0.992	19.738	0.768
MBS/PC/3	0.901	9.955	0.969	0.137	0.935	20.215	0.981	15.999	0.682
MBS/PC/4	0.988	3.759	0.995	0.042	0.883	7.455	0.997	4.613	0.864
MBP/CP/1	0.804	21.305	0.969	0.543	0.942	43.837	0.960	38.441	0.603
MBP/CP/2	0.924	18.660	0.983	0.383	0.947	37.829	0.996	29.546	0.692
MBP/CH/1	0.703	24.061	0.989	1.766	0.225	53.586	0.727	72.649	0.471
MBP/CH/2	0.856	22.790	0.989	0.956	0.839	48.927	0.882	55.765	0.492
MBT/CH/3	0.992	8.617	0.979	0.111	0.909	17.500	0.945	14.118	0.667
MBT/CH/4	0.987	5.396	0.990	0.063	0.988	11.188	0.990	10.717	0.534
MBC/CH/5	0.918	22.353	0.992	0.705	0.933	46.971	0.933	47.517	0.491
MBC/CH/6	0.986	19.408	0.986	0.439	0.977	39.877	0.994	35.225	0.598
MBP/GM/1	0.839	21.195	0.9571	0.516	0.915	43.313	0.947	35.911	0.647
MBP/GM/2	0.998	12.934	0.971	0.191	0.856	25.448	0.999	13.908	0.792

Table 5.9c: Data of drug release kinetics study of designed buccal mucoadhesive tablets made using HPMC/EC/EM

Formulation code	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	R^2	k_0 (mg%/h)	R^2	k_1 (h^{-1})	R^2	k_H ($h^{-0.5}$)	R^2	k_{KP} (h^{-n})	n-Value
MBP/HPMC/1	0.908	20.174	0.965	0.445	0.934	40.922	0.983	32.060	0.690
MBP/HPMC/2	0.957	18.135	0.965	0.349	0.924	36.462	0.995	26.232	0.753
MBP/EC/3	0.928	13.328	0.979	0.209	0.943	26.986	0.993	20.885	0.698
MBP/EC/4	0.851	7.626	0.921	0.097	0.959	15.620	0.983	13.385	0.621
MBP/EM/5	0.979	16.099	0.977	0.278	0.902	32.122	0.998	21.268	0.814
MBP/EM/6	0.933	12.283	0.991	0.185	0.940	24.848	0.995	19.028	0.707
MBN/EM/7	0.962	18.827	0.962	0.371	0.910	37.725	0.993	26.262	0.778
MBN/EC/8	0.922	19.787	0.968	0.426	0.934	40.057	0.987	30.852	0.703
MBN/EC/9	0.947	12.547	0.998	0.191	0.928	25.280	0.994	18.605	0.737

Table 5.10: First order degradation kinetic parameters of FDP in designed formulations

Formulation Code	CRT: 25 ± 2°C/60 ± 5 % RH			AT (40 ± 2°C/75 ± 5 % RH)		
	K_{deg}x 10^{-4} ($month^{-1}$)	$t_{90\%}$ (months)	R^2	K_{deg} x 10^{-4} ($month^{-1}$)	$t_{90\%}$ (months)	R^2
SD 1:10	22.57	46.52	0.8589	47.44	22.13	0.9776
NCP 1:10	32.47	32.34	0.9467	60.11	17.47	0.9758
NCS 1:6	23.49	44.70	0.8220	62.18	16.89	0.9993
MBP/HEC/2	33.39	31.44	0.8570	81.07	12.95	0.9594
MBS/HEC/4	42.38	24.78	0.9615	91.20	11.51	0.9989
MBP/EG/2	43.76	24.00	0.9122	78.99	13.29	0.9720
MBS/EG/4	47.90	21.92	0.9253	89.82	11.69	0.9553
MBP/AR/2	56.42	18.61	0.9146	123.90	8.47	0.99988
MBS/AR/4	68.86	15.25	0.9284	131.04	8.01	0.9764
MBP/PC/2	38.92	26.98	0.9328	98.11	10.70	0.9798
MBS/PC/4	46.75	22.46	0.9507	114.46	9.17	0.9961
MBP/CH/2	43.76	24.00	0.7569	101.79	10.32	0.8729
MBT/CH/4	50.90	20.63	0.8678	107.09	9.80	0.9811
MBP/HPMC/2	47.90	21.92	0.9412	100.87	10.41	0.9619
MBN/EC/9	49.74	21.11	0.9240	125.28	8.38	0.9544

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Chapter 6

In Vivo Bioavailability Studies

6.1 Introduction

In vivo studies in suitable animal models and/or human subjects of designed delivery systems along with in vitro evaluation are must to predict therapeutic efficacy of designed formulations. A buccal mucoadhesive drug delivery system should be clinically effective, non-sensitizing, non-irritating to the buccal mucus membrane. It should not pose difficulties in routine activities such as drinking, talking and eating. The in vivo studies in suitable animal models present valuable information about the pharmacokinetic behaviour and probable clinical efficacy of the active pharmaceutical ingredient. Generally, buccal formulations are designed to enhance bioavailability of drugs linked with poor oral bioavailability due to extensive first pass effect and/or 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. The in vivo pharmacokinetic evaluations of buccal drug delivery systems have been reported in variety of animal models like rabbits (Charde et al., 2008; Jaipal et al., 2013; Ahmed et al., 2014), humans (Ahmed et al., 2012; Kaseem et al., 2014), rats (Sakata and Onishi, 2013), pigs (Palem et al., 2011; Meng-Lund et al., 2014), hamsters (Aungst, 1994) and dogs (Ameye et al., 2005; Degim et al., 2006).

This chapter presents in vivo studies of designed buccal mucoadhesive tablets of felodipine. The bioavailability and pharmacokinetic parameters of FDP from designed formulations were assessed using New Zealand white rabbits as an animal model. The bioavailability of FDP from developed buccal formulations was compared with that of oral bioavailability of FDP from immediate release tablet. Effect of dissolution enhancement of FDP on pharmacokinetic profile of buccal tablets prepared with pure FDP, SD and NC was also studied by comparing the in vivo profile of buccal tablets made with pure FDP, and equivalent amount of SDs and NCs.

6.2 Materials

FDP was gifted by Ranbaxy laboratories Limited (New Delhi, India). Xyalzine and ketamine used for inducing anesthesia to rabbits were purchased from local markets. Other materials and reagents used were same as mentioned in chapter 4 and chapter 5.

