Design and Evaluation of Bioadhesive Buccal Delivery Systems of Buspirone Hydrochloride

THESIS

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By

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Dedication

This work is dedicated to my parents Arikotla Prashanth Kumar and Padma Laxmi
All I have and will accomplish are only possible due to their love and sacrifices

Table of Contents

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Jaipal A

Date

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	Microgram hour per liter
µg/mL	Microgram per milliliter
µm	Micro meter
λ_{max}	Wavelength of maximum absorbance
(R)	Rectus
(S)	Sinistrus
<	Less than
=	Equal to
µg/l	Microgram per liter
µg/ml	Microgram per milliliter
µl	Micro liter
ACN	Acetonitrile
AT	Accelerated temperature (40 ± 2^{o}C/ 75 ± 5 % RH)
ATP	Adenosine tri phosphate
$AUC_{(0-\infty)}$	Area under plasma concentration-time curve
AUMC	Area under first moments curve of plasma concentration-time profile
BP	Blood pressure
BS	Buspirone Hydrochloride
CA	Citric acid
Ca^{2+}	Calcium ion
$CaSO_4$	Calcium Sulfate
cm^2	Centimeter square
C_{max}	Maximum plasma concentration
CMC	Carboxy methyl cellulose

Conc.	Concentration
CO_2	Carbon dioxide
CRT	Controlled room temperature ($25 \pm 2^oC/60 \pm 5$ % RH)
CS	Calcium Sulfate
CYP	Cytochrome
DSC	Differential scanning calorimetry
EDTA	Ethylene di amine tetra acetic acid
et al.	Co-workers
F	Calculated or tabulated value of statistical test analysis of variance
F_r	Relative bioavailability
FT	Refrigerated temperature (5 ± 2^oC)
FTIR	Fourier transform infrared
g	Gram
GI	Gastro-intestinal
GRAS	Generally recognized as safe
h	Hour
HCl	Hydrochloric acid
HEC	Hydroxy ethyl cellulose
HPC	Hydroxy propyl cellulose
HPLC	High performance liquid chromatography
HPMC	Hydroxy propyl methyl cellulose
HPMC K100M	Hydroxy propyl methyl cellulose 100000 cPs
HPMC K15M	Hydroxy propyl methyl cellulose 15000 cPs
HQC	Higher quality control sample
ICH	International conference on harmonization
I.M	Intramuscular
IR	Infrared
I.V	Intravenous
J/g	Joules per gram
K	Release rate constant
K^+	Potassium
K_{deg}	Degradation rate constant
l	Liter
l/day	Liter per day

l/kg	Liter per kilo gram
LCMS	Liquid chromatography coupled with mass spectrophotometer
Log % RTD	Log percentage remaining to be degraded
Log P	Log of oil water partition coefficient
LQC	Lower quality control sample
M	Molar
MCC	Microcrystalline cellulose
MCG	Membrane coating granule
mg	Milligram
mg/day	Milligram per day
mg/mL	Milligram per milli liter
min	Minutes
mL	Milli liter
mL/min	Milli liter per minute
mM	Millimolar
mm	Millimeter
mm/sec	Millimeter per second
MQC	Medium quality control sample
MW	Molecular weight
MWn	Molecular weight number
N	Newton
$NaHCO_3$	Sodium bicarbonate
ng/mL	Nanogram per milli liter
NIR	Near infra red
nm	Nanometer
°C	Degree centigrade
PC	Polycarbophil
PEO	Polyethylene oxide
pH	Negative log to the base 10 of hydrogen ion concentration
$P_{o/w}$	Apparent partition coefficient

QC	Quality control
R	Correlation coefficient
R^2	Regression coefficient
R_f	Retention factor
RH	Relative humidity
RPM	Revolutions per minute
RT	Retention time
SBC	Sodium bicarbonate
SD	Standard deviation
Sec	Seconds
SSF	Simulated salivary fluid
$t_{1/2}$	Half life
$t_{50\%}$	Time taken for 50% of drug release from formulations
$t_{90\%}$	Time taken for drug concentration to come down to 90% of original concentration
TLC	Thin layer chromatography
T_{max}	Time to reach maximum concentration
UK	United Kingdom
USFDA	United States Food and Drug Administration
USP	United States Pharmacopoeia
UV	Ultraviolet
v/v	Volume by volume
w/v	Weight by volume
VIS	Visible
XG	Xanthan gum
σ	Standard deviation of *y* intercept of regression equation
cPs	Centi poise
cm^{-1}	Centimeter inverse
h^{-1}	Hour inverse

List of Tables

List of Figures

Abstract

The objective of the present work was to design and evaluate bioadhesive buccal discs formulations of Buspirone Hydrochloride (BS). BS is an anxiolytic agent belonging to azaspirodecanediones class and is a partial agonist for the serotonin 5-HT1A receptors and is an antagonist for the dopamine D2 auto receptors and has weak affinity to 5-HT2 receptors. Buspirone Hydrochloride (BS) is an anxiolytic drug and marketed as oral tablet formulations with brand name Buspar®. Orally administered BS has very poor bioavailability and erratic drug absorption in presence and absence of food in stomach. In the present study buccal discs of BS were designed using various bioadhesive polymers and process excipients. Prior to formulation design, analytical methods were developed and validated for estimation of drug in variety of samples like bulk, formulations, stability, in-vitro and in-vivo samples. Adequate preformulation studies were carried out using instruments like DSC and FTIR 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.

Bioadhesive buccal discs of BS with 10 mg loading were prepared by direct compression method. Formulations were designed using various bioadhesive polymers like xanthan gum, hydroxy propyl methyl cellulose, hydroxy propyl cellulose and polycarbophil in varying proportions. Effect of permeation enhancer on bioavailability was also assessed. The designed buccal discs 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 bioadhesion studies were performed using texture analyzer instrument. Effect of polymer type, polymer proportion and process excipients on drug release and bioadhesive behavior was studied for the designed bioadhesive buccal discs.

Further, in-vivo bioavailability studies were performed for the designed bioadhesive buccal discs using rabbit model and pharmacokinetic parameters were obtained using suitable techniques.

Results indicated that all the developed and validated methods were accurate and precise for estimation of BS in variety of samples. Preformulation studies

indicated Form-1 of buspirone hydrochloride polymorph was used in the complete study. Log P values obtained for BS indicated relatively higher partitioning towards lipophilic phase. Drug was found to be compatible with all the process excipients used in the study.

The designed buccal discs demonstrated good physical characteristics with acceptable limits. Drug release and bioadhesion behavior for the designed buccal discs were completely rely on polymer type, polymer proportion, hydrophilicity or lipophilicity characteristics of polymer, polymer combination and type and amount of process excipients used.

Buccal discs designed using xanthan gum polymer demonstrated interaction between calcium sulfate and xanthan gum. Increase in calcium sulfate concentration increased drug release rate and decreased bioadhesive behavior. These results clearly indicate the interaction of calcium ions released from calcium sulfate with xanthan gum polymer. Further, in rheological evaluation it was observed that viscosity of xanthan gum gel reduces with increasing concentration of calcium sulfate.

HPMC K15 buccal discs were designed using varying proportions of mannitol. The drug release rate from delivery systems decreased with increasing levels of HPMC in formulations. However, bioadhesive strength of formulations increased with increasing proportion of HPMC in buccal discs. Increased levels of mannitol resulted in faster rate of drug release and rapid in-vitro uptake of water due to formation of channels in the matrix.

In the present study controlled release effervescent buccal discs of BS were also designed using HPMC K15 as rate controlling and bioadhesive polymer. Sodium bicarbonate and citric acid were used in varying amounts as effervescence forming agents. Carbon dioxide evolved due to reaction of sodium bicarbonate and citric acid was explored for its potential as buccal permeation enhancer. It was observed that effervescent buccal discs have faster drug release compared to non-effervescent buccal discs in-vitro. However, the amount of acid and base used for generation of carbon dioxide should be selected with care as this may damage the integrity of bioadhesive dosage form.

BS buccal discs were designed using varying proportion of PEO 1 lakh and PEO 40 lakhs grades. Drug release studies demonstrated retarded release with

increased proportion of PEO 40 lakhs due to greater viscous matrix by high molecular weight number PEO (40 Lakhs). PEO buccal discs demonstrated higher bioadhesive behavior compared to formulations designed in this study.

Bioadhesive buccal discs designed using hydroxy propyl cellulose with varying proportions of mannitol. Increase in mannitol concentration has considerably increased the release rate due to formation of more pores or channels in the matrix system. Similar results were observed when mannitol is used as a varying factor in design of PC buccal discs

The release data fitted best in the first order kinetic model The drug release mechanism was found to be non-fickian anomalous type for the designed bioadhesive buccal discs based upon n-value obtained from Korsmeyer- Peppas model.

In-vivo studies performed for the selected formulations in rabbits demonstrated significant increase in bioavailability compared to reported value of oral bioavailability. Effervescent buccal discs demonstrated considerable increase in bioavailability compared to non effervescent formulations and can be used as an alternative to improve the drug permeation resulting in better bioavailability

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 discs was relatively simple and can easily be adopted in conventional manufacturing units on a commercial scale.

1.1 Background

Oral route is most common and convenient of the existing methods for systemic delivery of drugs. It affords high patient acceptability, compliance and ease of administration. Moreover, delivery of drugs using oral route is cost effective (Shojaei, 1998). However, oral route is not suitable for delivery of drugs exhibiting extensive first pass metabolism or drugs with poor and erratic drug absorption. Drugs that degrade in gastric environment and drugs resulting in gastric irritation also cannot be administered orally. These factors severely limit the delivery of biopharmaceutically compromised drugs by oral route (Rossi et al., 2005).

Drug delivery via parenteral route overcome most of these drawbacks associated with orally less efficient drugs but is expensive. Parenteral drug delivery sometimes leads to serious hazardous effects and is patient incompliant. These drawbacks associated with parenteral and oral drug delivery resulted in a significant interest in exploring alternative routes for systemic delivery of such drugs. So, mucosal linings of ocular, nasal, rectal, buccal, sublingual, vaginal cavities are being increasingly explored for systemic delivery of drugs that are orally less effective (Mao et al., 2004; Patil et al., 2006). Nasal cavity offers a potential route for systemic delivery of small and large molecules and many such drugs have already been explored through this route (Cho et al., 2008). However, delivery of drugs through nasal and pulmonary routes has limitation in delivering large and uniform doses. Moreover, use of expensive devices, irritation, sensitivity reactions and damage to the ciliary action limits use of this route for chronic therapy (Marttin et al., 1998; Wang et al., 2006).

Rectal, vaginal and ocular routes have poor patient compliance for delivery of drugs to systemic blood circulation. These routes are more likely suitable for local action. Due to these disadvantages, transmucosal routes of drug delivery through oral cavity are being explored extensively (Shojaei, 1998; Sudhakar et al., 2006). Transmucosal (buccal and sublingual) delivery offers ease of administration, rapid onset of action, high blood levels and avoidance of first pass metabolism. Moreover, this route can be used for local delivery of drugs for treating periodontal diseases (Rossi et al., 2005). Sublingual route has substantially faster onset of action for some of the drug molecules. Controlled drug delivery is a major limitation for sublingual

route , as sublingual region lacks an expanse of smooth and immobile mucosa and is constantly washed by a considerable amount of saliva, making adhesion of dosage form difficult to the site (Figueiras et al., 2010).

Buccal route has widely been reported for successful delivery of small and large molecules to the systemic circulation. Drug delivery via buccal route has advantages like avoidance of first pass metabolism, predictable drug absorption, special devices are not required and limited exposure of drug molecules to harsh gastric environment (pH and enzymatic activity) (Giovino et al., 2012; Salamat-Miller et al., 2005; Sudhakar et al., 2006). Buccal route provides an additional advantage of ease of application and removal of buccal dosage form compared to other non-invasive mucosal routes mentioned earlier (Harris & Robinson, 1992; Kianfar et al., 2013).

However, like any other delivery route this route has some disadvantages. Limited surface area available for absorption of drug molecules through buccal mucosa limits use of this route for some drugs especially drugs with poor permeability. Barrier properties of buccal epithelium due to membrane coating granules and basement membrane further limit permeation of drugs. Saliva present in oral cavity has abundance of various enzymes needed for digestion of food and some of the drugs are good substrates for these enzymes (Şenel et al., 1997).

1.2 Structure and functions of buccal mucosa

Buccal mucosa of oral cavity has different distinct patterns of epithelium when observed under light microscopy. The oral cavity is lined with epithelium, below which lies the supporting basement membrane. The basement membrane is in turn supported by connective tissues (Figure 1.1). The epithelium serves as a protective layer for the tissues beneath. The epithelium is further distinguished into non-keratinized and keratinized mucosa in the oral cavity. Non-keratinized surface is present in the mucosal lining of the soft palate, the ventral surface of the tongue, the floor of the mouth, alveolar mucosa, vestibule, lips and cheeks. Keratinized epithelium is found in the hard palate and non-flexible regions of the oral cavity (Squier, 1991). The epithelium of the buccal mucosa is about 40-50 cell layers thick, while that of the sublingual epithelium contains somewhat fewer. The epithelial cells

increase in size and become flatter as they move from the basal layers to the superficial layers.

Epithelium lining of buccal mucosa is non-keratinized stratified squamous epithelium that has thickness of approximately 500–800 μm with surface area of nearly 50 cm^2 (Harris & Robinson, 1992). Blood supply to buccal mucosa is supplied by maxillary artery. The blood supply is faster and richer (2.4 $mL/min/cm^2$) than sublingual, gingival and palatal regions, facilitating passive diffusion of drug molecules across the mucosa (Gandhi & Robinson, 1988). Basement membrane, lamina propria followed by the submucosa is present below the epithelial layer, lamina propria is rich with blood vessels and capillaries that open to the internal jugular vein (Mathiowitz et al., 1995; Salamat-Miller et al., 2005).

The major function of buccal epithelium is the protection of the underlying tissue. In nonkeratinized regions, lipid-based permeability barriers in the outer epithelial layers protect the underlying tissues against fluid loss and entry of potentially harmful environmental agents such as antigens, carcinogens, microbial toxins and enzymes from foods and beverages (Squier, 1991).

Oral epithelium is lined with a ground substance called as mucus with thickness varying from 40 to 300 μm secreted by buccal glands. Primary function of mucus is protection against mechanical abrasion (Shojaei, 1998; Sudhakar et al., 2006). Mucus is a complex viscous secretion which is synthesized by specialized goblet cells. These goblet cells are glandular columnar epithelium cells and line all organs that are exposed to the external environment (Andrews et al., 2009). Mucus also serves as an effective delivery vehicle by acting as a lubricant allowing cells to move relative to one another and is believed to play a major role in adhesion of bioadhesive drug delivery systems. Mucus is mainly composed of mucins and inorganic salts suspended in aqueous medium. Mucins are large molecules, glycosylated proteins composed of oligosaccharide chains attached to a protein core with molecular masses ranging from 0.5 to over 20 MDa. Mucins contain approximately 70 to 80% carbohydrates, 12 to 25 % proteins and up to 5% ester sulfates (Salamat-Miller et al., 2005). The intense presence of carbohydrates in the mucin imparts water holding capacity and presence of glycosylated protein core provide gel like characteristics to mucin (Andrews et al., 2009; Leung & Robinson, 1990; Smart, 2005). Monosaccharide residues (approximately 8 to 10) are attached to

the side chains of the oligosaccharides present in mucus. Five different types of monosaccharides identified are L-fucose, D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and sialic acid. Mucus is negatively charged at physiological salivary pH 5.8 to 7.4 due to presence of sialic acid and ester sulfates (Gandhi & Robinson, 1988; Sriamornsak et al., 2008). Mucin is stocked in both submucosal and goblet cells. The negatively charged mucin glycoproteins are shielded by calcium ions, making the molecules compactly packed. During the release of mucin into the luminal space, outflux of calcium exposes these negative charges resulting in electrostatic repulsion and an approximate 400-fold expansion of the molecule. The elongated negatively charged mucin chains entangle and form non-covalent interactions (such as hydrogen, electrostatic, and hydrophobic bonds) leading to the formation of a viscoelastic gel. In the presence of water, the released mucin chains begin to overlap to form a structured network and mechanically functions as mucus (Andrews et al., 2009; Kočevar-Nared et al., 1997; Willits & Saltzman, 2001).

1.3 Barriers for drug transport

The absorption potential of buccal mucosa is highly influenced by the lipid solubility, pH and molecular weight of the diffusant (Mørck Nielsen & Rømer Rassing, 2000). Primary barriers for drug absorption from the buccal epithelial membrane are membrane coating granules (MCG's) or cored granule or lamellar granules present in the outermost 200 μm of the superficial layer (Rossi et al., 2005; Squier, 1991). The membrane coating granules (MCG's) exist in both keratinized and non-keratinized epithelia. MCG's are spherical in shape, membrane bounded and measure about 0.2 μm in diameter (Squier, 1991). MCG's are present in the intermediate cell layers of stratified epithelia and migrate towards distal plasma membrane. These MCG's fuse with plasma membrane and the lipid extrude of these granules discharged into intracellular space acts as major barrier for transcellular and paracellular transport of therapeutic molecules. This barrier exists in the outermost 200 μm of the superficial layer. Although the superficial layers of the oral epithelium represent the primary barrier to the entry of substances from the exterior, it is evident that the basement membrane also plays a major role in limiting the passage of therapeutic drug molecules across the junction between epithelium and connective tissue (Sudhakar et al., 2006).

Salivary fluid is also one of the major barriers for buccal absorption of drugs. Saliva is secreted majorly from three glands, the parotid (40%), submandibular (40%) and sublingual glands (10%) and also from some minor salivary glands (10%). Salivary secretions also contain adequate digestive enzymes such as α-amylase, lingual lipase, kallikrein and several antimicrobial substances, such as immunoglobulin's (IgA, IgM and IgG), lysozymes and other antimicrobial proteins and peptides (Hearnden et al., 2012; Lam et al., 2014). Salivary fluids act as dissolution medium and drain the drug into the gastro intestinal tract, resulting in loss of drug and poor exposure to buccal epithelium. Presence of several enzymes in the salivary fluids lead to decreased drug passage into the systemic circulation due degradation of drug, especially protein and peptides (Rathbone & Tucker, 1993; Veuillez et al., 2001).

1.4 Mechanisms of drug transport

The drug transport mechanism from mucosal epithelium of buccal involves two major routes, that is transcellular (Intracellular) and paracellular (Intercellular) pathways by passive diffusion. The absorption potential of drug molecules is influenced by the lipid solubility and molecular weight of drug molecules (Sudhakar et al., 2006). Reports suggest that large and hydrophilic molecules predominantly permeate via transcellular pathway and small and lipophilic molecules via paracellular pathway (Kitano et al., 1998). However rate of drug molecule permeation largely depends on physicochemical characteristics of the drug molecules. Carrier pH has also been reported to have pronounced effect on drug permeation in in-vitro studies (Mørck Nielsen & Rømer Rassing, 2000). Non-ionized drug have been reported to permeate across buccal mucosa to a greater extent as compared to ionized compounds. Manipulation of microenvironment pH using drug delivery system helps in achieving desirable permeation rate by controlling the ratio of unionized and ionized species of drug (Mashru et al., 2005).

1.5 Bioadhesion

Bioadhesion is a process by which synthetic and natural polymers adhere to biological tissue or membrane (Woodley, 2001) and mucoadhesion is a process where the natural and synthetic polymers adhere to mucus secreting biological membrane specifically. However, both bioadhesion and mucoadhesion terms are used

interchangeably, as mucus membrane is also considered as a biological membrane. For attaining bioadhesive behavior of dosage forms, several types of bioadhesive polymers are used in designing buccal drug delivery systems. The bioadhesive polymers have ability either to solublilize or swell in presence of water to form viscous fluids. These polymers used in the drug delivery attract water from the biological membrane when present as dry form resulting in strong interaction with mucus membrane components (Gandhi & Robinson, 1988; Peppas & Sahlin, 1996; Sudhakar et al., 2006). Bioadhesive polymers should posses hydrogen bond forming groups, viscoelastic properties and flexibility for interpenetration with mucus layers (Andrews et al., 2009; Jaipal et al., 2013; Leung & Robinson, 1990; Smart, 2005; Woodley, 2001).

1.5.1 Theories of bioadhesion

Bioadhesion is an interfacial process involving classical colloids and surface science which involves several mechanisms and steps (Sudhakar et al., 2006). Various theories for bioadhesion are reported in literature, these include electronic theory, adsorption theory, wetting theory, diffusion-interlocking or diffusion interpenetration theory and fracture theory (Jiménez-castellanos et al., 1993). Whilst the most accepted theory among the previously proposed is diffusion-interpenetration theory (Andrews et al., 2009) and other presented theories are considered as supplementary processes involved in various stages of interaction between mucus composites and polymer/substrates used (Andrews et al., 2009; Duchêne & Ponchel, 1997; Mathiowitz et al., 1995; Peppas & Sahlin, 1996).

Electronic theory proposes mechanism of bioadhesion by transfer of electrons between mucus and bioadhesive substrate system resulting in formation of attractive forces. The attractive forces include Van der waals forces. This can further be subdivided in three components namely London dispersion forces, dipole-dipole interactions, Debye interactions. These van der waals forces are most prominent form of surface interaction in bioadhesion by forming a weaker and semi permanent bonds between adhesive polymer and mucus substance (Lam et al., 2014; Salamat-Miller et al., 2005).

Adsorption theory is defined as a surface interaction phenomenon caused due to formation of primary and secondary bonding between adhesive polymer and mucus

substrate. Primary bonding is caused due to ionic, covalent and metallic bonding, while the secondary bonding occurs due to van der waals forces (Shinkar et al., 2012; Sudhakar et al., 2006).

The wetting theory emphasizes spreadability of drug delivery systems on the biological membrane as one of the most important parameter for bioadhesion. The wetting theory hypothesizes penetration of adhesives substrate into the irregularities of biological membrane thereby resulting in bioadhesion with the surface. Substantial bioadhesive behavior is governed by the surface contact angle of the adhesive substrate. The surface contact angle defines the energy required to counter the surface tension at the interface between the adhesive substance and mucus substrate (Shojaei & Li, 1997; Ugwoke et al., 2005).

Diffusion interlocking theory proposes that bioadhesion occurs due to diffusion of the polymer chains into the glycoprotein chain network of the mucosal substrate or diffusion of mucus glycoprotein chains into bioadhesive drug delivery systems and interlocking of these chains (Duchêne & Ponchel, 1997; Gandhi & Robinson, 1988; Ludwig, 2005). Several properties of both the adhesive polymer and mucus glycoproteins are responsible for efficient bioadhesion by this mechanism; the critical properties include molecular weight, crosslinking density, chain mobility and flexibility, miscibility and expansion capacity of both the networks. Longer polymer chains may diffuse and entangle with mucus glycoprotein's leading to increase in bioadhesion. Intense penetration and crosslinking of the networks decrease the polymer chain mobility and interpenetration and hence bioadhesive strength (Jabbari & Peppas, 1995; Jaipal et al., 2013).

Fracture theory states that the work required to detach the adhesive bond between the bioadhesive polymer and mucus substrate can be correlated to bioadhesive strength. The longer the polymer strand or reduction in degree of crosslinking within the polymer system, the greater the work of fracture (Andrews et al., 2009). Fracture strength (σ) is determined by separation of two surfaces via its relationship to Young's modulus of elasticity (E), the fracture energy (ε) and the critical crack length (L) by using the following equation (Ahagon & Gent, 1975; Mathiowitz et al., 1995).

$$\sigma = \left(\frac{E \times \varepsilon}{L}\right)^{\frac{1}{2}} \quad \text{(Eq 1)}$$

1.5.2 Factors effecting bioadhesion

1.5.2.1 Effect of polymer functional group

Process of bioadhesion involves mainly interpenetration of mucus and polymer chains followed by formation of secondary non-covalent bonds mainly due to formation of hydrogen bonding. Many natural, synthetic and semi synthetic polymers, containing hydrophilic networks that contain numerous polar functional groups (such as -COOH, -OH, $-NH_2$ and SO_4), have been reported to have considerable bioadhesion (Smart, 2005). These functional groups have ability to interact with glycoprotein chains of mucin. The polymeric chains initially hydrolyze to form gel by diffusion of fluid from the surrounding environment and subsequently the polymeric chains entangle or interlock with glycoprotein chains of mucin at molecular level to form weak crosslinked bonds resulting in bioadhesion. The extent of bioadhesion depends on free polymeric chains available for interlocking and crosslinking (Andrews et al., 2009; Capra et al., 2007).

1.5.2.2 Degree of crosslinking

The degree of crosslinking within the polymer system considerably influences the chain mobility and resistance to dissolution. In presence of aqueous medium, linear hydrophilic polymer readily swells and disperses, whereas, crosslinked hydrophilic polymers swell in aqueous medium retaining their structure leading to enhanced bioadhesion. As cross-link density increases, chain mobility decreases and hence the effective chain length, which can penetrate into the mucus layer decreases, reducing bioadhesive strength. However, molecular weight of the polymer plays a significant role in bioadhesion (Peppas & Sahlin, 1996; Şenel et al., 1998). Increase in polymer molecular weight will substantially result in higher bioadhesion; moreover, increase in polymer chain length will decrease the bioadhesion ability due to poor flexibility. Polymer chain flexibility is one of the major considerations for interpenetration and interlocking with mucus substrate (Hearnden et al., 2012). Enhanced polymer chain mobility results in increased interpenetration of polymer chains into mucus and leading to greater extent of bioadhesion.

1.5.2.3 Degree of hydration

Hydration behavior of polymer is considered as one of the major factor for bioadhesive behavior. Several polymers exhibit bioadhesive behavior in the presence of limited water. Hydration is essential for relaxation of polymer and interpenetration of polymeric chain into mucosal surface. Excess hydration may also result in decreased bioadhesive behavior (Andrews et al., 2009; Hägerström et al., 2000; Sudhakar et al., 2006). Rate of hydration of polymer in the buccal drug delivery systems also depends on hydrophilic excipients such as mannitol, lactose used in the dosage form. These hydrophilic excipients forms channels or pores when in contact with water exposing the polymer to higher amount of aqueous medium (Kianfar et al., 2013; Woodley, 2001).

1.5.2.4 Polymer concentration

Amount of polymer in the delivery system has substantial influence on bioadhesive behavior. Increase in polymer concentration in solid buccal dosage forms has shown considerable increase in bioadhesive behavior. Whereas, in semisolid buccal dosage forms optimum polymer concentration exhibits good bioadhesion. Increase in polymer concentration beyond optimal concentration in semisolid dosage forms decreases bioadhesion behavior. This decreased adhesive behavior is may be due to decreased flexibility of polymeric chains (Ugwoke et al., 2005).

1.5.2.5 Charge and pH

Dissociation of polymer functional groups is influenced by the physiological pH of the mucosal environment (Mathiowitz et al., 1995). Ionized polyacrylic acid polymer systems repel and exhibit poor bioadhesion due to their negative charge similar to mucin (Guggi et al., 2004). Cationic polymers such as chitosan demonstrate positive charge and might form polyelectrolyte complexes with negatively charged mucin resulting in stronger bioadhesion (Peppas & Huang, 2004; Rai et al., 2011).

1.5.2.6 Environment and physiological factors affecting bioadhesion

Several environmental and physiological factors have discernible effect on bioadhesive behavior. Mucus turn over in the buccal cavity is one of the major factors affecting bioadhesion. The adhesion time of designed delivery system largely depend on the turnover time of mucosal substrate on the tissue (Peppas & Huang, 2004). Disease state is also critical factor in bioadhesion of polymer systems; several oral

infections will eventually influence the production of mucus secretion resulting in altered polymer mucin interactions (Ugwoke et al., 2005). Ionic strength of the surrounding mucus and excipients in the delivery system also influences the extent of bioadhesion (Jaipal et al., 2013). Increase in ionic concentration in mucus demonstrates decrease in bioadhesion strength; this effect is due to shielding of polymer chain function groups. However, it is also noted that some of the polymers like gellan gum demonstrated increased bioadhesion due to increase in viscosity in the presence of cations and elevated pH (Gohel et al., 2009).

1.6 Pharmaceutical factors in design of buccal dosage forms

Various factors need to be considered while designing appropriate buccal drug delivery systems. An ideal buccal dosage form should have desirable bioadhesive strength and should lead to proper permeation of drug being delivered. Controlled drug release while not mandatory is useful for drugs with short half life for reduction of dosing frequency and better prognosis (Guggi et al., 2004; Hauptstein et al., 2013; Kotagale et al., 2010; Rossi et al., 2005). Buccal bioadhesive drug delivery systems are intended either to deliver drug to systemic circulation (Jug et al., 2010; Martin et al., 2003) or for local action (Jones et al., 2000; Perumal et al., 2008; Preis et al., 2014; Şenel et al., 1998; Shinkar et al., 2012). Unidirectional design approach for buccal delivery systems prevent drug loss due to salivary clearance, one of such approach is Buccastem®, an adhesive antiemetic tablet with backing membrane containing prochlorperazine maleate (Hessell et al., 1989).

1.6.1 Permeation enhancers

The major limitations in delivering drugs via buccal route are permeability barrier of the mucosa and delivery of large doses due to lesser area available for absorption. Mucosal membrane characteristics can be altered by the use of permeation enhancers; this increases the rate of drug permeation and opportunity for designing higher dose therapeutic molecules via buccal route (Şenel & Hıncal, 2001; Veuillez et al., 2001). Ideal permeation enhancer used in buccal formulations should be non-toxic at their effective concentration and must not cause any permanent damage to mucosal membrane; these agents must be transient and reversible (Lam et al., 2014).

Several chemical substances as permeation enhancers to decrease the permeability barrier have been reported in the literature (Table 1.1). The proposed mechanisms of increased permeation is by

- Altering the mucus rheology, by reducing the viscosity and elasticity of mucus layer.
- Increasing membrane fluidity and facilitating transcellular transport through interaction with their lipidic or proteic membrane components.
- Facilitating paracellular transport.
- Overcoming the enzymatic barrier for peptides.

Based on the type, chemical permeation enhancers are classified into various classes such as surfactants, alcohols, chelators, fatty acids, bile salts and other miscellaneous agents.

1.6.1.1 Surfactants and bile salts

Surfactants are the class of permeation enhancer that is widely explored for many small and large molecules. Surfactants are believed to change the membrane characteristics by altering the lipid structures and expansion of intercellular spaces (Şenel & Hıncal, 2001; Veuillez et al., 2001). However the concentration and exposure time may lead to side effects, such as protein denaturation, swelling and irritation of mucosal tissue, extraction of lipid components depending on the type of surfactant used. Permeation of salicylic acid in presence of surfactants, such as sodium lauryl sulfate (SLS), cetylpyridinium chloride, polysorbate 80 and sodium taurocholate was studied at different pH values of 3.0, 4.0 and 7.0 in-vitro using a hamster cheek pouch. The results demonstrated concentration dependent permeation for SLS and cetylpyridinium chloride, decreased absorption of salicylic acid in the presence of polysorbate 80 was observed in the lower pH conditions and this phenomenon was explained by the decrease in the free fraction of salicylic acid. (Kurosaki et al., 1988). Buccal in-vitro permeation studies of triamcinolone acetonide reported enhanced permeation in presence of non ionic surfactants (polyoxyethy lene 2-stearyl ether, polyoxyethylene 2-oleyl ether, polyoxyethylene 23-lauryl ether) and bile salts (Sodium deoxycholate, sodium taurodeoxycholate, sodium cholate) in comparison to glycols (tetraethylene glycol , diethylene glycol) (Shin & Kim, 2000).

It was also observed that use of SLS as permeation enhancer caused significant removal of superficial mucosal cell, from this study it is evident that surfactants not only change the membrane characteristics, it also results in damage to mucosal surface (Gandhi & Robinson, 1992; Nicolazzo et al., 2004a).

Bile salts are semi synthetic surfactants widely explored for permeation studies. Bile salts acts as permeation enhancer due to solubilization of epithelial lipids by micellization leading to increased mucosal membrane permeability. The extent of permeability also dependent on bile salts concentration. Increase in rate and amount of absorption of buserelin was observed with higher concentration of glucodeoxycholate (Hoogstraate et al., 1996). Reports suggest that bile salts markedly increase the absorption of several protein and peptide molecules (Birudaraj et al., 2005; Nicolazzo et al., 2005; Şenel & Hıncal, 2001; Shin & Kim, 2000). The apparent permeability of the permeate is dependent on the concentration, degree of hydroxylation and type of conjugation to the bile salts (Nielsen & Rassing, 1999).

1.6.1.2 Fatty acids

Several fatty acids have been explored for enhancing drug permeation through buccal mucosa; some of the fatty acids are listed Table 1.1. Permeation effect of fatty acids depends on the presence and the position of double bonds, isomer type (cis- or trans-), chain length and the degree of branching. Unsaturated fatty acids have demonstrated more disruptive behavior than saturated fatty acids having the same carbon number (Aungst & Rogers, 1989; Veuillez et al., 2001). Sodium laurate demonstrated enhanced permeation of insulin (Aungst & Rogers, 1989) and myristate has shown increased calcitonin permeation in-vitro (Nakada et al., 1988).

1.6.1.3 Enzyme inhibitors

Peptidases present in buccal region limits the delivery of peptide therapeutic molecules. Several peptidases such as aminopeptidase, carboxypeptidases, dipeptidyl peptidase, elastase trypsin, serine endopeptidase have been identified in buccal tissue (Aungst & Rogers, 1989; Lee & Yamamoto, 1989). Aprotinin, a serine protease inhibitor, reduced the metabolism of insulin and proinsulin by approximately 70 to 80% within 2.5 h in homogenates of the albino rabbit buccal mucosa (Lee & Yamamoto, 1989). Peptidase inhibitors can be used either alone or in combination

with permeation enhancers to overcome the enzymatic barrier (Aungst & Rogers, 1989).

Other miscellaneous permeation enhancers such as azone (1-Dodecylazacycloheptan-2-one), a hydrophobic substance was also used to promote absorption of octreotide through oral mucosa (Merkle & Wolany, 1992). Solvents such as ethanol (Steward et al., 1994), lauric acid in propylene glycol have also been reported as permeation enhancer for peptides (Aungst & Rogers, 1989).

1.6.1.4 Gas Permeation enhancers

Carbon dioxide gas has been reported in literature as a permeation enhancer using both in vitro (Eichman & Robinson, 1998) and in vivo studies (Darwish et al., 2006a; Darwish et al., 2006b; Jaipal et al., 2014; Tadros, 2010; Wang & Tang, 2008). Increase in drug permeation across rabbit ileum has been reported in vitro when permeation experiment was performed by bubbling carbon dioxide (Eichman & Robinson, 1998). Fentanyl effervescent buccal tablets also resulted in rapid and significantly higher amount of drug in systemic circulation compared to non-effervescent buccal tablets in human volunteers (Darwish et al., 2006a; Darwish et al., 2006b). Enhanced permeation of Insulin using effervescent formulations in ex vivo studies have also been reported (Sadeghi et al., 2009).

1.6.2 Bioadhesive polymers

Bioadhesive polymers in the buccal dosage forms play an important role in delivery of the drugs to systemic circulation by adhering to the mucosal surface for longer duration of time and releasing the drug in a controlled fashion (Abruzzo et al., 2012; Andrews et al., 2009; Gandhi & Robinson, 1988; Rossi et al., 2005). Early or first generation bioadhesive polymers offer a non-specific interaction with the mucosal surface, usually through the hydrogen bonding or electrostatic interaction. The second or next generation of bioadhesive polymers results in specific and precise interactions with the mucosal surface (Lam et al., 2014; Langoth et al., 2003). Bioadhesive polymers are listed in Table 1.2, depending on source (natural, semi synthetic and synthetic), charge (non-ionic, anionic and cationic) and based on the solubility (hydrophilic or lipophilic).

Anionic polymers are characterized by the presence of carboxyl and sulfate functional groups that give rise to a net negative charge at pH values above the pKa

value of polymer. This type of polymers exhibit considerably high bioadhesive behavior and have been studied extensively for the design of buccal formulations (Andrews et al., 2009). Some of the representative polymers in this type include sodium carboxy methyl cellulose and crosslink derivatives of polyacrylic acid, such as carbopol (acrylic acid, crosslinked with an allyl ether pentaerythritol, allyl ether of sucrose or allyl ether of propylene) and polycarbophil (acrylic acid polymer crosslinked with divinyl glycol). Poly acrylic acid derivatives are available in variety of molecular weight and easily forms modified gel networks, these polymers are extensive used in many commercially available oral dosage forms due to well established safety profiles. Polyacrylic polymers are GRAS (Generally Recognized as Safe) listed substances (Ugwoke et al., 2005). Polycarbophil is a water insoluble polymer, but has swelling capacity in neutral pH medium and has widely been reported for its bioadhesive behavior (Ravi Kumar Reddy et al., 2013). The carboxy groups present in polycarbophil forms hydrogen bonding with mucin leading to strong bioadhesion (Eouani et al., 2001; Ludwig, 2005). Similarly, carbopol also demonstrates excellent bioadhesive behavior to that of polycarbophil, as both the polymers contain acrylic backbone and differ in type and degree of crosslinking (Andrews et al., 2009). Sodium carboxy methyl cellulose (NaCMC), a cellulose derivative also exhibits a bioadhesive behavior similar to that of poly acrylic acid (Le Bourlais et al., 1998). NaCMC has been widely reported for design of buccal delivery systems (Eouani et al., 2001; Mohammadi-Samani et al., 2005; Ravi Kumar Reddy et al., 2013). Xanthan gum is a high molecular weight anionic polysaccharide gum produced by aerobic fermentation of sugars by the bacterium xanthomonas campestris. Xanthan gum is a biopolymer with vast applications in food, cosmetic, agricultural, textile, petroleum and pharmaceutical industry (Garcia-Ochoa et al., 2000; Mirhosseini et al., 2008). USFDA has approved xanthan gum as GRAS (Generally Recognized as Safe) listed chemical for use in pharmaceutical, food and cosmetic preparations. Bioadhesive behavior of xanthan gum has been widely reported (Abu-Huwaij et al., 2011; Jaipal et al., 2013; Park & Munday, 2004).

Chitosan, a cationic biodegradable and biocompatible biopolymer and has been most extensively investigated for bioadhesive buccal formulations (Ayensu et al., 2012b; Ayensu et al., 2012c; Cid et al., 2012; Giovino et al., 2012, 2013; Pongjanyakul et al., 2013; Sander et al., 2013). Chitosan is a cationic polysaccharide,

produced by the deacetylation of chitin, the most abundant polysaccharide in the world, next to cellulose (Elsabee & Abdou, 2013; Geisberger et al., 2013). Reports suggest that bioadhesion mechanism of chitosan is by ionic interactions between primary amino functional groups and the sialic acid and sulphonic acid substructures of mucus (Hassan et al., 2010; Portero et al., 2007; Rossi et al., 2005). Chitosan can be tailored by adding various functional groups, using this modification chitosan can be customized to suit various formulation requirements (Geisberger et al., 2013; Martin et al., 2003; Sandri et al., 2005).

Several non-ionic polymers like Poly ethylene oxide (PEO) (El-Samaligy et al. 2004; Cappello et al. 2006; Miro et al. 2013), cellulose derivatives such as hydroxyl propyl cellulose (HPC)(Han et al., 1999; Kohda et al., 1997; Park & Munday, 2002), hydroxy propyl methyl cellulose (HPMC)(Kundu et al., 2008; Ravi Kumar Reddy et al., 2013), methyl cellulose (MC) (Govindasamy et al., 2013; Meher et al., 2013) and vinyl polymers such as polyvinyl pyrrolidone (PVP)(Diaz del Consuelo et al., 2007; Ravi Kumar Reddy et al., 2013) have been extensively reported for the design of various bioadhesive buccal dosage forms.

1.6.2.1 Second generation polymers

The second generation polymers refers to bioadhesive polymers with improved and specific chemical interaction or covalent bond with mucin or mucosal membrane exhibiting an immense bioadhesive behavior compared to that of older generation polymers (hydrogen bonding) (Andrews et al., 2009; Salamat-Miller et al., 2005; Shinkar et al., 2012; Smart, 2005). Lectins are considered as one of the second generation bioadhesive polymer. Lectin is a naturally occurring specific carbohydrate binding proteins that play a fundamental role in biological recognition phenomena involving cells and proteins (Berg et al., 2002). Lectins acts as bioadhesive substrate by potentially binding to the mucosal cells present on the oral mucosa surfaces of the mouth (Smart, 2005; Smart et al., 2002). Thiolated polymers are also considered as second generation polymers. Cysteine attached covalently to polycarbophil demonstrated enhanced bioadhesive strength up to 2 to 4 times to that of unmodified polycarbophil (Kast & Bernkop-Schnürch, 2002). Increased bioadhesive behavior of thiolated polymer is either due to formation of disulfide bonds between the thiomer and the mucus layer or may be due to in-situ crosslinking of thiomers with mucus glycoproteins (Bernkop-Schnürch, 2005).

1.7 Other excipients used in buccal dosage forms

Taste is a critical factor in achieving patient acceptability and oral cavity is the major site for taste sensing organ, tounge. Sweetening and flavoring agents (such as mannitol, menthol) should be used if necessary to mask the taste of the drug and make the buccal dosage form palatable (Chinna Reddy et al., 2011; Starokadomskyy & Dubey, 2006; Sudhakar et al., 2006).

1.8 Buccal dosage forms

In the recent past buccal dosage forms gained a significant interest in delivering orally inefficient drugs to the systemic circulation, this attention towards buccal route was due to non-invasive administration. Several buccal dosage forms are available as commercial products and some are in clinical stages of development (Table 1.3). Advantages and disadvantages of bioadhesive buccal formulations were mentioned in section 1.1 of this chapter. Bioadhesive polymers used in the design of buccal dosage forms play an important role in delivery of the drugs to systemic circulation by adhering to the mucosal surface for longer duration of time and releasing the drug in a controlled fashion (Ayensu et al., 2012a; Kianfar et al., 2012; Rossi et al., 2005; Salamat-Miller et al., 2005).

Various buccal dosage forms such as tablets (Choi & Kim, 2000), lozenges (Codd & Deasy, 1998), discs (Yehia et al., 2008), gels (Morishita et al., 2001), sprays (Pozzilli et al., 2005), solutions (Muchohi et al., 2008), patches or films or wafers (Ayensu et al., 2012b; Giovino et al., 2012; Khanna, 1997; Kianfar et al., 2012) have been reported for delivery of drugs to systemic and for local action. Overall these dosage forms can be classified as solid, semisolid and liquid buccal bioadhesive dosage forms

1.8.1 Bioadhesive buccal tablets

Commercially several buccal dosage forms are available and some are in their development stages (Chai et al., 2013). Tablets gained a significant interest due to accurate dose administration and are sufficiently robust to endure the physical forces experienced during handling and transportation.

Bioadhesive polymers and permeation enhancers are commonly employed in the oral tablet transmucosal delivery system to improve the bioavailability and onset of action. Buccal tablets are widely reported for the delivery of orally compromised

drugs into systemic circulation and in a controlled fashion (Boyapally et al., 2010; Kanjanabat & Pongjanyakul, 2011; Mumtaz & Ch'ng, 1995).

1.8.2 Buccal discs

Buccal discs are flat, thin solid unit non-flexible compacts; and are similar to buccal tablets. Buccal discs are designed to minimize the discomfort caused due to bulky tablet buccal dosage forms. Several buccal discs have been extensively reported for successful delivery of therapeutic drug molecules (El-Samaligy et al., 2004; Han et al., 1999; Jaipal et al., 2013; Jaipal et al., 2014; Sander et al., 2013; Yehia et al., 2008).

1.8.3 Buccal films

Buccal bioadhesive films offer several advantages over buccal tablets such as flexibility of dosage form reducing discomfort in oral cavity and with precise dose unlike gels and solutions (Elsabee & Abdou, 2013; Kianfar et al., 2012; Peh & Wong, 1999; Skulason et al., 2009). Buccal films designed using several natural and synthetic polymers were extensively reported for delivery of small and large molecules (Giovino et al., 2012; Pongjanyakul et al., 2013). Insulin loaded nanoparticles embedded in chitosan films has demonstrated good physical characteristics and sustained drug release in in-vitro (Giovino et al., 2013). Buccal films designed using poly ethylene oxide in combination with cyclodextrin has shown synergetic effect on drug solubility and complete absorption at the site of application (Miro et al., 2013). Tamarind seed xyloglucan, a natural polysaccharide polymer extracted from tamarind seeds was used to design buccal films for systemic delivery of rizatriptan benzoate (Avachat et al., 2013).

1.8.4 Buccal gels

Buccal bioadhesive gels or hydrogels have been widely investigated for delivery of drugs for both local (Jones et al., 2000; Needleman & Smales, 1995; Shinkar et al., 2012) and systemic conditions (Shin & Kim, 2000). Insulin loaded pluronic F-127 buccal gel was reported to investigate the hypoglycemic effect in normal rats (Morishita et al., 2001). Xerogels are also reported for delivery of therapeutic drug molecules, wet gels are often dried by evaporation or lypophilization to produce so called xerogels (Ayensu et al., 2012c).

1.8.5 Buccal sprays

The aerosolized spray is another suitable alternative to deliver drug onto the buccal mucosal surface. Delivery of accurate dose is a major problem and can be controlled using a metered dose measurement and loss of drug through saliva is unpredictable with fluctuations in drug absorption (Chinna Reddy et al., 2011). Report suggest that buccal spray formulation of insulin is as effective as subcutaneous route in lowering blood glucose levels (Pozzilli et al., 2005).

1.9 In-vitro characterization

In-vitro drug characterization of dosage form is a measure of product performance in simulated controlled laboratory environment to predict the in-vivo performance of the designed formulation. In development phase, in-vitro conditions are generally selected to simulate in-vivo conditions. In quality control it is used to assess conformance of a batch to pre-determined criteria at time of manufacture and to assess the long-term product stability. In this use, in-vitro test conditions are chosen to relate the changes in the drug product. It is an important tool in evaluating drug product performance for most dosage forms.

1.9.1 Bioadhesion/Mucoadhesion

Bioadhesion is adhesion of material to biological membrane or mucosal membrane. A prolonged contact of dosage form to biological membrane site will improve the bioavailability of the drug. Several drugs have very poor permeability from the buccal epithelial lining. Bioadhesive polymers adhere to the mucosal surface increasing the contact time of drugs with mucosal surface resulting in better absorption. A large number of methods found in the literature are based on the measurement of the force necessary to separate a bioadhesive material from a biological membrane. Peel, shear and tensile forces can be determined depending on the direction in which the mucoadhesive material is detached from the biological surface. Figure 1.2 depicts the various direction of force applied for tensile, shear and peel strengths determination (Duchêne & Ponchel, 1997; Gandhi & Robinson, 1988; Woodley, 2001).

1.9.1.1 Texture analyzer

Texture analyzer equipment has been used extensively in reports to measure the tensile strength as adhesive property for several types of dosage forms and

polymers, including buccal drug delivery systems. The instrument measures the force required to detach the dosage form from biological or mucosal membrane in presence of simulated buccal conditions and work of adhesion can also be calculated. However, some of the instrument variables such as contact force, contact time and the speed of withdrawal of probe from the tissue influence the bioadhesive behavior and need to be optimized. The maximum detachment force and total work of adhesion can be measured using texture analyzer equipment in several bioadhesive measurements of buccal dosage forms reported (Ayensu et al., 2012a; Giovino et al., 2013; Ivarsson & Wahlgren, 2012; Peh & Wong, 1999).

The texture analyzer equipment consists of a tissue holder, to mount or the fix selected mucosal tissue. On top of the tissue holder, there is a 14 mm diameter hole. Thus, the probe with 10 mm diameter can get in contact with the tissue. The test rig is placed in a beaker with simulated salivary fluid or medium and can be heated up to a defined body temperature. During measurements, the medium is stirred with a magnetic stir bar. The formulation is attached to the lower side of the probe using a double-sided adhesive tape. To start the experiment, the probe is lowered to the mucosa with a defined pretest speed. After reaching the trigger force, the probe with the bioadhesive formulation presses down to the mucosa for a defined time with a defined force. Subsequently, the probe moves up until the adhesive bond is broken (Charde et al., 2008; Giovino et al., 2013; Hauptstein et al., 2013; Needleman & Smales, 1995; Peh & Wong, 1999; Woertz et al., 2014).

1.9.1.2 Modified balance method

The force required to detach bioadhesive dosage form from the mucosal surface is used as a measure of the bioadhesive strength. Bioadhesive measurements are carried out on a specially fabricated physical balance assembly. The right side pan of the balance is glued with a buccal dosage form. The balance was adjusted for equal oscillation by keeping sufficient weight on the left pan and the dosage form was bought in contact with pre moistened mucosa. Subsequently the weights were increased in small increments on the left pan until the attachment breaks. The difference in final and initial weight is recorded as bioadhesive strength (Govindasamy et al., 2013; Pendekal & Tegginamat, 2012).

1.9.2 In-vitro drug release

In-vitro drug release testing is a measure of release of the active pharmaceutical ingredient (API) from the drug product matrix in controlled laboratory environment. It is also key evaluation in drug development and quality control. It involves subjecting the dosage form to a set of conditions that will induce drug release and quantitating the amount of drug released under those conditions, during this phase, in-vitro conditions are generally selected to simulate in-vivo condition (Heigoldt et al., 2010). The goals of a dissolution test include prediction of bioavailability (a surrogate parameter of the therapeutic efficacy), indication of the robustness of the dosage form (drug product safety) and implication of variations in the manufacturing process (Agoram et al., 2001; Borkar et al., 2014; Colombo, 1993; Siepmann & Peppas, 2012). USFDA recommends dissolution testing methods for some of the drugs designed as buccal dosage forms (Table 1.4). However, most of the reports suggest various in-vitro drug release methods using different USP types of dissolution apparatus and its modified versions using various buffer systems (Adhikari et al., 2010; Ayensu et al., 2012a; Charde et al., 2008; Giovino et al., 2012; Jaipal et al., 2013; Kaur & Kaur, 2012; Meher et al., 2013; Patel et al., 2012; Shidhaye et al., 2010).

Drug release rate from hydrophilic matrix systems depends on swelling behavior of the polymer, shape of the matrices, and diffusion and erosion properties of the polymer and dissolution characteristics of the drug. Dose and solubility of the drug, type and quantity of the fillers and the polymer characteristics influence the mechanism of the drug release (Baveja et al., 1988; Gurny et al., 1982; Korsmeyer et al., 1983a; Korsmeyer et al., 1983b; Peppas & Sahlin, 1996).

1.9.3 In-vitro permeation studies

In-vitro buccal permeation studies using animal buccal mucosa as a model for the purpose of predicating transbuccal drug absorption kinetics have been extensively reported in literature (Govindasamy et al., 2013; Jacobsen, 2001; Kitano et al., 1998; Langoth et al., 2005a). The criterion for selection of buccal mucosa is based on similarity of keratinization, thickness and lipid composition. Buccal tissue of rats (Figueiras et al., 2009; Tsagogiorgas et al., 2013), hamsters (Tsutsumi et al., 1998), pigs (Langoth et al., 2005b; Lee & Kellaway, 2000; Veuillez et al., 2002), dogs

(Voorspoels et al., 1996; Zhang et al., 1994) and monkeys (Mehta et al., 1991) are frequently used to investigate drug permeation. Buccal mucosa of rabbit is unevenly non-keratinized in parts but after careful isolation of excised mucosa, non-keratinized region was used for permeation studies (Chinna Reddy et al., 2011; Dowty et al., 1992; Xu et al., 2002). Mucosal membrane of dog and monkey are non-keratinized but is anatomically vary in thickness with human mucosa (Nair et al., 2013; Nicolazzo et al., 2005; Patel et al., 2012). Buccal mucosa of porcine shows most anatomical resemblance with human mucosa and hence considered as best suitable model in permeation studies. However, viability and integrity are the major issue often encounter with excised buccal mucosa (Dowty et al., 1992).

The drawback of isolated tissue models such as those described above is the maintenance of tissue viability and integrity. The complexity involved in excision of the animal tissue has lead to the evolution of in-vitro buccal epithelial cell culture models for testing drug permeation and metabolism. The TR146 cell culture model demonstrated appropriate differentiation patterns seen in human non-keratinized epithelium (Mørck Nielsen & Rømer Rassing, 2000; Nielsen & Rassing, 1999). The TR146 cells originate from a human buccal carcinoma and the cell lines were extensively studied for buccal permeation of drugs. However, the TR146 cell culture model has less of a barrier nature when compared to human and porcine buccal epithelium (Mørck Nielsen & Rømer Rassing, 2000; Nielsen & Rassing, 1999; Patel et al., 2012; Sander et al., 2013). Cultures of human oral mucosal keratinocytes obtained from healthy adults develop similar permeability properties and barrier lipid composition to their site of origin (Selvaratnam et al., 2001).

1.9.4 In-vivo models

In-vivo drug permeation using perfusion cells were extensively reported in the literature (Adrian et al., 2006; Rathbone, 1991a, b). Perfusion cells applied on the buccal mucosa allow for in-vivo studies of buccal drug permeability. A drug solution is perfused through the cell and the drug permeability is calculated from the amount of drug disappeared from the solution over a certain period of time. A major disadvantage of using this method is that the disappearance of drug from the perfusion solution is not necessarily equal to the drug appearance in the systemic circulation (Rathbone & Hadgraft, 1991).

In-vivo pharmacokinetic study in animal models and /or human subjects is most followed method to propose the drug pharmacokinetic profile such as absorption, distribution, metabolism and elimination. Pharmacokinetic parameters can be calculated from the plasma concentration versus time profile. (Holm et al., 2013; Kassem et al., 2014; Kim et al., 1995; Onishi et al., 2014; Sawicki & Janicki, 2002).

1.10 Objectives of the research

Buspirone hydrochloride (BS) was selected as a candidate drug for the preparation of buccal discs. BS is an anxiolytic agent belonging to azaspirodecanediones and is a partial agonist for the serotonin 5-HT1A receptors and is an antagonist for the dopamine D2 auto receptors and has weak affinity to 5-HT2 receptors. BS is devoid of anticonvulsant, sedative and muscle-relaxant properties associated with other anxiolytics and animal studies suggest it also lacks potential for abuse.

Presently, conventional oral tablets of BS are available in the market (5, 10, 15 and 30 mg). Orally administered BS is completely and rapidly absorbed from gastro intestinal (GI) tract. However, oral bioavailability of the drug is approximately 4% due to extensive first pass metabolism. BS has been reported for interaction with food leading to erratic and unpredictable absorption resulting in fluctuation of plasma drug concentration. These pharmacokinetic properties (extensive first pass metabolism and erratic oral absorption) make BS an ideal candidate for buccal administration.

The current research work thus aimed at developing controlled release bioadhesive buccal delivery systems of BS using various bioadhesive and rate controlling polymers to attain improved bioavailability. Research work was carried out in following stages for achieving this broad objective.

- Suitability of controlled release buccal delivery systems to improve the bioavailability of buspirone hydrochloride was evaluated and examined.
- Buccal dosage forms were designed by selecting appropriate formulation additives on the basis of preformulation studies.
- Buccal bioadhesive controlled release systems for BS were developed with good physical characteristics by optimization of various process variables.
- Possibility of effervescent controlled release buccal formulations of buspirone hydrochloride for probable improved drug permeability was evaluated.

- In-vitro drug release and bioadhesive behavior of designed formulations were evaluated.
- Selected formulations were evaluated for in-vivo pharmacokinetic performance in rabbit model.

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

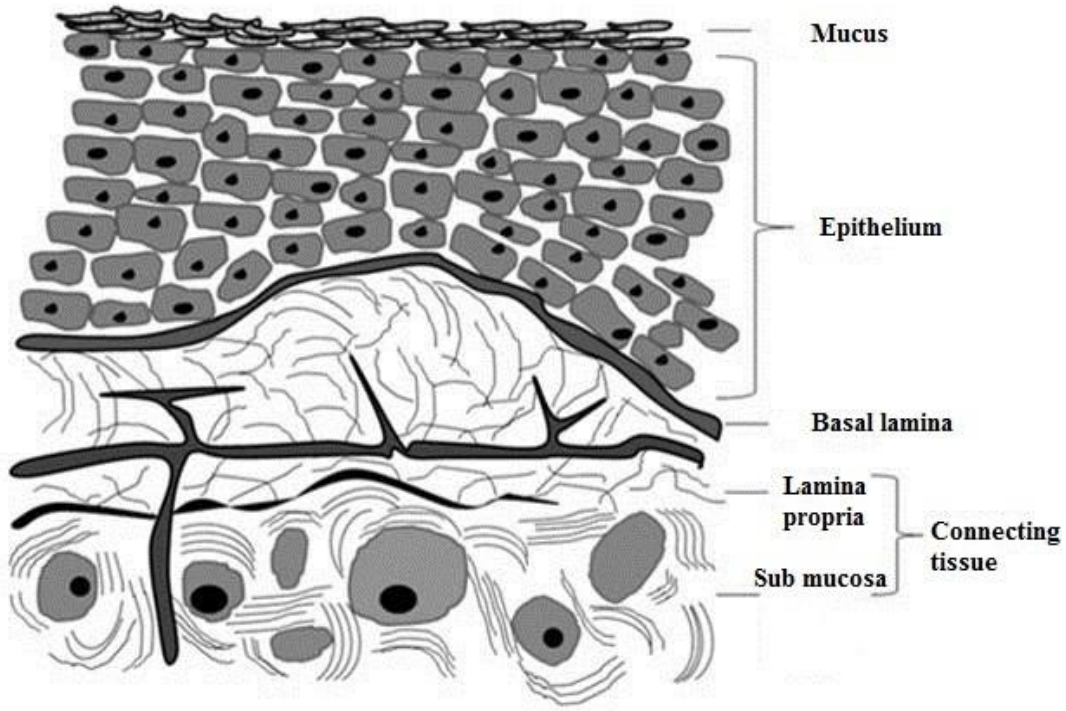


Figure 1.1: Structure of buccal mucosa (Salamat-Miller et al., 2005)

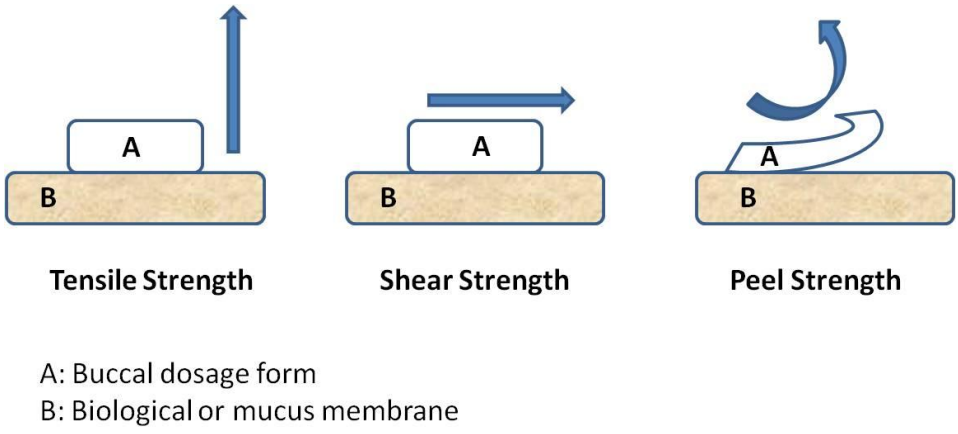


Figure 1.2: Various direction of force applied for tensile, shear and peel strengths

Table 1.1: Classification of permeation enhancers used in various buccal dosage forms

Classification	***Examples***
Anionic Surfactants	Sodium lauryl sulfate (Gandhi & Robinson, 1992; Nicolazzo et al., 2004a; Shidhaye et al., 2010) Laureth-9 (Aungst & Rogers, 1989)
Nonionic Surfactants	Polyoxyethylene-9-lauryl ether (PLE) (Hosny et al., 2002) Tween80 (Kurosaki et al., 1988; Rai et al., 2011) BriJ (Rai et al., 2011) Plunoric F27 (Das et al., 2012)
Cationic Surfactants	Cetylpyridinium chloride (Kurosaki et al., 1988)
Polymers	Chitosan (Portero et al., 2007) 5-methyl-pyrrolidinone chitosan, chitosan and a partially reacetylated chitosan (Rossi et al., 2005) N-trimethyl chitosans(Rossi et al., 2005)
Fatty acids and derivatives	Oleic acid (Ganem-Quintanar et al., 1998) Oleic acid together with polyethylene glycol 200 (PEG 200) incorporated into the cubic liquid crystalline phase of glyceryl monooleate (Lee & Kellaway, 2000). Lauric acid (Van Der Bijl et al., 2000) Sucrose laurate (Ganem-Quintanar et al., 1998) Sodium caprate, (Maher et al., 2009) Oleic acid (Lee & Kellaway, 2000) Sodium myristate (Nakada et al., 1988)
Bile salts and derivatives	Sodium deoxycholate (Langoth et al., 2005a) Sodium taurocholate (Artusi et al., 2003; Steward et al., 1994) Sodium taurodihydrofusidate (STDHF) (Lee & Choi, 2003) Sodium glycocholate (Mørck Nielsen & Rømer Rassing, 2000; Şenel et al., 1997)

Table 1.1: Classification of permeation enhancers used in various buccal dosage forms (Contd.)

Classification	***Examples***
Sulfoxides	Dimethyl sulfoxide (DMSO) (Veuillez et al., 2001)
Chelating agents	EDTA (de Vries et al., 1991)
Polyols	Polyethylene glycol (Rambharose et al., 2014), Propylene glycol (Birudaraj et al., 2005)
Enzyme Inhibitors	Glutathione (Palermo et al., 2011) Bestatin (Stratford Jr & Lee, 1986) Puromycin (Stratford Jr & Lee, 1986)
Others (non-surfactants)	Urea and derivative (Shidhaye et al., 2010) Azone(1-dodecylazacycloheptan-2-one) (Nicolazzo et al., 2004b) β-cyclodextrin and methyl-β-cyclodextrin (Figueiras, Hombach et al. 2009) *l*-menthol (Kitano et al., 1998) Cod-liver oil extract (Tsutsumi et al., 2002) Ethanol (Veuillez et al., 2002)

Table 1.2: Classification of bioadhesive polymers used in design of various buccal dosage forms

Classification	***Category***	***Examples***
Source	Natural	Agarose, Chitosan, Gelatin, Hyaluronic acid Various Gums (Guar, Xanthan, Gellan, Carragenan, Pectin, Sodium Alginate, Hakea)
	Semi synthetic	Cellulose Derivatives (Carboxy methyl cellulose (CMC), Thiolated CMC, Hydroxy ethyl cellulose, Hydroxy propyl cellulose, Hydroxy propyl methyl cellulose, Methyl cellulose, Methyl hydroxy ethyl cellulose)
	Synthetic	Polyacrylic acid based Polymers (Carbopol, Polycarbophil, Polyacrylates, Polymethacrylate, copolymers of acrylic acid and Polyethylene glycol)
Aqueous Solubility	Water Soluble	Carbopol, Hydroxy ethyl cellulose, Hydroxy propyl cellulose, Hydroxy propyl methyl cellulose, Sodium CMC, Sodium alginate, Polyethylene oxide, Chitosan, Polycarbophil, Xanthan gum, Tamarind seed xyloglucan
	Water Insoluble	Ethyl cellulose, Eudragit RS100
Charge	Cationic	Chitosan, Aminodextran
	Anionic	Carbopol, Polycarbophil, Sodium alginate, Sodium CMC, CMC.
	Non ionic	Polyvinyl alcohol, Hydroxy propyl cellulose, Polyethylene oxide
Potential Bioadhesive Forces	Covalent	Cyanoacrylate
	Hydrogen Bond	Carbopol, Polycarbophil, Polyvinyl alcohol, Acrylates
	Electrostatic	Chitosan derivatives (trimethyl chitosan, thiolated chitosan)

Table 1.3: Buccal dosage forms available commercially and in clinical development stages

Commercial Brand	***Dosage form***	***Indication***
Onsolis	Fentanyl buccal soluble film	Break through Pain management in cancer.
Fentora	Fentanyl buccal tablet	Break through Pain management in cancer.
Actiq	Fentanyl on plastic stick (lollipop)	Break through Pain management in cancer.
Oravig	Miconazole buccal tablets	Local treatment of oropharyngeal candidiasis.
Lauriad*	Miconazole buccal tablets (Phase III)	Local treatment of oropharyngeal candidiasis.
Sitavig	Acyclovir buccal tablets	Recurrent herpes labialis.
Striant	Testosterone buccal adhesive system	Hormone replacement therapy.
Buccastem M	Prochlorperazine	Control of severe nausea and vomiting, schizophrenia.
Generex Oral-lyn	Insulin spray	Diabetes mellitus.
Buccolam	Buccal Solution	Treatment of prolonged, acute, convulsive seizures in infants, children and adolescents.
Suscard	Glyceryl trinitrate	Treatment of angina
Xylonor	Lidocaine and citrimide Gingival/buccal gel	Topical anesthesia in the buccal cavity.
Sativex	Tetranabinex and Nabidiolex Buccal Spray	Adjunctive analgesic treatment in adult patients with advanced cancer.

* In clinical development stage

Table 1.4: USFDA recommended dissolution methods for buccal dosage forms

Drug Name	***Dose (mg)***	***Buccal Dosage Form***	***USP Apparatus***	***Speed (RPMs)***	***Dissolution medium***	***Dissolution medium Volume (mL)***	***Recommended Sampling Times***
Fentanyl Citrate	0.1 and 0.4	Tablet	II (Paddle) small volume dissolution apparatus	100	Phosphate Buffered Saline solution, pH 7.0	100	3, 5, 7.5, 10, 15 and 20 min
Fentanyl Citrate	0.2, 0.3, 0.6 and 0.8	Tablet	II (Paddle) small volume dissolution apparatus	100	Phosphate Buffered Saline solution, pH 7.0	200	3, 5, 7.5, 10, 15 and 20 min
Fentanyl Citrate	0.2, 0.4, 0.6 and 0.8	Film	I (Basket) 100 mL dissolution vessel	100	25-mM Phosphate Buffer, pH 6.4	60	5, 10, 15, 20, 30 and 45 min
Fentanyl Citrate	1.2	Film	I (Basket) 100 mL dissolution vessel	100	25-mM Phosphate Buffer, pH 6.4	100	5, 10, 15, 20, 30 and 45 min
Miconazole	50	Tablet	I (Basket)	60	0.5% SDS in water-pH adjusted to 6.5 ± 0.5	1000	1, 2, 4, 6, 8, 10 and 12 h
Testosterone	30	Extended Release Tablet	II (Paddle, may use sinker)	60	1% sodium dodecyl sulfate in double distilled water	1000	1, 2, 4, 6, 10, 12 and 24 h

References

Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., & Luppi, B. (2012). Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. Carbohydr. Polym., 87(1), 581-588.

Abu-Huwaij, R., Obaidat, R., Sweidan, K., & Al-Hiari, Y. (2011). Formulation and In Vitro Evaluation of Xanthan Gum or Carbopol 934-Based Mucoadhesive Patches, Loaded with Nicotine. AAPS PharmSciTech., 12(1), 21-27.

Adhikari, S. N., Nayak, B. S., Nayak, A. K., & Mohanty, B. (2010). Formulation and evaluation of buccal patches for delivery of atenolol. AAPS PharmSciTech., 11(3), 1038-1044.

Adrian, C. L., Olin, H. B. D., Dalhoff, K., & Jacobsen, J. (2006). In vivo human buccal permeability of nicotine. Int. J. Pharm., 311(1–2), 196-202.

Agoram, B., Woltosz, W. S., & Bolger, M. B. (2001). Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv. Drug Deliv. Rev., 50, Supplement 1(0), S41-S67.

Ahagon, A., & Gent, A. N. (1975). Effect of interfacial bonding on the strength of adhesion. J. Polym. Sci., Polym. Phys. Ed., 13(7), 1285-1300.

Andrews, G. P., Laverty, T. P., & Jones, D. S. (2009). Mucoadhesive polymeric platforms for controlled drug delivery. Eur. J. Pharm. Biopharm., 71(3), 505-518.

Artusi, M., Santi, P., Colombo, P., & Junginger, H. E. (2003). Buccal delivery of thiocolchicoside: in vitro and in vivo permeation studies. Int. J. Pharm., 250(1), 203-213.

Aungst, B. J., & Rogers, N. J. (1989). Comparison of the effects of various transmucosal absorption promoters on buccal insulin delivery. Int. J. Pharm., 53(3), 227-235.

Avachat, A. M., Gujar, K. N., & Wagh, K. V. (2013). Development and evaluation of tamarind seed xyloglucan-based mucoadhesive buccal films of rizatriptan benzoate. Carbohydr. Polym., 91(2), 537-542.

Ayensu, I., Mitchell, J. C., & Boateng, J. S. (2012a). Development and physico-mechanical characterisation of lyophilised chitosan wafers as potential protein drug delivery systems via the buccal mucosa. Colloids Surf., B., 91, 258-265.

Ayensu, I., Mitchell, J. C., & Boateng, J. S. (2012b). Effect of membrane dialysis on characteristics of lyophilised chitosan wafers for potential buccal delivery of proteins. Int. J. Biol. Macromol., 50(4), 905-909.

Ayensu, I., Mitchell, J. C., & Boateng, J. S. (2012c). In vitro characterisation of chitosan based xerogels for potential buccal delivery of proteins. Carbohydr. Polym., 89(3), 935-941.

Baveja, S. K., Ranga Rao, K. V., Singh, A., & Gombar, V. K. (1988). Release characteristics of some bronchodilators from compressed hydrophilic polymeric matrices and their correlation with molecular geometry. Int. J. Pharm., 41(1–2), 55-62.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (Eds.). (2002). Biochemistry

Bernkop-Schnürch, A. (2005). Thiomers: A new generation of mucoadhesive polymers. Adv. Drug Deliv. Rev., 57(11), 1569-1582.

Birudaraj, R., Berner, B., Shen, S., & Li, X. (2005). Buccal permeation of buspirone: mechanistic studies on transport pathways. J. Pharm. Sci., 94(1), 70-78.

Borkar, N., Xia, D., Holm, R., Gan, Y., Müllertz, A., Yang, M., & Mu, H. (2014). Investigating the correlation between in vivo absorption and in vitro release of fenofibrate from lipid matrix particles in biorelevant medium. Eur. J. Pharm. Sci., 51(0), 204-210.

Boyapally, H., Nukala, R. K., Bhujbal, P., & Douroumis, D. (2010). Controlled release from directly compressible theophylline buccal tablets. Colloids Surf., B., 77(2), 227-233.

Capra, R. H., Baruzzi, A. M., Quinzani, L. M., & Strumia, M. C. (2007). Rheological, dielectric and diffusion analysis of mucin/carbopol matrices used in amperometric biosensors. Sens. Actuators, B., 124(2), 466-476.

Chai, J., Wong, C. Y. G., & Ho, P. C. (2013). A randomized clinical trial comparing the short-term side effects of sublingual and buccal routes of misoprostol administration for medical abortions up to 63 days' gestation. Contraception, 87(4), 480-485.

Charde, S., Mudgal, M., Kumar, L., & Saha, R. (2008). Development and Evaluation of Buccoadhesive Controlled Release Tablets of Lercanidipine. AAPS PharmSciTech., 9(1), 182-190.

Chinna Reddy, P., Chaitanya, K. S., & Madhusudan Rao, Y. (2011). A review on bioadhesive buccal drug delivery systems: current status of formulation and evaluation methods. Daru, 19(6), 385-403.

Cho, E., Gwak, H., & Chun, I. (2008). Formulation and evaluation of ondansetron nasal delivery systems. Int. J. Pharm., 349(1–2), 101-107.

Choi, H.-G., & Kim, C.-K. (2000). Development of omeprazole buccal adhesive tablets with stability enhancement in human saliva. J. Control. Release., 68(3), 397-404.

Cid, Y. P., Pedrazzi, V., de Sousa, V. P., & Pierre, M. B. (2012). In vitro characterization of chitosan gels for buccal delivery of celecoxib: influence of a penetration enhancer. AAPS PharmSciTech., 13(1), 101-111.

Codd, J. E., & Deasy, P. B. (1998). Formulation development and in vivo evaluation of a novel bioadhesive lozenge containing a synergistic combination of antifungal agents. Int. J. Pharm., 173(1–2), 13-24.

Colombo, P. (1993). Swelling-controlled release in hydrogel matrices for oral route. Adv. Drug Deliv. Rev., 11(1–2), 37-57.

Darwish, M., Kirby, M., Robertson Jr, P., Tracewell, W., & Jiang, J. G. (2006a). Pharmacokinetic properties of fentanyl effervescent buccal tablets: A phase I, open-label, crossover study of single-dose 100, 200, 400, and 800 μg in healthy adult volunteers. Clin. Ther., 28(5), 707-714.

Darwish, M., Tempero, K., Kirby, M., & Thompson, J. (2006b). Relative bioavailability of the fentanyl effervescent buccal tablet (FEBT) 1080 pg versus Oral transmucosal fentanyl citrate 1600 pg and dose proportionality of FEBT 270 to 1300 μg: A single-dose, randomized, open-label, three-period study in healthy adult volunteers. Clin. Ther., 28(5), 715-724.

Das, N., Parshotam, M., & Senshang, L. (2012). Statistical optimization of insulin-loaded Pluronic F-127 gels for buccal delivery of basal insulin. Pharm. Dev. Technol., 17(3), 363-374.

de Vries, M. E., Boddé, H. E., Verhoef, J. C., Ponec, M., Craane, W. I. H. M., & Junginger, H. E. (1991). Localization of the permeability barrier inside porcine buccal mucosa: a combined in vitro study of drug permeability, electrical resistance and tissue morphology. Int. J. Pharm., 76(1–2), 25-35.

Diaz del Consuelo, I., Falson, F., Guy, R. H., & Jacques, Y. (2007). Ex vivo evaluation of bioadhesive films for buccal delivery of fentanyl. J. Control. Release., 122(2), 135-140.

Dowty, M., Knuth, K., Irons, B., & Robinson, J. (1992). Transport of Thyrotropin Releasing Hormone in Rabbit Buccal Mucosa in Vitro. Pharm. Res., 9(9), 1113-1122.

Duchêne, D., & Ponchel, G. (1997). Bioadhesion of solid oral dosage forms, why and how? Eur. J. Pharm. Biopharm., 44(1), 15-23.

Eichman, J. D., & Robinson, J. R. (1998). Mechanistic Studies on Effervescent-Induced Permeability Enhancement. Pharm. Res., 15(6), 925-930.

El-Samaligy, M. S., Yahia, S. A., & Basalious, E. B. (2004). Formulation and evaluation of diclofenac sodium buccoadhesive discs. Int. J. Pharm., 286(1–2), 27-39.

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. Mater. Sci. Eng. C: Mater. Biol. Appl., 33(4), 1819-1841.

Eouani, C., Piccerelle, P., Prinderre, P., Bourret, E., & Joachim, J. (2001). In-vitro comparative study of buccal mucoadhesive performance of different polymeric films. Eur. J. Pharm. Biopharm., 52(1), 45-55.

Figueiras, A., Hombach, J., Veiga, F., & Bernkop-Schnürch, A. (2009). In vitro evaluation of natural and methylated cyclodextrins as buccal permeation enhancing system for omeprazole delivery. Eur. J. Pharm. Biopharm., 71(2), 339-345.

Figueiras, A., Pais, A. A., & Veiga, F. J. (2010). A comprehensive development strategy in buccal drug delivery. AAPS PharmSciTech., 11(4), 1703-1712.

Gandhi, R., & Robinson, J. (1992). Mechanisms of penetration enhancement for transbuccal delivery of salicylic acid. Int. J. Pharm., 85(1–3), 129-140.

Gandhi, R. B., & Robinson, J. R. (1988). Bioadhesion in drug delivery. Indian J. Pharm. Sci., 50(3), 145-152.

Ganem-Quintanar, A., Quintanar-Guerrero, D., Falson-Rieg, F., & Buri, P. (1998). Ex vivo oral mucosal permeation of lidocaine hydrochloride with sucrose fatty acid esters as absorption enhancers. Int. J. Pharm., 173(1–2), 203-210.

Garcia-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: production, recovery, and properties. Biotechnol. Adv., 18(7), 549-579.

Geisberger, G., Gyenge, E. B., Maake, C., & Patzke, G. R. (2013). Trimethyl and carboxymethyl chitosan carriers for bio-active polymer–inorganic nanocomposites. Carbohydr. Polym., 91(1), 58-67.

Giovino, C., Ayensu, I., Tetteh, J., & Boateng, J. S. (2012). Development and characterisation of chitosan films impregnated with insulin loaded PEG-b-PLA nanoparticles (NPs): a potential approach for buccal delivery of macromolecules. Int. J. Pharm., 428(1-2), 143-151.

Giovino, C., Ayensu, I., Tetteh, J., & Boateng, J. S. (2013). An integrated buccal delivery system combining chitosan films impregnated with peptide loaded PEG-b-PLA nanoparticles. Colloids Surf., B., 112C, 9-15.

Gohel, M. C., Parikh, R. K., Nagori, S. A., Shah, S. N., & Dabhi, M. R. (2009). Preparation and Evaluation of Soft Gellan Gum Gel Containing Paracetamol. Indian J. Pharm. Sci., 71(2), 120-124.

Govindasamy, P., Kesavan, B. R., & Narasimha, J. K. (2013). Formulation of unidirectional release buccal patches of carbamazepine and study of permeation through porcine buccal mucosa. Asian Pac. J. Trop. Biomed., 3(12), 995-1002.

Guggi, D., Marschütz, M. K., & Bernkop-Schnürch, A. (2004). Matrix tablets based on thiolated poly(acrylic acid): pH-dependent variation in disintegration and mucoadhesion. Int. J. Pharm., 274(1–2), 97-105.

Gurny, R., Doelker, E., & Peppas, N. A. (1982). Modelling of sustained release of water-soluble drugs from porous, hydrophobic polymers. Biomaterials., 3(1), 27-32.

Hägerström, H., Paulsson, M., & Edsman, K. (2000). Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using a rheological method. Eur. J. Pharm. Sci., 9(3), 301-309.

Han, R.-Y., Fang, J.-Y., Sung, K. C., & Hu, O. Y. P. (1999). Mucoadhesive buccal disks for novel nalbuphine prodrug controlled delivery: effect of formulation variables on drug release and mucoadhesive performance. Int. J. Pharm., 177(2), 201-209.

Harris, D., & Robinson, J. R. (1992). Drug delivery via the mucous membranes of the oral cavity. J. Pharm. Sci., 81(1), 1-10.

Hassan, N., Ahad, A., Ali, M., & Ali, J. (2010). Chemical permeation enhancers for transbuccal drug delivery. Expert Opin. Drug Deliv., 7(1), 97-112.

Hauptstein, S., Hintzen, F., Muller, C., Ohm, M., & Bernkop-Schnurch, A. (2013). Development and in vitro evaluation of a buccal drug delivery system based on preactivated thiolated pectin. Drug Dev. Ind. Pharm (doi:10.3109/03639045.2013.836213).

Hearnden, V., Sankar, V., Hull, K., Juras, D. V., Greenberg, M., Kerr, A. R., Lockhart, P. B., Patton, L. L., Porter, S., & Thornhill, M. H. (2012). New developments and opportunities in oral mucosal drug delivery for local and systemic disease. Adv. Drug Deliv. Rev., 64(1), 16-28.

Heigoldt, U., Sommer, F., Daniels, R., & Wagner, K.-G. (2010). Predicting in vivo absorption behavior of oral modified release dosage forms containing pH-dependent poorly soluble drugs using a novel pH-adjusted biphasic in vitro dissolution test. Eur. J. Pharm. Biopharm., 76(1), 105-111.

Hessell, P. G., Lloyd-Jones, J. G., Muir, N. C., Parr, G. D., & Sugden, K. (1989). A comparison of the availability of prochlorperazine following i.m. buccal and oral administration. Int. J. Pharm., 52(2), 159-164.

Holm, R., Meng-Lund, E., Andersen, M. B., Jespersen, M. L., Karlsson, J.-J., Garmer, M., Jørgensen, E. B., & Jacobsen, J. (2013). In vitro, ex vivo and in vivo examination of buccal absorption of metoprolol with varying pH in TR146 cell culture, porcine buccal mucosa and Göttingen minipigs. Eur. J. Pharm. Sci., 49(2), 117-124.

Hoogstraate, A. J., Verhoef, J. C., Tuk, B., Pijpers, A., van Leengoed, L. A. M. G., Verheijden, J. H. M., Junginger, H. E., & Boddé, H. E. (1996). Buccal delivery of fluorescein isothiocyanate-dextran 4400 and the peptide drug buserelin with

glycodeoxycholate as an absorption enhancer in pigs. J. Control. Release., 41(1–2), 77-84.

Hosny, E. A., Elkheshen, S. A., & Saleh, S. I. (2002). Buccoadhesive tablets for insulin delivery: in-vitro and in-vivo studies. Boll. Chim. Farm., 143(3), 210-217.

Ivarsson, D., & Wahlgren, M. (2012). Comparison of in vitro methods of measuring mucoadhesion: Ellipsometry, tensile strength and rheological measurements. Colloids Surf., B., 92(0), 353-359.

Jabbari, E., & Peppas, N. A. (1995). A model for interdiffusion at interfaces of polymers with dissimilar physical properties. Polymer, 36(3), 575-586.

Jacobsen, J. (2001). Buccal iontophoretic delivery of atenolol·HCl employing a new in vitro three-chamber permeation cell. J. Control. Release., 70(1–2), 83-95.

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., 111C, 644-650.

Jaipal, A., Pandey, M. M., Charde, S., sadhu, N., Ajjarapu, S., & Reddy, P. G. (2014). Controlled Release Effervescent Buccal Discs of Buspirone Hydrochloride: In-vitro and In-Vivo Evaluation Studies. Drug Delivery, 1-7 (DOI: 10.3109/10717544.10712014.10917388).

Jiménez-castellanos, M. R., Zia, H., & Rhodes, C. T. (1993). Mucoadhesive Drug Delivery Systems. Drug Dev. Ind. Pharm., 19(1-2), 143-194.

Jones, D. S., Woolfson, A. D., Brown, A. F., Coulter, W. A., McClelland, C., & Irwin, C. R. (2000). Design, characterisation and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease. J. Control. Release., 67(2–3), 357-368.

Jug, M., Maestrelli, F., Bragagni, M., & Mura, P. (2010). Preparation and solid-state characterization of bupivacaine hydrochloride cyclodextrin complexes aimed for buccal delivery. J. Pharm. Biomed. Anal., 52(1), 9-18.

Kanjanabat, S., & Pongjanyakul, T. (2011). Preparation and characterization of nicotine-magnesium aluminum silicate complex-loaded sodium alginate matrix tablets for buccal delivery. AAPS PharmSciTech., 12(2), 683-692.

Kassem, M. A. A., ElMeshad, A. N., & Fares, A. R. (2014). Enhanced bioavailability of buspirone hydrochloride via cup and core buccal tablets: Formulation and in vitro/in vivo evaluation. Int. J. Pharm., 463(1), 68-80.

Kast, C. E., & Bernkop-Schnürch, A. (2002). Polymer–cysteamine conjugates: new mucoadhesive excipients for drug delivery? Int. J. Pharm., 234(1–2), 91-99.

Kaur, A., & Kaur, G. (2012). Mucoadhesive buccal patches based on interpolymer complexes of chitosan-pectin for delivery of carvedilol. Saudi Pharm J, 20(1), 21-27.

Khanna, R., Agarwal S P, Ahuja A. . (1997). Preparation and evaluation of muco adhesive buccal films of clotrimazole for oral candida infections. Indian J. Pharm. Sci., 59(6), 299-305.