6.3 Animal model

New Zealand white rabbits weighing 2.0 to 2.5 kg were supplied by the Central Animal Facility of Birla Institute of Technology and Science, Pilani, Pilani campus. The mean weight of the animals used in the study was 2.23 ± 0.21 kg. A prior approval from Institution Animal Ethics Committee was obtained for carrying out the study (Protocol approval number: IAEC/RES/16/04). The study was performed with respect to the guidelines provided by the Institutional Animal Ethics Committee and under the supervision of a registered veterinarian. Animals were kept in standard cages in light controlled room at 25 ± 2 ^{o}C and 50 ± 5% RH. For the experiment, rabbits were issued and acclimatized one week before the experimentation. Animals were kept on standard pellet diet and water ab libitum during period of acclimatization. Animals were kept on fasting 6 h prior to the actual start of the experimentation. The rabbits were not provided food and water till 4 h after the start of the experiment.

6.4 Preparation of formulation

Immediate release oral tablet containing 5 mg of FDP was prepared. Drug was mixed with lactose and magnesium stearate and was directly compressed using 4 mm punch. Lactose and magnesium stearate were used as diluent and lubricant respectively for the preparation of the tablets. Five tablets were taken and assayed for the determination of FDP content using analytical method 1 of chapter 3. Fresh batches of MBP/HEC/2, MBS/HEC/4, MBP/PC/2, MBS/PC/4, MBP/EM/6 and MBN/EM/7 were prepared before the experiment. The composition of the tablets are given in Table 5.7a, 5.7b and 5.7c of chapter 5. All the quality control tests mentioned in chapter 5 were carried out for immediate release and freshly prepared buccal mucoadhesive tablets before proceeding with animal dosing.

6.5 Dosing

Prior to experimentation, rabbits were anaesthetized by an intramuscular (i.m.) injection of 1:5 mixture of xylazine (1.5 mg/kg) and ketamine (9.0 mg/kg). The light plane of anesthesia was maintained by administering one third of initial dose of xyalzine and ketamine intramuscularly as needed. Designed buccal mucoadhesive tablets were pre-moistened by dipping the tablet in millipore water for 5 sec. The rabbit mouth was opened using specially designed mouth restrainer 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. For oral dosing, mouth of rabbit was opened using mouth restrainer and tablet was placed using a forceps at the end of oral cavity. Water (5 ml) was immediately administered using syringe to ensure that the tablet was swallowed. The entire study was carried out in triplicate. Each rabbit was dosed with specific dose (5 mg) of FDP without taking weight of the rabbit into consideration.

6.6 Blood sample collection and processing

In each study, 1 ml blood samples was withdrawn from the marginal ear vein of the animals at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 9.0, 12.0, 18.0, 24.0, 36.0 and 48.0 h post dosing using a 21 G needle. Blood sample was also collected prior to dosing from all the rabbits. The blood was collected in 2 ml centrifuge tubes containing 100 µl of EDTA solution (1.0 mg/ml) and centrifuged at 4000 rpm for 4 min at 4 °C (Eppendorf centrifuge-5702R). The plasma supernatant obtained was collected and stored at -20 °C till further processing for analysis.

6.7 Sample analysis

Frozen plasma samples were thawed by keeping the sealed tubes at room temperature (25 ± 2 ^{o}C) for at least 60 min. The protein present in the plasma samples was precipitated with acetonitrile. For this, 300 µl of plasma samples were taken and 1.5 ml of acetonitrile was added to it and vortex mixed. The mixture was then centrifuged for 20 min at 13000 rpm at 4°C. The supernatant was carefully taken and evaporated to dryness using vacuum concentrator. The dried residue was further reconstituted with a solvent system containing 1:4 (% v/v) of methanol and phosphate buffer (pH 6.8). Finally, the samples were analyzed using analytical method 3 of chapter 3. The plasma drug concentration at various time points of the study was thus measured.

6.8 Data analysis

The plasma drug concentration versus time data of FDP 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.

6.9 Results and discussion

In vivo pharmacokinetic study of FDP was conducted by administering it in form of oral and buccal mucoadhesive modified release tablets. The plasma concentration versus time profile of FDP following administration of various formulations are given in Fig. 6.1, 6.2 and 6.3. The summary of the pharmacokinetic parameters determined with non-compartmental data analysis are presented in Table 6.1, 6.2 and 6.3.

On administration of oral tablet FDP showed maximum plasma concentration (C_{max}) of 29.98 ng/ml, 4.00 h post dosing (Fig. 6.1). $AUC_{(0-\infty)}$ was found to be 435.25 ± 42.63 ng h/ml. The elimination rate constant of was found to be 0.06 ± 0.02 h^{-1}. Table 6.1 presents summary of the results.

Pharmacokinetic profile of designed buccal tablets were compared with that of orally administered tablets. In order to see the effect of SD and NC, the pharmacokinetic parameters of the buccal tablets prepared with pure FDP, SD and NC were also compared. Fig. 6.1 exhibits comparison of plasma profile of FDP for the oral tablet, MBP/HEC/2 and MBS/HEC/4. The buccal tablet prepared with HEC and pure FDP (MBP/HEC/2) demonstrated C_{max} of 154.27 ± 50.41 ng/ml after 6 h of dosing. In contrast to it, buccal tablet prepared with HEC and SD (MBS/HEC/4) containing equivalent amount of drug showed considerably greater C_{max} of 333.71 ± 110.23 ng/ml. This concentration was achieved after 3 h of dosing which can be attributed to the dissolution enhancement of FDP in SD. $AUC_{(0-\infty)}$ value for the buccal tablets MBP/HEC/2 and MBS/HEC/4 was found to be 1889.48 ± 540.27 and 4246.39 ± 1325.27 ng h/ml respectively. The difference in C_{max} and $AUC_{(0-\infty)}$ values following administration of oral tablet and both MBP/HEC/2 and MBS/HEC/4 was found to be statistically significant at 5% level of significance. The difference in the AUC is probably due to incomplete absorption of FDP from the buccal tablets containing pure drug during the anaesthetized period (6 h) of the rabbits. After becoming conscious, the animal might have swallowed the left over portions of tablets resulting in first pass metabolism of the remaining drug and finally leading to lower AUC values. Bioavailability of FDP from MBP/HEC/2 and MBS/HEC/4 was found to be 4.34 and 9.76 times with respect to that of oral tablets (Table 6.1).

The comparison of plasma concentration of FDP from oral and buccal tablets (MBP/PC/2 and MBS/PC/4 containing pure drug and SD respectively) is shown in Fig. 6.2. The C_{max} values for the tablets MBP/PC/2 and MBS/PC/4 were found to be

119.13 ± 38.19 ng/ml and 42.14 ± 14.38 ng/ml respectively. The $AUC_{(0-\infty)}$ values obtained for the formulations were 1304.37 ± 390.18 and 661.21 ± 178.31 ng h/ml. So, the presence of SD in tablets has caused a decrease in the C_{max} and $AUC_{(0-\infty)}$ values. The difference in C_{max} and $AUC_{(0-\infty)}$ values following administration of oral tablet and both MBP/PC/2 and MBS/PC/4 was found to be statistically significant at 5% level of significance. This result is in agreement with that of the in vitro dissolution study of the tablets as presented in Fig. 5.27 of chapter 5. Bioavailability of FDP from MBP/PC/2 and MBS/PC/4 was 3.00 and 1.52 with respect to the oral tablets (Table 6.2).

In order to see the effect of dissolution enhancement of FDP by nanocrystal technology on the pharmacokinetic profile of FDP, in vivo study of buccal tablets, MBP/EM/6 and MBN/EM/7 prepared with pure drug and NC respectively, were conducted. The comparison of plasma profiles of the oral tablet, MBP/EM/6 and MBN/EM/7 is presented in Fig. 6.3. Table 6.3 exhibits summary of the pharmacokinetic parameters of the drug from the aforesaid tablets. For Buccal tablet MBP/EM/6, C_{max} obtained was 130.58 ± 34.28 ng/ml after 4.0 h of dosing. The $AUC_{(0-\infty)}$ value for the tablet was observed to be 2001.23 ± 569.18 ng h/ml. The formulation MBN/EM/7 exhibited an $AUC_{(0-\infty)}$ value of 4458.68 ± 1410.91 ng h/ml and C_{max} value of 318.48 ± 106.84 ng/ml. The maximum plasma concentration of this formulation was achieved after 3.00 h of dosing. The difference in C_{max} and $AUC_{(0-\infty)}$ values following administration of oral tablet and both MBP/EM/6 and MBN/EM/7 was found to be statistically significant at 5% level of significance. Bioavailability of FDP from MBP/EM/6 and MBN/EM/7 was 4.60 and 10.24 with respect to the oral tablets.

The designed buccal tablets resulted in higher C_{max} and $AUC_{(0-\infty)}$ values in comparison to oral tablets. The tablets made using solid dispersion (1:10) and nanocrystals (1:10) showed a further enhancement in the bioavailability when compared to tablets containing same proportion of identical polymer. This enhancement may probably be due to rapid dissolution and subsequent permeation of released drug from tablets prepared using SD and NC. The buccal tablet made with SD and PC as a mucoadhesive polymer was exception of this result. Furthermore, other drawbacks such as erratic oral absorption and interaction with food can also be considerably avoided by designed buccal dosage forms.

6.10 In vitro in vivo correlation

Although animal studies were planned only to evaluate effect of buccal delivery of felodipine on bioavailability in rabbits. IVIV correlation development should be preplanned in order to achieve a successful correlation (Van Buskirk et al., 2014). However, an attempt was made to develop IVIV correlation for designed buccal formulations.

6.10.1 Level A correlation

The comparative in vitro release and in vivo profile of the designed buccal formulations are shown in Fig. 6.4 and 6.5 respectively. The rank order of formulations for in vitro release rate was found to be MBS/HEC/4 > MBN/EM/7 > MBP/PC/2 > MBP/EM/6 > MBP/HEC/2 > MBS/PC/4. While in case of in vivo studies with respect to C_{max}, the rank order of formulations was found to be MBS/HEC/4 > MBN/EM/7 > MBP/HEC/2 > MBP/EM/6 > MBP/PC/2 > MBS/PC/4. It was observed from the profiles that the rank order of buccal tablets does not match. For a good IVIV correlation, data should show a rank order correlation (Van Buskirk et al., 2014). So, Level A of IVIV correlation was not possible for the formulations.

6.10.2 Level B correlation

In order to establish level B correlation, the mean in vitro dissolution time (MDT) was compared with mean in vivo residence time (MRT) of the designed and developed buccal formulations. This comparison produced a regression coefficient (R^2) value of 0.2872 indicating poor level B correlation for the formulations. The result is shown in Fig. 6.6.

6.10.3 Level C correlation

In this level of IVIV correlation, one or more dissolution time point ($t_{50\%}$, $t_{60\%}$, $t_{90\%}$, etc.) is compared with one or more relevant mean pharmacokinetic parameter (AUC, t_{max} or C_{max}). In our study, we attempted combination of several in vitro-in vivo parameters such as C_{max} and $t_{50\%}$, t_{max} and $t_{50\%}$, $AUC_{0-\infty}$ and $t_{50\%}$, C_{max} and $t_{60\%}$, tmax and $t_{60\%}$, $AUC_{0-\infty}$ and $t_{60\%}$ etc. We also tried to see correlation between $AUC_{0-\infty}$ and $t_{90\%}$. The results are shown in Table 6.4. The results indicated poor IVIV correlation for the parameters used in the study.

6.10.4 Factors governing in vitro in vivo results

The study demonstrated poor correlation at Level A, Level B and Level C. This poor correlation can probably be attributed to the various factors. A detailed discussion of the factors are given in the next sections.

6.10.4.1 Dissolution study related factors

The conditions and apparatus used for in vitro drug release study do not mimic the in vivo conditions. Although we have taken all possible measures which were feasible to come up with in vitro release methodology mimicking in vivo environment, it seems that in vitro testing methodology used was not adequately simulating in vivo conditions. In vitro release studies were carried out using basket rotated at 25 rpm to simulate the in vivo environment. Since, the hydrodynamics and product-medium interactions are significantly different hence, it is difficult to mimic the in vivo stirring environment with modified apparatus (Repta, 1999; Qureshi, 2004). Moreover, in order to expect good IVIV correlation for designed buccal formulations, biorelevant dissolution media need to be used along with suitable dissolution testing apparatus simulating in vivo conditions. The media used during the study might not have mimicked the conditions prevalent in oral cavity.

6.10.4.2 Animal study related issues

For IVIVC study, in vivo data should be obtained using at least 6 subjects per group (US FDA, 1997). Bioavailability enhancing capability of designed buccal formulations was assessed by performing pharmacokinetic study in rabbits (three rabbits per group). High data variation could be possible due to less number of animals per group which can distort the mean data and in turn the deconvolution. Moreover, presence of salivary juice and digestive enzymes in oral cavity, effect of anesthetic agents and formulation excipients may have also influenced in vivo drug release. The lack of steady absorption of the dissolved FDP might have also been caused due to the less availability of surface area (rabbit's mucosal surface area). Some of the dissolved drug might have been swallowed by the animals resulting in poor IVIV correlation.