Kianfar, F., Antonijevic, M., Chowdhry, B., & Boateng, J. S. (2013). Lyophilized wafers comprising carrageenan and pluronic acid for buccal drug delivery using model soluble and insoluble drugs. Colloids Surf., B., 103, 99-106.

Kianfar, F., Chowdhry, B. Z., Antonijevic, M. D., & Boateng, J. S. (2012). Novel films for drug delivery via the buccal mucosa using model soluble and insoluble drugs. Drug Dev. Ind. Pharm., 38(10), 1207-1220.

Kim, S., Snipes, W., Hodgen, G. D., & Anderson, F. (1995). Pharmacokinetics of a single dose of Buccal testosterone. Contraception, 52(5), 313-316.

Kitano, M., Maitani, Y., Takayama, K., & Nagai, T. (1998). Buccal absorption through golden hamster cheek pouch in vitro and in vivo of 17β-estradiol from hydrogels containing three types of absorption enhancers. Int. J. Pharm., 174(1–2), 19-28.

Kočevar-Nared, J., Kristl, J., & Šmid-Korbar, J. (1997). Comparative rheological investigation of crude gastric mucin and natural gastric mucus. Biomaterials., 18(9), 677-681.

Kohda, Y., Kobayashi, H., Baba, Y., Yuasa, H., Ozeki, T., Kanaya, Y., & Sagara, E. (1997). Controlled release of lidocaine hydrochloride from buccal mucosa-adhesive films with solid dispersion. Int. J. Pharm., 158(2), 147-155.

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983a). Mechanisms of potassium chloride release from compressed, hydrophilic, polymeric matrices: Effect of entrapped air. J. Pharm. Sci., 72(10), 1189-1191.

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983b). Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm., 15(1), 25-35.

Kotagale, N. R., Patel, C. J., Parkhe, A. P., Khandelwal, H. M., Taksande, J. B., & Umekar, M. J. (2010). Carbopol 934-Sodium Alginate-Gelatin Mucoadhesive Ondansetron Tablets for Buccal Delivery: Effect of pH Modifiers. Indian J. Pharm. Sci., 72(4), 471-479.

Kundu, J., Patra, C., & Kundu, S. C. (2008). Design, fabrication and characterization of silk fibroin-HPMC-PEG blended films as vehicle for transmucosal delivery. Mater. Sci. Eng. C: Mater. Biol. Appl., 28(8), 1376-1380.

Kurosaki, Y., Hisaichi, S.-i., Hamada, C., Nakayama, T., & Kimura, T. (1988). Effects of surfactants on the absorption of salicylic acid from hamster cheek pouch as a model of keratinized oral mucosa. Int. J. Pharm., 47(1–3), 13-19.

Lam, J. K. W., Xu, Y., Worsley, A., & Wong, I. C. K. (2014). Oral transmucosal drug delivery for pediatric use. Adv. Drug Deliv. Rev., 73(0), 50-62.

Langoth, N., Bernkop-Schnürch, A., & Kurka, P. (2005a). In Vitro Evaluation of Various Buccal Permeation Enhancing Systems for PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide). Pharm. Res., 22(12), 2045-2050.

Langoth, N., Kalbe, J., & Bernkop-Schnürch, A. (2003). Development of buccal drug delivery systems based on a thiolated polymer. Int. J. Pharm., 252(1–2), 141-148.

Langoth, N., Kalbe, J., & Bernkop-Schnürch, A. (2005b). Development of a mucoadhesive and permeation enhancing buccal delivery system for PACAP (pituitary adenylate cyclase-activating polypeptide). Int. J. Pharm., 296(1–2), 103-111.

Le Bourlais, C., Acar, L., Zia, H., Sado, P. A., Needham, T., & Leverge, R. (1998). Ophthalmic drug delivery systems—Recent advances. Prog. Retin. Eye Res., 17(1), 33-58.

Lee, J., & Choi, Y. (2003). Enhancedex vivo buccal transport of propranolol: Evaluation of phospholipids as permeation enhancers. Arch. Pharm. Res., 26(5), 421-425.

Lee, J., & Kellaway, I. W. (2000). Combined effect of oleic acid and polyethylene glycol 200 on buccal permeation of [D-Ala2, D-Leu5]enkephalin from a cubic phase of glyceryl monooleate. Int. J. Pharm., 204(1–2), 137-144.

Lee, V. H. L., & Yamamoto, A. (1989). Penetration and enzymatic barriers to peptide and protein absorption. Adv. Drug Deliv. Rev., 4(2), 171-207.

Leung, S.-H. S., & Robinson, J. R. (1990). Polymer structure features contributing to mucoadhesion. II. J. Control. Release., 12(3), 187-194.

Ludwig, A. (2005). The use of mucoadhesive polymers in ocular drug delivery. Adv. Drug Deliv. Rev., 57(11), 1595-1639.

Maher, S., Leonard, T. W., Jacobsen, J., & Brayden, D. J. (2009). Safety and efficacy of sodium caprate in promoting oral drug absorption: from in vitro to the clinic. Adv. Drug Deliv. Rev., 61(15), 1427-1449.

Mao, S., Chen, J., Wei, Z., Liu, H., & Bi, D. (2004). Intranasal administration of melatonin starch microspheres. Int. J. Pharm., 272(1–2), 37-43.

Martin, L., Wilson, C. G., Koosha, F., & Uchegbu, I. F. (2003). Sustained buccal delivery of the hydrophobic drug denbufylline using physically cross-linked palmitoyl glycol chitosan hydrogels. Eur. J. Pharm. Biopharm., 55(1), 35-45.

Marttin, E., Schipper, N. G. M., Verhoef, J. C., & Merkus, F. W. H. M. (1998). Nasal mucociliary clearance as a factor in nasal drug delivery. Adv. Drug Deliv. Rev., 29(1–2), 13-38.

Mashru, R., Sutariya, V., Sankalia, M., & Sankalia, J. (2005). Transbuccal delivery of lamotrigine across porcine buccal mucosa: in vitro determination of routes of buccal transport. J. Pharm. Pharm. Sci., 8(1), 54-62.

Mathiowitz, E., Chickering, I. D. E., & Lehr, C.-M. (Eds.). (1995). Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development. Marcel Dekker, Inc.

Meher, J. G., Tarai, M., Yadav, N. P., Patnaik, A., Mishra, P., & Yadav, K. S. (2013). Development and characterization of cellulose-polymethacrylate mucoadhesive film for buccal delivery of carvedilol. Carbohydr. Polym., 96(1), 172-180.

Mehta, M., Kemppainen, B. W., & Stafford, R. G. (1991). In vitro penetration of tritium-labelled water (THO) and [3H]PbTx-3 (a red tide toxin) through monkey buccal mucosa and skin. Toxicol. Lett., 55(2), 185-194.

Merkle, H. P., & Wolany, G. (1992). Buccal delivery for peptide drugs. J. Control. Release., 21(1–3), 155-164.

Mirhosseini, H., Tan, C. P., Hamid, N. S. A., & Yusof, S. (2008). Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion. Food Chem., 107(3), 1161-1172.

Miro, A., d'Angelo, I., Nappi, A., La Manna, P., Biondi, M., Mayol, L., Musto, P., Russo, R., Rotonda, M. I. L., Ungaro, F., & Quaglia, F. (2013). Engineering poly(ethylene oxide) buccal films with cyclodextrin: A novel role for an old excipient? Int. J. Pharm., 452(1–2), 283-291.

Mohammadi-Samani, S., Bahri-Najafi, R., & Yousefi, G. (2005). Formulation and in vitro evaluation of prednisolone buccoadhesive tablets. Il Farmaco, 60(4), 339-344.

Mørck Nielsen, H., & Rømer Rassing, M. (2000). TR146 cells grown on filters as a model of human buccal epithelium: V. Enzyme activity of the TR146 cell culture model, human buccal epithelium and porcine buccal epithelium, and permeability of leu-enkephalin. Int. J. Pharm., 200(2), 261-270.

Morishita, M., Barichello, J. M., Takayama, K., Chiba, Y., Tokiwa, S., & Nagai, T. (2001). Pluronic® F-127 gels incorporating highly purified unsaturated fatty acids for buccal delivery of insulin. Int. J. Pharm., 212(2), 289-293.

Muchohi, S. N., Kokwaro, G. O., Ogutu, B. R., Edwards, G., Ward, S. A., & Newton, C. R. J. C. (2008). Pharmacokinetics and clinical efficacy of midazolam in children with severe malaria and convulsions. Br. J. Clin. Pharmacol., 66(4), 529-538.

Mumtaz, A. M., & Ch'ng, H.-S. (1995). Evaluation of bioadhesive buccal tablets containing triamcinolone acetonide in healthy volunteers. Int. J. Pharm., 121(2), 249-254.

Nair, A. B., Kumria, R., Harsha, S., Attimarad, M., Al-Dhubiab, B. E., & Alhaider, I. A. (2013). In vitro techniques to evaluate buccal films. J. Control. Release., 166(1), 10-21.

Nakada, Y., Awata, N., Nakamichi, C., & Sugimoto, I. (1988). The effect of additives on the oral mucosal absorption of human calcitonin in rats. J. Pharmacobiodyn., 11(6), 395-401.

Needleman, I. G., & Smales, F. C. (1995). In vitro assessment of bioadhesion for periodontal and buccal drug delivery. Biomaterials., 16(8), 617-624.

Nicolazzo, J. A., Reed, B. L., & Finnin, B. C. (2004a). Assessment of the effects of sodium dodecyl sulfate on the buccal permeability of caffeine and estradiol. J. Pharm. Sci., 93(2), 431-440.

Nicolazzo, J. A., Reed, B. L., & Finnin, B. C. (2004b). Modification of buccal drug delivery following pretreatment with skin penetration enhancers. J. Pharm. Sci., 93(8), 2054-2063.

Nicolazzo, J. A., Reed, B. L., & Finnin, B. C. (2005). Buccal penetration enhancers—How do they really work? J. Control. Release., 105(1–2), 1-15.

Nielsen, H. M., & Rassing, M. R. (1999). TR146 cells grown on filters as a model of human buccal epithelium: III. Permeability enhancement by different pH values, different osmolality values, and bile salts. Int. J. Pharm., 185(2), 215-225.

Onishi, H., Yumoto, K., & Sakata, O. (2014). Preparation and evaluation of ritodrine buccal tablets for rational therapeutic use. Int. J. Pharm., 468(1–2), 207-213.

Palermo, A., Napoli, N., Manfrini, S., Lauria, A., Strollo, R., & Pozzilli, P. (2011). Buccal spray insulin in subjects with impaired glucose tolerance: the prevoral study. Diabetes Obes. Metab., 13(1), 42-46.

Park, C. R., & Munday, D. L. (2002). Development and evaluation of a biphasic buccal adhesive tablet for nicotine replacement therapy. Int. J. Pharm., 237(1–2), 215-226.

Park, C. R., & Munday, D. L. (2004). Evaluation of Selected Polysaccharide Excipients in Buccoadhesive Tablets for Sustained Release of Nicotine. Drug Dev. Ind. Pharm., 30(6), 609-617.

Patel, V. F., Liu, F., & Brown, M. B. (2012). Modeling the oral cavity: In vitro and in vivo evaluations of buccal drug delivery systems. J. Control. Release., 161(3), 746-756.

Patil, S. B., Murthy, R. S. R., Mahajan, H. S., Wagh, R. D., & Gattani, S. (2006). Mucoadhesive polymers: Means of improving drug delivery. Pharma times, 38, 25-28.

Peh, K. K., & Wong, C. F. (1999). `Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. J. Pharm. Pharm. Sci., 2, 53-61.

Pendekal, M., S. , & Tegginamat, P., K. . (2012). Formulation and evaluation of a bioadhesive patch for buccal delivery of tizanidine. Acta Pharmaceutica Sinica B, 2(3), 318-324.

Peppas, N. A., & Huang, Y. (2004). Nanoscale technology of mucoadhesive interactions. Adv. Drug Deliv. Rev., 56(11), 1675-1687.

Peppas, N. A., & Sahlin, J. J. (1996). Hydrogels as mucoadhesive and bioadhesive materials: a review. Biomaterials., 17(16), 1553-1561.

Perumal, V. A., Lutchman, D., Mackraj, I., & Govender, T. (2008). Formulation of monolayered films with drug and polymers of opposing solubilities. Int. J. Pharm., 358(1–2), 184-191.

Pongjanyakul, T., Khunawattanakul, W., Strachan, C. J., Gordon, K. C., Puttipipatkhachorn, S., & Rades, T. (2013). Characterization of chitosan-magnesium aluminum silicate nanocomposite films for buccal delivery of nicotine. Int. J. Biol. Macromol., 55, 24-31.

Portero, A., Teijeiro-Osorio, D., Alonso, M. J., & Remuñán-López, C. (2007). Development of chitosan sponges for buccal administration of insulin. Carbohydr. Polym., 68(4), 617-625.

Pozzilli, P., Manfrini, S., Costanza, F., Coppolino, G., Cavallo, M. G., Fioriti, E., & Modi, P. (2005). Biokinetics of buccal spray insulin in patients with type 1 diabetes. Metabolism., 54(7), 930-934.

Preis, M., Woertz, C., Schneider, K., Kukawka, J., Broscheit, J., Roewer, N., & Breitkreutz, J. (2014). Design and evaluation of bilayered buccal film preparations for local administration of lidocaine hydrochloride. Eur. J. Pharm. Biopharm., 86(3), 552-561.

Rai, V., Tan, H. S., & Michniak-Kohn, B. (2011). Effect of surfactants and pH on naltrexone (NTX) permeation across buccal mucosa. Int. J. Pharm., 411(1–2), 92-97.

Rambharose, S., Ojewole, E., Mackraj, I., & Govender, T. (2014). Comparative buccal permeability enhancement of didanosine and tenofovir by potential multifunctional polymeric excipients and their effects on porcine buccal histology. Pharm. Dev. Technol., 0(0), 1-9.

Rathbone, M. J. (1991a). Human buccal absorption. I. A method for estimating the transfer kinetics of drugs across the human buccal membrane. Int. J. Pharm., 69(2), 103-108.

Rathbone, M. J. (1991b). Human buccal absorption. II. A comparative study of the buccal absorption of some parahydroxybenzoic acid derivatives using the buccal absorption test and a buccal perfusion cell. Int. J. Pharm., 74(2–3), 189-194.

Rathbone, M. J., & Hadgraft, J. (1991). Absorption of drugs from the human oral cavity. Int. J. Pharm., 74(1), 9-24.

Rathbone, M. J., & Tucker, I. G. (1993). Mechanisms, barriers and pathways of oral mucosal drug permeation. Adv. Drug Deliv. Rev., 12(1–2), 41-60.

Ravi Kumar Reddy, J., Indira Muzib, Y., & Chowdary, K. P. R. (2013). Development and in-vivo characterization of novel trans buccal formulations of Amiloride hydrochloride. J. Pharm. Res.,, 6(6), 647-652.

Rossi, S., Sandri, G., & Caramella, C. M. (2005). Buccal drug delivery: A challenge already won? Drug Discov. Today. Technol., 2(1), 59-65.

Sadeghi, A. M. M., Avadi, M. R., Ejtemaimehr, S., Abashzadeh, S., Partoazar, A., Dorkoosh, F., Faghihi, M., Rafiee-Tehrani, M., & Junginger, H. E. (2009). Development of a Gas Empowered Drug Delivery system for peptide delivery in the small intestine. J. Control. Release., 134(1), 11-17.

Salamat-Miller, N., Chittchang, M., & Johnston, T. P. (2005). The use of mucoadhesive polymers in buccal drug delivery. Adv. Drug Deliv. Rev., 57(11), 1666-1691.

Sander, C., Nielsen, H. M., & Jacobsen, J. (2013). Buccal delivery of metformin: TR146 cell culture model evaluating the use of bioadhesive chitosan discs for drug permeability enhancement. Int. J. Pharm., 458(2), 254-261.

Sandri, G., Rossi, S., Bonferoni, M. C., Ferrari, F., Zambito, Y., Colo, G. D., & Caramella, C. (2005). Buccal penetration enhancement properties of N-trimethyl chitosan: Influence of quaternization degree on absorption of a high molecular weight molecule. Int. J. Pharm., 297(1–2), 146-155.

Sawicki, W., & Janicki, S. (2002). Pharmacokinetics of verapamil and its metabolite norverapamil from a buccal drug formulation. Int. J. Pharm., 238(1–2), 181-189.

Selvaratnam, L., Cruchley, A. T., Navsaria, H., Wertz, P. W., Hagi-Pavli, E. P., Leigh, I. M., Squier, C. A., & Williams, D. M. (2001). Permeability barrier properties of oral keratinocyte cultures: a model of intact human oral mucosa. Oral Dis., 7(4), 252-258.

Şenel, S., Çapan, Y., Sargon, M. F., Giray, C. B., & Hıncal, A. A. (1998). Histological and bioadhesion studies on buccal bioadhesive tablets containing a penetration enhancer sodium glycodeoxycholate. Int. J. Pharm., 170(2), 239-245.

Şenel, S., Çapan, Y., Sargon, M. F., İkinci, G., Şolpan, D., Güven, O., Boddé, H. E., & Hincal, A. A. (1997). Enhancement of transbuccal permeation of morphine sulfate by sodium glycodeoxycholate in vitro. J. Control. Release., 45(2), 153-162.

Şenel, S., & Hıncal, A. A. (2001). Drug permeation enhancement via buccal route: possibilities and limitations. J. Control. Release., 72(1–3), 133-144.

Shidhaye, S. S., Thakkar, P. V., Dand, N. M., & Kadam, V. J. (2010). Buccal Drug Delivery of Pravastatin Sodium. AAPS PharmSciTech., 11(1), 416-424.

Shin, S.-C., & Kim, J.-Y. (2000). Enhanced permeation of triamcinolone acetonide through the buccal mucosa. Eur. J. Pharm. Biopharm., 50(2), 217-220.

Shinkar, D. M., Dhake, A. S., & Setty, C. M. (2012). Drug delivery from the oral cavity: a focus on mucoadhesive buccal drug delivery systems. PDA J. Pharm. Sci. Technol., 66(5), 466-500.

Shojaei, A. H. (1998). Buccal Mucosa as a route for systemic drug delivery: a review. J. Pharm. Pharm. Sci., 1(1), 15-30.

Shojaei, A. H., & Li, X. (1997). Mechanisms of buccal mucoadhesion of novel copolymers of acrylic acid and polyethylene glycol monomethylether monomethacrylate. J. Control. Release., 47(2), 151-161.

Siepmann, J., & Peppas, N. A. (2012). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv. Drug Deliv. Rev., 64, Supplement(0), 163-174.

Skulason, S., Asgeirsdottir, M. S., Magnusson, J. P., & Kristmundsdottir, T. (2009). Evaluation of polymeric films for buccal drug delivery. Pharmazie, 64(3), 197-201.

Smart, J. D. (2005). The basics and underlying mechanisms of mucoadhesion. Adv. Drug Deliv. Rev., 57(11), 1556-1568.

Smart, J. D., Nantwi, P. K. K., Rogers, D. J., & Green, K. L. (2002). A quantitative evaluation of radiolabelled lectin retention on oral mucosa in vitro and in vivo. Eur. J. Pharm. Biopharm., 53(3), 289-292.

Squier, C. A. (1991). The Permeability of Oral Mucosa. Crit. Rev. Oral Biol. Med., 2(1), 13-32.

Sriamornsak, P., Wattanakorn, N., Nunthanid, J., & Puttipipatkhachorn, S. (2008). Mucoadhesion of pectin as evidence by wettability and chain interpenetration. Carbohydr. Polym., 74(3), 458-467.

Starokadomskyy, P. L., & Dubey, I. Y. (2006). New absorption promoter for the buccal delivery: Preparation and characterization of lysalbinic acid. Int. J. Pharm., 308(1–2), 149-154.

Steward, A., Bayley, D. L., & Howes, C. (1994). The effect of enhancers on the buccal absorption of hybrid (BDBB) α-interferon. Int. J. Pharm., 104(2), 145-149.

Stratford Jr, R. E., & Lee, V. H. L. (1986). Aminopeptidase activity in homogenates of various absorptive mucosae m the albino rabbit: implications in peptide delivery. Int. J. Pharm., 30(1), 73-82.

Sudhakar, Y., Kuotsu, K., & Bandyopadhyay, A. K. (2006). Buccal bioadhesive drug delivery — A promising option for orally less efficient drugs. J. Control. Release., 114(1), 15-40.

Tadros, M. I. (2010). Controlled-release effervescent floating matrix tablets of ciprofloxacin hydrochloride: Development, optimization and in vitro–in vivo evaluation in healthy human volunteers. Eur. J. Pharm. Biopharm., 74(2), 332-339.

Tsagogiorgas, C., Theisinger, S., Holm, P., Thiel, M., Quintel, M., & Holm, R. (2013). Buccal absorption of propofol when dosed in 1-perfluorobutylpentane to anaesthetised and conscious Wistar rats and Göttingen mini-pigs. Eur. J. Pharm. Biopharm., 85(3, Part B), 1310-1316.

Tsutsumi, K., Obata, Y., Nagai, T., Loftsson, T., & Takayama, K. (2002). Buccal absorption of ergotamine tartrate using the bioadhesive tablet system in guinea-pigs. Int. J. Pharm., 238(1–2), 161-170.

Tsutsumi, K., Obata, Y., Takayama, K., Loftsson, T., & Nagai, T. (1998). Effect of the cod-liver oil extract on the buccal permeation of ionized and nonionized forms of ergotamine using the keratinized epithelial-free membrane of hamster cheek pouch mucosa. Int. J. Pharm., 174(1–2), 151-156.

Ugwoke, M. I., Agu, R. U., Verbeke, N., & Kinget, R. (2005). Nasal mucoadhesive drug delivery: Background, applications, trends and future perspectives. Adv. Drug Deliv. Rev., 57(11), 1640-1665.

Van Der Bijl, P., Penkler, L., & Van Eyk, A. D. (2000). Permeation of Sumatriptan Through Human Vaginal and Buccal Mucosa. Headache., 40(2), 137-141.

Veuillez, F., Kalia, Y. N., Jacques, Y., Deshusses, J., & Buri, P. (2001). Factors and strategies for improving buccal absorption of peptides. Eur. J. Pharm. Biopharm., 51(2), 93-109.

Veuillez, F., Rieg, F. F., Guy, R. H., Deshusses, J., & Buri, P. (2002). Permeation of a myristoylated dipeptide across the buccal mucosa: topological distribution and evaluation of tissue integrity. Int. J. Pharm., 231(1), 1-9.

Voorspoels, J., Remon, J.-P., Eechaute, W., & De Sy, W. (1996). Buccal Absorption of Testosterone and Its Esters Using a Bioadhesive Tablet in Dogs. Pharm. Res., 13(8), 1228-1232.

Wang, J., Lu, W.-L., Liang, G.-W., Wu, K.-C., Zhang, C.-G., Zhang, X., Wang, J.-C., Zhang, H., Wang, X.-Q., & Zhang, Q. (2006). Pharmacokinetics, toxicity of nasal cilia and immunomodulating effects in Sprague–Dawley rats following intranasal delivery of thymopentin with or without absorption enhancers. Peptides, 27(4), 826-835.

Wang, L., & Tang, X. (2008). A novel ketoconazole bioadhesive effervescent tablet for vaginal delivery: Design, in vitro and 'in vivo' evaluation. Int. J. Pharm., 350(1–2), 181-187.

Willits, R. K., & Saltzman, W. M. (2001). Synthetic polymers alter the structure of cervical mucus. Biomaterials., 22(5), 445-452.

Woertz, C., Preis, M., Breitkreutz, J., & Kleinebudde, P. (2014). Assessment of test methods evaluating mucoadhesive polymers and dosage forms: An overview. Eur. J. Pharm. Biopharm., 85(3), 843-853.

Woodley, J. (2001). Bioadhesion. Clin. Pharmacokinet., 40(2), 77-84.

Xu, H.-B., Huang, K.-X., Zhu, Y.-S., Gao, Q.-H., Wu, Q.-Z., Tian, W.-Q., Sheng, X.-Q., Chen, Z.-X., & Gao, Z.-H. (2002). Hypoglycaemic effect of a novel insulin buccal formulation on rabbits. Pharmacol. Res., 46(5), 459-467.

Yehia, S., El-Gazayerly, O., & Basalious, E. (2008). Design and In Vitro/In Vivo Evaluation of Novel Mucoadhesive Buccal Discs of an Antifungal Drug: Relationship Between Swelling, Erosion, and Drug Release. AAPS PharmSciTech., 9(4), 1207-1217.

Zhang, J., Niu, S., Ebert, C., & Stanley, T. H. (1994). An in vivo dog model for studying recovery kinetics of the buccal mucosa permeation barrier after exposure to permeation enhancers: apparent evidence of effective enhancement without tissue damage. Int. J. Pharm., 101(1–2), 15-22.

2.1 Buspirone Hydrochloride

2.1.1 Chemistry

Chemically, buspirone hydrochloride (BS) is 8-[4-[4-(2-pyrimidinyl)-1piperazinyl] butyl]-8-azaspiro decane-7, 9-dione monohydrochloride. The empirical formula $C_{21}H_{31}N_5O_2 \cdot HCl$ is represented by the following structural formula (Figure 2.1) with molecular weight 422.0 (Sheikhzadeh et al., 2007b; Sheikhzadeh et al., 2007c)

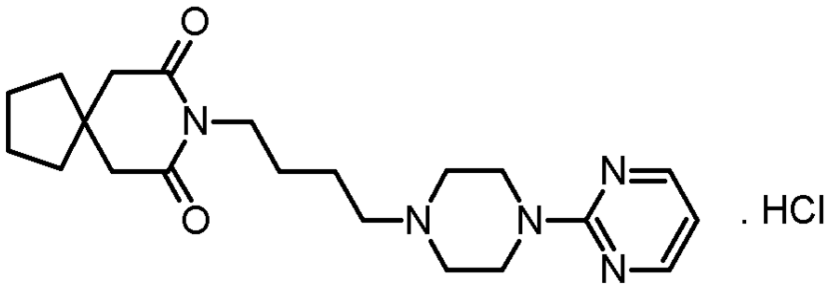


Figure 2.1: Structure of Buspirone Hydrochloride

2.1.2 Polymorphism

BS has several polymorphs and extensive polymorph screening of BS has shown that close to 90% of the experiments results in the production of two main polymorphs, namely Form 1 with a melting point at 188 °C and Form 2 with a melting point at 203 °C (Sheikhzadeh et al., 2007a; Sheikhzadeh et al., 2007b). These two crystal structures are enantiotropes and the transformation temperature from Form 1 to Form 2 is at 95 ºC (Sheikhzadeh et al., 2007a; Sheikhzadeh et al., 2007b). The inter-conversion of two polymorphs can occur in the salt formation step which is the last step in the synthesis of BS. Polymeric inter-conversion also depends on pH, co-solvent ratio and amount of solvent (Sheikhzadeh et al., 2007a; Sheikhzadeh et al., 2006).

2.1.3 Solubility

Form 1 and 2 of BS are white crystalline and has solubility in both water and alcohol. Both the forms of BS have low solubility in isopropyl alcohol compared to that of water. Further literature reports of BS suggest that the Form 2 has higher solubility than Form 1 in both water and isopropyl alcohol (Sheikhzadeh et al.,

2007c). BS is very soluble in 0.1N HCl (approx. 7.306 g/mL) and 0.1N NaOH solution (5.607 g/mL) (Sheikhzadeh et al., 2007c). The dissociation constants reported for the BS are 1.93 and 7.64 (Shalaeva et al., 2008).

2.2 Pharmacodynamic Profile

2.2.1 Mechanism of action

Buspirone hydrochloride (BS) is an anxiolytic agent and a serotonin receptor partial agonist belonging to the azaspirodecanedione class of compounds. BS is a partial agonist for the serotonin 5-HT1A receptors and is an antagonist for the dopamine D2 auto receptors and has weak affinity to 5-HT2 receptors (Blier & Ward, 2003; Ortiz et al., 1987; Sramek et al., 1997; Sramek et al., 2002). BS is as effective as benzodiazepines for the treatment of generalized anxiety and superior to placebo (Goldberg, 1984). The mechanism of action for the drug buspirone is unknown; it clearly does not act on the same receptor systems that are affected by benzodiazepines. BS does not possess anticonvulsant or muscle relaxant properties and also does not cause sedative or physical dependence like other anxiolytics (Riblet et al., 1983; Taylor et al., 1985) and has little or no sedative effect and has been reported to be safe even when given in very high doses (Erhorn, 2007).

2.2.2 Treatment in generalized anxiety disorder (GAD)

BS is clinically proven in the management of anxiety disorders or the short-term relief of the symptoms of anxiety at doses as low as 7.5 mg (Erhorn, 2007; Frackiewicz & Tigel, 2001; Loane & Politis, 2012; Riblet et al., 1983; Taylor et al., 1985). Clinical trials have demonstrated that BS is effective in the treatment of anxiety with efficacy and dosage comparable to diazepam or chlorazepate (Taylor et al., 1985).

2.2.3 Other effects

BS has been reported to be helpful in some of the neurological and psychiatric disorders (Loane & Politis, 2012) such as tardive diskineia (Ross, 1987), social phobia (Condren et al., 2002; Munjack et al., 1991), olivopontocerebellar atrophy (OPCA) (Heo et al., 2008). Reports suggest that BS may be useful in detoxification of the alcohol and can also be used in alcohol withdrawal management (Dougherty & Gates, 1990).

2.3 Pharmacokinetic Profile

The pharmacokinetic profile of BS has been evaluated by randomized, double blind study in patients with generalized anxiety disorder (GAD), commonly called persistent anxiety and the studies demonstrated that BS is effective in the treatment of anxiety and anxiety in the presence of depression (Riblet et al., 1983; Salazar et al., 2001; Sramek et al., 1997). A multiple-dose study carried in 15 subjects suggests that buspirone has nonlinear pharmacokinetics. Thus, dose increases and repeated dosing may lead to somewhat higher blood levels of unchanged buspirone than would be predicted from results of single-dose studies.

2.3.1 Absorption

BS is rapidly and completely absorbed in man and undergoes extensive first pass metabolism resulting is very low oral bioavailability of approximately 4% (Mahmood & Sahajwalla, 1999). Following oral administration, plasma concentrations of unchanged BS are very low and variable between subjects. Peak plasma levels of 1 ng/mL to 6 ng/mL have been observed at 40 to 90 minutes after single oral doses of 20 mg. The single-dose bioavailability of unchanged buspirone when taken as a tablet is on the average about 90% of an equivalent dose of solution, but there is large variability (Lilja et al., 1998; Ratey et al., 1989; Realmuto et al., 1989). Peak plasma concentration of BS varies with and without food (Lilja et al., 1998; Mahmood & Sahajwalla, 1999). The effect of food on the pharmacokinetics of buspirone was evaluated in 8 healthy individuals in a 2-way crossover design study following an oral dose of buspirone 20mg. Buspirone was administered 15 minutes after the meal following an overnight fast. The results of the study indicated that food increased the area under the concentration- time curve (AUC) and the C_{max} of buspirone almost 2-fold, whereas there was negligible change in t_{max} and half-life between fed and fasting states (Gammans et al., 1986; Gammans et al., 1985; Gammans et al., 1989).

2.3.2 Distribution

The volume of distribution of buspirone is 5.3 L/kg. Plasma protein binding of buspirone is >95% and buspirone is bound to albumin and α1-acid glycoprotein (Gammans et al., 1986).

2.3.3 Metabolism and elimination

BS is metabolized primarily by cytochrome P450 3A4 (CYP 3A4). BS is oxidized to several hydroxylated derivatives and a pharmacologically active metabolite, 1-pyrimidinylpiperazine (1-PP). In animal models predictive of anxiolytic potential, 1-PP has about one quarter of the activity of buspirone, but is present in up to 20-fold greater amounts (Zhu et al., 2005). However, this is not important in humans as blood samples from humans chronically exposed to marketed BS oral dosage form (BuSpar®) demonstrated high levels of 1-PP; mean values are approximately 3 ng/mL and the highest human blood level recorded among 108 chronically dosed patients was 17 ng/mL, less than 1/200th of 1-PP levels found in animals given large doses of buspirone without signs of toxicity.

In a single-dose study using 14C-labeled buspirone, 29% to 63% of the dose was excreted in the urine within 24 hours, primarily as metabolites; fecal excretion accounted for 18% to 38% of the dose. The average elimination half-life of unchanged buspirone after single doses of 10 mg to 40 mg is about 2 to 3 hours.

2.4. Dosage and administration

Commercially BS is supplied as conventional oral tablets with dose ranging from 5 to 30 mg. The recommended initial dose is 15 mg daily (7.5 mg b.i.d.). To achieve an optimal therapeutic response, at intervals of 2 to 3 days the dosage may be increased 5 mg per day, as needed. The maximum daily dosage should not exceed 60 mg per day. In clinical trials allowing dose titration, divided doses of 20 mg to 30 mg per day were commonly employed. The bioavailability of buspirone increases when given with food as compared to the fasted state. Consequently, patients should take buspirone in a consistent manner with regard to the timing of dosing; either always with or always without food (Mahmood & Sahajwalla, 1999).

References

Blier, P., & Ward, N. M. (2003). Is there a role for 5-HT1A agonists in the treatment of depression? Biol. Psychiatry, 53(3), 193-203.

Condren, R. M., Dinan, T. G., & Thakore, J. H. (2002). A preliminary study of buspirone stimulated prolactin release in generalised social phobia: evidence for enhanced serotonergic responsivity? Eur. Neuropsychopharmacol., 12(4), 349-354.

Dougherty, R. J., & Gates, R. R. (1990). The role of buspirone in the management of alcohol withdrawal: A preliminary investigation. J. Subst. Abuse Treat., 7(3), 189-192.

Erhorn, S. (2007). Buspirone. In S. J. E. Editors-in-Chief: & B. B. David (Eds.). xPharm: The Comprehensive Pharmacology Reference (pp. 1-5). New York: Elsevier.

Frackiewicz, E. J., & Tigel, P. D. (2001). Pharmacokinetics and tolerability of buspirone during oral administration to children and adolescents with anxiety disorder and normal healthy adults. Eur. Neuropsychopharmacol., 11, Supplement 3(0), S314.

Gammans, R. E., Mayol, R. F., & LaBudde, J. A. (1986). Metabolism and disposition of buspirone. Am. J. Med., 80(3B), 41-51.

Gammans, R. E., Mayol, R. F., Mackenthun, A. V., & Sokya, L. F. (1985). The relationship between buspirone bioavailability and dose in healthy subjects. Biopharm. Drug Dispos., 6(2), 139-145.

Gammans, R. E., Westrick, M. L., Shea, J. P., Mayol, R. F., & LaBudde, J. A. (1989). Pharmacokinetics of buspirone in elderly subjects. J. Clin. Pharmacol., 29(1), 72-78.

Goldberg, H. L. (1984). Buspirone hydrochloride: a unique new anxiolytic agent. Pharmacokinetics, clinical pharmacology, abuse potential and clinical efficacy. Pharmacotherapy, 4(6), 315-324.

Heo, J.-H., Lee, S.-T., Chu, K., & Kim, M. (2008). The efficacy of combined estrogen and buspirone treatment in olivopontocerebellar atrophy. J. Neurol. Sci., 271(1–2), 87-90.

Lilja, J. J., Kivisto, K. T., Backman, J. T., Lamberg, T. S., & Neuvonen, P. J. (1998). Grapefruit juice substantially increases plasma concentrations of buspirone[ast]. Clin. Pharmacol. Ther., 64(6), 655-660.

Loane, C., & Politis, M. (2012). Buspirone: what is it all about? Brain Res., 1461, 111-118.

Mahmood, I., & Sahajwalla, C. (1999). Clinical pharmacokinetics and pharmacodynamics of buspirone, an anxiolytic drug. Clin. Pharmacokinet., 36(4), 277-287.

Munjack, D. J., Bruns, J., Baltazar, P. L., Brown, R., Leonard, M., Nagy, R., Koek, R., Crocker, B., & Schafer, S. (1991). A pilot study of buspirone in the treatment of social phobia. J. Anxiety Disord., 5(1), 87-98.

Ortiz, A., Pohl, R., & Gershon, S. (1987). Azaspirodecanediones in generalized anxiety disorder: buspirone. J. Affect. Disord., 13(2), 131-143.

Ratey, J. J., Sovner, R., Mikkelsen, E., & Chmielinski, H. E. (1989). Buspirone therapy for maladaptive behavior and anxiety in developmentally disabled persons. J. Clin. Psychiatry, 50, 382-384.

Realmuto, G. M., August, G. J., & Garfinkel, B. D. (1989). Clinical effect of buspirone in autistic children. J. Clin. Psychopharmacol., 9, 122-125.

Riblet, L. A., Eison, A. S., Eison, M. S., Newton, R. E., Taylor, D. P., & Temple Jr, D. L. (1983). Buspirone: An anxioselective alternative for the management of anxiety disorders. Prog. Neuropsychopharmacol. Biol. Psychiatry, 7(4–6), 663-668.

Ross, C. A. (1987). Buspirone in the treatment of tardive dyskinesia. Med. Hypotheses, 22(3), 321-328.

Salazar, D. E., Frackiewicz, E. J., Dockens, R., Kollia, G., Fulmor, I. E., Tigel, P. D., Uderman, H. D., Shiovitz, T. M., Sramek, J. J., & Cutler, N. R. (2001). Pharmacokinetics and tolerability of buspirone during oral administration to children and adolescents with anxiety disorder and normal healthy adults. J. Clin. Pharmacol., 41(12), 1351-1358.

Shalaeva, M., Kenseth, J., Lombardo, F., & Bastin, A. (2008). Measurement of dissociation constants (pKa values) of organic compounds by multiplexed capillary electrophoresis using aqueous and cosolvent buffers. J. Pharm. Sci., 97(7), 2581-2606.

Sheikhzadeh, M., Murad, S., & Rohani, S. (2007a). Response surface analysis of solution-mediated polymorphic transformation of buspirone hydrochloride. J. Pharm. Biomed. Anal., 45(2), 227-236.

Sheikhzadeh, M., Rohani, S., Jutan, A., & Manifar, T. (2007b). Quantitative and molecular analysis of buspirone hydrochloride polymorphs. J. Pharm. Sci., 96(3), 569-583.

Sheikhzadeh, M., Rohani, S., Jutan, A., Manifar, T., Murthy, K., & Horne, S. (2006). Solid-state characterization of buspirone hydrochloride polymorphs. Pharm. Res., 23(5), 1043-1050.

Sheikhzadeh, M., Rohani, S., Taffish, M., & Murad, S. (2007c). Solubility analysis of buspirone hydrochloride polymorphs: Measurements and prediction. Int. J. Pharm., 338(1–2), 55-63.

Sramek, J. J., Frackiewicz, E. J., & Cutlet, N. R. (1997). Efficacy and safety of two dosing regimens of buspirone in the treatment of outpatients with persistent anxiety. Clin. Ther., 19(3), 498-506.

Sramek, J. J., Zarotsky, V., & Cutler, N. R. (2002). Generalised Anxiety Disorder: Treatment Options. Drugs, 62(11), 1635-1648.

Taylor, D. P., Eison, M. S., Riblet, L. A., & Vandermaelen, C. P. (1985). Pharmacological and clinical effects of buspirone. Pharmacol. Biochem. Behav., 23(4), 687-694.

Zhu, M., Zhao, W., Jimenez, H., Zhang, D., Yeola, S., Dai, R., Vachharajani, N., & Mitroka, J. (2005). Cytochrome P450 3a-mediated metabolism of buspirone in human liver microsomes. Drug Metab. Dispos., 33(4), 500-507.

3.1 Introduction

Development of a validated analytical method is utmost important stage for the design of dosage forms. The validated method provides qualitative and quantitative information about the drug of interest in various samples like bulk powders, formulations, in-vitro release samples, stability samples and biological samples.

3.2 Analytical Methods for Estimation of Buspirone Hydrochloride

Several methods have been reported for estimation of BS in bulk, formulations and biological samples. Estimation of BS and its degradation products in serum by HPLC method using UV detector has been reported (Kristjansson, 1991). The method reported uses a solid phase extraction procedure for the extraction of BS from biological matrix and separation was performed on a C18 column. Retention of BS on the column was based on ionic interactions due to the presence of sodium lauryl sulphate in the mobile phase (Kristjansson, 1991). Stability indicating HPLC assay for the analysis of BS and its potential impurities and degradation products using C18 column has also been reported. The analysis of samples was carried out using a photo-diode array detector (Khedr & Sakr, 1999). HPLC method to determine the plasma concentrations of BS and its active metabolite 1-pyrimidinyl-piperazine (1-PP) by UV detector has been reported (Du et al., 2003). Literature report for estimation of BS in human plasma using HPLC by C18 column is also available, initially plasma sample cleanup was performed using solid phase extraction cartridges and BS was monitored by UV detector (Foroutan et al., 2004). A stability indicating HPLC method using UV detector for estimation of BS in bulk and pharmaceutical formulations has also been reported. The chromatographic separation was performed using C18 column (Azeem et al., 2009). A method reported for estimation of BS and it's metabolites in human plasma using LC/MS technique was also found to be precise and accurate (Nägele & Fandino, 2007). Another method for estimation of BS and its metabolite (1-PP) using electrochemical detector has also been described in the literature (Betto et al., 1992). Estimation of BS and its metabolite in rat plasma using HPLC has been reported as well. This method uses an ion paired HPLC solid extraction scheme for estimation of BS and its metabolite (Aparicio et al., 1988). A selective and sensitive HPLC method with coulometric detection has been described for quantitation of BS and its active

metabolite (1-PP) in plasma samples of mice (Betto et al., 1992). Simultaneous estimation of BS along with other drugs and its metabolites using HPLC has also been reported (Bianchi & Caccia, 1988). An isocratic reversed-phase HPLC with coulometric end-point detection for estimation of BS and its metabolite in plasma utilizing solid phase extraction column has also been reported (Odontiadis & Franklin, 1996). Determination of BS and its metabolites using LC-MS with electrospray time-of-flight (ESI-TOF) have also been reported (Nägele & Fandino, 2007). Estimation of BS using LC-MS in rat samples was also reported as well (Kerns et al., 1997).

An exhaustive survey of literature revealed that none of the reported methods were suitable for routine analysis of BS in formulations and in in-vitro release samples of present research endeavor. In general, the method of analysis should be simple, cost effective and less time consuming apart from being sensitive, accurate, precise and stability indicating. Simple spectrophotometric methods are found to be very suitable for routine analysis of formulations for content uniformity and analysis of in-vitro release samples. The methods reported for estimation of BS in biosamples, although found to be very sensitive, precise and accurate but mostly uses LC-MS technique (Chew et al., 2006; Gammans et al., 1985; Kerns et al., 1997; Nägele & Fandino, 2007; Zayed et al., 2007; Zhu et al., 2013). Such methods are not practical in laboratories with relatively modest infrastructure. Moreover, extensive literature survey did not reveal any method for estimation of BS rabbit plasma. As rabbit was selected as animal model for in-vivo studies of developed formulations, it was planned to develop a simple, sensitive and accurate HPLC method for estimation of BS in rabbit serum.

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

These developed and validated methods were used for estimation of BS in bulk, formulations, in-vitro release samples, stability samples and biological samples.

3.3 Materials

Buspirone hydrochloride (BS) was provided as gift sample by Astron Research limited, Gujarat, India. Analytical grade potassium dihydrogen orthophosphate, sodium hydroxide, acetonitrile (HPLC grade) and orthophosphoric acid were purchased form Sigma, India. Xanthan gum (XG) was supplied as gift sample by Signet Chemical Corporation Pvt. Ltd., India. Lactose and mannitol were purchased from CDH fine chemicals, India. Deionized water used for analytical studies was obtained using a Millipore water purification system (Milli-Q). Buffers were filtered through nylon membrane filters of pore diameter 0.22 µm purchased from Millipore® India. Solid phase extraction cartridges (HyperSep Retain PEP 30 mg, 1 mL capacity) were purchased from Thermo Scientific India Limited, India.

3.4 Reagents

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

Orthophosphoric acid: Ortho phosphoric acid (6.78 mL) was diluted to 1000 mL using deionized water.

Phosphate buffer (pH 3.0): Potassium dihydrogen orthophosphate (1.36 g) was dissolved in deionized water and the volume was made up to 1000 mL using deionized water. The pH of the solution was adjusted to 3.0 using 0.1 M orthophosphoric acid.

3.5 Analytical Method 1: Ultraviolet (UV) Spectrophotometric Method for Estimation of BS in Bulk, Formulations and In-Vitro Drug Release Samples

3.5.1 Instrumentation

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

3.5.2 Selection of media

The criterion for selection of media was based on the solubility, stability and selectivity. The buccal salivary pH reported in various reports was pH 6.5 to 7.0, an optimum pH of 6.8 (Aframian et al., 2006; Campisi et al., 2010; Smart, 1993) was selected to analyze the BS in bulk, formulations and in-vitro drug release samples.

3.5.3 Calibration curve

A stock solution of 100 µg/mL was prepared by dissolving 5 mg of BS in 50 mL of pH 6.8 phosphate buffer. The λ_{max} was determined and selected by scanning a suitable dilution of stock using spectrophotometer. From the above stock solution suitable dilutions were prepared to obtain calibration concentration solutions of 2.5, 5, 10, 15, 20 and 25 µg/mL and the absorbance was measured for all the dilutions at the selected λ_{max} of the drug.

To establish linearity of the proposed method, eight separate calibration sets were prepared and analyzed. The stability of drug solution during analysis was assessed by analyzing samples at different time intervals on the same day and the subsequent day by storing at 25 ± 2 ^{o}C.

3.5.4 Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996).

Specificity and selectivity of the method was assessed by scanning a known concentration of drug solution (20 µg/mL) from the stock solution of pure drug. Drug solutions with and without excipients (mannitol, lactose and XG) used in the design of buccal formulations were prepared and analyzed for any change in the absorbance spectra of BS.

For determining the accuracy of the proposed method, different quality control (QC) levels of drug concentrations [lower quality control samples (LQC) = 4 µg/mL, medium quality control samples (MQC) = 12.5 µg/mL, and higher quality control samples (HQC) = 22.5 µg/mL] were prepared independently from stock solution and analyzed (n = 6). Accuracy was assessed by calculating mean percentage recovery and percentage bias (% bias). % bias was calculated as, % bias = [(Predicted conc.-Nominal Conc.) / Nominal Conc.] x 100. Further, different concentrations of pure drug (5, 10 and 15 µg/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] × 100, where Cv is the total drug concentration measured after standard addition, Cu is the drug concentration in the formulation, and Ca is the drug concentration added to the formulation solution.

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

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

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

3.5.5 Estimation of drug content in commercial tablets and in-house designed buccal discs

Commercially available oral tablet brand of BS containing 5 mg of drug (Brand: Buspin-5, Manufacturer: Intas Pharmaceuticals) was obtained from the local pharmacy market for estimation of total drug content per tablet. Tablets (20) were

weighed and finely powdered using mortar and pestle. An accurately weighed aliquot amount (equivalent to 5 mg of BS) was transferred to a series of 100 mL volumetric flasks (n=3) and in pH 6.8 phosphate buffer was added to this. The samples were then subjected to bath sonication process for complete solubility of BS, finally the volume was made up to 100 mL. An aliquot of this solution was filtered through syringe filter and was diluted suitably to obtain a concentration of 5 µg/mL with the media selected and the samples were analyzed using proposed method.

3.6 Results and Discussion

3.6.1 Selection of media

BS is available as its salt form and has considerably good solubility in aqueous and organic phase. BS is very soluble in 0.1N HCl (approx. 7.306 g/mL), 0.1N NaOH solution (5.607 g/mL) (Sheikhzadeh et al., 2007) and freely soluble in commonly used organic solvents like methanol, acetonitrile, isopropyl alcohol and ethanol. Absorbance (λ_{max} 238 nm) of drug was found to be stable at least for 24 h in phosphate buffer pH 6.8 and was selected as solvent system on the basis of selectivity and stability.

3.6.2 Calibration curve

The spectrum of BS showed a distinct λ_{max} at 238 nm (Figure 3.1). The absorbance of samples at 238 nm was found to be stable for at least 24 h at 25 ± 2 °C, indicating stability of the drug in the selected media. Absorbance values for different drug concentrations are shown in Table 3.1. At all concentration levels the SD was low and the % RSD did not exceed 1.87. The predicted concentrations were nearly matching with nominal concentrations. Linearity range was found to be 2.5-25 µg/mL. The linear regression equation obtained was Absorbance= [0.0512 x Concentration in µg/mL] + 0.0097; with excellent regression coefficient of 0.9999.

Individual values of slopes and intercepts obtained from replicate measurements were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (7.98×10^{-5}) and standard error of intercept (1.81×10^{-3}) in addition to lower calculated *F*-value [calculated *F* value (7, 40) of 4.25×10^{-4} and critical *F*-value of 2.25 at $p = 0.05$] further confirmed precision of the method.

3.6.3 Analytical method validation

Estimation of BS in formulations and comparison of pure drug spectrum with that of drug spectrum in presence of various common excipients used in the buccal formulations confirmed lack of interference at the selected wave length employed (λ_{max} 238 nm) in the present method. Absence of interference confirmed selectivity and specificity of the proposed method. Figure 3.2 shows overlaid spectra of solutions of pure drug and solutions containing xanthan gum (XG) and drug in 1:1 ratio in the selected media.

All three QC levels (LQC, MQC, HQC) showed an accuracy (% bias) ranging from -0.19 to 1.44. The high (nearly 100%) mean percent recovery values and their low SD values (SD < 1.15) represented the accuracy of the method (Table 3.2). In the standard addition method, the mean percentage analytical recoveries (± SD) for LQC, MQC and HQC levels were found to be 100.07 (± 0.82), 99.68 (± 1.08) and 101.17 (± 0.96) respectively. This result further established the validity and reliability of the proposed method.

In repeatability study, the % RSD ranged from 0.32 to 1.27 (Table 3.3). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 1.27% and inter-day variation less than 1.08% (Table 3.3). Lower % RSD values indicated the repeatability and intermediate precision of the method.

LOD and LOQ were found to be 0.23 µg/mL and 0.77 µg/mL respectively. The mean percentage recovery (± SD) of 0.77 µg/mL (LOQ) in triplicate was found to be 100.73 (± 2.70) representing the accuracy and precision of the method. The method was found to be robust as variation of pH of the selected media by ± 0.5 units.

Estimation of BS from the marketed oral tablet and in-house designed buccal formulations by the proposed method was found 100.65 ± 1.84 and 99.15 ± 1.38 respectively (Table 3.4). This indicated absence of interference of excipients.

3.7 Analytical method 2: Liquid chromatographic method for estimation of BS in bulk and formulations.

3.7.1 Instrument

The chromatographic instrument used was Shimadzu Japan, equipped with a binary flow pump (Model LC-10AT VP Shimadzu LC, Japan), auto sampler (SIL-HTA, Shimadzu, Japan), UV-Visible detector (Model SPD-10A VP-Shimadzu Shimadzu LC, Japan) and column oven (CTO-10AS Shimadzu LC, Japan). Data collection and integration was accomplished using LC solutions software.

3.7.2 Chromatographic conditions

Chromatographic separation was performed on a C8 column (LiChroCART®; 250mm x 4.6mm ID, 5µm particle size, Merck, Germany) equipped with a guard column of same packing material. Mobile phase consisted of pH 3.0 phosphate buffer (0.025 M potassium dihydrogen phosphate buffer in distilled water, pH adjusted to 3.0 using 0.1 M orthophosphoric acid) and acetonitrile (75:25 v/v) maintained at 25 ºC using a column oven. The buffer was filtered through 0.22 µm membrane using a vacuum filtration assembly (Millipore). The HPLC system was run for minimum 1 h at 1 mL/min flow rate for system equilibration through baseline monitoring, prior to actual analysis. BS was monitored at wavelength of 238 nm with mobile phase flow rate of 1 mL/min. The injection volume was 50 µL.

3.7.3 Selection of mobile phase

For mobile phase optimization various buffers of different pH and in varying combination with acetonitrile and methanol were investigated. Main purpose was to develop a simple, precise, sensitive and selective HPLC method for quantitation of BS in bulk, dosage forms and stability samples. Mobile phase finally selected consisted of an aqueous phase (0.025 M potassium dihydrogen orthophosphate buffer in distilled water, pH adjusted to 3.0 using 0.1 M orthophosphoric acid) and acetonitrile (75:25 v/v). For the selection of mobile phase, the criteria employed were peak properties (retention time and asymmetric factor), sensitivity (height and area), ease of sample preparation and applicability of the method for various purposes.

3.7.4 Preparation of calibration curve

Primary stock of BS was prepared by dissolving 5 mg of BS in mobile phase and making up the volume to 50 mL to obtain a concentration of 100 µg/mL using same solvent system. Secondary stock of 10 µg/mL concentration was prepared by appropriate dilution of primary stock by mobile phase. From the secondary stock solution, calibration standards of 25, 50, 100, 250, 500, 1000, 1500 ng/mL concentrations were made by suitable dilution with mobile phase for the purpose of calibration curve. 50 µl of each concentration was injected automatically using a program controlled auto sampler and the area of the peak at 238 nm was determined. To establish linearity of the proposed method, six separate calibrations sets were prepared and analyzed.

3.7.5 Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996).

To study selectivity of the method, BS stock solutions (100 µg/mL) were separately prepared in a mobile phase with and without common excipients (mannitol, lactose and XG). All the solutions were diluted suitably with the mobile phase to get a drug concentration of 100 ng/mL and were analyzed. A blank solution containing only excipients was also injected and interference near the drug peak was checked.

For determining the accuracy of the proposed method, different QC levels of drug concentrations [lower quality control samples (LQC) = 30 ng/mL, medium quality control samples (MQC) = 750 ng/mL and higher quality control samples (HQC) = 1250 ng /mL] were prepared independently from stock solution and analyzed (n = 6). Accuracy was assessed by calculating mean percentage recovery and percentage bias (% bias). % bias was calculated as, % bias = [(Predicted conc.-Nominal Conc.)/ Nominal Conc.] x 100. Further, different concentrations of pure drug (100, 200 and 500 ng/mL) were added to a known pre-analyzed formulation sample and analyzed using the proposed method (n = 3) to check analytical recovery. The percent analytical recovery of the added pure drug was calculated as, % Analytical Recovery = [(Cv – Cu)/Ca] × 100, where Cv is the total drug concentration measured after standard addition, Cu is the drug concentration in the formulation, and Ca is the drug concentration added to formulation solution.

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

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

Robustness study was conducted by making small but deliberate changes to the optimized method parameters. Robustness of the developed method was determined by varying the flow rate and percentage of organic phase. Bench top and stock solution stability of BS was established by storing the samples at controlled room temperature (CRT) of 25 ± 2 ^{o}C for 24 h.

3.7.6 Estimation of drug content in commercial tablets

Commercially available tablet brand of BS (Buspin 5, contains 5 mg of drug) was purchased from local market and in-house designed buccal discs were analyzed for total drug content per formulation was estimated by proposed method. Tablets (20) are weighed, finely powdered and mixed using a clean mortar and pestle. An accurately weighed aliquot amount (equivalent to 5 mg of BS) was transferred to a 50 mL volumetric flasks (n=6) and dissolved in mobile phase solvent system by sonication (20 min) and volume was made up to 50 mL. The aliquot of resulting solution was filtered through 0.45 µm syringe filters and was diluted suitably with the mobile phase to obtain a secondary stock of concentration 10 µg/mL. An aliquot of secondary stock was suitably diluted with mobile phase to obtain a concentration 750 ng/mL and the samples were analyzed using the proposed method.

3.8 Results and Discussion

3.8.1 Selection of mobile phase

Optimization of mobile phase consisting of aqueous phase (0.025 M potassium dihydrogen orthophosphate buffer, pH adjusted to 3.0 using 0.1 M orthophosphoric acid) and acetonitrile (75:25 v/v), was based on peak properties (retention time and asymmetric factor) and sensitivity (height and area). With optimized mobile phase retention time of BS was found to be 8.50 ± 0.41 min with an asymmetric factor of 1.11 ± 0.08 (Figure 3.3). The retention time of BS increased to 14.12 min with decrease in proportion of acetonitrile from 25% v/v to 20% v/v in the mobile phase. However, there was no effect on peak area, peak height and asymmetric factor. Use of methanol (25% v/v) instead of acetonitrile (25% v/v) in the mobile phase increased retention time of BS to 18.11 min and asymmetric factor was more than 2.0. Change in pH of aqueous phase above 3.0 affected asymmetric factor of peak. Decrease in molar concentration resulted in increase asymmetric factor. Thus, aqueous phase (0.025 M potassium dihydrogen phosphate buffer, pH adjusted to 3.0 using 0.1 M orthophosphoric acid) and acetonitrile (75:25 v/v) was finally selected as mobile phase.

3.8.2 Calibration curve

Different concentrations and their corresponding area at 238 nm are shown in the Table 3.5. At all the concentration levels, the SD of the area was low and the % RSD did not exceed 1.91. Overlaid chromatograms of blank and selected concentration in the calibration range are shown in Figure 3.3. Total run time for single injection was 10 min for the proposed method. The predicted concentrations were nearly matching with the nominal concentrations. The linear regression equation obtained was Peak Area = [145.94 x Concentration in ng/mL] + 64.98; with excellent regression coefficient of 0.9999. Individual values of slopes and intercepts obtained from replicate measurements were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (9.95×10^{-1}) and standard error of intercept (150.75) in addition to lower calculated *F*-value [calculated *F* value (5, 36) of 1.36×10^{-3} and critical *F*-value of 2.47 at $p = 0.05$] further confirmed precision of the method.

3.8.3 Analytical method validation

Overlaid chromatograms of pure BS and combination of BS with XG in 1:1 proportion is shown in Figure 3.4. Estimation of BS in formulations and comparison of pure drug peak with that of drug peak in presence of common excipients used in formulations confirmed lack of interference at the retention time of BS. The blank samples of excipients did not show any interference near the drug peak. In the presence of excipients, peak characteristics of the drug (retention time, area, and asymmetric factor) were not affected. This indicated that there is no significant interference of excipients in the estimation of the drug by the proposed method. This confirmed the specificity and selectivity of the method.

All three QC levels showed an accuracy (% bias) ranging from 1.15 to 1.76 (Table 3.6). The high (nearly 100%) mean % recovery values and their low SD values (SD < 1.87) represented the accuracy of the method. In the standard addition method, the mean percentage analytical recoveries (± SD) for 100, 200 and 500 ng/mL concentrations were found to be 100.31 (± 0.42), 100.42 (± 0.92) and 100.86 (± 0.68) respectively. This result further established the validity and reliability of the proposed method.

In repeatability study, the % RSD ranged from 0.39 to 1.63 (Table 3.7). Lower % RSD values indicated the repeatability and intermediate precision of the method.

LOD and LOQ were found to be 8.35 and 23.55 ng/mL respectively. The mean percentage recovery (± SD) of 25 ng/mL (LOQ) in triplicate was found to be 99.17 (± 1.16) representing the accuracy and precision of the method. The method was found to be robust as variation in proportion of organic component and flow rate did not have any significant effect on peak height, peak area and asymmetric factor. Different concentrations of bench-top BS solutions and stock solutions of BS showed % RSD values less than 1.71%, indicating stability of BS. These solutions exhibited no change in chromatographic characters (retention time, asymmetric factor, and area) at least for 24 h at room temperature. During this period no extra peaks were observed in the chromatograms across all concentrations indicating stability of BS.

Estimation of BS in pharmaceutical formulations by the proposed method was 100.75 ± 1.43 and 98.89 ± 1.70 for marketed oral tablet and in-house designed buccal discs respectively (Table 3.8). Assay values of formulations were very close to the

label claim. This indicated absence of interference of excipient matrix in estimation of BS by the proposed method. The estimated drug content with low values of SD further established precision of the proposed method (Table 3.8).

3.9 Analytical method 3: Liquid Chromatographic Method for Estimation of BS in Rabbit Plasma

3.9.1 Instrumentation and chromatographic conditions

Liquid chromatographic instrument and chromatographic condition used were same as mentioned in analytical method 2.

Other instruments used in the method development and validation include cyclo mixer (Remi, India), bath sonicator (Bransonic Cleaning Company, USA), Millipore® filtration assembly (Waters, USA), refrigerated centrifuge (Eppendorf centrifuge-5702R).

3.9.2 Collection of blood and separation of plasma

Blood was collected from marginal ear vein of male New Zealand white rabbits weighing between 1.5-2.0 kg. Collection of blood was carried out with permission of Institutional Animal Ethics Committee (Protocol approval no. IAEC/RES/16/04) BITS-Pilani, Pilani campus. Blood samples were collected in 1.5 mL centrifuge tubes (Eppendorf, India) containing 100µL of EDTA solution (1.0 mg/mL). The tubes containing blood and EDTA were then centrifuged at 4000 rpm for 4 min at 4°C (Eppendorf centrifuge-5702R, India). The supernatant plasma obtained was collected and stored at -20 °C.

3.9.3 Selection of mobile phase

For mobile phase optimization using various buffers of different pH and in varying combination with acetonitrile or methanol was investigated. Main purpose was to develop a simple, precise, sensitive and selective HPLC method for quantitation of BS in plasma samples. Mobile phase finally selected consisted of an aqueous phase (0.025 M potassium dihydrogen orthophosphate buffer in distilled water, pH was adjusted to 3.0 using 0.1 M orthophosphoric acid) and acetonitrile (75:25 v/v). For the selection of mobile phase, the criterion employed were peak properties (retention time and asymmetric factor), sensitivity (height and area), ease

of sample preparation, non interference from the bio matrix and applicability of the method for in-vivo studies in rabbits.

3.9.4 Calibration curve

Primary stock of BS was prepared by dissolving 5 mg of BS in mobile phase (pH 3.0 potassium hydrogen orthophosphate buffer and acetonitrile (75:25)) and making up the volume to 100 mL using the same solvent system to obtain a concentration of 50 µg/mL. Secondary stock of 10µg/mL concentration was prepared by appropriate dilution of primary stock by mobile phase. Plasma standards were prepared by spiking appropriate amount of secondary stock of BS in rabbit plasma to obtain solutions of 10, 50, 100, 250, 500, 1000 and 1500 ng/mL concentration.

Solid phase extraction (SPE) process was employed for extraction of BS from plasma samples. SPE cartridges (HyperSep Retain PEP® with 30mg, 1cc) were used for separation of BS from the plasma samples. SPE cartridges were conditioned (1 mL methanol, 2500 rpm) and equilibrated (25mM potassium dihydrogen orthophosphate, 2500 rpm) before loading the sample. Sample was loaded and centrifuged at 2000 rpm to remove interfering components and matrix, washing step was performed using 3% v/v methanol (1 mL, 2500 rpm) to remove the remaining interfering components and finally elution was carried out using 0.025 M phosphate buffer pH 3.0 and acetonitrile (75:25, 1mL, 3000 RPM) solvent system. The separation was performed using a centrifuge (Remi, India) and elutes were collected in 5 mL disposable tubes. Flow chart for the process is represented in Figure 3.5.

Plasma and analytical standards (100 µL) were injected on to the column for analysis. The peaks obtained for both plasma and analytical standards were integrated and peak area was calculated for each concentration. To establish linearity of the proposed method, eight separate sets of plasma standards were prepared and analyzed. Percent absolute drug recovery from plasma sample was calculated by using the formula [(Peak area of plasma standard/ peak area of analytical standard of same concentration) x 100].

3.9.5 Analytical method validation

The developed method was validated according to standard guidelines (International Conference on Harmonization, 1996). Various validation parameters of the developed method were determined as per standard guidelines.

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

For determining the accuracy of the proposed method, different quality control (QC) levels of drug concentrations in plasma [lower quality control samples (LQC) = 20 ng/mL, medium quality control samples (MQC) = 750 ng/mL, and higher quality control samples (HQC) = 1300 ng /mL] were prepared independently and analyzed (n = 6). Accuracy was assessed by calculating mean percentage recovery and percentage bias (% bias). % bias was calculated as, % bias = [(Predicted conc.-Nominal Conc.)/ Nominal Conc.] x 100.

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

Limit of quantitation (LOQ) is defined as minimum concentration of BS in plasma sample that can be quantified with less than 15% RSD (International Conference on Harmonization, 1996). In order to determine LOQ, three independent plasma samples containing 20 ng/mL of BS were prepared and analyzed using developed method. The peaks were integrated and concentrations were back calculated using calibration equation. Mean concentration and % RSD for these three estimates were determined.

In order to evaluate long term stability, aliquots of QC samples were frozen at -20 °C for 30 days, then thawed and analyzed against fresh samples. The difference between the starting concentration and the concentration after 30 days was observed to assess the stability of drug in plasma samples. Freeze thaw stability involved estimation of analyte after three freeze thaw cycles. Bench-top stability of the spiked samples was checked after 12 hours. The stock solution stability of the drug was evaluated at refrigerated condition for 15 days by comparing the response of the

stability samples with fresh stock. All stability studies were carried out using six replicates of LQC, MQC and HQC samples and the results were compared with freshly spiked calibration curve standards and fresh QC samples.

3.10 Results and Discussion

3.10.1 Selection of mobile phase

Optimization of mobile phase consisting of aqueous phase (0.025 M potassium dihydrogen orthophosphate buffer, pH adjusted to 3.0 using 0.1 M orthophosphoric acid) and acetonitrile (75:25 v/v) was based on peak properties (retention time and asymmetric factor), sensitivity (height and area) and separation of peak from protein impurities present in plasma. With optimized mobile phase retention time of BS was found to be 8.72 ± 0.81 min with an asymmetric factor of 1.63 ± 0.18 (Figure 3.5). The retention time of BS increased to 14.86 min with decrease in proportion of acetonitrile from 25% v/v to 20% v/v in the mobile phase. However, there was no effect on peak area, peak height and asymmetric factor. Use of methanol (25% v/v) instead of acetonitrile (25% v/v) in the mobile phase increased retention time of BS to 12.03 min and asymmetric factor was more than 2.0. Change in pH of aqueous phase above 3.0 resulted in increase of asymmetric factor of peak. Decrease in molar concentration has also resulted in increased asymmetric factor (Tailing). Thus, aqueous phase (0.025 M potassium dihydrogen phosphate buffer, pH adjusted to 3.0 using 0.1 M orthophosphoric acid) and acetonitrile (75:25 v/v) was finally selected as mobile phase.

3.10.2 Calibration curve

Different concentrations and their corresponding areas are shown in the Table 3.9. At all the concentration levels, the SD of the area was acceptable and the % RSD did not exceed 6.24. Overlaid chromatograms of blank plasma, plasma standard are shown in Figure 3.6 and in-vivo test sample is shown Figure 3.7. Retention time of BS was found to be 8.72 ± 0.81 min (Figure 3.6) in the selected mobile phase. Peak was having good resolution with asymmetric factor of 1.63 ± 0.18. Total run time for single injection was 11.5 min for the proposed method. The linearity range in the selected mobile phase was found to be 10–1500 ng/mL. According to a linear regression analysis, the slope (± standard error) and intercept (± standard error) were found to be 142.35 (± 2.99) and -333.80 (± 89.86), respectively with a regression

coefficient value of 0.9997. The absolute recovery of BS from the spiked rabbit plasma samples when compared with analytical standards of same concentration was within 94.98 to 98.75% with maximum SD of 6.03 (Table 3.9). Thus, the proposed solid phase extraction technique employed was found to be accurate and precise with high recovery values precluding the use of internal standard. Individual values of slopes and intercepts obtained from replicate measurements were within 95% confidence limits of mean values of slope and intercept. Lower values of standard error of slope (9.00×10^{-1}) and standard error of intercept (144.52) in addition to lower calculated *F*-value [calculated *F* value (7, 48) of 5.09×10^{-4} and critical *F*-value of 2.43 at $p = 0.05$] further confirmed precision of the method.

3.10.3 Analytical method validation

Simple and efficient solid phase extraction technique was used to separate BS from rabbit plasma. The technique was found to be suitable for estimation of BS from biological matrix with no interference from endogenous protein impurities. No additional peaks resulting from metabolism or degradation of the drug were observed in the near vicinity of drug peak (Figure 3.7). Blank plasma sample also showed absence of any interference near the retention time of the drug (Figure 3.6). Thus, the proposed method is specific and selective for the estimation of BS in rabbit plasma.

All three quality control samples [lower quality control samples (LQC) = 20 ng/mL, medium quality control samples (MQC) = 750 ng/mL, and higher quality control samples (HQC) = 1300 ng /mL] showed an accuracy (% bias) ranging from -1.75% to 0.66% (Table 3.10). The high (nearly 100%) mean percent recovery values and low SD values (SD < 5.0) further established the accuracy of the method (Table 3.10).

In repeatability study, the % RSD ranged from 2.16 to 9.37 (Table 3.11). % RSD values were significantly low for intermediate precision, with intra-day variation not more than 8.03% and inter-day variation less than 9.37% (Table 3.11). Lower % RSD values indicated the repeatability and intermediate precision of the method.

The mean concentration of three independent samples of 20 ng/mL, calculated using calibration equation was found to be 19.16 ng/mL with % RSD value of 9.46. Hence, the concentration of 20 ng/mL was considered as limit of quantitation for the proposed method.

Results of bench top stability at three QC levels (LQC MQC and HQC) of BS was indicated to be stable in rabbit plasma. Low % RSD values obtained in response up to 12 h as compared to response obtained from freshly prepared samples further confirm the stability of BS in rabbit plasma. BS was found to be stable in rabbit plasma at -20 °C at QC levels, as there was no major difference between response of the standard at zero time and at the end of 30 days. The deviation observed was within the acceptable limit (% RSD < 15). There was no significant degradation was observed in the QC standards up to 3 freeze thaw cycles. Results are demonstrated as % recovery, which was found to be 96.13%, 99.50 % and 99.31 % at LQC, MQC and HQC respectively. Percent deviation calculated for all stability studies were within the acceptable limit of ± 15% at LQC, MQC and HQC levels exhibiting good stability of the of BS under the various conditions of the study to be conducted. Summary of the results are presented in Table 3.12

3.10.4 Conclusions

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

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

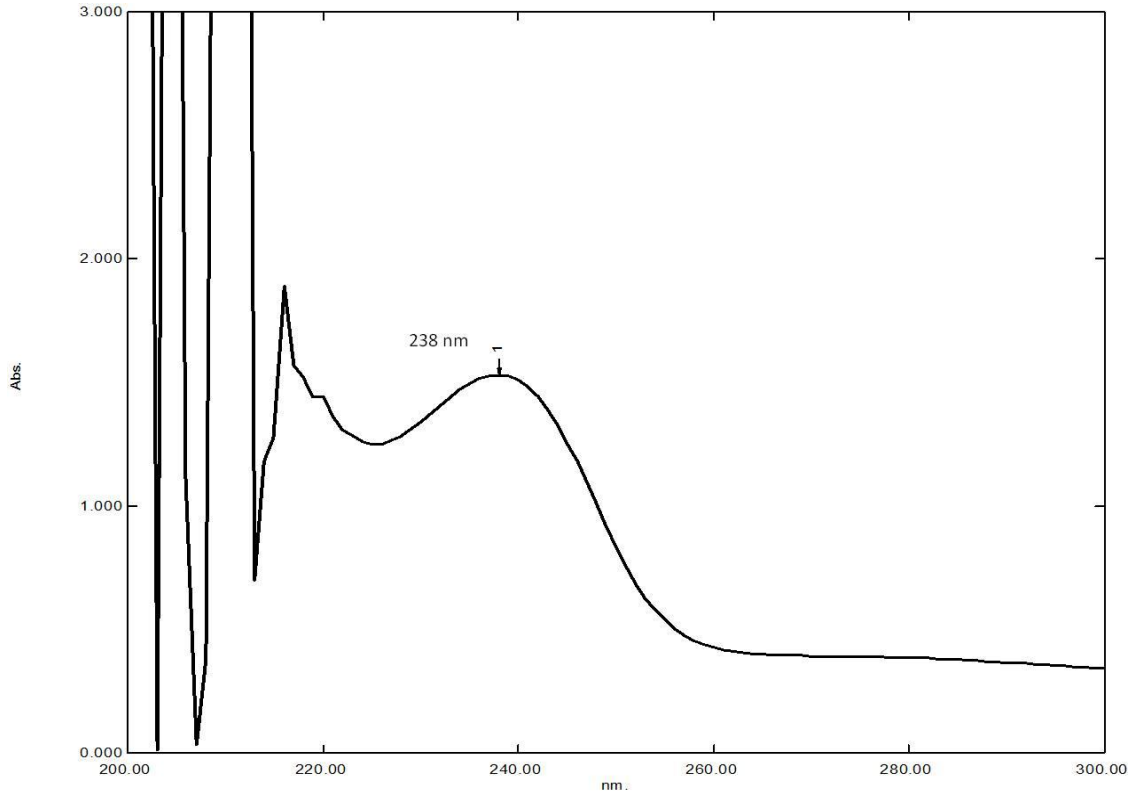


Figure 3.1: Representative UV absorption spectra of BS in pH 6.8 Phosphate buffer using analytical method 1

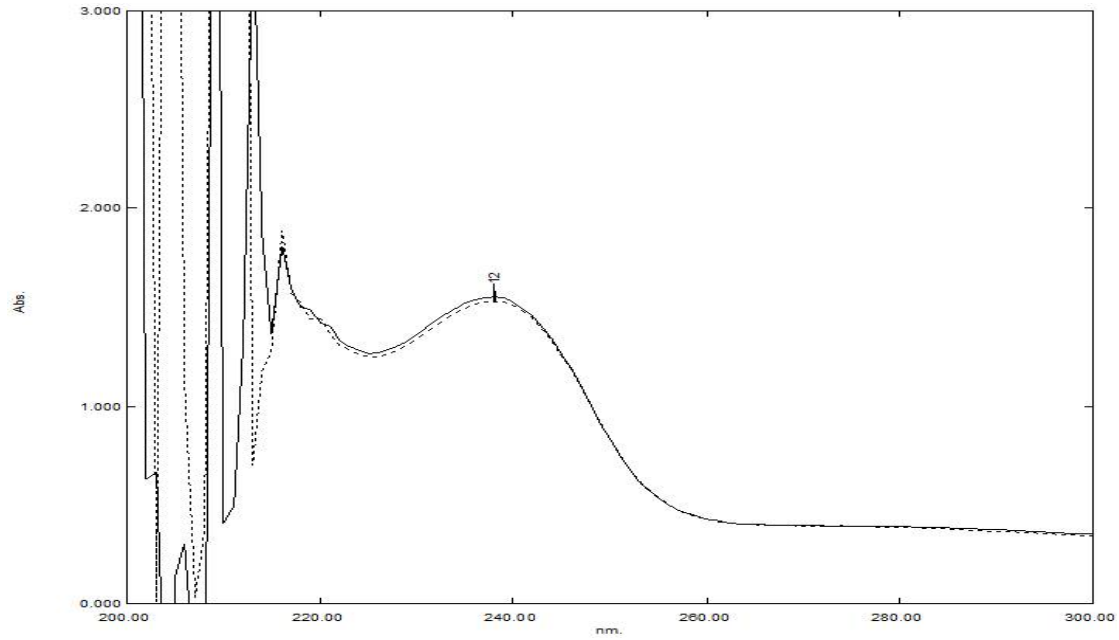


Figure 3.2: Overlaid spectra of pure drug solution and solution containing drug and XG in 1:1 ratio obtained using analytical method 1

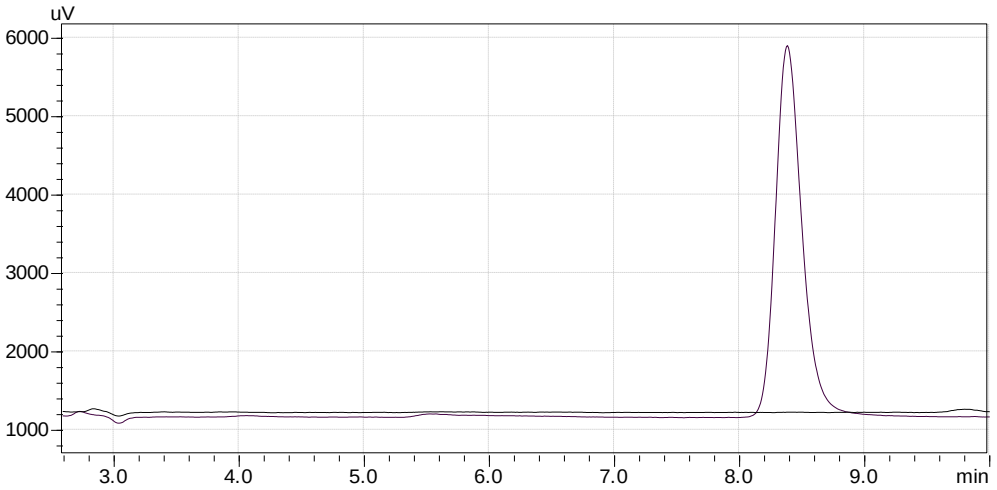


Figure 3.3: Overlaid chromatogram of blank (mobile phase) and pure BS (500 ng/mL) obtained using method 2

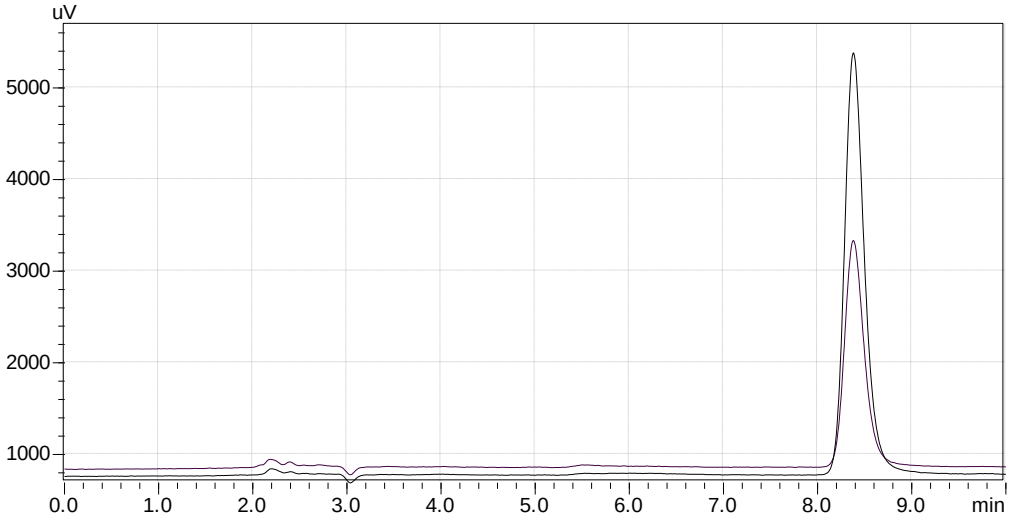


Figure 3.4: Overlaid chromatograms of pure BS (500 ng/mL) and combination of BS (250 ng/mL) with XG 1:1 proportion obtained using method 2

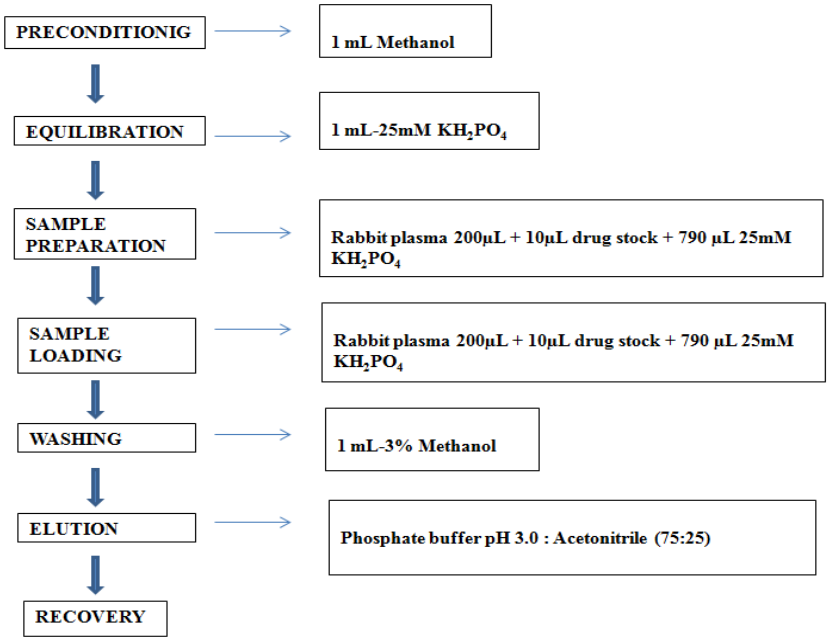


Figure 3.5: Flow chart for plasma sample processing using SPE cartridges in method 3

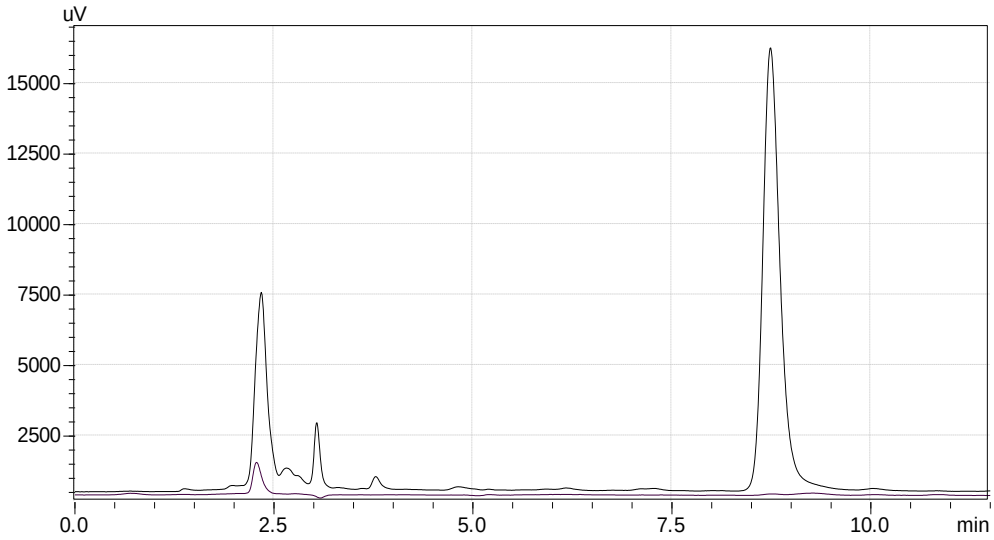


Figure 3.6: Representative chromatogram of blank and BS (1500 ng/mL) in rabbit plasma samples obtained using method 3

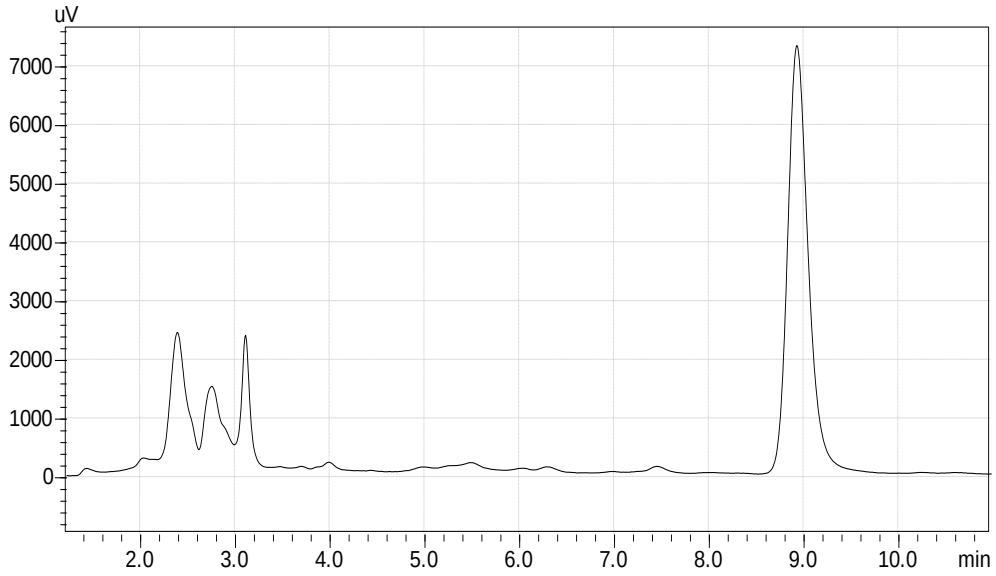


Figure 3.7: Representative chromatogram of BS in in-vivo test rabbit plasma sample obtained using method 3