6.10.4.3 Formulation related issues

Buccal tablets that have been studied in vivo were prepared with lots of variable factors that might have led to the in vivo variability. The formulations tested in vivo have very few things in common amongst them as either the polymer (quantity or type) or drug form (pure drug, solid dispersion or nanocrystals) is different. Lack of good correlation can also be attributed to this reason. The final in vivo pharmacokinetic parameters of drug from the designed formulations is not solely defined by drug release rate but also by the time for which the delivery system is retained in buccal cavity which in turn depends upon polymer type and amount.

Possible involvement of all these factors might have resulted in poor IVIV correlation.

6.11 Conclusions

The in vivo studies of the selected buccal formulations (MBP/HEC/2, MBS/HEC/4, MBP/PC/2, MBS/PC/4, MBP/EM/6 and MBN/EM/7) conducted in rabbits exhibited considerable increase in the bioavailability of felodipine. The enhancement in bioavailability can be attributed to the avoidance of first pass metabolism of the drug. Buccal tablets made with solid dispersion and nanocrystal showed a further enhancement which may be due to the complete release of FDP from the formulation within 6-8 h of time. Mucoadhesive polymers used in the tablet matrices also played a significant role in absorption enhancement and bioavailability. Hence, it can be concluded that the designed mucoadhesive buccal tablets are promising and may result in substantial dose reduction, more predictable plasma concentration and prolonged duration of action of FDP as compared to the conventional marketed delivery systems.

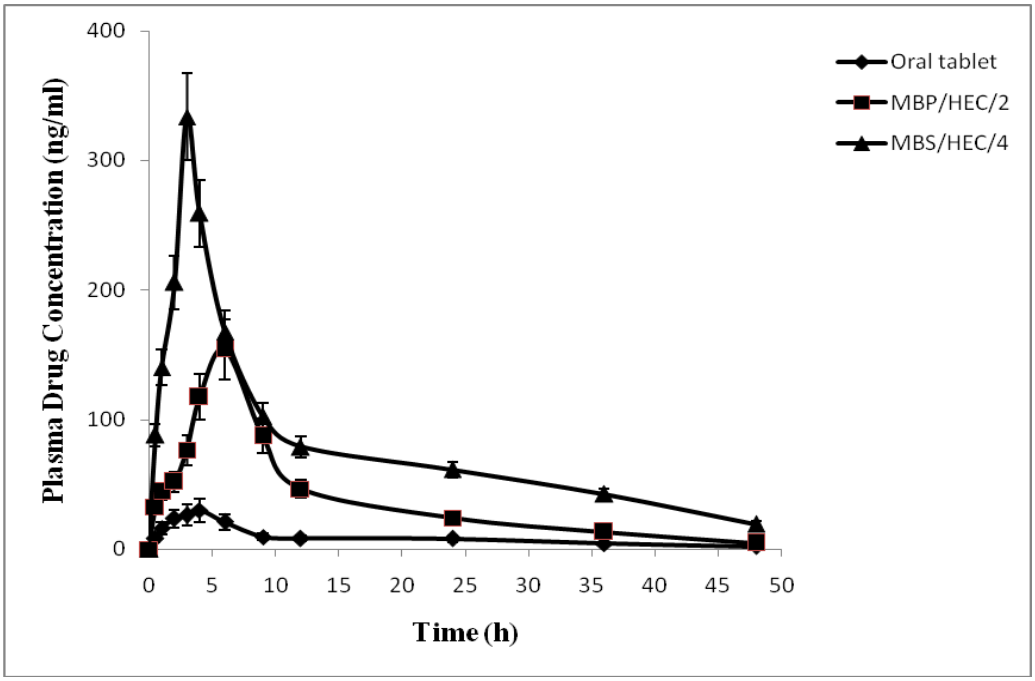

Fig. 6.1. In vivo profiles of FDP following administration of immediate release oral tablet and buccal tablet prepared using varying proportions of HEC

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

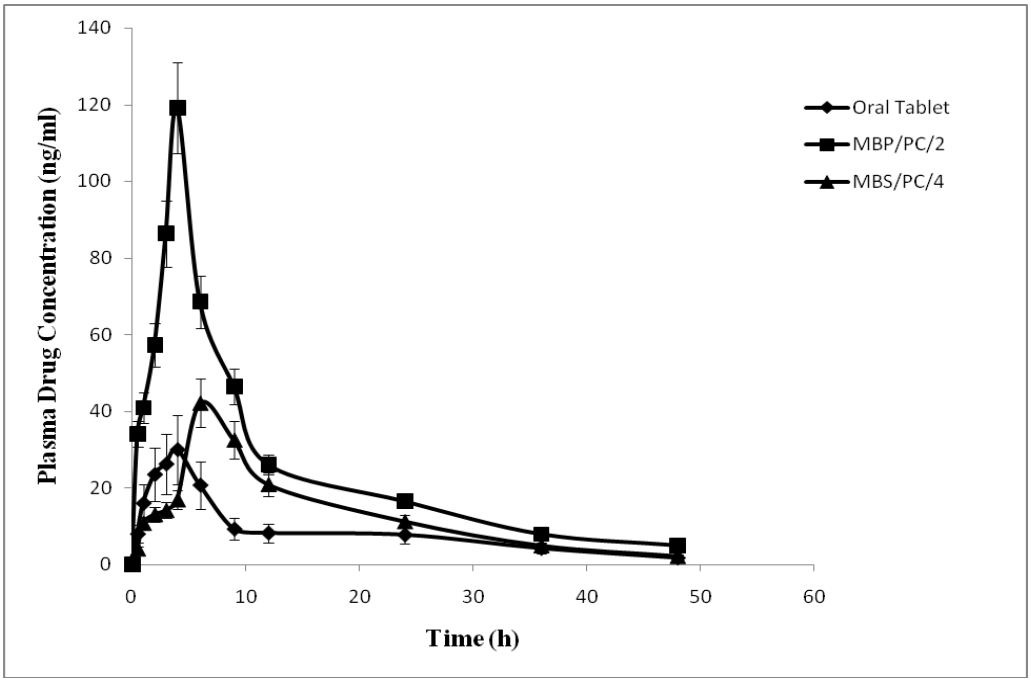

Fig. 6.2. In vivo profiles of FDP following administration of immediate release oral tablet and buccal tablet prepared using varying proportions of PC