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

Conc. (µg/mL)	***Mean Absorbance[a] (± SD)***	***% RSD[b]***	***Predicted Conc.[c] (µg/mL)***
2.5	0.1391 ± 0.0026	1.87	2.53
5.0	0.2687 ± 0.0018	0.67	5.06
10.0	0.5246 ± 0.0061	1.16	10.06
15.0	0.7784 ± 0.0044	0.57	15.01
20.0	1.0404 ± 0.0074	0.71	20.13
25.0	1.2857 ± 0.0083	0.65	24.92

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

Table 3.2: Accuracy and precision data for analytical method 1

Level	***Predicted Conc.[a] (µg/mL)***			***Mean % Recovery[b] (± SD)***	***% Bias[c]***
	Range	***Mean[b] (±SD)***	***% RSD***		
LQC	3.97 - 4.12	3.993 ± 0.06	1.50	99.81 ± 0.26	-0.19
MQC	12.62 - 12.94	12.68 ± 0.23	1.81	101.44 ± 0.76	1.44
HQC	22.42 - 22.96	22.79 ± 0.35	1.54	101.28 ± 1.01	1.28

[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	1.09	0.74	0.83	0.76
	0.58	0.49	0.38	
MQC	0.94	0.57	0.91	0.89
	0.72	0.42	1.27	
HQC	0.55	0.97	0.39	1.08
	1.06	0.32	0.83	

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

Products	***Mean Amount Found (mg) (Mean ± SD, n=5)***	***% Assay (Mean ± SD, n=5)***
Buspin 5 *(Commercial oral Tablet*-5 mg *)*	5.03 ± 0.09	100.65 ± 1.84
Buccal discs *(In-house designed-5 mg)*	4.95 ± 0.07	99.15 ± 1.38

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

Conc. (ng/mL)	***Mean Area[a] (µv-sec) (± SD)***	***% RSD[b]***	***Predicted Conc.[c] (ng/mL)***
25	3596.13 ± 67	1.86	24.36
50	7030.13 ± 134	1.91	48.06
100	14108.73 ± 211	1.50	96.89
250	36181.67 ± 586	1.62	249.18
500	72511.07 ± 619	0.85	499.83
1000	147700.00 ± 1074	0.73	1018.59
1500	215747.60 ± 2411	1.12	1488.08

[a] Each value is mean of six 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] (± SD)***	***% Bias[c]***
	Range	***Mean[b] (± SD)***	***% RSD***		
LQC	28.91 - 30.89	30.12 ± 0.53	1.76	100.40 ± 1.87	1.04
MQC	743.16 - 756.06	747.26 ± 8.64	1.15	99.90 ± 1.73	-0.10
HQC	943.22 - 958.04	952.38 ± 14.94	1.57	100.25 ± 0.55	0.25

[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***
	Day 1	**Day 2**	**Day 3**	***(% RSD) (n=18)***
LQC	0.87	1.12	1.61	1.08
	1.24	1.63	0.76	
MQC	0.54	0.82	1.11	0.67
	0.49	0.69	0.89	
HQC	1.01	0.88	0.46	0.84
	0.39	0.53	1.33	

Table 3.8: Determination of BS in marketed formulation using analytical method 2

Commercial Products	***Mean Amount Found[a] (mg) (± SD)***	***% Assay[a] (± SD)***
Buspin 5 *(Commercial oral Tablet*-5 mg *)*	5.04 ± 0.07	100.75 ± 1.43
Buccal discs *(In-house designed-5 mg)*	4.94 ± 0.09	98.89 ± 1.70

[a]Mean of five independent determinations

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

Conc. (ng/mL)	***Mean Area[a] (µv-sec) (± SD)***	***% RSD[b]***	***% Recovery[c] (± SD)***
10	1340.167 ± 83.00	6.24	96.95 ± 5.12
50	7091.75 ± 168.22	2.16	98.75 ± 3.26
100	13979.67 ± 234.48	1.57	96.90 ± 3.12
250	34515.42 ± 1699.75	4.92	95.43 ± 5.24
500	69621.25 ± 1414.73	2.03	96.16 ± 3.46
1000	137595.9 ± 4951.79	3.60	94.98 ± 6.03
1500	206475.1 ± 3758.16	1.74	95.00 ± 4.13

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

Table 3.10: Accuracy and precision data for analytical method 3

	Predicted Conc.[a] (ng/mL)			***Mean % Recovery[b] (± SD)***	***% Bias[c]***
Level	***Range***	***Mean[b] (± SD)***	***% RSD***		
LQC	19.11 - 21.33	19.65 ± 0.88	4.49	98.25 ± 3.74	-1.75
MQC	746.48 - 753.64	747.57 ± 1.59	0.21	99.67 ± 3.08	-0.33
HQC	1251.87 - 1351.28	1308.69 ± 30.78	2.35	100.66 ± 2.39	0.66

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

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

Level	***Intra-day repeatability (% RSD) (n=3)***			***Inter-day repeatability (% RSD) (n=18)***
	Day 1	***Day 2***	***Day 3***	
LQC	5.17	6.43	6.12	5.68
	6.14	5.38	2.16	
MQC	3.61	2.84	3.91	9.37
	2.18	3.22	8.03	
HQC	7.11	5.47	6.69	7.13
	3.78	3.91	7.34	

Table 3.12: Stability data obtained using method 3

Storage period and storage conditions	***Nominal conc. (ng/mL)***	***Mean[a] ± SD***	***% RSD***	***% Recovery***
Stock solution - 15 days, (Refrigerated temp.)	30.00	29.24 ± 1.67	5.72	97.47
	750.00	746.03 ± 5.15	0.73	99.47
	1300.00	1287.83 ± 3.44	0.26	99.06
Bench top ~ 12 hours, room temp.	30.00	29.18 ± 2.06	7.05	97.26
	750.00	744.63 ± 6.18	0.83	99.28
	1300.00	1293.36 ± 12.54	0.96	99.49
Freeze thaw cycle (3 cycles)	30.00	28.84± 1.94	6.72	96.13
	750.00	746.28 ± 8.26	1.10	99.50
	1300.00	1291.07 ± 5.64	0.43	99.31
Long term stability (- 20 °C for 30 days)	30.00	30.47± 1.73	5.67	101.56
	750.00	748.61 ± 3.18	0.42	99.81
	1300.00	1289.91 ± 4.26	0.33	99.22

References

Aframian, D. J., Davidowitz, T., & Benoliel, R. (2006). The distribution of oral mucosal pH values in healthy saliva secretors. Oral Dis., 12(4), 420-423.

Aparicio, X., Campos, A., Gras, J., Fernandez, E., & Gelpi, E. (1988). Determination of a buspirone metabolite in plasma samples. J. Pharm. Biomed. Anal., 6(6-8), 945-948.

Azeem, A., Rizwan, M., Ahmad, F., Iqbal, Z., Khar, R., Aqil, M., & Talegaonkar, S. (2009). Development and validation of a stability-indicating LC-UV method for rapid analysis of buspirone in pharmaceutical dosage forms. Acta Chromatogr., 21(2), 283-297.

Betto, P., Meneguz, A., Ricciarello, G., & Pichini, S. (1992). Simultaneous high-performance liquid chromatographic analysis of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine in plasma using electrochemical detection. J. Chromatogr., 575(1), 117-121.

Bianchi, G., & Caccia, S. (1988). Simultaneous determination of buspirone, gepirone, ipsapirone and their common metabolite 1-(2-pyrimidinyl)piperazine in rat plasma and brain by high-performance liquid chromatography. J. Chromatogr., 431(2), 477-480.

Campisi, G., Paderni, C., Saccone, R., Di Fede, O., Wolff, A., & Giannola, L. I. (2010). Human buccal mucosa as an innovative site of drug delivery. Curr. Pharm. Des., 16(6), 641-652.

Chew, W. M., Xu, M.-J., Cordova, C. A., & Chow, H. H. S. (2006). Quantification of a cytochrome P450 3A4 substrate, buspirone, in human plasma by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B, 844(2), 235-239.

Du, Q., Sun, J., Kang, Li-juan, Yan, & Guo-wei. (2003). Determination of Buspirone Hydrochloride and Its Active Metabolite in Plasma by HPLC. J. Pharm. Analysis., 0(1), 56-58.

Foroutan, S. M., Zarghi, A., Shafaati, A. R., & Khoddam, A. (2004). Simple high-performance liquid chromatographic determination of buspirone in human plasma. Il Farmaco, 59(9), 739-742.

Gammans, R. E., Kerns, E. H., & Bullen, W. W. (1985). Capillary gas chromatographic—mass spectrometric determination of buspirone in plasma. J. Chromatogr., 345(0), 285-297.

Kerns, E. H., Rourick, R. A., Volk, K. J., & Lee, M. S. (1997). Buspirone metabolite structure profile using a standard liquid chromatographic-mass spectrometric protocol. J. Chromatogr. B. Biomed Sci Appl., 698(1-2), 133-145.

Khedr, A., & Sakr, A. (1999). Stability-indicating high-performance liquid chromatographic assay of buspirone HCl. J. Chromatogr. Sci., 37(12), 462-468.

Kristjansson, F. (1991). Sensitive determination of buspirone in serum by solid-phase extraction and two-dimensional high-performance liquid chromatography. J. Chromatogr., 566(1), 250-256.

Nägele, E., & Fandino, A. S. (2007). Simultaneous determination of metabolic stability and identification of buspirone metabolites using multiple column fast liquid chromatography time-of-flight mass spectrometry. J. Chromatogr. A, 1156(1–2), 196-200.

Odontiadis, J., & Franklin, M. (1996). Simultaneous quantitation of buspirone and its major metabolite 1-(2-pyrimidinyl)piperazine in human plasma by high-performance liquid chromatography with coulometric detection. J. Pharm. Biomed. Anal., 14(3), 347-351.

Sheikhzadeh, M., Rohani, S., Taffish, M., & Murad, S. (2007). Solubility analysis of buspirone hydrochloride polymorphs: Measurements and prediction. Int. J. Pharm., 338(1–2), 55-63.

Smart, J. D. (1993). Drug delivery using buccal-adhesive systems. Adv. Drug Deliv. Rev., 11(3), 253-270.

Zayed, M. A., Fahmey, M. A., Hawash, M. A., & El-Habeeb, A. A. (2007). Mass spectrometric investigation of buspirone drug in comparison with thermal analyses and MO-calculations. Spectrochim. Acta, Part A, 67(2), 522-530.

Zhu, L., Yang, X., Zhou, J., Tang, L., Xia, B., Hu, M., Zhou, F., & Liu, Z. (2013). The exposure of highly toxic aconitine does not significantly impact the activity and expression of cytochrome P450 3A in rats determined by a novel ultra performance liquid chromatography–tandem mass spectrometric method of a specific probe buspirone. Food Chem. Toxicol., 51(0), 396-403.

4.1 Introduction

The drug and pharmaceutical materials must be compatible with one another to produce a drug product that is stable, efficacious, easy to administer and safe. Most drug substances in use today are solid materials and pure chemical compounds of either crystalline or amorphous constitution. The purity of the chemical substance is essential for its identification and for evaluation of its chemical, physical, and biologic properties (Hsu & Lin, 2009). A comprehensive preformulation study of a drug molecule leads to development of suitable dosage form by selecting appropriate excipients for improved bioavailability and also to improve the product stability during storage conditions (Picker-Freyer, 2009). A lack of understanding may lead to changes in solubility, polymorph and or dissolution that can affect the drug absorption and product stability (Brittain, 2011). Preformulation stage guide the formulation strategy for the selected drug and is essentially carried out for chemical and physical characterization using suitable techniques (van Dooren & Müller, 1984).

The preliminary drug degradation studies are needful to guide the formulation of stable product (Wen & Park, 2011). Typical preformulation studies of pharmaceuticals include characterization and determination of drug physicochemical properties like solubility of drug in various solvents (water, pH buffers and organic solvents), pH stability, dissociation constants, partition coefficient and drug-excipient compatibility (Brittain, 2001).

Physicochemical properties of BS like solubility, dissociation coefficient are widely reported in the scientific literature and are mentioned in section 2.1.2 of chapter 2. Extensive survey revealed lack of literature on partition coefficient of BS.

In the current research work, extensive preformulation studies were carried out to investigate solid and solution state stability of BS. Compatibility of B\S with various excipients selected for design of buccal bioadhesive formulations was assessed. Partition coefficient of BS was also determined during the course of research work.

4.2 Experimental

4.2.1 Materials

Buspirone hydrochloride (BS) was provided as a gift sample by Astron Research Limited, India. Xanthan gum (XG) and hydroxy propyl cellulose (HPC)

were supplied as gift sample by Signet Chemical Corporation Pvt. Ltd., India. Hydroxy propyl methyl cellulose (HPMC K15) and calcium sulfate (CS) was purchased from Sigma-Aldrich® Corporation., India. Avicel PH101, a cellulose microcrystalline (MCC) grade was purchased from FMC BioPolymer., USA. Polycarbophil (PC) was obtained as gift samples from Noveon Inc., USA. HPLC grade acetonitrile and methanol were purchased from Merck, India, and deionized water used for in-vitro and analytical studies was obtained using a Millipore water purification system (Milli-Q). Nylon membrane filters of pore diameter 0.22 µm were purchased from Millipore, India.

4.2.2 Equipments/Instruments

A water bath shaker (MAC instruments, India) was used for partition coefficient determination studies. All pH measurements were performed using a digital pH meter (Eutech Instruments, Singapore). Polymorphic form of drug was investigated using differential scanning calorimetry (DSC, Shimadzu®, Japan, integrated with TA 60WS software) and Fourier transform infrared spectroscopy (FTIR, Shimadzu®-Prestige 21, Japan, equipped with IR solutions, version 1.0). Drug excipient compatibility studies were performed using DSC (Shimadzu®) instrument mentioned above. A sensitive five digital balance (Mettler Toledo®, Switzerland) was used for all weighing purpose.

4.3 Methods

4.3.1 Identification of BS and characterization of polymorphic forms

Identification of drug was carried out by comparing experimental melting point and infra red (IR) spectra with that of reported values. Melting point was determined using Differential Scanning Calorimetry (DSC). Fourier Transformed Infrared Spectroscopic (FTIR) analysis was performed to identify the functional groups related to BS. Characterization of polymorphic form of BS used during the current research was carried out using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR)

For DSC analysis, pure drug Sample (2-4 mg) was weighed accurately in to a tared standard aluminum pans and sealed using an aluminum lid. Analysis was carried out over a temperature range of 30 to 300 °C with a heating rate of 10 °C per minute in a nitrogen gas environment (30 mL/min). FTIR spectroscopic studies were carried

out by appropriately diluting the drug sample with dried potassium bromide and acquiring infra red (IR) spectrum in the range of 400 to 4000 cm^{-1} with 2 cm^{-1} resolution. The results obtained from the above tests were compared with the values reported in literature for identification of drug and determination of polymorphic form.

4.3.2 Determination of partition coefficient

Partition coefficient of BS was determined in n-octanol/aqueous buffer solvent system (phosphate buffer pH 6.8) by shake flask method. n-Octanol was presaturated with aqueous buffer solvent by shaking on a rotary flask shaker for 24 h at room temperature (25 °C ± 2 °C). Organic n-octanol was separated from the pre saturated mixture of n-octanol and aqueous buffer using a glass separating funnel and was used to carry out further experiments. To 4 mL of presaturated n-octanol, 4 mL of pre analyzed solution of drug in aqueous phosphate buffer pH 6.8 (100µg/ml) was added and allowed for shaking on a rotary flask shaker at room temperature. Analysis was performed using an analytical method previously mentioned in method 2 of chapter 3. One mL of aqueous phase was withdrawn after 24 h end point and centrifuged at 4000 rpm for 15 min. The sample acquired was diluted suitably and analyzed by analytical method 2 of chapter 3. The experiment was carried out in triplicate. Apparent partition coefficient was calculated using the equation given below and log P was calculated by taking logarithm to the base 10 of partition coefficient.

$$P_{o/w} = (A_i - A_f)/A_f \qquad \text{(Eq-1)}$$

Where, $P_{o/w}$ = Apparent partition coefficient; A_i = Initial amount of drug in aqueous phase; A_f = Final amount of drug in aqueous phase.

4.3.3 Determination of stability

4.3.3.1 Solution state stability

Stability profile in solution state for the drug BS was established in various buffered solutions of varying pH (pH 1.2, 2.0, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.4, 8.0, 9.0). A stock solution (1 mg/mL) of BS was prepared in distilled water. 1 mL of this stock solution was added to buffered solutions of varying pH and volume was made up to 10 mL to achieve a final concentration of 100 µg/mL. All samples were kept at 25 ± 2 ^{o}C in closed containers. The entire experiment was carried out in triplicates. Samples

were withdrawn at different time points, suitably diluted and were analyzed by analytical method 2 of chapter 3.

4.3.3.2 Solid state stability

Solid state stability of BS and compatibility with various process excipients proposed to be used for the design of buccal dosage forms was studied. The compatibility screening studies involve the use of physical mixtures of drug with excipient. The proportion of drug in the mixtures is usually kept high (drug: excipient,1:1, w/w) as compared to that in the formulation to maximize the proportion of reacting species, thereby increasing the chance of spotting incompatibility (Chadha & Bhandari, 2014). This study is essential to understand the roles of bound water and exposed high temperature in case of solid samples (Chadha & Bhandari, 2014). Various process excipients used for the study were lactose, mannitol, calcium sulfate, XG, HPMC K15, PC, MCC, HPC, PEO 1L, PEO 40L and talc.

DSC study was carried out for pure BS, individual excipient and combination of BS with different excipients (mixed in 1:1 ratio). Mixed samples of drug and excipients were analyzed by analytical method 2 of chapter 3 for content uniformity. Sample (2-4 mg) was weighed accurately in to tarred aluminum pans and analysis was carried out as mentioned previously in section 4.3.1. All the samples were stored at controlled room temperature (CRT 25 ± 2 °C and 60 ± 5 % RH) and the study was repeated after 12 months.

FTIR study was also carried out for pure BS, individual excipient and combination of BS and excipient (mixed in 1:1 ratio) as mentioned in section 4.3.1. All the samples were stored at controlled room temperature (CRT 25 ± 2 °C and 60 ± 5 % RH) and the study was repeated after 12 months.

BS (passed through 80 #) and various excipients were physically mixed in 1:1 ratio. The physical mixtures were prepared carefully with geometric mixing and analyzed for content uniformity using analytical method 2 given in chapter 3. The prepared mixtures were filled in vials and kept at different temperature conditions of controlled room temperature (CRT: 25 ± 2^{o}C/ 60 ± 5 % RH) and at accelerated condition (AT: 40 ± 2^{o}C/ 75 ± 5 % RH). At predetermined time intervals, samples (in triplicates) were taken and analyzed for drug content by analytical method 2 of chapter 3 after suitable dilution.

4.4 Results and Discussion

4.4.1 Identification of BS and characterization of polymorphic forms

DSC thermogram of pure drug was used for identification of drug and confirmation of polymorphic form of drug used during the current research work. DSC thermogram of pure drug showed two distinct endothermic peaks at 189.89 °C and 204.43 °C with -55.22 and -36.32 J/g enthalpy values respectively and one exothermic peak at 192.18 °C with 33.16 J/g enthalpy value (Figure 4.1). These two crystal structures are enantiotropes and the transition temperature from Form 1 to Form 2 is 95 °C. The values obtained were close to values reported in the literature (Sheikhzadeh et al., 2007b). The endothermic peak at 189.89 °C was due to melting of pure drug sample. The exothermic peak at 192.18 °C was due to conversion of polymorphic Form 1 of drug to polymorphic Form 2. The last endothermic peak at 204.43 °C was due to melting of recrystallized Form 2 of the drug.

BS has several polymorphs including Form 1 and Form 2. In the pharmaceutical industry, different polymorphs are usually prepared by crystallization from solution employing various solvents, temperature regimes, synthetic route etc (Sheikhzadeh et al., 2007a). Extensive polymorph screening on BS has shown that close to 90% of the experiments result in the two main polymorphs of this compound, namely Form 1 and Form 2. Form 1 exhibits one endotherm at 189 °C, the melting point of Form 1, an exotherm at 192 °C for crystallization to Form 2, and another endotherm at 203 °C for melting of the recrystallized Form 2 and Form 2 yields a single melting point at 203 °C. Report suggests that Form 2 of BS has a distinct special characteristic peak at 1156/cm when analyzed by Fourier transform infrared spectroscopy (FTIR) (Sheikhzadeh et al., 2007a; Sheikhzadeh et al., 2007b).

FTIR spectroscopic analysis of pure drug revealed peaks specific to functional groups of BS. IR bands were obtained at 2949 cm^{-1} for aromatic C-H stretching of pyrimidine, 1724 cm^{-1} for C=O stretching. Moreover, bands were also obtained at 1454 cm^{-1} for CH2 bending of piperazine, 1338 cm^{-1} for CN stretch, 980 cm^{-1} and 802 cm^{-1} for aromatic C-H bending (Table 4.1). Distinct special characteristic peak at 1156/cm was not observed in the spectra obtained from pure BS indicating polymorphic form 1 of BS (Figure 4.2). Similar results were reported for Form 1 of BS (Sheikhzadeh et al., 2007b). These results further confirmed that Form 1 of BS was used for the present study.

4.4.2 Determination of partition coefficient

The equilibrium partition coefficient of BS was determined in n-octanol/deionized water solvent system by shake flask method. The partition coefficient of BS was found to be 41.16 ± 4.66. Log P of BS was found to be 1.61 (± 0.051). The reported computational value of Log P is 2.18 (Alelyunas et al., 2010).This difference between theoretical and experimental value of Log P may be ascribed due to sensitivity of the method for determination of Log P (shake flask method)

4.4.3 Determination of stability

4.4.3.1 Solution state stability

BS was found to be stable over the pH range of 1.2 to 9.0 and the log % RTD (Remaining to degrade) versus time profiles of BS at various pH range did not show any sign of degradation for at least 3 days (Figure 4.3). Degradation rate constant (K_{deg}) values obtained were ranging from 4.61 x 10^{-4} h^{-1} (pH 1.2) to 9.21 x 10^{-4} h^{-1} (pH 9.0) and t90% values obtained were ranging from 1.14 x 10^{2} to 2.28 x 10^{2} days (Table 4.2). From Figure 4.3 it becomes further evident that the drug has minimal degradation at neutral pH range of 6 to 7.4 with higher degradation at acidic pH.

4.4.3.2 Solid state stability

DSC study was carried out for pure BS, individual excipient and combination of BS and various excipients (1:1 ratio). DSC thermogram of pure drug showed two endothermic peaks at 189.89 °C and 204.43 °C with enthalpy values of -55.22 and -36.32 J/g respectively and one exothermic peak at 192.18 °C (Figure 4.1) with enthalpy value of 33.16 J/g (Table 4.3). Endothermic and exothermic peaks recorded during pure drug analysis were retained in almost all the cases of physical mixture of drug and excipients. However a slight change in peak shape with little broadening and shifting to higher or lower temperature was observed in all physical mixtures, which could be attributed to mixing process that lowers the purity of each of the component (Verma & Garg, 2004).

In DSC thermogram for pure calcium sulfate, a wide sharp peak at 137.02 °C was observed (Figure 4.4). All the peaks of drug and calcium sulfate were well retained in the physical mixture of BS and calcium sulfate, indicating absence of drug excipient interaction (Figure 4.4). DSC thermogram of pure xanthan gum has revealed

a wide and blunt peak in between 50 to 120 °C. In physical mixtures of BS and XG, all the drug peaks were also well retained. The DSC of XG and calcium sulfate was performed to investigate the degree of polymer interaction with calcium sulfate. The DSC thermogram obtained from the analysis of the physical mixture clearly illustrated the peaks of XG and calcium sulfate (Figure 4.4). However, calcium sulfate peak shape was well preserved with a shift in melting point from 137.02 °C to 143.56 °C (Figure 4.4); this implies nonexistence of XG and Calcium sulfate interaction in solid state analysis.

Similarly, thermograms obtained from the DSC analysis carried individually for the polymers HPMC K15 (Figure 4.5), MCC (Figure 4.5), PC (Figure 4.6) and HPC (Figure 4.7) has revealed broad and blunt peaks over a temperature range from 50 to 120 °C. All the drug peaks were well preserved in the physical mixtures of drug with different selected polymers (HPMC K15, MCC, PC and HPC) indicating lack of drug excipient interaction.

DSC analysis of pure lactose revealed a sharp endothermic peak at 146.60 °C, this event can be attributed to loss of bound water (Araújo et al., 2003) followed by melting endotherm at around 217.46 °C (Figure 4.8). All the peaks were retained in DSC thermogram physical mixture of drug and lactose with a minor variation in peak shape, this results clearly indicate lack of interaction between drug and lactose. All the drug peaks were also well preserved in the thermogram recorded from the DSC analysis of physical mixture of drug and talc (Figure 4.8).

DSC thermogram obtained for PEO 1L and PEO 40L revealed sharp peaks at 66.78 and 69.20 °C respectively. Drug peaks were retained in physical mixture of BS along with PEO 1L and BS with PEO 40L indicating lack of interactions (Fig 4.9).

In DSC thermogram of pure mannitol, a sharp endothermic peak was observed at 168.41°C very near to that of the drug with an enthalpy value of -207.31 J/g. In physical mixture of drug and mannitol, a single, wide endothermic peak was observed which can be attributed to interaction of BS with mannitol (Figure 4.6). To further confirm the interaction between BS and mannitol, FTIR and HPLC studies were performed. FTIR results obtained from physical mixture of BS and mannitol by suitable dilution with potassium bromide have clearly demonstrated all the peaks persistent to BS (Figure 4.10) and HPLC analysis of BS in presence of mannitol did

not alter peak characteristics and did not reveal any degradation peaks, peak area was also well retained with respect to analysis of pure BS. On basis of this observation it can be concluded that drug is stable in presence of mannitol.

In FTIR study, the IR bands that can be attributed to drug are presented in Table 4.1. In all the drug-excipient mixtures studied, these bands were retained representing absence of chemical interaction between the drug and excipients. FTIR study further established that there is no chemical interaction between drug and excipients studied.

Similar results were obtained when DSC and FTIR studies were repeated on the samples stored at CRT for 12 months.

Physical mixture of BS prepared in 1:1 ratio with various excipients showed good content uniformity between 99.11 to 101.96 % with maximum SD of 1.91. Table 4.4 gives the degradation kinetics of drug alone and combination of drug with various excipients. At controlled room temperature (CRT: 25 ± 2 °C/ 60 ± 5 % RH) and at accelerated conditions (AT: 40 ± 2 °C/ 75 ± 5 % RH), the log % RTD versus time profiles were linear indicating first order degradation kinetics. R^2 value close to 1 further established linear relationship between log% RTD versus time (Table 4.4). The degradation rate constant (K_{deg}) for pure drug was found to be 20.73 x 10^{-4} and 59.88 x 10^{-4} $month^{-1}$ at CRT and AT respectively. The t_{90}% of drug at CRT and AT was found to be 50.66 and 17.54 months respectively.

The K_{deg} values for all the mixtures were ranging from 20.73 x 10^{-4} to 138.18 x 10^{-4} $month^{-1}$ when stored at CRT. The highest degradation rate constant was observed with magnesium stearate and lowest degradation rate constant was observed with PC and HPC. At these storage condition t_{90}% were ranging from 7.60 to 50.66 months.

When stored at accelerated condition, the K_{deg} values for all the drug excipient mixtures were ranging from 62.18 x 10^{-4} to 175.03 x 10^{-4} $month^{-1}$. The highest degradation rate constant was observed with magnesium stearate and lowest degradation rate constant was observed with PC. At these storage condition t_{90}% were ranging from 6.00 to 16.89 months. BS alone and in combination with various excipients was stable for at least 6 months at this condition.

4.5 Conclusions

In the present study, polymorphic Form 1 of BS was used and was confirmed by DSC and FTIR data obtained from the experimental results. Log P values of BS suggests the hydrophobic nature of the drug. BS was found to be compatible and stable with all the proposed excipients in solid state stability studies. BS was found stable over a pH range of 1.2 to 9.0 at 25 °C. The results obtained from the above preformulation studies were helpful in the design and development of buccal bioadhesive formulations.

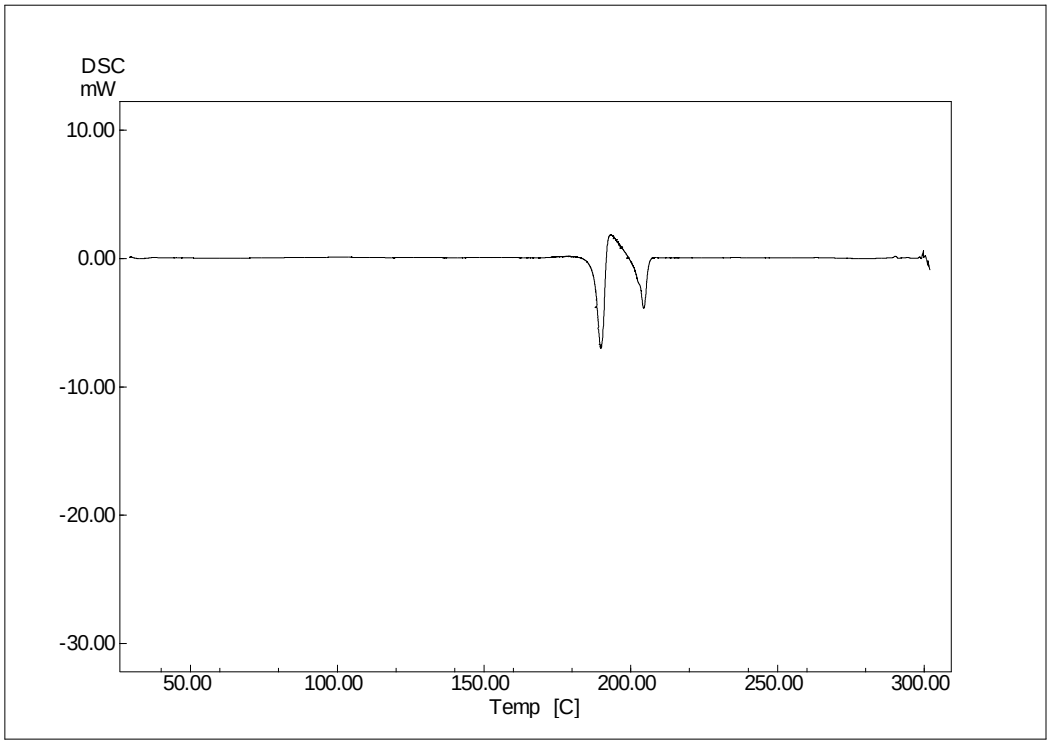


Figure 4.1: DSC thermogram of pure BS

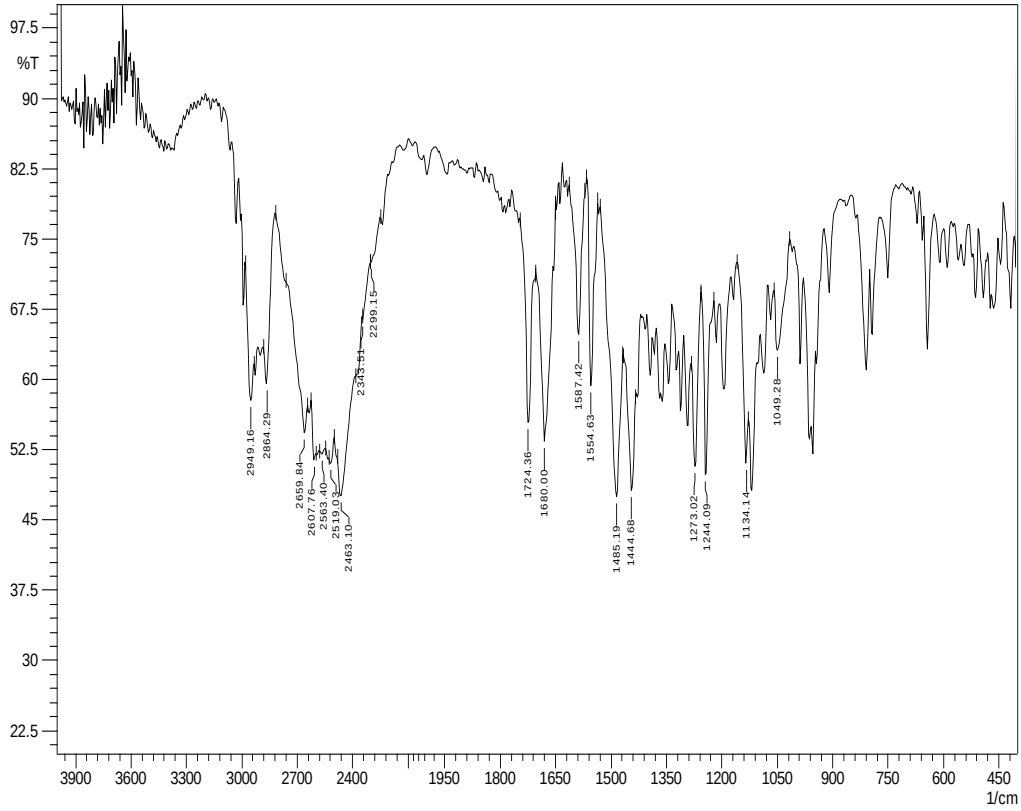


Figure 4.2: FTIR spectrum of pure BS

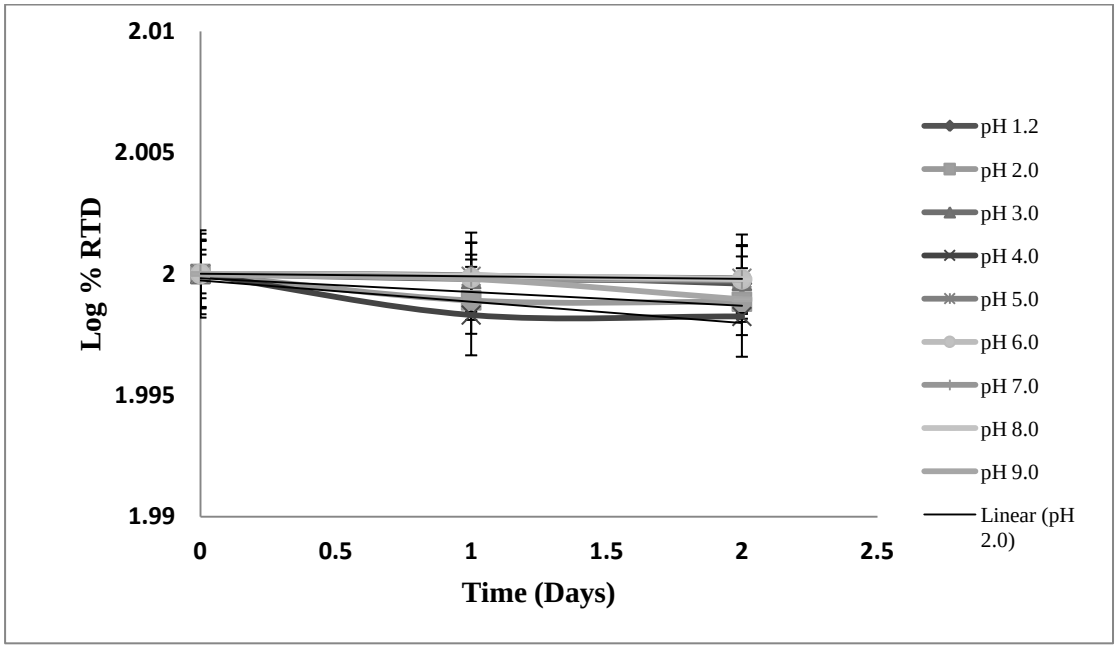


Figure 4.3: Solution stability profile of BS in various pH buffered media

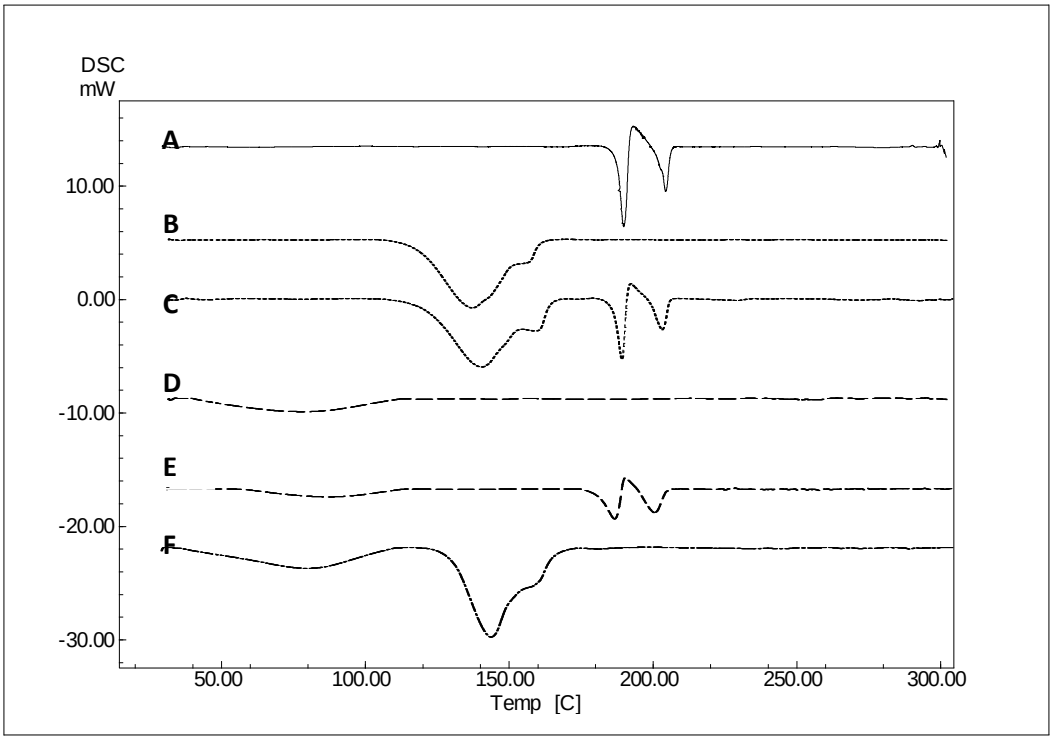


Figure 4.4: DSC of Pure BS (A), Calcium Sulfate (B), mixture of BS and calcium Sulfate (C), XG (D), mixture of BS and XG (E) and mixture of XG and Calcium sulfate (F)

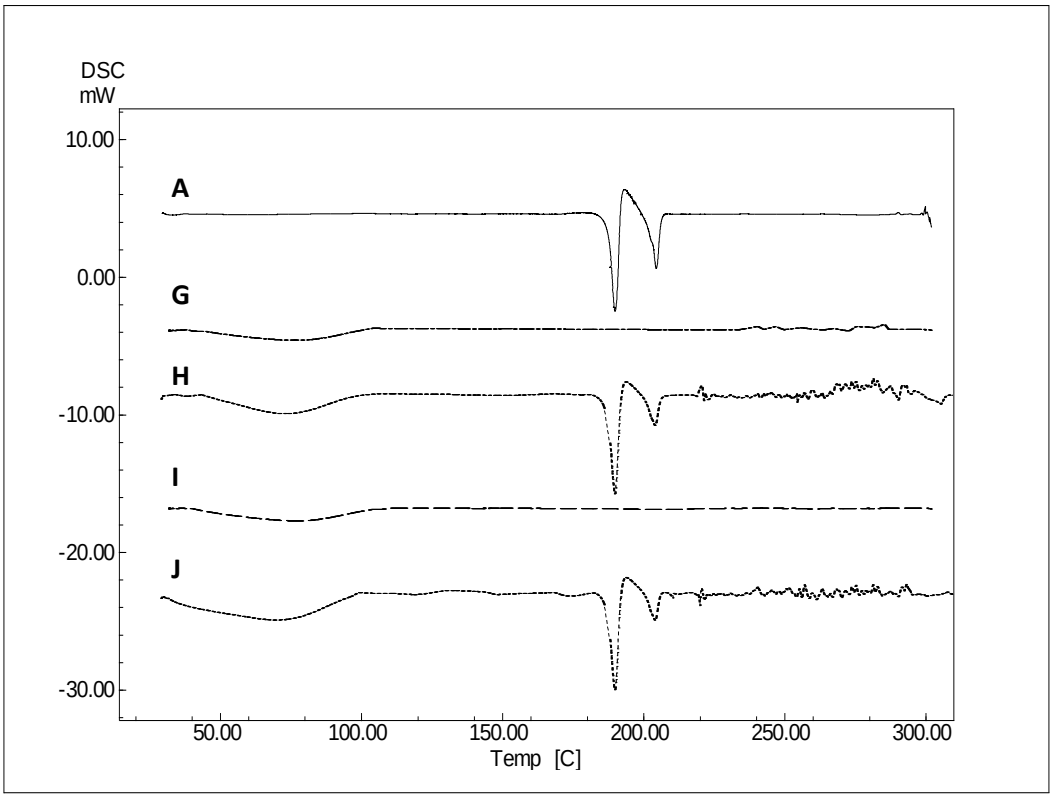


Figure 4.5: DSC of Pure BS (A), HPMC K15 (G), mixture of BS and HPMC K15 (H), MCC (I) and mixture of BS and MCC (J)