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

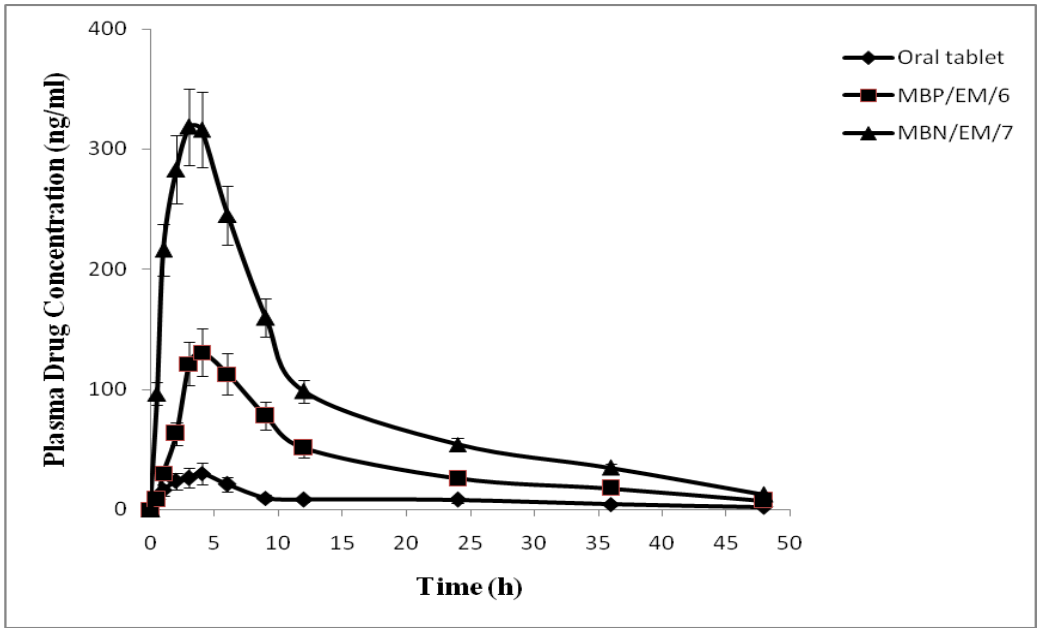

Fig. 6.3. In vivo profiles of FDP following administration of immediate release oral tablet and buccal tablet prepared using varying proportions of EM

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

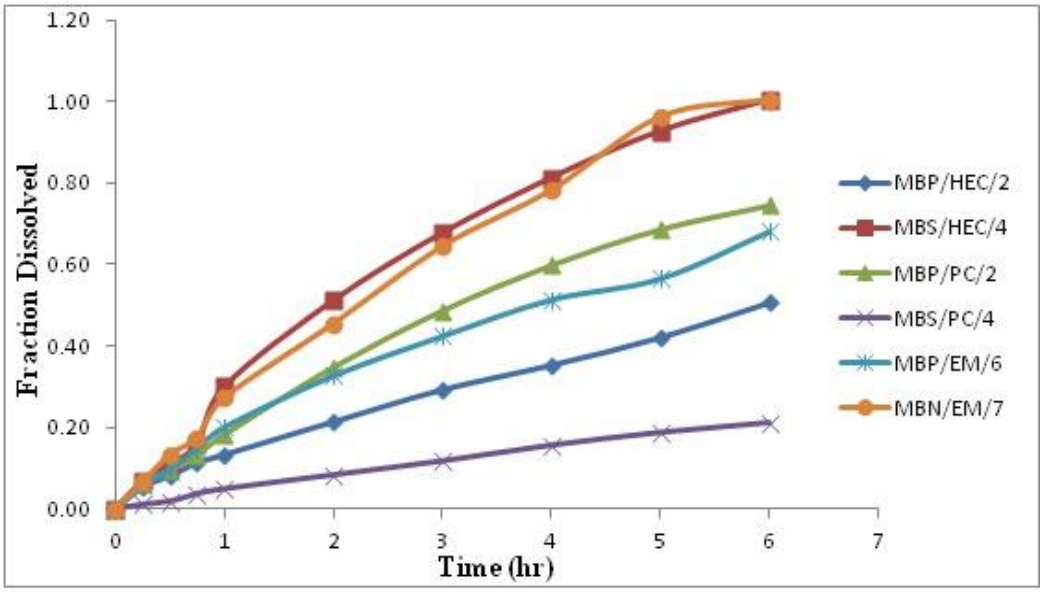

Fig. 6.4. Comparative in vitro release profile of buccal tablets

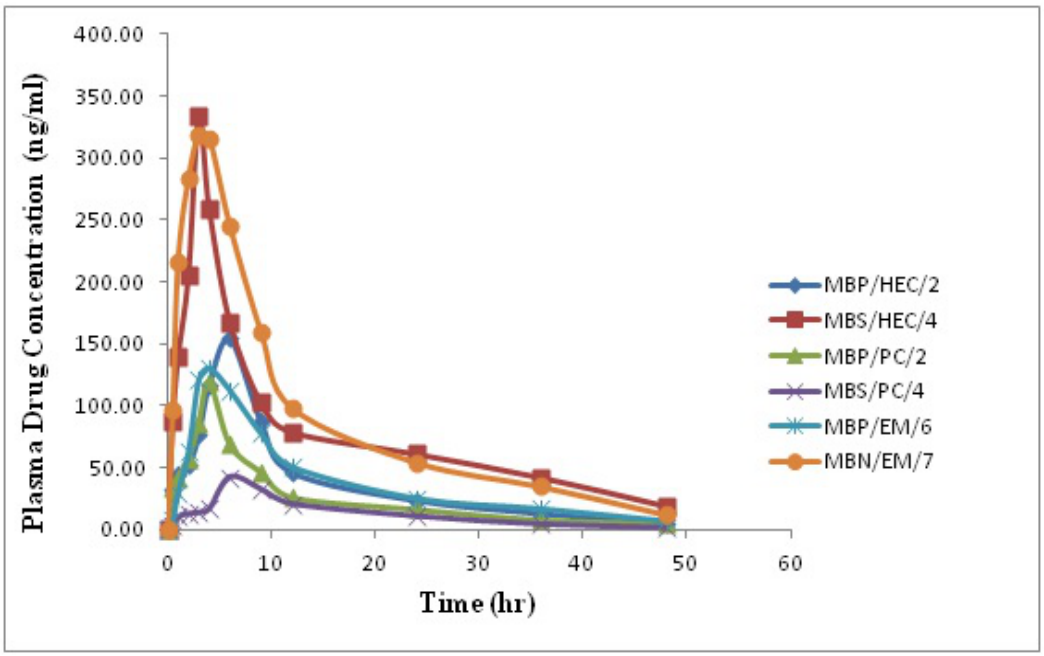

Fig. 6.5. Comparative in vivo profile of buccal tablets

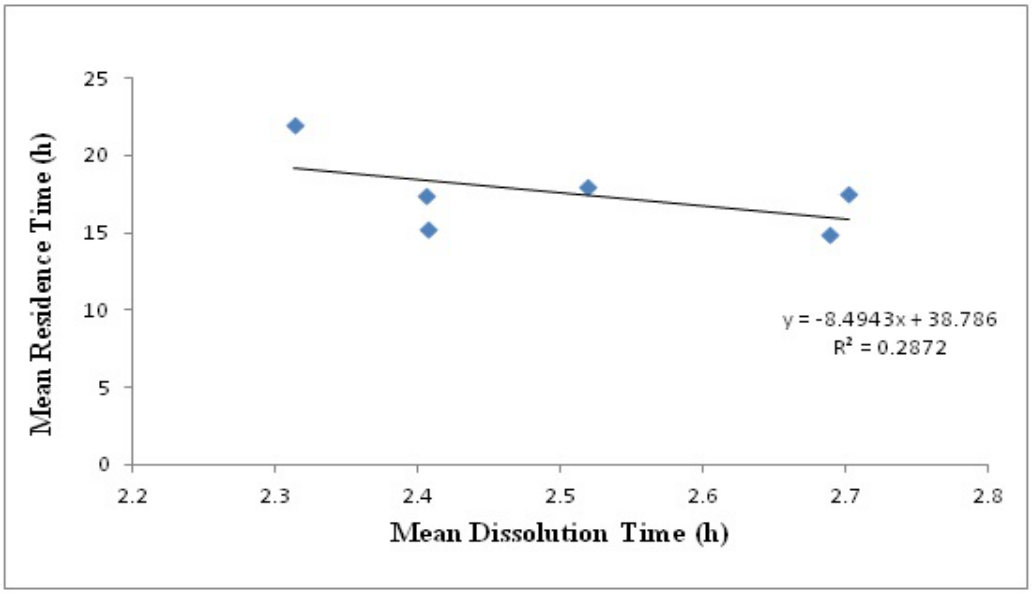

Fig. 6.6. Level B IVIV correlation profile of mean residence time (MRT) vs mean 333dissolution time (MDT)

Table 6.1: Summary of pharmacokinetic parameters of FDP following administration of immediate release oral tablet and buccal tablet prepared using varying proportions of HEC (Mean ± STDEV for 3 rabbits)

Pharmacokinetic Parameters	**FDP oral tablet (5 mg)**	**MBP/HEC/2**	**MBS/HEC/4**
C_{max} (ng /ml) [a]	29.98 ± 9.60	154.27 ± 50.41*	333.71 ± 110.23*
t_{max} (h) [b]	4.00	6.00	3.00
Elimination Rate Constant[c] (h^{-1})	0.06 ± 0.02	0.06 ± 0.03	0.04 ± 0.02
$AUC_{(0-\infty)}$ [d] (ng h/ml)	435.25 ± 42.63	1889.48 ± 540.27*	4246.39 ± 1325.27*
$AUMC_{(0-\infty)}$ [e] (ng h^2/ml)	8025.28	28222.41	93467.89
$T_{1/2}$ (h)	11.21 ± 0.23	10.90 ± 0.31	17.88 ± 0.17
MRT [f] (h)	18.44	14.94	22.01
F_r [g]	--	4.34	9.76

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

Table 6.2: Summary of pharmacokinetic parameters of FDP following administration of immediate release oral tablet and buccal tablet prepared using varying proportions of PC (Mean ± STDEV for 3 rabbits)

Pharmacokinetic Parameters	**FDP oral tablet (5 mg)**	**MBP/PC/2**	**MBS/PC/4**
C_{max} (ng /ml) [a]	29.98 ± 9.60	119.13 ± 38.19*	42.14 ± 14.38*
t_{max} (h) [b]	4.00	4.00	6.00
Elimination Rate Constant[c] (h^{-1})	0.06 ± 0.02	0.05 ± 0.02	0.07 ± 0.01
$AUC_{(0-\infty)}$ [d] (ng h/ml)	435.25 ± 42.63	1304.37 ± 390.18*	661.21 ± 178.31*
$AUMC_{(0-\infty)}$ [e] (ng h^2/ml)	8025.28	22651.66	11552.58
$T_{1/2}$ (h)	11.21 ± 0.23	14.62 ± 0.27	10.14 ± 0.21
MRT [f] (h)	18.44	17.37	17.47
F_r [g]	--	3.00	1.52

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

Table 6.3: Summary of pharmacokinetic parameters of FDP following administration of immediate release oral tablet and buccal tablet prepared using varying proportions of EM (Mean ± STDEV for 3 rabbits)

Pharmacokinetic Parameters	**FDP oral tablet (5 mg)**	**MBP/EM/6**	**MBN/EM/7**
C_{max} (ng /ml) [a]	29.98 ± 9.60	130.58 ± 34.28*	318.48 ± 106.84*
t_{max} (h) [b]	4.00	4.00	3.00
Elimination Rate Constant[c] (h^{-1})	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.1
$AUC_{(0-\infty)}$ [d] (ng h/ml)	435.25 ± 42.63	2001.23 ± 569.18*	4458.68 ± 1410.91*
$AUMC_{(0-\infty)}$ [e] (ng h^2/ml)	8025.28	36043.79	67833.38
$T_{1/2}$ (h)	11.21 ± 0.23	13.37 ± 0.26	11.63 ± 0.25
MRT [f] (h)	18.44	18.01	15.21
F_r [g]	--	4.60	10.24

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

Table 6.4: Regression coefficient (R^2) values for various in vitro and in vivo parameters used for level C IVIV correlation

In vitro Parameter	In vivo Parameter	Regression Coefficient (R^2)
$t_{50\%}$ (h)	C_{max} (ng/ml)	0.5302
	t_{max} (h)	0.6912
	$AUC_{0-\infty}$ (ng h/ml)	0.4923
$t_{60\%}$ (h)	C_{max} (ng/ml)	0.5418
	t_{max} (h)	0.7238
	$AUC_{0-\infty}$ (ng h/ml)	0.5051
$t_{90\%}$ (h)	$AUC_{0-\infty}$ (ng h/ml)	0.5305

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Chapter 7

Conclusions and Future Scope of Work

7.1 Conclusions

Buccal drug delivery systems are among the popular drug delivery systems designed to combat bioavailability problems of drug undergoing extensive first pass metabolism when administered orally. The factors responsible in making buccal delivery system, a highly sought after delivery systems, include the ease of accessibility, administration and withdrawal, good retention. Moreover, low enzymatic activity, cost effectiveness and better patient compliance of buccal delivery systems are added advantages. Furthermore, the potential drawback of parenteral drug delivery systems such as patient incompliance, higher costs, hazardous effects due to inability of drug withdrawal, requirement of skilled professional during administration has also worked in favour of the buccal drug delivery systems.