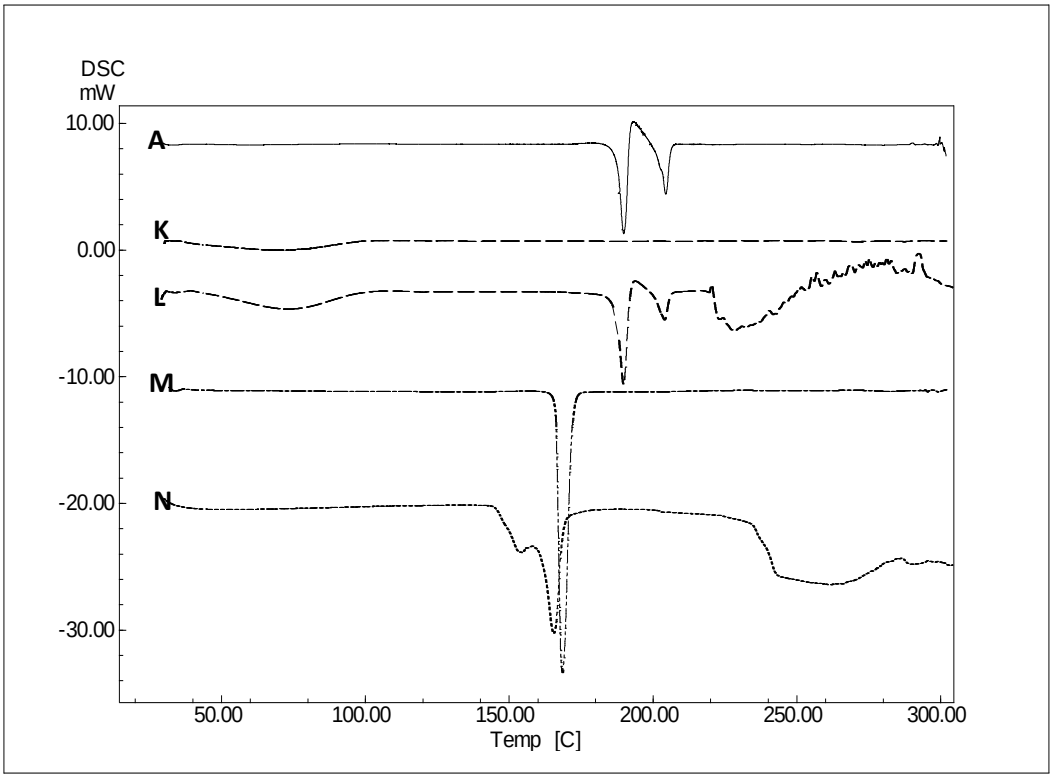


Figure 4.6: DSC of Pure BS (A), PC (K), mixture of BS and PC (L), mannitol (M) and mixture of BS and mannitol (N)

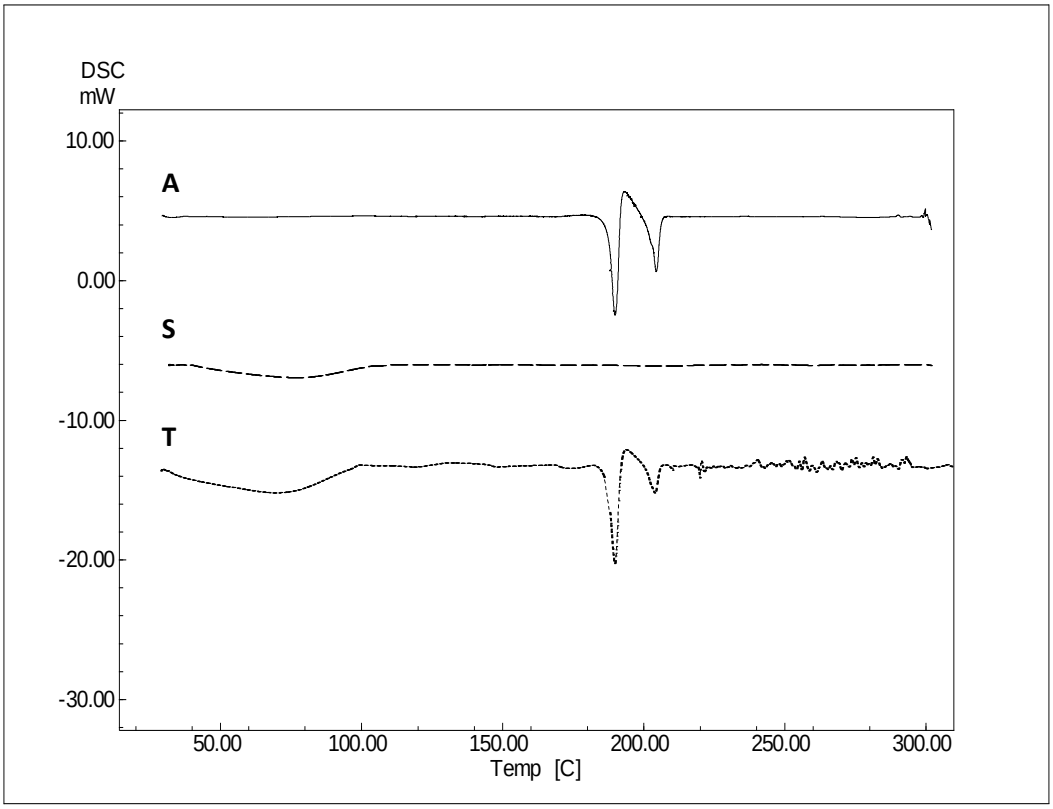


Figure 4.7: DSC thermogram of pure BS (A), HPC (O) and mixture of BS and HPC (P)

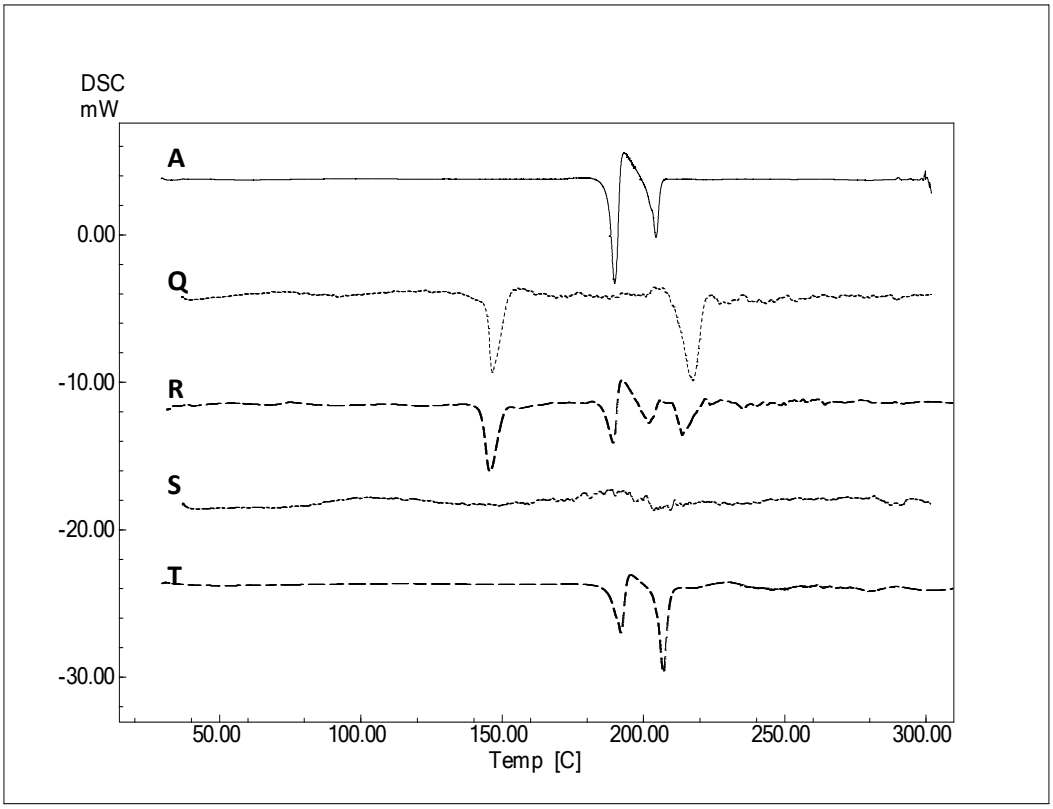


Figure 4.8: DSC thermogram of pure BS (A), lactose (Q), mixture of BS and lactose (R), talc (S) and mixture of BS and talc (T)

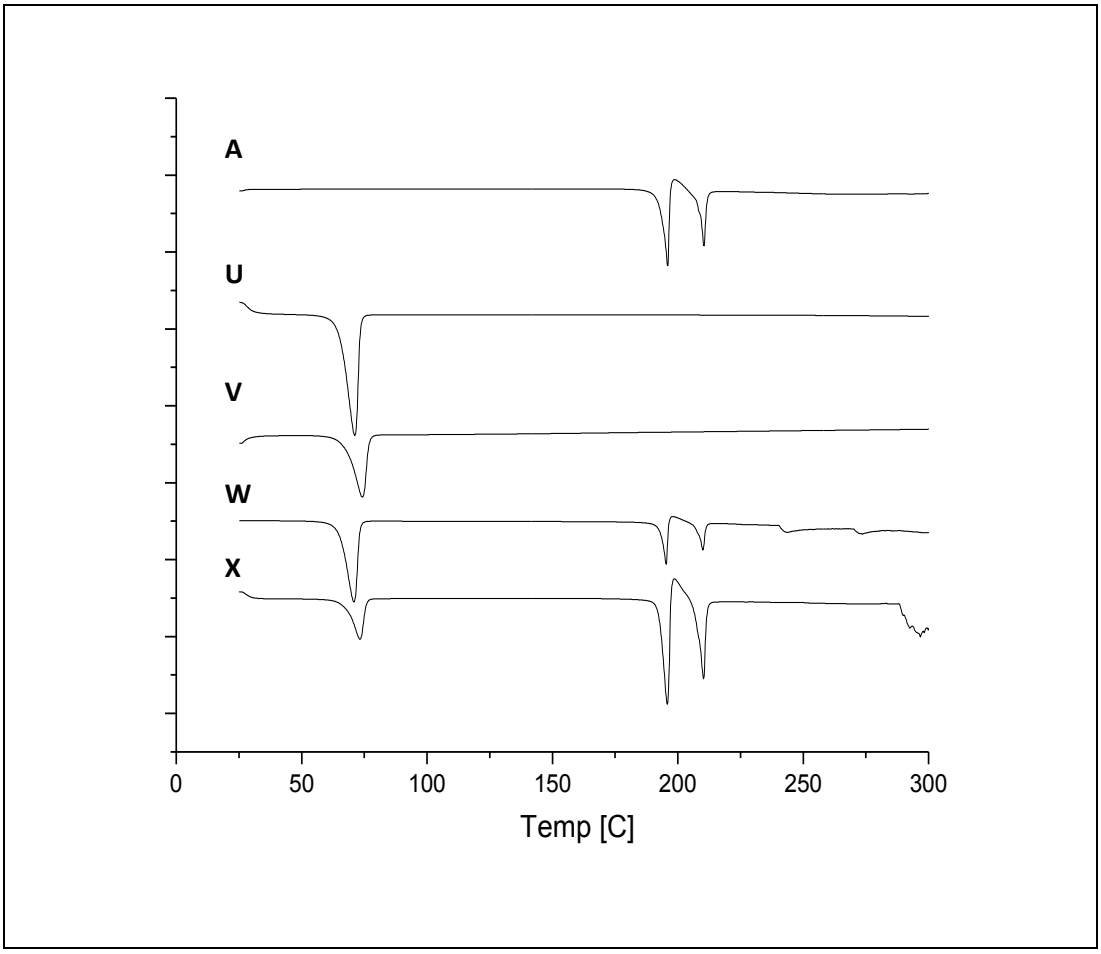
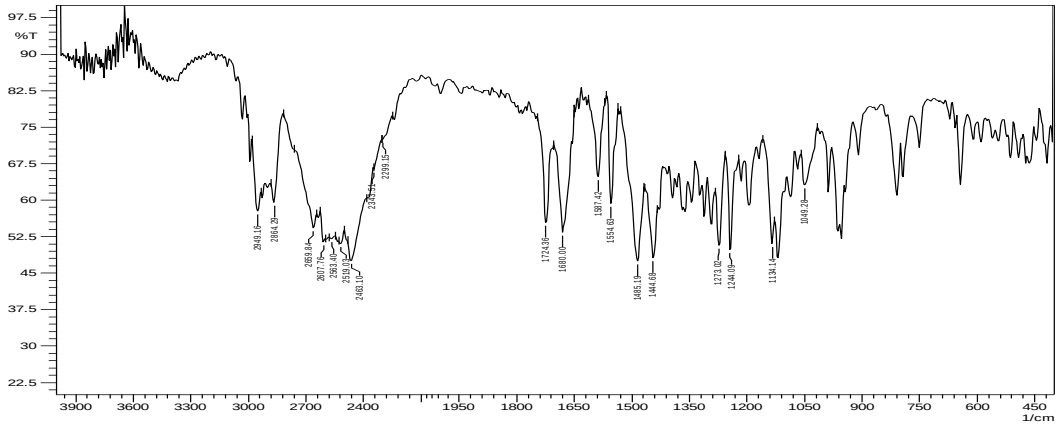
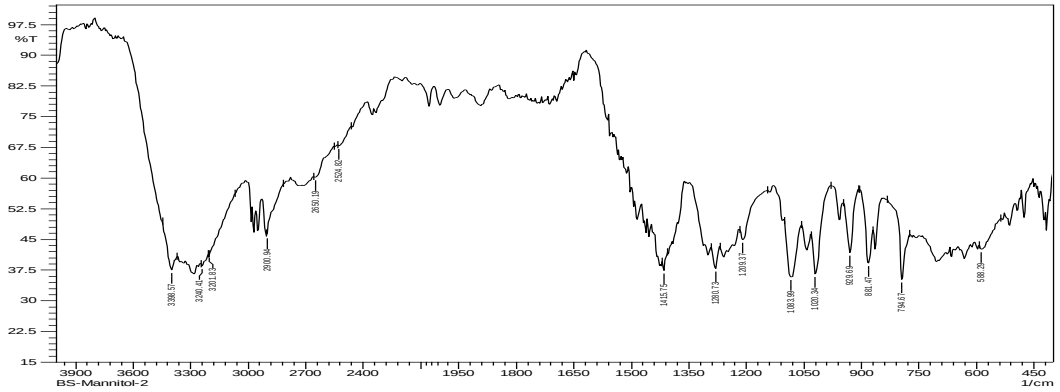


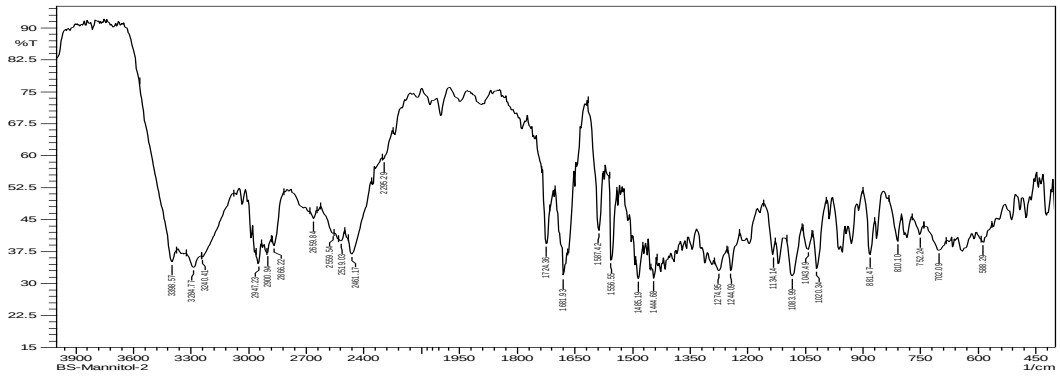
Figure 4.9: DSC thermogram of pure BS (A), PEO 1L (U), PEO 40L (V), mixture of BS and PEO 1L (W) and mixture of BS and 40L (X).



(A) Pure BS



(B) Pure Mannitol



(C) Physical mixture of BS and mannitol

Figure 4.10: FTIR spectrum of Pure BS (A), pure mannitol (B) and physical mixture of BS and mannitol

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

Wavelength (cm^{-1})	**Origin**
1338	C-N stretching
1724.36, 1681.93	C=O stretching vibration
1554.63	C=C stretching vibration
1454	CH2 bending
2949.16	C-H In aromatic ring
980	C-H bending

Table 4.2: First order degradation kinetics of BS in buffered media of varying pH at 25 ± 2 °C

pH	***Degradation Rate Constant*** $K_{deg} \times 10^{-4}(Days^{-1})$	$t_{90\%}$ $(Days \times 10^{2})$	R^2
1.2	6.91	1.52	0.9604
2.0	4.61	2.28	0.9765
3.0	6.91	1.52	0.9642
4.0	9.21	1.14	0.7754
5.0	6.91	1.52	0.9561
6.0	4.61	2.28	0.9031
6.8	6.91	1.52	0.9522
7.0	9.21	1.14	0.8691
8.0	4.61	2.28	0.8936
9.0	6.91	1.52	0.9256

Table 4.3: Thermal properties of drug and excipients in alone or in combination

Sample	Peak onset (ºC)	Peak (ºC)	Peak End set (ºC)	Heat (J/g)
Buspirone HCl (BS)	188.26	189.89	191.44	-55.22
	191.61	192.18	200.28	33.16
	202.97	204.43	205.82	-36.32
Xanthan Gum	---	---	---	---
BS + Xanthan gum	186.49	189.13	190.28	-52.16
	190.32	191.76	199.87	31.38
	201.39	203.34	205.88	-37.23
BS + HPMC K15	58.82	86.63	166.01	-51.35
	181.05	186.49	188.86	-23.00
	188.94	191.23	197.84	35.31
	201.84	200.39	204.24	-20.16
PC	--	--	--	--
BS + PC	187.68	190.08	191.14	-54.26
	191.28	192.76	199.21	33.05
	200.26	202.98	204.97	-40.19
HPC	--	--	--	--
BS + HPC	187.07	190.26	191.22	-53.63
	191.36	192.36	200.16	36.84
	201.43	204.08	205.74	40.11
Calcium sulfate	120.39	136.91	160.17	-273.18
BS + calcium Sulfate	187.31	190.00	190.47	53.65
	190.72	192.17	198.22	31.46
	199.75	202.98	205.37	36.85
	124.69	140.23	163.52	-265.96
Mannitol	166.38	168.41	171.41	-207.31

Table 4.3: Thermal properties of drug and excipients in alone or in combination (Contd.)

Sample	Peak onset (ºC)	Peak (ºC)	Peak Endset (ºC)	Heat (J/g)
BS + mannitol	160.53	165.35	168.20	-151.48
Lactose	144.70	146.80	151.93	-53.96
	211.89	217.46	221.63	-87.25
BS + lactose	187.14	189.68	191.79	-50.91
	191.86	192.38	200.53	31.68
	200.97	204.11	205.31	34.19
	144.16	146.23	150.79	-47.66
	210.53	215.31	220.71	-87.25
Microcrystalline Cellulose	---	---	---	---

Table 4.4: First order reaction kinetics of incompatibility study of BS with different excipients

BS + Excipient (1:1)	***CRT(25 ± 2^{o}C/60 ± 5 % RH)***			***AT (40 ± 2^{o}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**
BS	20.73	50.66	0.8394	59.88	17.54	0.9012
BS + HPMC K15	29.94	35.07	0.9937	85.21	12.32	0.9264
BS + XG	23.03	45.59	0.8435	69.09	15.20	0.9765
BS + PC	20.73	50.66	0.8777	62.18	16.89	0.9927
BS + HPC	20.73	50.66	0.8198	82.91	12.66	0.9993
BS + PEO	27.64	37.99	0.8950	73.70	14.25	0.9953
BS + Caso4	25.33	41.45	0.8340	96.73	10.86	0.9923
BS + Mannitol	27.63	37.99	0.9994	69.09	15.20	0.9958
BS + Magnesium Stearate	138.18	7.60	0.9845	175.03	6.00	0.9953
BS + MCC	23.03	45.59	0.9234	62.18	16.89	0.9992

References

Alelyunas, Y. W., Empfield, J. R., McCarthy, D., Spreen, R. C., Bui, K., Pelosi-Kilby, L., & Shen, C. (2010). Experimental solubility profiling of marketed CNS drugs, exploring solubility limit of CNS discovery candidate. Bioorg. Med. Chem. Lett., 20(24), 7312-7316.

Araújo, A. A. S., Storpirtis, S., amp, x, lvia, Mercuri, L. P., Carvalho, F. M. S., Filho, M. d. S., & Matos, J. R. (2003). Thermal analysis of the antiretroviral zidovudine (AZT) and evaluation of the compatibility with excipients used in solid dosage forms. Int. J. Pharm., 260(2), 303-314.

Brittain, H. G. (2001). Solid-state analysis. In A. Satinder & S. Stephen (Eds.). Sep. Sci. Technol. (Vol. Volume 3, pp. 57-84): Academic Press.

Brittain, H. G. (2011). 2 - Characterization of Pharmaceutical Compounds in the Solid State. In A. Satinder & S. Stephen (Eds.). Sep. Sci. Technol. (Vol. Volume 10, pp. 11-58): Academic Press.

Chadha, R., & Bhandari, S. (2014). Drug–excipient compatibility screening—Role of thermoanalytical and spectroscopic techniques. J. Pharm. Biomed. Anal., 87(0), 82-97.

Hsu, C.-H., & Lin, S.-Y. (2009). Rapid examination of the kinetic process of intramolecular lactamization of gabapentin using DSC–FTIR. Thermochim. Acta, 486(1–2), 5-10.

Picker-Freyer, K. M. (2009). M. Adeyeye, H. Brittain (Eds.), Preformulation in Solid Dosage Form Development, Taylor and Francis, London, 2008, 596 pp., ISBN: 978-0-8247-5809-7. Eur. J. Pharm. Biopharm., 71(2), 404.

Sheikhzadeh, M., Murad, S., & Rohani, S. (2007a). Response surface analysis of solution-mediated polymorphic transformation of buspirone hydrochloride. J. Pharm. Biomed. Anal., 45(2), 227-236.

Sheikhzadeh, M., Rohani, S., Jutan, A., & Manifar, T. (2007b). Quantitative and molecular analysis of buspirone hydrochloride polymorphs. J. Pharm. Sci., 96(3), 569-583.

van Dooren, A. A., & Müller, B. W. (1984). Purity determinations of drugs with differential scanning calorimetry (DSC)—a critical review. Int. J. Pharm., 20(3), 217-233.

Verma, R. K., & Garg, S. (2004). Compatibility studies between isosorbide mononitrate and selected excipients used in the development of extended release formulations. J. Pharm. Biomed. Anal., 35(3), 449-458.

Wen, H., & Park, K. (2011). Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice. Wiley.

5.1 Introduction

In the recent past buccal dosage forms gained a significant interest in delivering orally inefficient drugs to the systemic circulation. Amongst various buccal dosage forms, bioadhesive drug delivery system have been resported to achieve reproducible plasma drug profile (Abruzzo et al., 2012). Buccal route has widely been reported for successful delivery of small and large molecules to the systemic circulation. Advantages like ease of application and removal makes buccal delivery a more preferred route of drug administration when compared to other non-invasive mucosal routes such as nasal, pulmonary and rectal (Harris & Robinson, 1992; Kianfar et al., 2013). Drug delivery via buccal route has advantages like avoidance of first pass metabolism, predictable drug absorption and limited exposure of drug molecule to harsh gastric environment (pH and enzymatic activity) (Giovino et al., 2012; Salamat-Miller et al., 2005; Sudhakar et al., 2006). Bioadhesive polymers in the buccal dosage forms play an important role in delivery of the drugs to systemic circulation by adhering to the mucosal surface for longer duration of time and releasing the drug in a controlled fashion (Ayensu et al., 2012b; Charde et al., 2008; Kianfar et al., 2013; Rossi et al., 2005; Salamat-Miller et al., 2005).

Several polymers have been reported for the delivery of large and small therapeutic drug molecules through buccal route in a controlled release manner. Various bioadhesive and controlled release polymers have been discussed in chapter 1 with classification presented in Table 1.

Variety of dosage forms like tablets (Boyapally et al., 2010; Kanjanabat & Pongjanyakul, 2011), discs (Jaipal et al., 2013; Jaipal et al., 2014; Yehia et al., 2008), patches (Abu-Huwaij et al., 2011; Govindasamy et al., 2013; Kaur & Kaur, 2012) and gels (Ayensu et al., 2012a; Bueno et al., 2013; Das et al., 2012) have been reported in the literature for buccal delivery of drugs. Buccal tablets are extensively reported for both immediate release and controlled release; however buccal tablets are less patient compliant due to non-flexibility and large size. Buccal patches and films resolve the flexibility problems associated with buccal tablets but due to presence of high water content, chances of microbial contamination and drug instability are high. Moreover scale-up related issues make buccal patches less preferable. Buccal gels and sprays have the limitation of precise dose delivery to the desired site. To address most of

these drawbacks related to buccal tablets, patches, gels and sprays, buccal discs are designed. Buccal discs are flat, thin solid unit non-flexible compacts; and are similar to buccal tablets. Buccal discs are designed to minimize the discomfort caused due to bulky tablet buccal dosage forms. Several buccal discs have been extensively reported for successful delivery of therapeutic drug molecules (El-Samaligy et al., 2004; Han et al., 1999; Jaipal et al., 2013; Sander et al., 2013; Yehia et al., 2008).

Bioadhesive polymers in the buccal dosage forms play an important role in delivery of the drugs to systemic circulation by adhering to the mucosal surface for longer duration of time and releasing the drug in a controlled fashion (Ayensu et al., 2012b; Kianfar et al., 2012; Rossi et al., 2005; Salamat-Miller et al., 2005). Polymers, permeation enhancers and other excipients reported for buccal delivery of drugs have been discussed in Chapter 1. However, here further details of polymers, permeation enhancers and excipients used during current research work have been presented.

Xanthan gum is a biopolymer with vast applications in food, cosmetic, agricultural, textile, petroleum and pharmaceutical industry (Garcia-Ochoa et al., 2000; Mirhosseini et al., 2008). Xanthan gum is a high molecular weight anionic polysaccharide gum produced by aerobic fermentation of sugars by the bacterium *xanthomonas campestris*. USFDA has approved xanthan gum as GRAS (Generally Recognized as Safe) listed chemical for use in some of the pharmaceutical, food and cosmetic preparations. The primary structure of xanthan gum contains repeated polysaccharide units formed by two D-glucopyranosyl units, two D-mannopyranosyl units and one glucopyranosyluronic acid unit in the molar ratio 2.8 : 2.0 : 2.0 respectively. Use of xanthan gum as viscosity enhancer and stabilizer in suspensions and emulsions has been reported (Jian et al., 2012; Talukdar et al., 1998). Xanthan gum has also been widely investigated for sustained release behavior in oral tablets (Talukdar & Kinget, 1995). Xanthan gum in combination with other polymers, such as starch (Shalviri et al., 2010), sodium alginate (Zeng, 2004) has also been reported for the design of sustained release formulations. Bioadhesive behavior of xanthan gum has been widely reported (Abu-Huwaij et al., 2011; Park & Munday, 2004).

Hydroxy propyl methyl cellulose (HPMC) is a semi synthetic and inert bioadhesive polymer. HPMC is GRAS (generally recognized as safe) listed ingredient and included in FDA's Inactive Ingredient database used in manufacturing of variety of dosage forms available commercially. HPMC is available in a wide range of

viscosity grades (3 mPa to 100,000 mPa) (Nafee et al., 2004). Biocompatible and biodegradable nature of HPMC along with bioadhesive and release rate retarding properties of HPMC make it a suitable excipient for designing buccal discs (Mumtaz & Ch'ng, 1995; Narendra et al., 2005; Taylan et al., 1996).

Polyethylene oxide (PEO) is a water soluble non-ionic homopolymer synthesized by heterogeneous catalytic polymerization of ethylene oxide. PEO has wide application in drug delivery (Almeida et al., 2012), mining (Gong et al., 2010), paper making (van de Ven et al., 2007) etc. PEO is commercially available with varying molecular weight numbers (100000-8000000). Several buccal dosage forms for various therapeutic drug molecules of PEO has been previously reported such as films (Miro et al., 2013), tablets (Cappello et al., 2006; Charde et al., 2008) and discs (El-Samaligy et al., 2004). Owing to an advantage of PEO availability in different molecular weight numbers, blend of these grades are utilized to design an optimized buccal formulation with desired release profiles.

Hydroxy propyl cellulose (HPC) is an aqueous and organic soluble neutral polysaccharide derived from cellulose. HPC is an ether of cellulose in which some of the hydroxyl groups in the repeating glucose units have been hydroxypropylated forming $-OCH_2CH(OH)CH_3$ groups using propylene oxide (Mezdour et al., 2007). HPC also exhibits considerable controlled release and bioadhesive properties suitable for design of buccal dosage forms (Repka & McGinity, 2001).

Polycarbophil (PC) is a high-molecular-weight acrylic acid polymer cross-linked with polyalkenyl ethers or divinylglycol. There is a large number of carboxyl groups (COOH) on the molecular chain. It is insoluble in aqueous media but in the neutral pH conditions, it has a good swelling capacity, allowing high levels of entanglement within the mucus layer. Comprehensive adhesion and the inherent characteristics of PC, the bioadhesive effect is produced by the carboxylic acid groups binding to the mucosal surfaces via hydrogen bonding interaction (Zhu et al., 2013). Retarded release formulations using PC have been widely reported (Hosny & Al-Angary, 1995; Repka & McGinity, 2001).

Other excipients such as fillers, lubricants and permeations enhancers also serve as important factors for the design of buccal disc formulations.

Mannitol is a white crystalline polyol synthesized industrially by catalytic hydrogenation of fructose or glucose syrup. Mannitol is water soluble, non toxic and non hygroscopic ingredient extensively used in food and pharmaceutical preparations (Debord et al., 1987). Mannitol is a GRAS (generally recognized as safe) listed ingredient and is used in many dosage forms available commercially. It has been reported that mannitol enhances dissolution of drug from the dosage form as it forms pores within the dosage form matrix (Holgado et al., 1995).

Calcium sulfate is an inorganic and inactive ingredient used as filler or diluent in many solid unit dosage forms. USFDA has listed calcium sulfate as GRAS (Generally Recognized as Safe) additive approved for food and pharmaceutical products. Calcium sulfate was selected as an excipient in the design of buccal discs due to its excellent physical properties favorable for direct compression method. Direct compression method offers several advantages over wet granulation method such as prevention of drug excipient incompatibility and prevention of drug crystal bridges formation due to use of aqueous and non aqueous solvents resulting in improved stability. Calcium sulfate is a free flowing, non-hygroscopic, odorless and directly compressible excipient and has been reported for demonstration of enhanced stability compared to that of organic excipients such as lactose with respect to impurities after one month stability trial (Eyjolfsson, 2004).

Permeation enhancers have been reported in the literature (Lee & Kellaway, 2000; Nicolazzo et al., 2005a, b; Oh et al., 2011; Şenel et al., 1997; Tsutsumi et al., 1998) that alter the characteristics of mucosal membranes resulting in better permeability of drugs across buccal membrane. Classification of permeation enhancers are discussed in Table 1.1 of chapter 1. Most of the permeation enhancers reported cause irritation and obnoxious taste thereby reducing compatibility of drug delivery systems (Şenel & Hıncal, 2001). Hence, newer agents are being explored for permeation enhancement.

Carbon dioxide gas has been reported in literature as a permeation enhancer using both in-vitro (Eichman & Robinson, 1998) and in-vivo studies (Darwish et al., 2006a; Darwish et al., 2006b; Tadros, 2010; Wang & Tang, 2008). Increase in drug permeation across rabbit ileum has been reported in-vitro when permeation experiment was performed by bubbling carbon dioxide (Eichman & Robinson, 1998). Fentanyl effervescent buccal tablets also resulted in rapid and significantly higher

amount of drug in systemic circulation compared to non-effervescent buccal tablets in human volunteers (Darwish et al., 2006a; Darwish et al., 2006b). Enhanced permeation of Insulin using effervescent formulations in *ex-vivo* studies have also been reported (Sadeghi et al., 2009).

The present chapter deals with the design and development of controlled release bioadhesive buccal disc matrices of BS for probable improvement in bioavailability. The designed buccal matrices were prepared using various bioadhesive and release retarding polymers either alone or in combination along with several process excipients by direct compression method. Effect of polymer concentration and process excipients on drug release and bioadhesive behavior was studied. Effervescent buccal discs were also designed to study the effect of carbon dioxide on in-vitro drug release and bioadhesion behavior. Required quality control tests were also carried out for the designed buccal dosage forms. Stability of designed formulations was studied at various conditions of temperature and humidity.

5.2 Materials and Reagents

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

5.3 Equipments

BS buccal discs were prepared using a 8 station compression machine (Rimek Mini Press, India) equipped with 4mm punches. Digital analytical balance (Mettler Toledo TA 215D, India) was used for all weighing purposes. In-vitro drug release studies were performed using in-house modified USP type-I dissolution apparatus. Bioadhesion studies for the designed formulations were carried out using texture analyzer instrument (Stable Micro Systems TA-XT Plus, UK). Rheological measurements were carried out using Brookfield viscometer (Model DV-III+ Pro, USA) using spindle number-3. pH measurements were performed using a digital pH meter (Eutech Instruments, Singapore) Friability was assessed using USP friability test apparatus (Campbell Electronics, India). Humidity chamber (MAC Instruments, India) was used for water uptake studies. Analytical instruments used in the present study are mentioned in chapter 3.

5.4 Methods

5.4.1 Preparation of bioadhesive buccal discs

Bioadhesive buccal discs were prepared using various bioadhesive and controlled release polymers either alone or in combination. Various polymers used for the design of buccal discs are xanthan gum (XG), hydroxy propyl methyl cellulose (HPMC K15), polyethylene oxide, hydroxy propyl cellulose (HPC) and polycarbophil (PC). Buccal discs (4 mm) of BS with 10 mg drug loading were prepared by direct compression method. Drug and all the process ingredients were passed through sieve (18#) and mixed in a geometric ratio; finally magnesium stearate (2 %w/w) was added as lubricant in all batches of designed buccal discs. The resulting mix was compressed using a 8 station compression machine (Remik Mini Press, 4 mm, round shape punch and die set). Composition of all the designed formulations is presented in Table 5.1.

5.4.2 Effect of various formulation parameters

Buccal discs were prepared using different types of polymers like XG, HPMC K15, PEO (MWn: 1 and 40 Lakhs), HPC and PC. Effect of polymer type, proportion and process excipients used on in-vitro drug release and bioadhesive behavior was studied. Effect of ionic nature of polymer on in-vitro drug release rate and bioadhesion was studied by using anioinic (PC and HPC) and non-ionic polymers (PEO). The polymers selected were from natural (XG), semi-synthetic (HPMC, HPC) and synthetic origin (PC, PEO).

Effect of various process excipients on in-vitro drug release and bioadhesive behavior was also studied. Calcium sulfate, mannitol and lactose were used as diluents in designed buccal discs to assess impact on drugs release and bioadhesion.

5.4.3 Evaluation of designed bioadhesive buccal dosage forms

5.4.3.1 Weight Variation

Randomly selected 20 buccal discs from each batch were evaluated for weight variation using an electronic digital weighing balance (Mettler Toledo TA 250D, India). Mean weight of buccal discs and percentage deviation was calculated for all the batches.

5.4.3.2 Thickness

Thickness of 20 buccal discs selected randomly from each batch was measured in millimeter using digital Vernier Caliper (Mitutoyo Digimatic Caliper, Japan). The mean thickness of buccal discs and percent standard deviation was calculated.

5.4.3.3 Friability

Friability of 20 buccal discs was assessed using friability test apparatus (Campbell Electronics, India) by standard procedure mentioned in USP29-NF24 (25 rpm for 4 min). The percentage loss on friability was calculated.

5.4.3.4 Surface pH

Acidic or alkaline pH of the dosage form could cause uneasiness in the oral cavity and may usher irritation. Surface pH for all the batches was determined in triplicate using a digital pH meter (Eutech Instruments, Singapore). The buccal discs were hydrated in 100 mL distilled water for 20 min. The electrode upon contact with the hydrated buccal discs surface was allowed to equilibrate for 1 min and the pH was recorded immediately. All the readings were recorded in triplicate.

5.4.3.5 Drug content

Randomly selected 10 buccal discs were crushed using mortar and pestle. Powder equivalent to 2 mg of BS was weighed accurately and BS was extracted using 10 mL of solvent system (acetonitrile and phosphate buffer pH 3.0; 1:3 v/v). The resultant mix was centrifuged at 3000 rpm (Remi-R8C laboratory centrifuge) and 1 mL of supernatant was collected and suitably diluted for determination of drug content using analytical method 2 of chapter 3.

5.4.3.6 In-vitro drug release studies

In-vitro drug release studies were performed using in-house modified USP type-I dissolution apparatus. The method uses 50 mL capacity screw cap tubes arranged on a dissolution vessel using an in-house designed aluminum holder. Single unit of buccal disc was loaded in the basket attached to the instrument shaft and allowed to immerse in the prearranged tubes containing 50 mL phosphate buffer (pH 6.8) maintained at 37 °C. The instrument was operated at 25 rpm and samples were collected at predetermined time intervals and replenished with fresh buffer. The

samples collected were analyzed for drug content using HPLC method described previously in method 2 of chapter 3. Percentage cumulative drug release was calculated at each time point after application of appropriate correction factor. All the drug release studies were performed in triplicate.

Data obtained using in-vitro release study 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; Peppas & Sahlin, 1996; Ritger & Peppas, 1987). Drug release rate from hydrophilic matrix systems depends on swelling behavior of the polymer, shape of the matrices, diffusion and erosion properties of the polymer and dissolution characteristics of the drug. Dose and solubility of the drug, type and quantity of the fillers and the polymer characteristics influence 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).

5.4.3.7 Rheological evaluation

Viscosity studies for XG polymer mucilage in the presence and absence of calcium sulfate was investigated using Brookfield viscometer (Model DV-III+ Pro) using spindle number-3 to understand the effect of calcium sulfate on xanthan gum mucilage. Varying amounts of calcium sulfate (0.2%, 0.4% and 0.5% w/v) was added to XG mucilage (0.6% w/v in phosphate buffer) and viscosity was measured at varying spindle speed of 10, 15, 20 and 75 rpm at 25 °C. Rheological measurement was performed in triplicate and percent standard deviation was calculated.

5.4.3.8 Bioadhesion studies

Bioadhesion studies of designed formulations were carried out using texture analyzer instrument (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 at room temperature before the study. The tissue was placed in simulated salivary fluid and stored at -20 °C till further usage. The simulated salivary fluid (SSF) comprised of sodium chloride (0.8% w/v),

potassium phosphate monobasic (0.019% w/v) and sodium phosphate dibasic (0.238% w/v) (Gohel et al., 2009).

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 disc was attached to the base of the texture analyzer movable probe (SMSP/10) using a double sided adhesive tape. The probe was lowered at 0.5 mm/sec till the buccal disc was in contact with mucosal membrane and upon of disc with mucosal membrane, a 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 disc from the mucosal surface was recorded in triplicate.

5.4.3.9 In-vitro water uptake studies

Method 1: Water uptake behavior of the designed XG buccal discs was assessed by immersing the dosage form in 600 mL phosphate buffer (pH 6.8) maintained at 37 °C and 40 % RH in a humidity chamber. Initial weight and final weight of dosage form was recorded at predetermined time intervals of 0.5, 1, 2, 3, 4 and 6 h. All the readings were recorded in triplicates for each time point. Percent water uptake was calculated for all the batches using the Eq. 1.

Method 2: Water uptake of the designed buccal discs (except XG) was performed on 2% w/v aqueous agar plates maintained at 37 °C and 40% RH in a humidity chamber. The amount of water taken up was observed at different time points. All the readings were recorded in triplicate for each time point. Percent water uptake was calculated for all the batches using the Eq. 1

$$Percent\ water\ uptake = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} \times 100 \qquad \text{(Eq 1)}$$

5.4.3.10 Stability studies

Representative formulations 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^oC/ 60 ± 5 % RH) and accelerated condition (AT: 40 ± 2^oC/ 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 was calculated at different storage conditions for all the formulations.

5.5 Results and Discussion

5.5.1 Evaluation of buccal bioadhesive controlled release discs

5.5.1.1 *Physical characteristics of designed buccal discs*

Bioadhesive buccal discs of BS were designed using various polymers like XG. HPMC K15, PEO, HPC and PC were reported for controlled release and bioadhesive behavior. Other excipients like microcrystalline cellulose, mannitol and lactose were used as diluent and mannitol additionally served as a sweetener. Magnesium stearate was used as an anti-adherent and lubricant. The composition of all the batches is shown in Tables 5.1.

All the batches of designed buccal discs was well within permissible limit (less than 10 % for solid unit dosage forms weighing less than 130 mg according to USP) of percent deviation for weight variation (0.15 to 0.81 %). This also indicated by the low SD value (< 0.61 mg) (Table 5.2). The weight variation in case of all the formulations was within ± 1.5 % of theoretical buccal disc weight indicating the formulations are within the acceptance criteria.

Friability observed for all the batches of buccal discs was less than 1 % (weight loss of not more than 1% according to USP29-NF24) indicating the suitability of process and method adopted for the design and manufacture of bioadhesive buccal discs (Table 5.2).

Thickness measurements recorded for all the batches with digital vernier calipers demonstrated maximum thickness of 2.55 mm with a maximum SD of 0.04 mm. Thickness of buccal discs prepared using XG (JB/XG/00 to JB/XG/50) decreased with increase in calcium sulfate amount. This can be attributed to higher bulk density of calcium sulfate (Table 5.2). Surface pH values observed was around 7.0 for all the batches of designed buccal discs.

Drug content values of designed formulations were found to be acceptable with a range of 98 to 102 % of theoretical claim (according to USP29-NF24 acceptable limit is 85 to 115 % for solid unit dosage forms weighing less than 130mg) with maximum SD of 0.83 mg (Table 5.2). Hence, the designed buccal discs prepared using different polymers demonstrated good physical characteristics indicating the suitability of the process and excipients used.

5.5.1.2 In-vitro drug release studies

In-vitro drug release is a predictable tool for understanding the drug release behavior in in-vivo. It is a key evaluation parameter in dosage form development and quality control. The main objective for the design of buccal discs was to improve bioavailability and retard the release of drug for at least 4-6 h, for maintaining the steady plasma levels of drug for longer duration in in-vivo.

Xanthan gum based buccal discs retarded the release of drug for at least 6 h (Figure 5.1). This retardation of drug release can be ascribed to swelling of polymer matrix caused due to elastic retraction of acetyl side chains of polymer network due to hydration. The drug release rate from xanthan gum based discs increased significantly with increasing proportion of calcium sulfate (Figure 5.1). This can be attributed to change in xanthan gum molecular structure conformation due to presence of divalent calcium ions (Ca^{2+}) (Baumgartner et al., 2008; Bergmann et al., 2008; Higiro et al., 2006; Jaipal et al., 2013; Lambert et al., 1985; Mohammed et al., 2007; Zhong et al., 2013). Formation of intra molecular complexes due to binding of divalent calcium ions to the side chains of pyruvate residue of xanthan gum has been reported (Bergmann et al., 2008). This increase in drug release rate is due to a reduction of hydrodynamic volume of xanthan gum because of formation of orderly structure (collapse of side chains in to the xanthan gum backbone) in the presence of calcium ions (Higiro et al., 2006). Interaction of calcium ions with polymeric side chains of xanthan gum might have led to density fluctuation and change of network morphology to an orderly form resulting in formation of larger pores. The size and number of these pores will directly be related to degree of calcium ion interaction (amount of calcium ions). This pore formation might have enhanced diffusion of water in the disc matrix and decreased water holding capacity thereby resulting in faster drug release.

Increased drug release may also be attributed to decrease in viscosity of xanthan gum mucilage in the presence of calcium sulfate. Interaction of calcium ions with ionized groups of polymer can also be related to decrease in hydration behavior (Rochefort & Middleman, 1987).

Bioadhesive buccal discs were designed using varying proportions of HPMC K15 and mannitol. Designed buccal discs using HPMC and mannitol retarded drug

release for at least 5 h (Figure 5.2). Changes in HPMC and mannitol proportion have significantly influenced the drug release and bioadhesive behavior for the designed formulations. The drug release rate decreased with increasing proportion of HPMC in buccal disc matrix. An increase in polymer proportion increases the viscosity of gel layer and also results in gel layer with longer diffusional path length resulting in greater retardation of drug release (Pygall et al., 2009; Shakya et al., 2013; Siepmann & Peppas, 2001) (Figure 5.2). Increase in proportion of mannitol resulted in faster dissolution of drug and faster drug release rate. This can be ascribed to formation of pore in buccal disc matrix due to higher and faster solubility of mannitol, through which the media can enter the swollen polymer matrix and dissolves the drug resulting in for faster drug release rate.

Controlled release effervescent buccal discs were designed using HPMC K15 polymer, a controlled release and bioadhesive polymer. The main objective for the design of controlled release effervescent formulations was to improve the drug permeability across the buccal mucosal membrane in in-vivo. Carbon dioxide gas has been reported in literature as a permeation enhancer using both in-vitro (Eichman & Robinson, 1998) and in-vivo studies (Darwish et al., 2006a; Darwish et al., 2006b; Tadros, 2010; Wang & Tang, 2008). Sodium bicarbonate and citric acid were used as effervescent agents. Other excipients like lactose and MCC were used as diluents. Designed effervescent HPMC buccal discs (JB/EF/01 to JB/EF/06) clearly demonstrated faster drug release rate in formulations containing sodium bicarbonate and citric acid as effervescence forming agents (Figure 5.3). The formulation containing only citric acid (JB/EF/03) have demonstrated faster drug release rate as compared to the formulations containing only sodium bicarbonate (JB/EF/02) and lower concentration of effervescence forming agents (JB/EF/04) (Figure 5.3). This result clearly indicates that the drug release from HPMC matrices was directly influenced by citric acid either by creating acidic microenvironment pH favoring dissolution of weakly basic drug BS (AlKhatib et al., 2008) or by weakening the gel strength of HPMC (Espinoza et al., 2000; Martínez González & Villafuerte Robles, 2003; Pygall et al., 2009).

Drug release from HPMC matrices is usually due to swelling and erosion of polymer matrix (Asare-Addo et al., 2013). Upon contact with in-vitro release media, a gel layer forms on the surface of HPMC matrices. This surface gel layer is followed

by glassy layer of swollen polymer in the middle whereas the core remains dry. However, with passage of time, the core of matrix also gets hydrated. The drug release from this swollen matrix is due to disentanglement of surface polymeric chains resulting in erosion and due to diffusion of media into the discs through the swollen matrix (Asare-Addo et al., 2013; Chen et al., 2010; Pajander et al., 2012; Tajarobi et al., 2009). Due to solubilization of components of matrix, the number of pores and pore diameter in swollen matrix increases thereby resulting in further drug release.

In the present study, effervescent buccal discs exhibited faster drug release in comparison with non-effervescent buccal discs. Moreover, the release rate increased as the amount of effervescence forming agents increased in the designed buccal discs. This can be attributed to formation of more pores in the HPMC disc due to rapid escape of carbon dioxide evolved during the reaction of sodium bicarbonate and citric acid. Lactose used as diluent in the buccal discs might have also enhanced the formation of channels for escape of carbon dioxide gas and the drug. The formation of more pores in the effervescent buccal discs might have resulted in the increased hydration rate of HPMC, thereby resulting in lesser strength of gel formed at the surface. This in turn would have resulted in faster erosion of polymer layer resulting in faster drug release.

Bioadhesive buccal discs were designed using two molecular weight numbers of PEO *viz* 1 lakh and 40 lakhs. Designed PEO buccal discs retarded drug release for a minimum of 4 h (Figure 5.4). Increase in proportion of higher molecular weight number PEO polymer resulted in retardation of drug release for longer duration. This retardation of drug release can be ascribed to formation of relatively greater viscous matrix by high molecular weight number PEO (40 Lakhs), thus making the dosage form relatively less soluble compared to disc prepared using lower molecular weight number PEO (1 Lakh). Owing to an advantage of this drug release behavior, varying the proportions of PEO blends (1 lakh and 40 lakhs) can be used to tailor the optimized buccal discs for desired release profile.

Buccal discs designed using HPC with varying proportions of polymer concentration and mannitol also retarded drug release for at least 4 h (Figure 5.5). Increase in polymer concentration has retarded drug release and with increase in mannitol proportion has increased drug release rate. This release behavior is due to

formation of viscous hydrogels with increased polymer concentration. Increase in mannitol concentration has considerably increased the release rate due to formation of more pores or channels in the matrix system. Similar results were observed when mannitol is used as a varying factor in design of PC buccal discs (Figure 5.6). All the formulations has shown faster drug release in first 30 min due to presence of surface drug, this initial burst might be helpful in achieving the target drug concentration in-vivo.

Overall, from the percent cumulative drug release profiles against time obtained from in-vitro drug release data for the designed buccal discs clearly indicate, that the polymer concentration is inversely proportion to drug release rate and presence of water soluble excipients has marked effect on increased drug release behavior.

The release data fitted best in the first order kinetic model. The values of release rate constant (K), diffusion exponent (n) and regression coefficient (R^2) for zero order and first order release kinetics for all the designed formulations are tabulated in Table 5.3. Drug release data was also fitted to Higuchi and Korsmeyer-Peppas equations for the prediction of drug release mechanisms (Table 5.3). The drug release mechanism was found to be non-fickian anomalous type for the designed bioadhesive buccal discs based upon n-value obtained from Korsmeyer- Peppas model (Eq. 2). The n-values obtained were ranged from 0.45 to 0.89 indicating diffusion, polymer relaxation and erosion as predominant mechanism of drug release (Korsmeyer et al., 1983a) (Table 5.3).

$$\frac{M_t}{M_\infty} = Kt^n \qquad \text{(Eq. 2)}$$

Where, M_t/M_∞ is fraction of drug released at time "t", "K" is the diffusion rate constant and "n" is the release exponent indicative of mechanism of drug release. If the "n" value obtained is 0.89, it corresponds to zero order drug release (case II transport) wherein the drug release rate is independent of time and the rate controlling factors are polymer relaxation and erosion. If "n" has a value of 0.45, then Fickian diffusion will be the rate controlling factor. If the "n" value lies between 0.45 and 0.89 the drug release is governed by diffusion, polymer relaxation and erosion and the release mechanism is termed to be non-fickian anomalous.

5.5.1.3 Rheological evaluation

To further explore the reason for faster drug release from XG buccal matrices in presence of calcium sulfate, viscosity measurement of XG mucilage was carried out in presence and absence of calcium sulfate. Viscosity of XG mucilage reduced with increasing concentration of calcium sulfate (Figure 5.7). This decrease in viscosity may be due to interaction of divalent calcium ions with negatively charged acetyl groups on polymeric chains of xanthan gum resulting in charge screening thereby reducing hydration behavior of xanthan gum resulting in reduced hydrodynamic radii (Baumgartner et al., 2008; Bergmann et al., 2008; Rochefort & Middleman, 1987). XG molecule elongates in aqueous solution without calcium ions resulting in the formation of strong columbic repulsions between like charges along the polyelectrolyte backbone stretch and in the presence of calcium ions the ionic charges are neutralized resulting in decrease of viscosity (Zhong et al., 2013). Overall weak interaction with water molecules as indicated by hydration studies and reduction in viscosity of XG in the presence of calcium ions as indicated by rheological studies results in faster rate of diffusion and drug release.

5.5.1.4 Bioadhesion studies

In-vitro bioadhesion studies for the designed buccal discs was performed using porcine cheek mucosa using texture analyzer. The force required for detachment of the buccal disc from the biological membrane was recorded. Many polysaccharide polymers, containing hydrophilic networks that contain numerous polar functional groups (such as -COOH, -OH, $-NH_2$ and SO_4) and have been reported to have a considerable bioadhesive behavior (Smart, 2005). In the present study water soluble/swellable polymers were used to design bioadhesive buccal discs of BS. These polymers possesses hydrophilic -COOH and -OH functional groups that interact with glycoprotein chains of mucin. The polymeric chains initially hydrolyze to form gel by diffusion of fluid from the surrounding environment and subsequently the polymeric chains entangle or interlock with glycoprotein chains of mucin at molecular level to form weak crosslinked bonds resulting in bioadhesion.

XG is a water soluble biopolymer and was used to for its efficient controlled release and bioadhesive behavior. XG buccal discs were designed with and without calcium sulfate as one of the diluent. This amount of calcium sulfate in the

formulation has shown marked effect on bioadhesion behavior. XG buccal discs without calcium sulfate showed a higher force of detachment as compared to the formulations containing calcium sulfate (Table 5.2). Force of detachment of XG buccal discs decreased with increasing proportion of calcium sulfate (Figure 5.8). Thus bioadhesive strength of formulations decreased with increasing concentration of calcium sulfate in formulations. This can be attributed to lesser strength of gel formed due to in situ interaction of xanthan gum with calcium ions as demonstrated by rheological measurements discussed in the previous section of rheological evaluation *5.5.1.3*. Moreover, poor hydration of XG buccal discs in the presence of calcium sulfate may have resulted in poorer bioadhesion. The collapse of XG side chains in to the polymer backbone in the presence of calcium ions may have resulted in lesser availability of polymeric side chains for entanglement with glycoprotein chains of mucous (Bergmann et al., 2008; Dentini et al., 1984; Lambert et al., 1985; Launay et al., 1997; Mohammed et al., 2007). Reduction in viscosity of mucilage accompanied by poorer hydration and swelling of xanthan gum in presence of calcium sulfate as indicated by studies mentioned above also plays a major role in reduction of bioadhesion.

HPMC is also a hydrophilic polymer with many polar functional groups. The polymeric chains of HPMC upon hydration form a hydrogel favoring suitable conditions for bioadhesion. HPMC polymer chains entangle or interlock with the glycoprotein chains of the mucous at molecular level resulting in bioadhesion (Andrews et al., 2009; Jaipal et al., 2013). Increase in polymer proportion in the designed formulation has predominantly improved the bioadhesive strength in in-vitro bioadhesion measurements (Figure 5.9), this effect might be due to availability of more polymeric chains for interlocking with glycoprotein chains of mucus (Table 5.2).

In controlled release effervescent buccal discs, HPMC buccal discs without effervescent agents demonstrated higher force of detachment and hence demonstrated better bioadhesion as compared to effervescent formulations (Figure 5.10). This can be ascribed to formation of porous and hydrated gel that erodes rapidly in case of effervescent buccal discs. This in turn would have resulted in poorer interaction between polymeric chains and glycoprotein chains of mucin. The release of carbon dioxide gas might also have hindered with the process of bioadhesion.

PEO buccal discs demonstrated higher bioadhesive behavior compared to other polymeric buccal discs (XG, HPMC, HPC and PC) designed in the present study (Table 5.2). This effect is due to higher hydrophilic nature of the polymer due to abundance of polar functional groups. The series of stages related to higher bioadhesion are primarily due faster hydration and gel formation of buccal discs prior to bioadhesion event resulting in stronger bioadhesion (Figure 5.11).

Buccal discs designed with HPC also demonstrated higher bioadhesion with higher polymers concentration (Figure 5.12). PC buccal discs have shown a marked bioadhesive strength compared to other polymers except PEO based buccal discs (Figure 5.13).

However, the degree of bioadhesion depends on type and amount of polymer, excipients used in the dosage form, degree of hydration, polymer chain length and molecular weight of polymer. The extent of bioadhesion also depends on free polymeric chains and reactive polar groups available for interlocking and crosslinking with mucins (Andrews et al., 2009; Capra et al., 2007). Instrument variable factors such as contact force and time of dosage form to biological membrane also serves as an important criterion (Wong et al., 1999). Literature report suggests that a bioadhesive force higher than 4.5×10^{-5} N/cm^2 ensures attachment of delivery system to buccal mucosa for 4 h (Choi et al., 2000; Choi & Kim, 2000). All the formulations were found to have acceptable bioadhesive strength.

5.5.1.5 Water Uptake Studies

Water uptake studies for the designed buccal discs were performed as described in method 1 of water uptake studies section.

Water uptake behavior of XG based buccal discs has shown a pronounced effect due to presence of calcium sulfate excipient. Increase in calcium sulfate concentration demonstrated significant decrease in water uptake behavior of xanthan gum buccal discs. The poor hydration behavior of xanthan gum buccal discs containing calcium sulfate may be ascribed to partial replacement of water molecules by calcium ions in the hydrated state. Conversely, the binding sites of XG are more hydrated in xanthan gum buccal discs without calcium sulfate (Bergmann et al., 2008). XG buccal discs formulation without calcium sulfate (JB/XG/00) demonstrated maximum percent water uptake of 2411.21 ± 2.01 (Figure 5.14). As the proportion of

calcium sulfate was increased in formulations relative to BS, reduction in water uptake capability of formulations was observed. The results clearly shows decline in water uptake behavior due to interaction of calcium ions with polymeric side chains. This interaction results in elastic retraction of polymeric chains leading to reduction in conformational entropy and hydrodynamic volume. Finally this resulted in conversion of polymer to a compact state from an originally coiled state (Dário et al., 2011; Dentini et al., 1984). XG buccal discs demonstrated marked increase in water uptake (Figure 5.14) due to complete immersion of disc in the phosphate buffer media. Whereas, water uptake studies performed for other buccal discs designed were studied on 2 %w/w agar plates with only one side exposed to wet surface. Complete immersion of buccal discs was performed to assess the effect of calcium sulfate on overall hydration behavior of XG hydrogel.

In in-vitro water uptake studies for the designed HPMC buccal discs (JB/HPMC15/1 to JB/HPMC15/9) were performed using method-2 described in water uptake studies in methods section 5.4.3.9. Percent water uptake of designed buccal discs increased with increasing proportion of HPMC (Figure 5.15). Increase in mannitol proportion also contributed to increased rate of water uptake (Figure 5.15). This increased hydration behavior with increasing proportion of mannitol relative to HPMC can be attributed to faster permeability of aqueous medium in to the matrix due to formation of pores or channels caused due to higher solubility of mannitol.

Water uptake study for the designed buccal discs of PEO was performed by method-2 described in water uptake studies in section 5.4.3.9. The designed PEO buccal discs have also demonstrate direct relation with polymer proportion. Initially percent water uptake was ranged near close but increased water uptake behavior was observed over the time for buccal discs with higher proportion of polymer content (Figure 5.16). Similarly buccal discs designed with HPC also demonstrated increase in water uptake behavior with increase in polymer proportion (Figure 5.17).

Water uptake behavior of PC buccal discs performed by method-2 described in water uptake studies in methods section 5.4.3.9. The designed PC buccal discs contain same concentration of polymer and varying concentration of mannitol. Increase in mannitol concentration has shown significant increase in water uptake initially for two hours and later over a period of 6 h water uptake observed was in close proximity (Figure 5.18).

Overall, the results obtained from the water uptake studies clearly indicate the influence of polymer concentration. Presence of water soluble excipient in equal proportion of polymer has shown faster water uptake in the initial time intervals and final percent water uptake over a period of time is nearly similar and was completely dependent on polymer concentration.

5.5.2 Stability studies

Results of stability studies carried out on the designed formulations at different condition of temperature and humidity like room temperature (CRT: 25 ± 2 °C/ 60 ± 5% RH) and accelerated condition (AT: 40 ± 2°C/ 75 ± 5% RH) are shown in Table 5.4.

At accelerated condition, the maximum degradation rate constant for the drug was found to be 124.36 x 10^{-4} $month^{-1}$ for effervescent formulations prepared using HPMC K15 with predicted t_{90}% values of 8.44 months. The minimum degradation rate constant of 39.15 x 10^{-4} $month^{-1}$ was obtained for formulation prepared using HPC and PEO with predicted t_{90}% value of 26.82 months. In vitro drug release profile from the aged samples was similar to zero time profiles for all the designed formulations (data not given). All the formulations were stable for entire study duration (6 months) with no apparent change in physical characteristics and in vitro release and mucoadhesive behavior.

5.6 Conclusions

Buccal discs designed using various polymers (XG, HPMC, HPC and PC) were successfully prepared using direct compression method and were evaluated for physical characterization, drug release behavior and release kinetics.

The drug release and bioadhesion from XG matrix buccal discs was strongly influenced by the presence of calcium ions. Calcium ions change the polymer conformation resulting in faster drug release due to reduced mucilage viscosity, polymer hydration and swelling. This effect is due to interaction of calcium ions of calcium sulfate with xanthan gum rather than crosslinking. Addition of small amounts of calcium salts in the xanthan gum matrix can alter the drug release behavior and can be used to fabricate the optimized controlled release dosage form.

Effect of mannitol and HPMC on drug release and mucoadhesive behavior from the designed buccal discs was studied successfully. Increase in mannitol

proportion resulted in faster drug release rate and drug release rate decreased with increasing proportion of HPMC in buccal disc. Bioadhesive strength of designed discs increased with increasing proportion of HPMC in buccal discs. From the above study it can be concluded that the drug release rate can be adjusted by adding water soluble mannitol as release modifier.

Effervescent and non-effervescent buccal disc of BS using HPMC were also successfully prepared using direct compression method. Drug release rate from effervescent buccal discs was directly proportional to amount of effervescence forming agent. The idea of designing effervescent controlled release buccal discs was to evaluate the potential as release modifier and permeation enhancer in in-vivo for improved bioavailability.

PEO polymer based buccal discs designed using two molecular weight number in varying proportions have demonstrated considerable controlled drug release and bioadhesion behavior. The drug release behavior from the designed discs can be tailored by selecting appropriate polymer concentration. Moreover, PEO buccal discs demonstrated better bioadhesion compared to other bioadhesive polymers selected in the present study.

Formulations showing optimum drug release behavior between 4-5 h with good bioadhesion values were considered and in-vivo pharmacokinetic studies were carried out for the selected formulations.

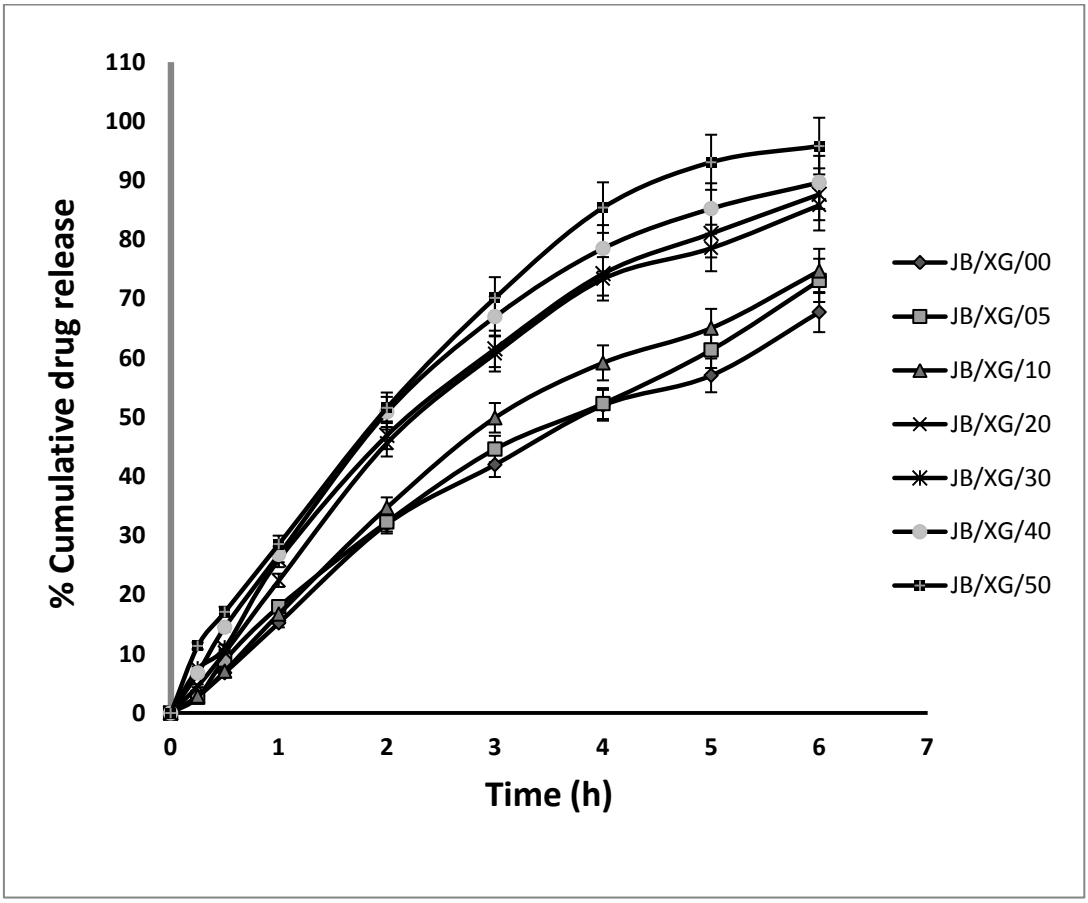


Figure 5.1: In-vitro release profile of BS buccal discs prepared using XG and varying proportions of calcium sulfate

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

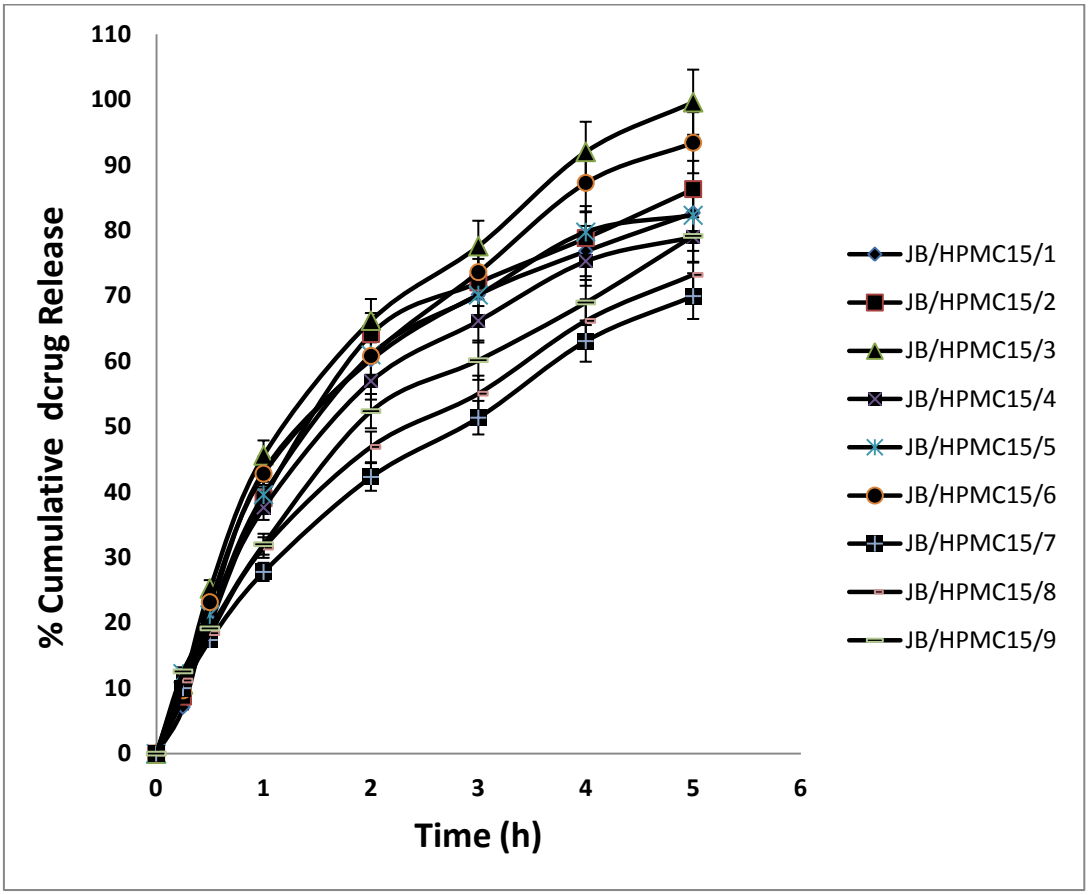


Figure 5.2: In-vitro release profile of BS buccal discs prepared using varying proportions of HPMC K15 and mannitol

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

.

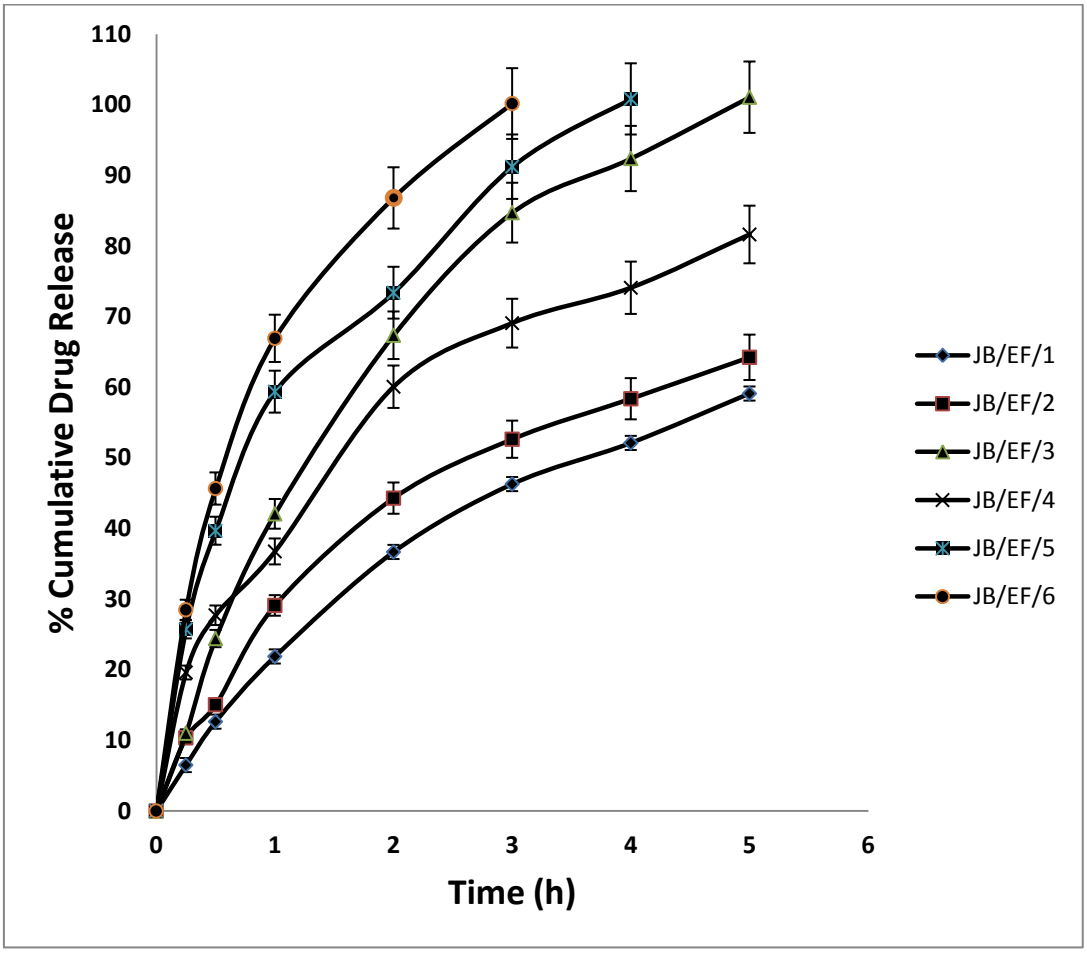


Figure 5.3: In-vitro release profile of BS effervescent buccal discs prepared using HPMC K15 polymer with varying proportions of effervescent forming agent

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

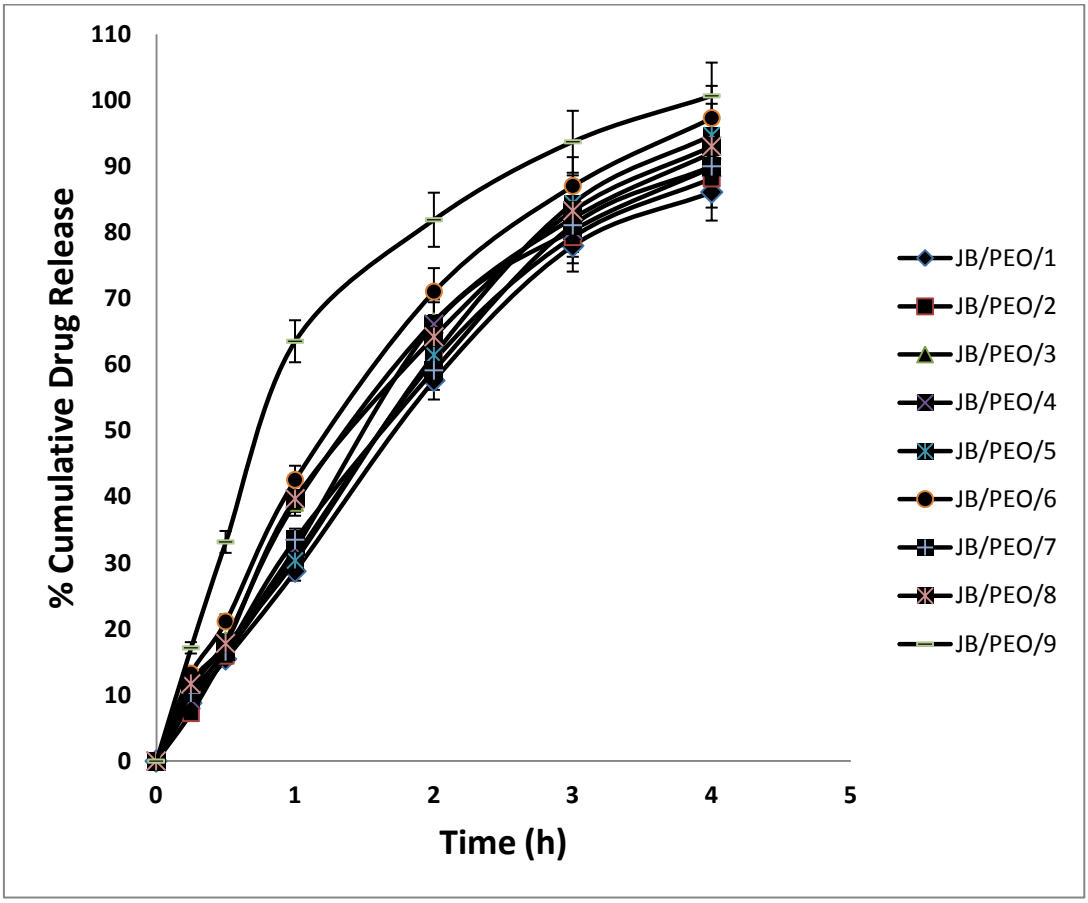


Figure 5.4: In-vitro release profile of BS buccal discs prepared using combination of PEO 1L and PEO 40L polymers in varying proportion

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

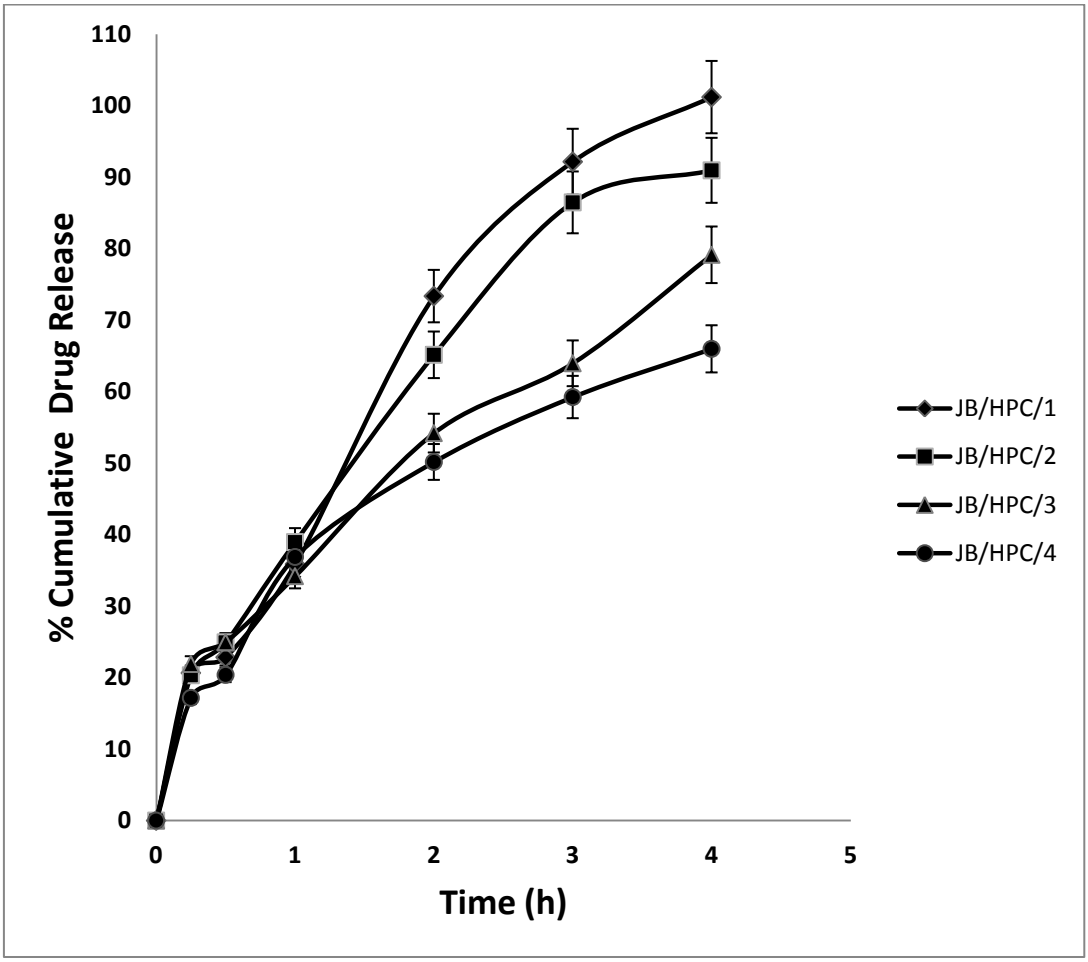


Figure 5.5: In-vitro release profile of BS buccal discs prepared using varying proportions of HPC

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

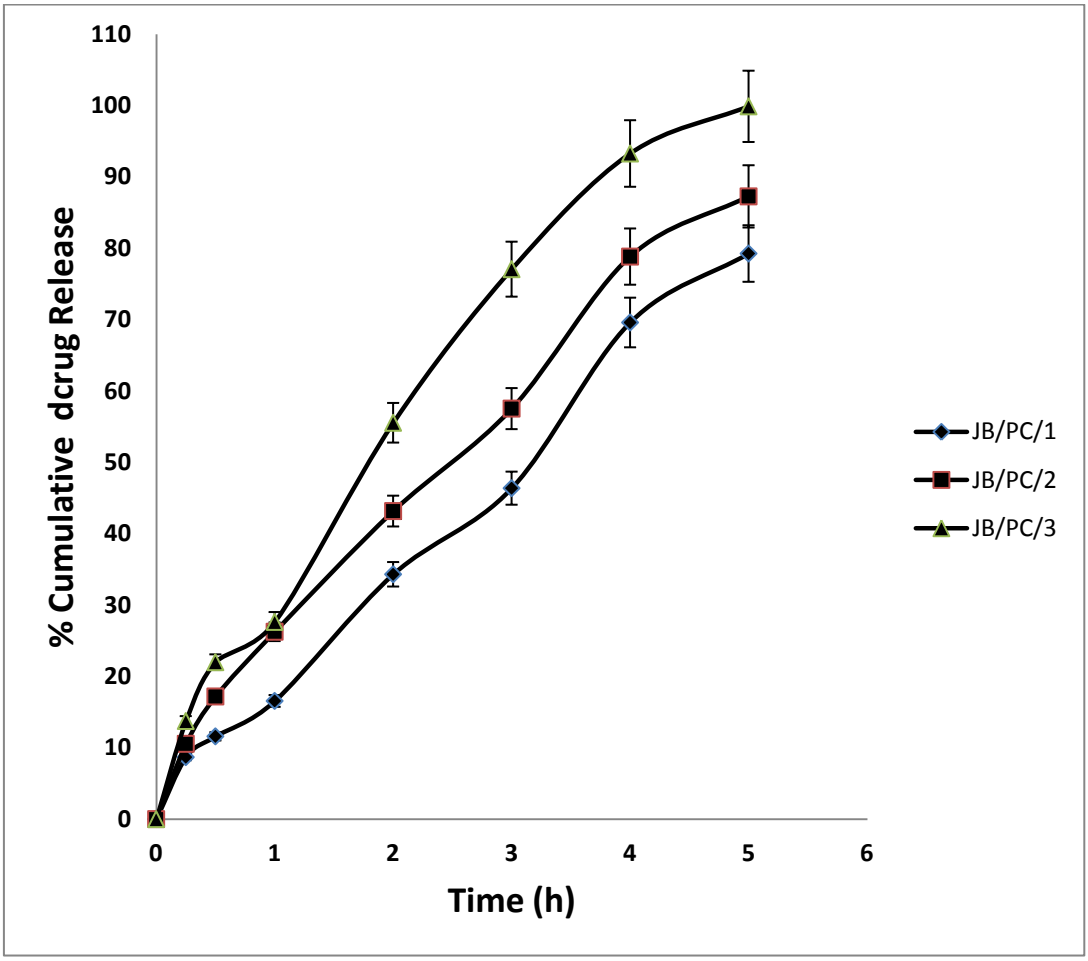


Figure 5.6: In-vitro release profile of BS buccal discs prepared using varying proportions of PC

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

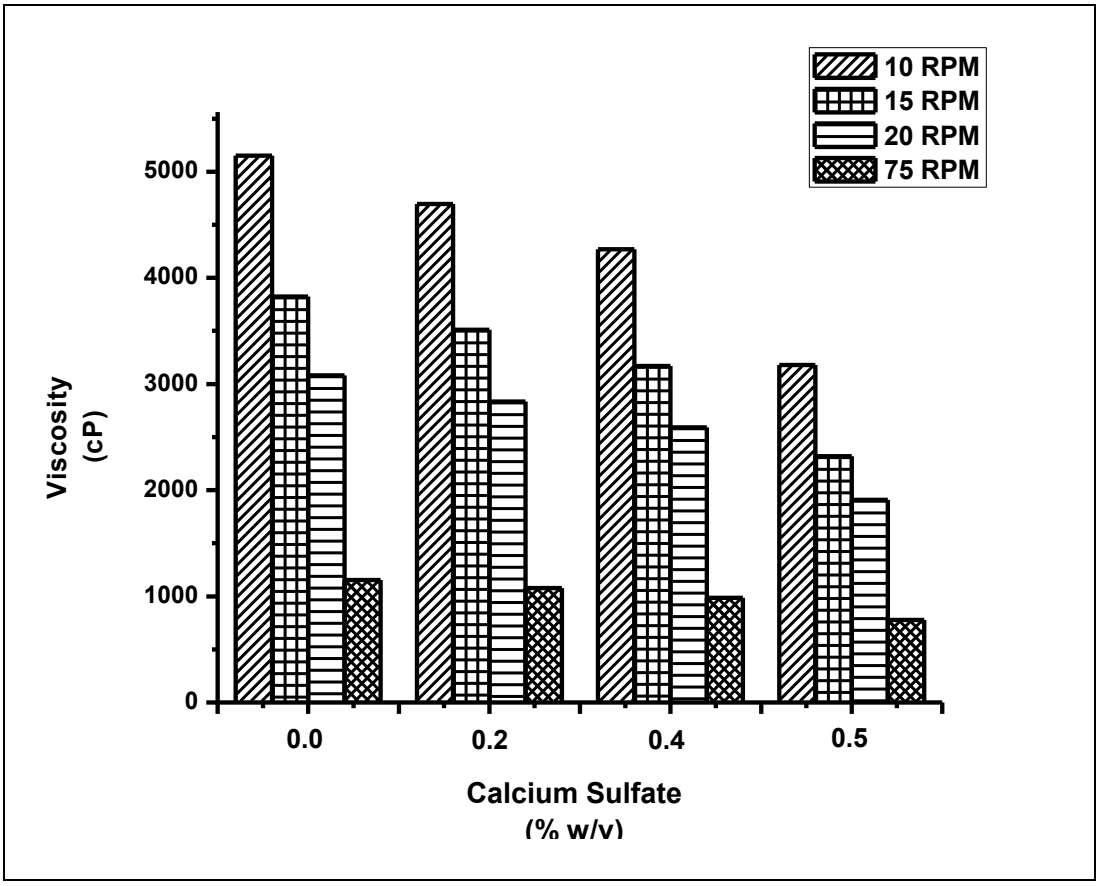


Figure 5.7: Effect of calcium sulfate on viscosity of xanthan gum mucilage carried out using Brookfield viscometer