FDP, a 1,4-dihydropyridine derivative, is a vasoselective calcium antagonist widely used in the treatment of angina pectoris and hypertension. It undergoes extensive first pass metabolism leading to oral bioavailability of only 15%. It is practically insoluble in water. It also shows variation in absorption when administered in fed and unfed conditions leading fluctuations in plasma drug concentration.

For analysis of FDP in variety of samples like bulk, formulation, stability, in vitro and bio samples, suitable analytical methods using UV-Visible spectrophotometer and HPLC were developed and validated. In vivo performance of the designed formulations was assessed using an in house developed and validated HPLC bioanalytical method. All the methods were found to be simple, sensitive, specific and suitable for the current research work and routine work as well.

Preformulation studies revealed Form I polymorph of FDP was used during entire research work when analyzed using DSC and FT-IR techniques. The dissociation constant of the drug was found to be 5.07 as calculated from the experiments. FDP demonstrated good stability in solution state at varying pH with t_{90}% values ranging from 2.81 to 8.44 days. Solid state stability studies indicated FDP to be compatible and stable with process excipients used in the design of buccal dosage forms.The aqueous solubility of FDP was substantially enhanced by the solid dispersion and nanocrystal technology. Solid dispersions of the drug were prepared using a novel hydrophilic polymer soluplus®. The amorphous nature of FDP with particle size in nanometer range indicated suitability of polymer and method used for the formulation of solid dispersion. FDP nanocrystals with enhanced rate and extent of dissolution were prepared with various stabilizers. The amorphous nature and drug

particle size below 50 nm in the optimized nanocrystals indicated appropriateness of the process and components used in the manufacturing process.

Buccal mucoadhesive modified release tablets of FDP were prepared using solid dispersions, nanocrystals and pure forms of the drug. Mucoadhesive polymer of various types like anionic (CP, PC), cationic (CH) and nonionic (HEC, EC, HPMC) were used in the preparation of buccal tablets of FDP. The designed buccal tablets were found to possess good physical characteristics indicating suitability of the direct compression method employed in the manufacturing process. The designed formulations were found to be stable for at least one year when stored at CRT indicating that excipients, process and packaging material used were appropriate and compatible with drugs. There was no change in physical characters and release profiles over the aforesaid period of time.

In vitro release of FDP from the designed buccal tablets was affected by form of drug (pure, solid dispersion or nanocrystal) used in the formulation. The amount, swelling behaviour and hydophilicity or hydrophobicity of the mucoadhesive polymer used in the formulation also played a crucial role in the release of the drug. The optimized formulations were found to control release of FDP for a period of 6-8 h. The designed buccal formulations were found to follow first order release kinetics. The release mechanism of almost all formulations was anomalous non-Fickian transport. The formulations showed an initial rapid release followed by a decelerated one with passage of time indicating possibility of achieving target concentration without loading dose. The mucoadhesive strength of the designed formulations was affected by polymer proportion, viscosity of gel formed by the polymer, flexibility of polymeric chains and swelling behaviour of the polymer.

In vivo experiments of selected buccal formulations in rabbits demonstrated substantial increase in bioavailability of FDP in comparison to that of oral tablets. The reason behind this is the reduction in the first pass metabolism by the buccal tablets. The buccal formulation containing solid dispersions and nanocrystals of FDP showed further enhancement in bioavailability of the drug as compared to that made with pure drug. This enhancement might probably be due to the rapid dissolution and subsequent permeation of released drug from tablets prepared using solid dispersions and nanocrystals.

The study suggested that the designed buccal mucoadhesive tablet formulations possess potential credentials for commercial use. The formulation would

cause considerable dose reduction and better predictable plasma profile of FDP in comparison to conventional marketed preparations. The method employed for the formulation was found to be simple and can easily be followed in general formulation manufacturing unit on a commercial scale.

7.2 Future scope of the research work

Further, the optimized designed buccal formulations can be scaled up and can be tested clinically in human volunteers for final proof of concept. Process variables for manufacturing of buccal tablets with an optimum dose need to be optimized to obtain a desirable drug release profile and plasma drug concentration in humans. Other bioadhesive polymers either alone or in combination and excipients should be explored to understand the FDP release and bioadhesion, and any interactions of excipients with the drug should be established.

The optimized buccal formulations need to be studied clinically in humans for acceptability on the front of irritation caused by polymers and excipients and swelling behavior in the oral cavity.

Designed modified release buccal formulations containing solid dispersions and nanocrystals have shown improved bioavailability of FDP in comparison to that of pure drug buccal tablets. There must not be any issue in the scale up of the manufacturing process of the solid dispersions but this may not be the case with the method of preparation of nanocrystals. So, issues in scale up of the nanocrystal preparation need to be addressed.

Moreover, permeation enhancers alone or in combination need to be investigated for improved drug permeation and better understanding of permeation mechanisms.

Appendix

List of Publications from Thesis

1. **Pandey, M.M.**, Jaipal, A., Kumar, A., Malik, R., Charde, S.Y., 2013. Determination of pk_a of felodipine using UV-Visible spectroscopy. Spectrochim. Acta A Mol. Biomol. Spectrosc., 115, 887-890.
2. **Pandey, M.M.,** Jaipal, A., Charde, S.Y., Goel, P., Kumar, L., 2015. Dissolution enhancement of felodipine by amorphous nanodispersions using an amphiphilic polymer: Insight into the role of drug-polymer interactions on drug dissolution. Pharmaceutical Development and Technology. (Early online, DOI: 10.3109/10837450.2015.1022785).
3. **Pandey, M.M.**, Charde, S.Y., Jaipal, A., Raut, P.P., Goel, P., Kumar, L., Development and validation of a simple high-performance liquid chromatography method for estimation of felodipine in rabbit plasma: Application to pharmacokinetic study. Under review (Current Pharmaceutical Analysis).
4. **Pandey, M.M.**, Charde, S.Y., Jaipal, A., Sihag, A., Singh, R., Singh, S., Preparation and in vitro/in vivo characterization of felodipine nanocrystal. Communicated (Drug Development and Industrial Pharmacy).

Poster Presentations at National and International Conferences from Thesis

1. **Pandey, M.M.**, Raut, P.P, Charde, S.Y. Modified release from directly compressible felodipine buccal tablets. Fifteenth international symposium of controlled release society - Indian chapter, 2016, Mumbai, India.
2. **Pandey, M.M.**, Gajja, A., Jaipal, A., Charde, S.Y., Dissolution rate enhancement of felodipine by solid dispersions using novel amphiphilic polymer soluplus®. CRS Annual Meeting and Exposition, 2013, Honolulu, USA.
3. **Pandey, M.M.**, Jaipal, A., Goel, P., Kumar, L., Charde, S.Y., Estimation of felodipine in bulk and formulations by high performance liquid chromatographic method using fluorescence detection. AAPS Annual Meeting and Exposition 2013, San Antonio, Texas, USA.
4. **Pandey, M.M**., Raut, P., Jaipal, A., Charde, S.Y. Development of a high performance liquid chromatographic method for the estimation of felodipine in

bulk and formulation. 5th International Conference on "Clinical Pharmacology-Discovery, Development & Beyond, 2011, Mumbai, India.

5. **Pandey, M.M.**, Krishna Kumar, G., Khandelwal, R., Jaipal, A., Charde, S.Y., Solubility enhancement of felodipine by solid dispersion technique. APTI 16th Annual National Convection, 2011, Moga, India.
6. **Pandey, M.M.**, Yadav Laxmiharika, T., Gajja, A., Malik, R., Kumar, A., Parwani, M., Charde, S.Y., UV-Visible spectrophotometric analysis of felodipine in bulk and formulation. 3rd World Congress on Bioavailability and Bioequivalence, 2012, Hyderabad, India.
7. **Pandey, M.M.**, Jaipal, A., Kumar, A., Malik, R., Parwani, M., Charde, S.Y., Formulation and in vitro evaluation of buccal tablets of felodipine using polycarbophil, a mucoadhesive polymer. APTI 17th Annual National Convention, 2012, Manipal, India.

List of Publications outside Thesis

1. Jaipal, A., **Pandey, M.M.**, Abhishek, A., Vinay, S., Charde, S.Y., 2013. Interaction of calcium sulfate with xanthan gum: Effect on in vitro bioadhesion and drug release behavior from xanthan gum based buccal discs of buspirone. Colloids Surf. B Biointerfaces, 111, 644-650.
2. Jaipal, A., **Pandey, M.M.**, Charde, S.Y., Nilanjan, S., Srinivas, A., Prasad, R.G., 2014. Controlled release effervescent buccal discs of buspirone hydrochloride: In-vitro and in-vivo evaluation studies. Drug Delivery (Early online, DOI: 10.3109/10717544.2014.917388).
3. Jaipal, A., **Pandey, M.M.**, Charde, S.Y., Raut, P.P., Prasanth, K.V., Prasad, R.G., 2014. Effect of HPMC and mannitol on drug release and bioadhesion behavior of buccal discs of buspirone hydrochloride: In vitro and in vivo pharmacokinetic studies. Saudi Pharmaceutical Journal. (Early online, DOI:10.1016/j.jsps.2014.11.012).

Poster Presentations at National and International Conferences outside Thesis

1. Jaipal, A., **Pandey, M.M.**, Shailaja, P., Charde, S.Y., Design of buccal mucoadhesive drug delivery systems of buspirone: Effect of HPMC and mannitol on drug release and mucoadhesion behavior using factorial design approach. AAPS Annual Meeting and Exposition 2013, San Antonio, Texas, USA.
2. **Pandey, M.M.**, Jaipal, A., Malik, R., Kumar, A., Charde, S.Y., Dissolution rate enhancement of pioglitazone hydrochloride by solid dispersions using poloxamer 188. APTI 17th Annual National Convention, 2012, Manipal, India.
3. Jaipal, A., **Pandey, M.M.**, Charde, S.Y., Vinay, S., Design and evaluation of buccal tablets of buspirone HCl : Effect of HPC and mannitol on drug release. APTI 17th Annual National Convention, 2012, Manipal, India.
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6. **Pandey, M.M.**, Ramakrishna, R., Singh, D., Raut, P., Charde, S.Y., Solubility enhancement of felodipine using beta-cyclodextrin inclusion complexes. APTI 16th Annual National Convection, 2011, Moga, India.
8. **Pandey, M.M.**, Raut, P., Jaipal, A., Charde, S.Y., Spectrophotometric and spectrofluorometric study for the estimation of felodipine in bulk and formulation. 62nd Indian Pharmaceutical Congress, 2010, Manipal, India.

Biography of Shrikant Charde

Prof. Shrikant Y. Charde is Associate Professor and Head of Pharmacy Department at BITS Pilani, Hyderabad Campus. He completed his Bachelor of Pharmacy from University Department of Pharmaceutical Sciences, Nagpur in the year 1999 and Master of Pharmacy and Ph.D. from BITS Pilani. He has more than 13 years of teaching and research experience and is currently supervising four doctoral candidates. He has been associated with BITS Pilani as faculty since 2001. He has published research articles in renowned journals and presented papers in conferences in India and abroad. Prof. Shrikant Charde has successfully completed several government and industry sponsored projects. Presently he is undertaking UGC and DST projects. He is a life time member of Association of Pharmaceutical Teachers of India (APTI).

Biography of Murali Monohar Pandey

Mr. Murali Monohar Pandey has completed his Bachelor of Pharmacy from Department of Pharmacy, Jadavpur University, Kolkata, West Bengal in the year 2001 and Master of Technology (Biotech.) from Department of Life Science and Biotechnology of the same university in 2003. He served as a faculty member in various pharmacy institutions till 2007 and later joined as Lecturer in Department of Pharmacy, BITS Pilani, Pilani Campus in 2007. He registered in doctoral program of BITS Pilani in 2009. He has published research articles in reputed international journals and presented papers in national and international conferences. He is also a life time member of Association of Pharmaceutical Teachers of India (APTI).