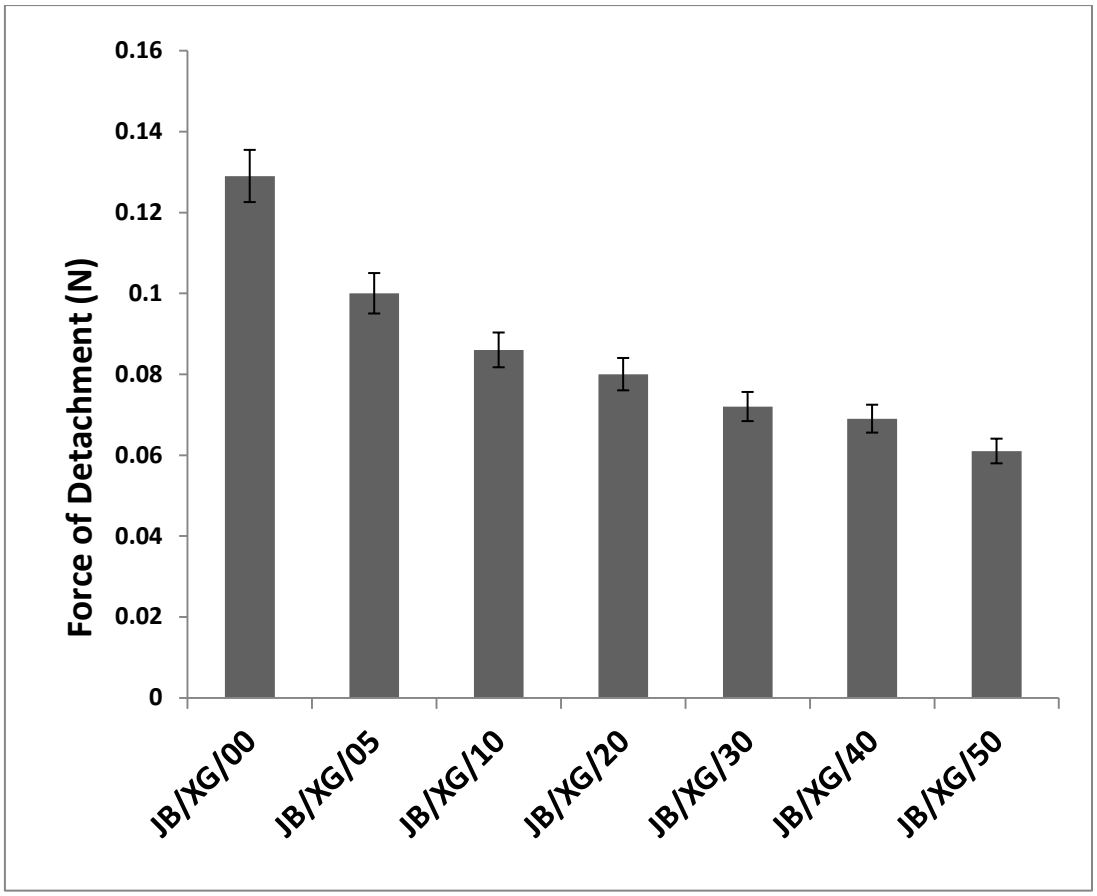


Figure 5.8: In-vitro bioadhesion of xanthan buccal discs in presence of various proportions of calcium sulfate

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

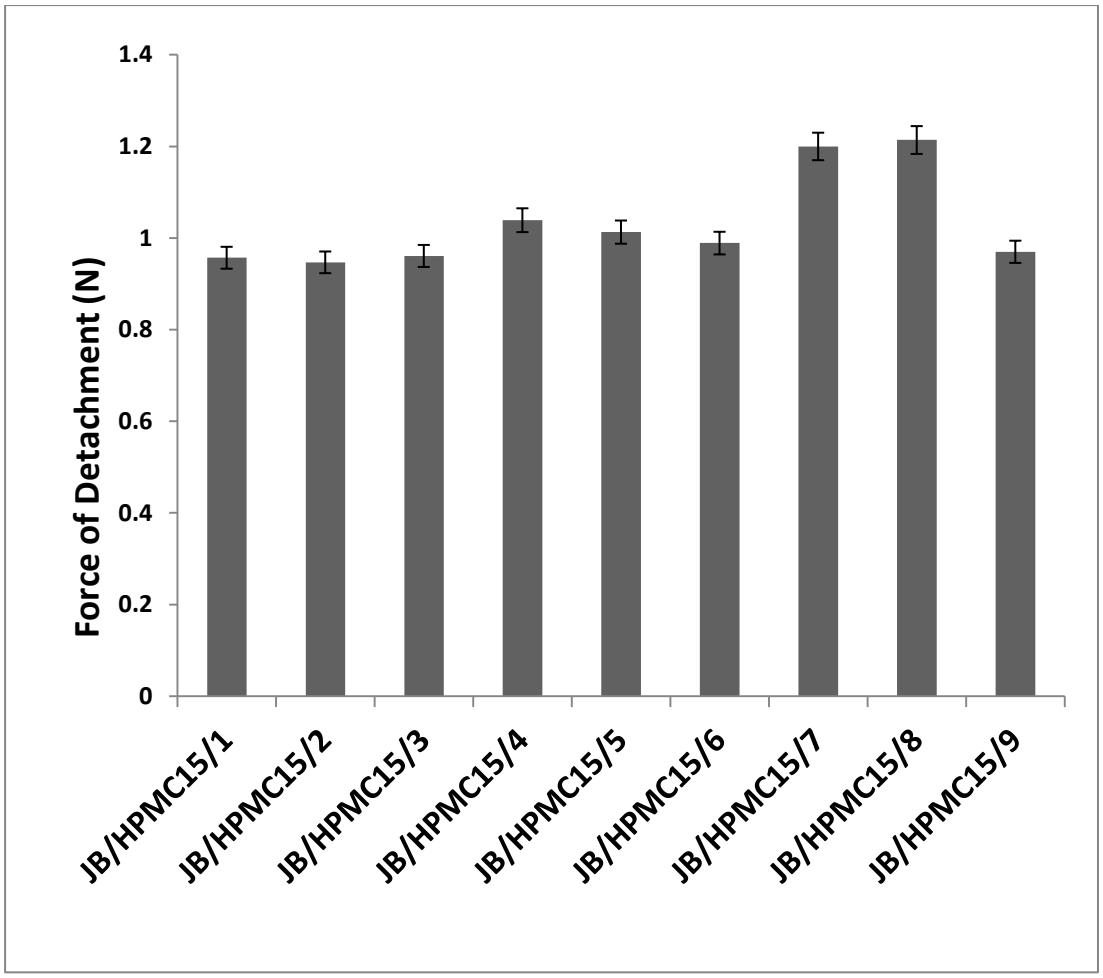


Figure 5.9: In-vitro bioadhesion of HPMC 15K buccal discs

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

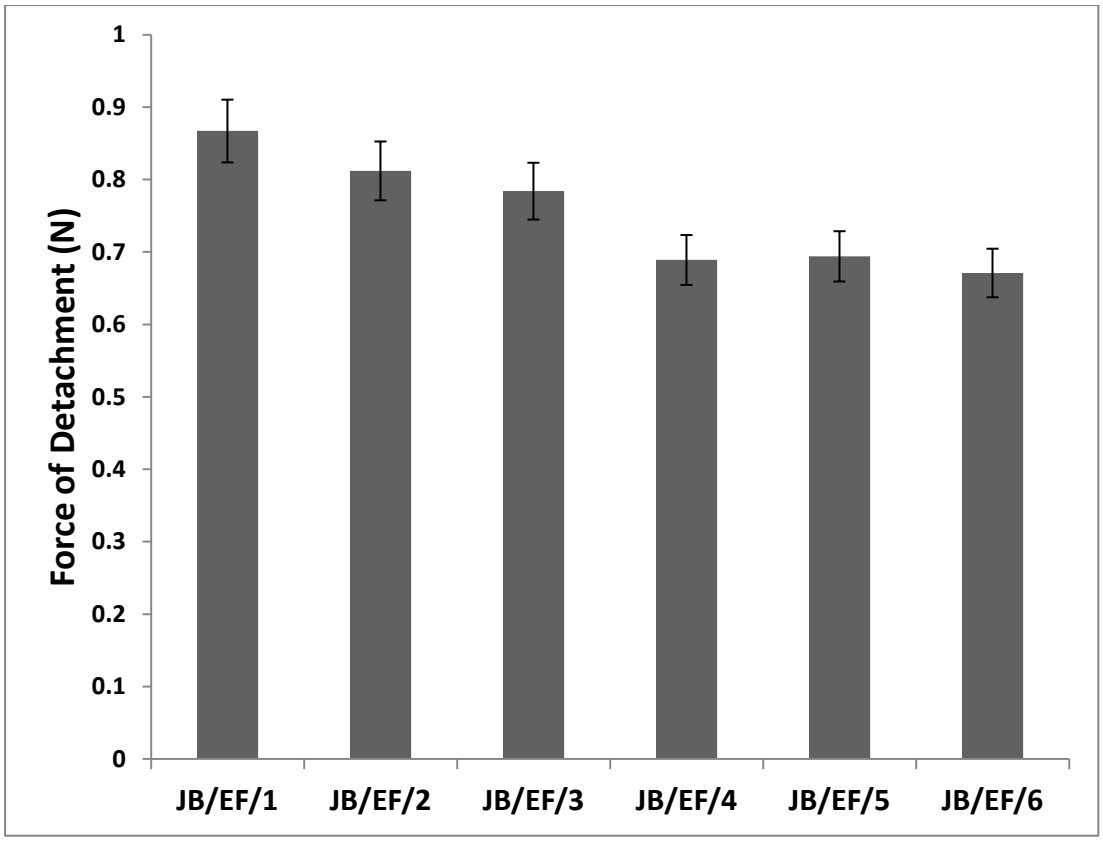


Figure 5.10: In-vitro bioadhesion of effervescent buccal discs

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

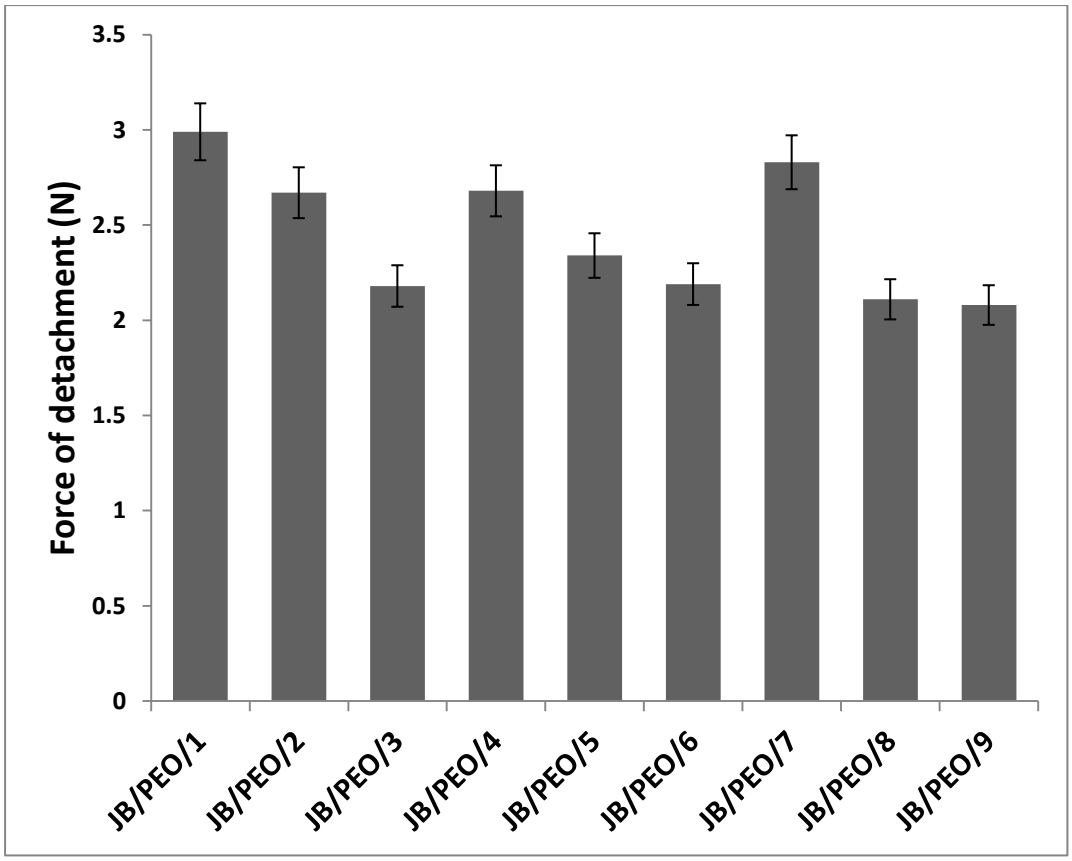


Figure 5.11: In-vitro bioadhesion of PEO buccal discs

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

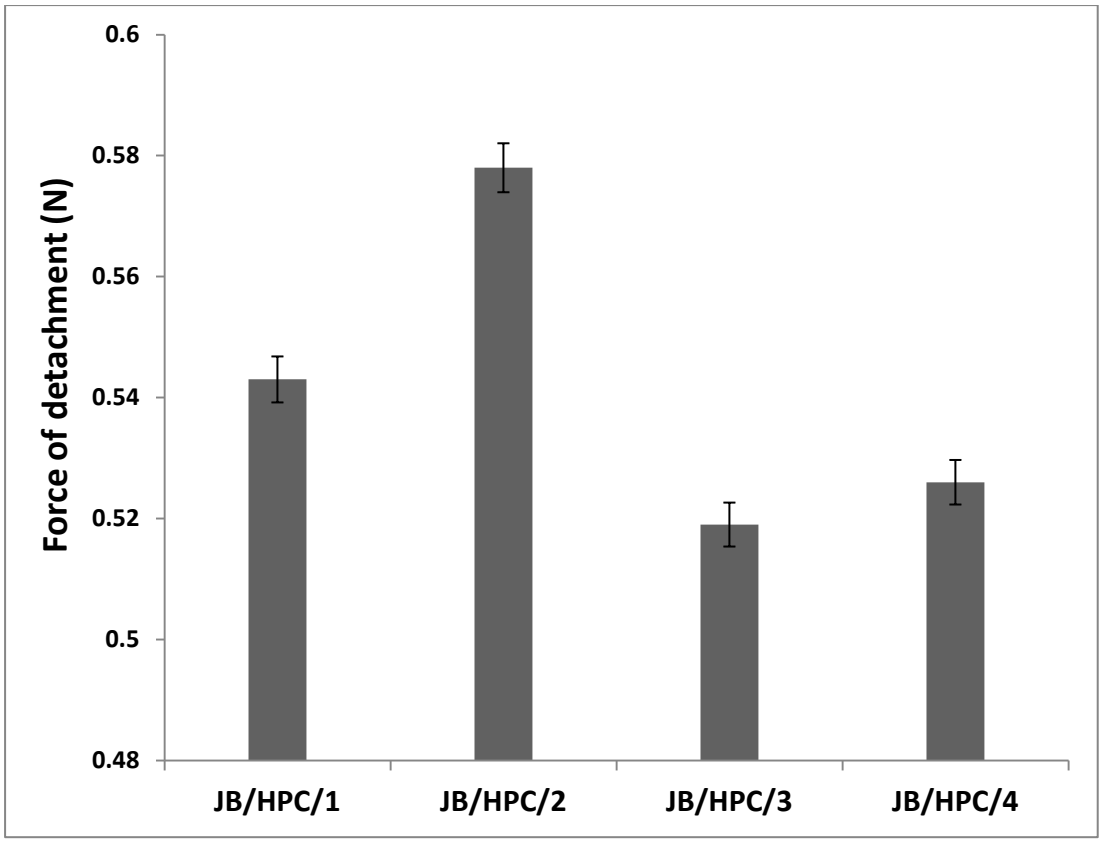


Figure 5.12: In-vitro bioadhesion of HPC buccal discs

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

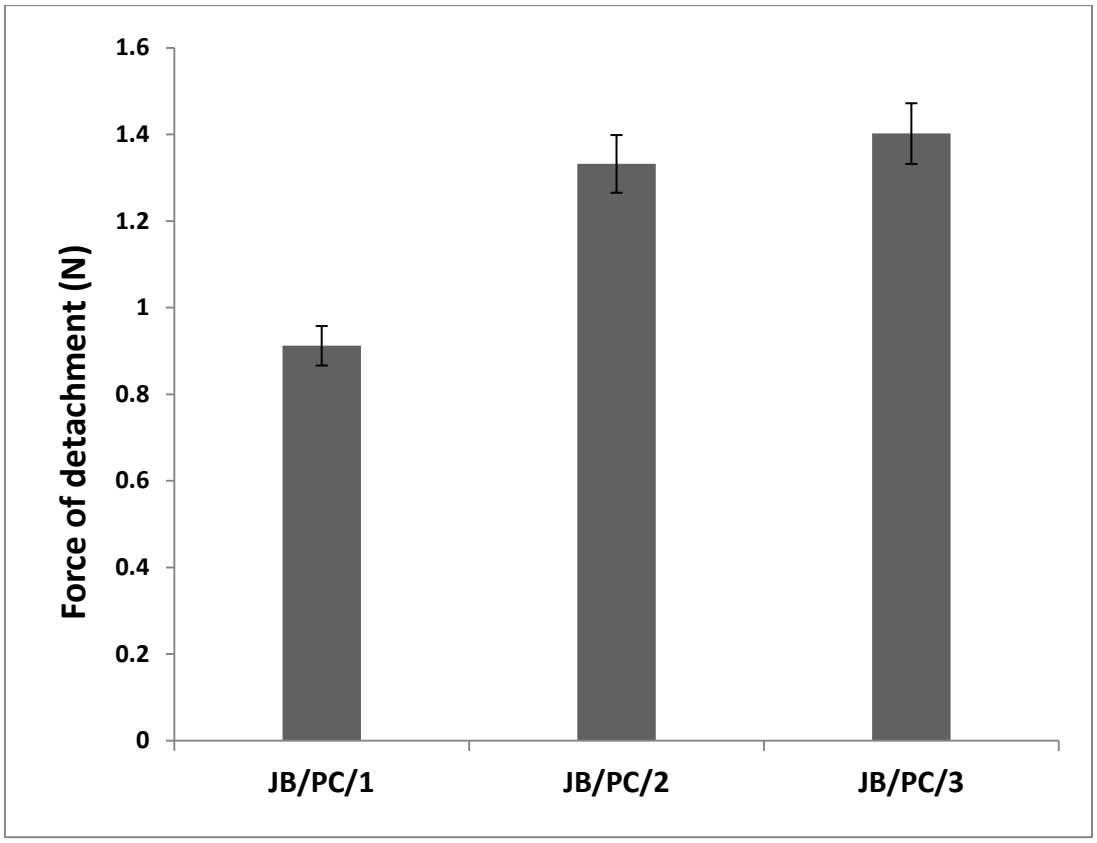


Figure 5.13: In-vitro bioadhesion of PC buccal discs

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

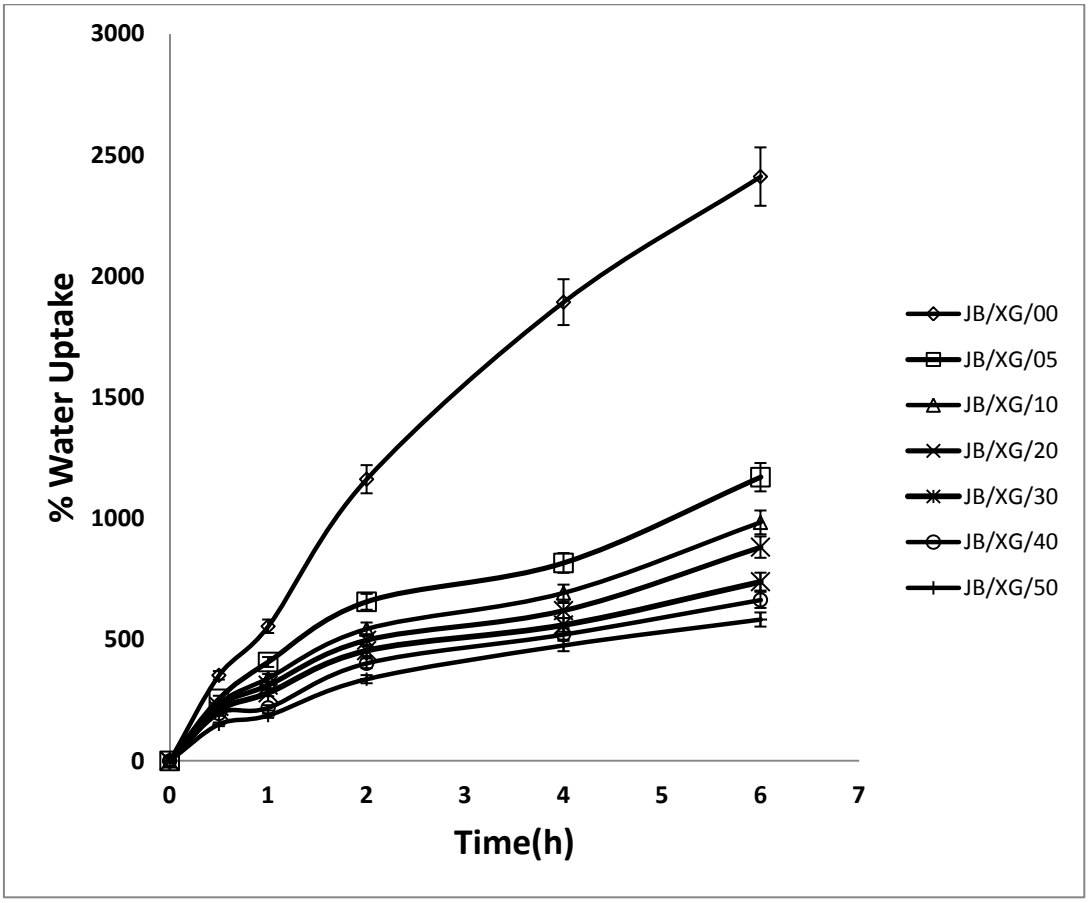


Figure 5.14: In-vitro water uptake studies of BS buccal discs prepared using XG with varying proportions of calcium sulfate

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

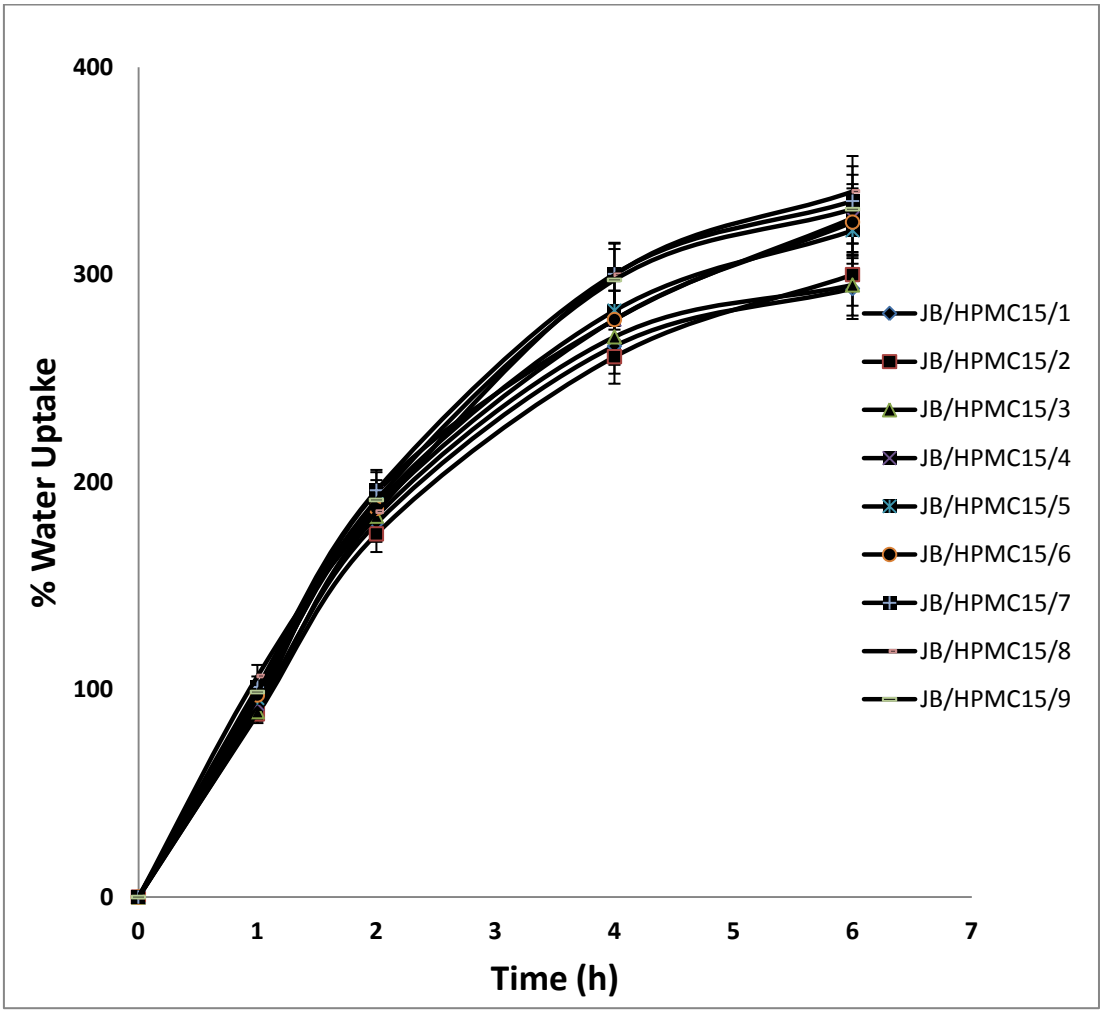


Figure 5.15: In-vitro water uptake studies of BS buccal discs prepared using varying proportions of HPMC K15 polymer

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

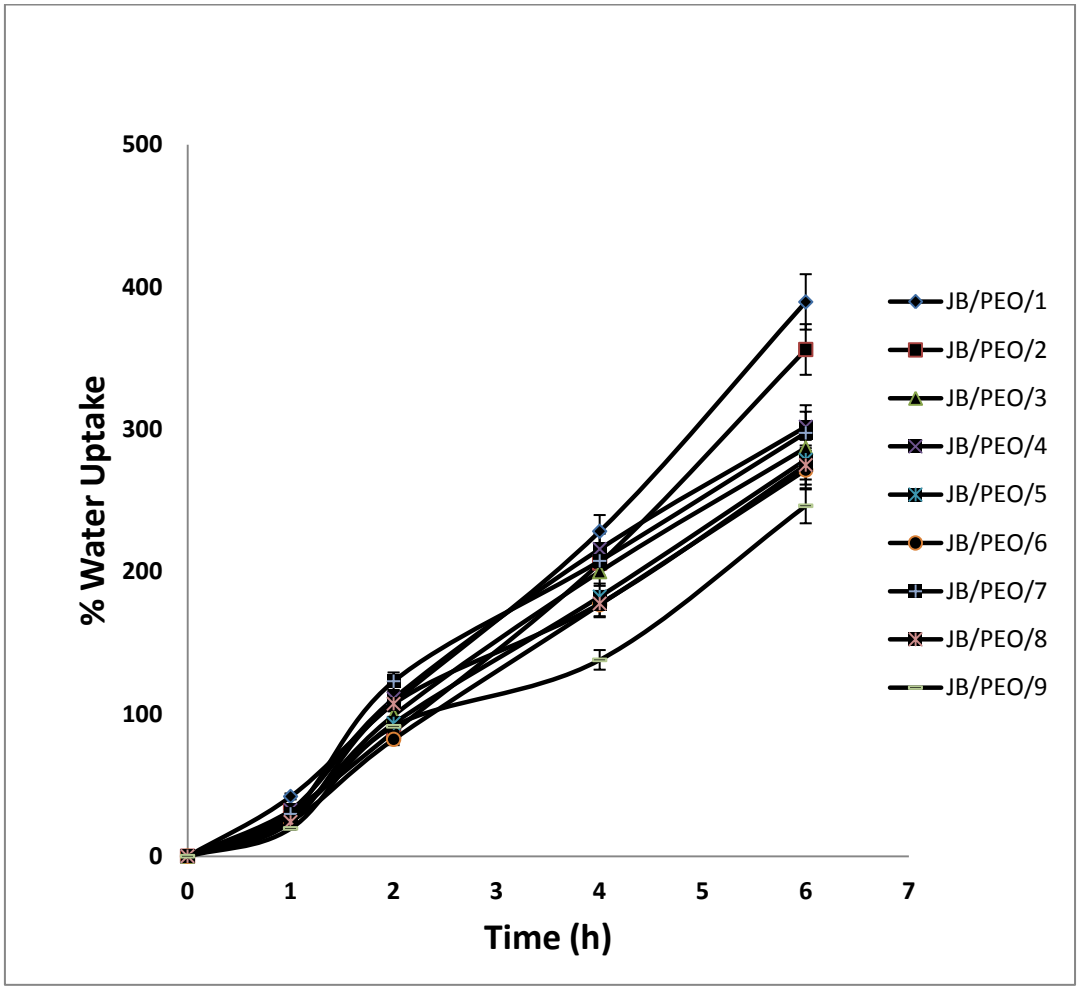


Figure 5.16: In-vitro water uptake studies of BS buccal discs prepared using combination of PEO 1L and PEO 40L polymers in varying proportion

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

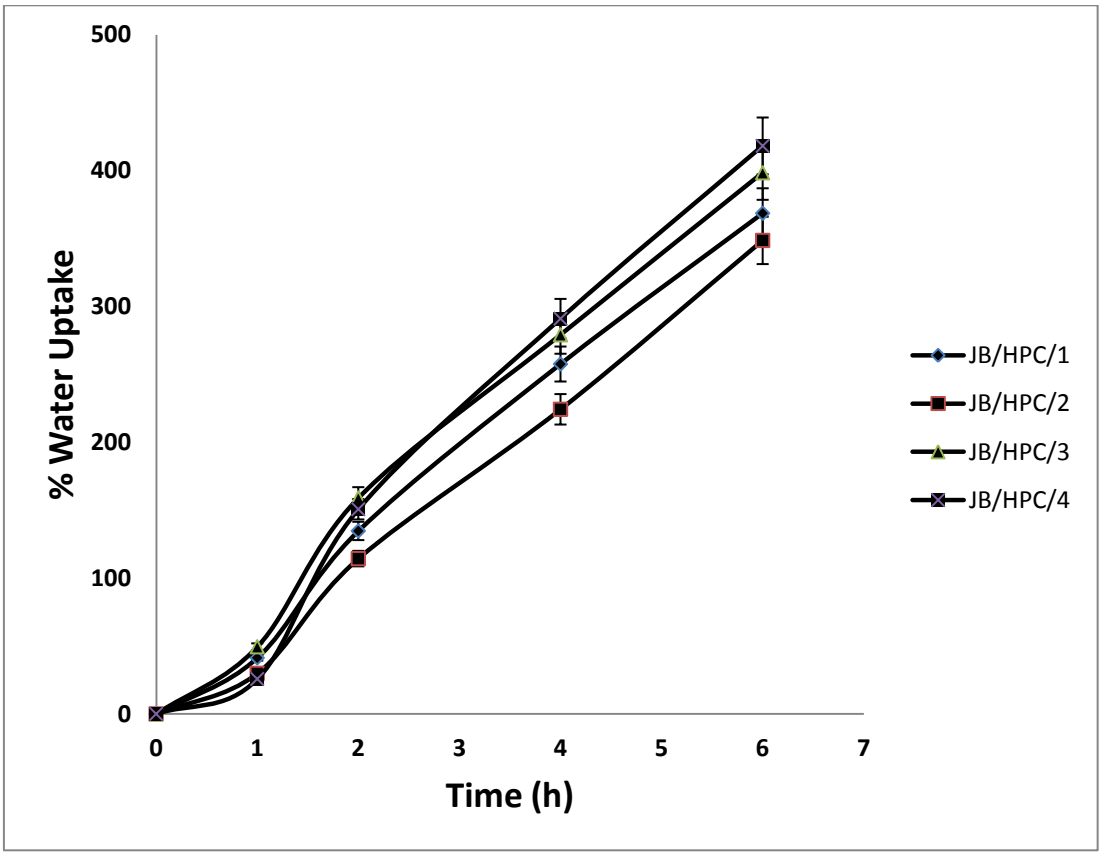


Figure 5.17: In-vitro water uptake studies of BS buccal discs prepared using varying proportions of HPC

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

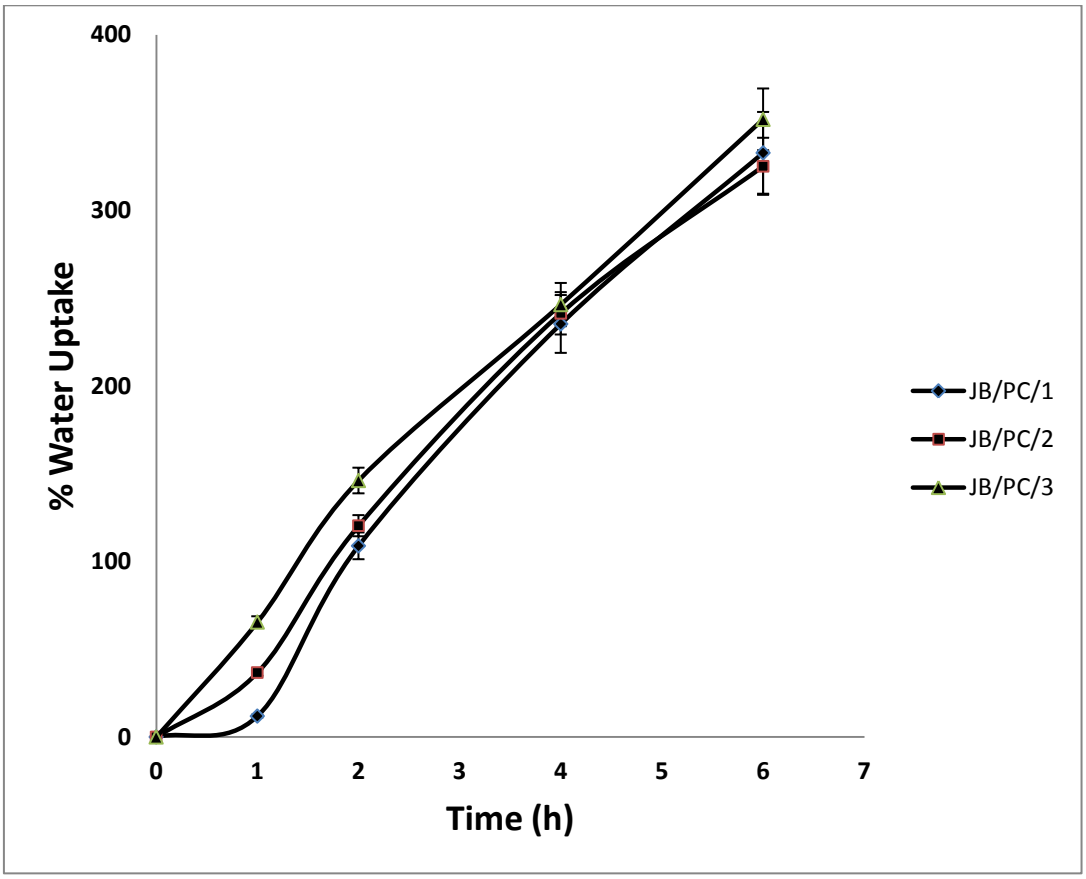


Figure 5.18: In-vitro water uptake studies of BS buccal discs prepared using varying proportions of PC

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

Table 5.1: Composition of controlled release bioadhesive buccal discs

Formulation Code[#]	Formulation Composition (% w/w)											
	Lactose	**Mannitol**	**MCC**	**SBC**	**CA**	**CS**	**XG**	**HPMC K15M**	**HPC**	**PC**	**PEO 1 Lakh**	**PEO 40 Lakhs**
JB/XG/00	-	-	54.67	-	-	0.00	40.00	-	-	-	-	-
JB/XG/05	-	-	49.67	-	-	5.00	40.00	-	-	-	-	-
JB/XG/10	-	-	44.67	-	-	10.00	40.00	-	-	-	-	-
JB/XG/20	-	-	34.67	-	-	20.00	40.00	-	-	-	-	-
JB/XG/30	-	-	24.67	-	-	30.00	40.00	-	-	-	-	-
JB/XG/40	-	-	14.67	-	-	40.00	40.00	-	-	-	-	-
JB/XG/50	-	-	4.67	-	-	50.00	40.00	-	-	-	-	-
JB/HPMC15/1	-	30.00	34.67	-	-	-	-	20.00	-	-	-	-
JB/HPMC15/2	-	40.00	24.67	-	-	-	-	20.00	-	-	-	-
JB/HPMC15/3	-	50.00	14.67	-	-	-	-	20.00	-	-	-	-
JB/HPMC15/4	-	30.00	29.67	-	-	-	-	25.00	-	-	-	-
JB/HPMC15/5	-	40.00	19.67	-	-	-	-	25.00	-	-	-	-
JB/HPMC15/6	-	50.00	9.67	-	-	-	-	25.00	-	-	-	-
JB/HPMC15/7	-	30.00	24.67	-	-	-	-	30.00	-	-	-	-
JB/HPMC15/8	-	40.00	14.67	-	-	-	-	30.00	-	-	-	-
JB/HPMC15/9	-	50.00	4.67	-	-	-	-	30.00	-	-	-	-

[#] All formulations contain 13.33%w/w BS and 2% w/w magnesium stearate

Table 5.1: Composition of controlled release bioadhesive buccal discs (contd.)

Formulation Code[#]	Formulation Composition (% w/w)											
	Lactose	**Mannitol**	**MCC**	**SBC**	**CA**	**CS**	**XG**	**HPMC K15M**	**HPC**	**PC**	**PEO 1Lakh**	**PEO 40 Lakhs**
JB/EF/1	51.33	-	6.67	0.00	0.00	-	-	26.67	-	-	-	-
JB/EF/2	23.33	-	6.67	28.00	0.00	-	-	26.67	-	-	-	-
JB/EF/3	30.00	-	6.67	16.00	5.33	-	-	26.67	-	-	-	-
JB/EF/4	19.33	-	6.67	24.00	8.00	-	-	26.67	-	-	-	-
JB/EF/5	14.00	-	6.67	28.00	9.33	-	-	26.67	-	-	-	-
JB/EF/6	42.00	-	6.67	0.00	9.33	-	-	26.67	-	-	-	-
JB/PEO/1	13.33	-	16.00	-	-	-	-	-	-	-	35.33	20.00
JB/PEO/2	13.33	-	21.00	-	-	-	-	-	-	-	35.33	15.00
JB/PEO/3	13.33	-	26.00	-	-	-	-	-	-	-	35.33	10.00
JB/PEO/4	13.33	-	26.33	-	-	-	-	-	-	-	25.00	20.00
JB/PEO/5	13.33	-	31.33	-	-	-	-	-	-	-	25.00	15.00
JB/PEO/6	13.33	-	36.33	-	-	-	-	-	-	-	25.00	10.00
JB/PEO/7	13.33	-	36.33	-	-	-	-	-	-	-	15.00	20.00
JB/PEO/8	13.33	-	41.33	-	-	-	-	-	-	-	15.00	15.00
JB/PEO/9	13.33	-	46.33	-	-	-	-	-	-	-	15.00	10.00

[#] All formulations contain 13.33%w/w BS and 2% w/w magnesium stearate

Table 5.1: Composition of controlled release bioadhesive buccal discs (contd.)

Formulation Code[#]	Formulation Composition (% w/w)											
	Lactose	**Mannitol**	**MCC**	**SBC**	**CA**	**CS**	**XG**	**HPMC K15M**	**HPC**	**PC**	**PEO 1 Lakh**	**PEO 40 Lakhs**
JB/HPC/1	-	14.67	40.00	-	-	-	-	-	30.00	-	-	-
JB/HPC/2	-	9.67	45.00	-	-	-	-	-	30.00	-	-	-
JB/HPC/3	-	14.67	35.00	-	-	-	-	-	35.00	-	-	-
JB/HPC/4	-	9.67	40.00	-	-	-	-	-	35.00	-	-	-
JB/PC/1	-	20.00	24.67	-	-	-	-	-	-	40.00	-	-
JB/PC/2	-	25.00	19.67	-	-	-	-	-	-	40.00	-	-
JB/PC/3	-	30.00	14.67	-	-	-	-	-	-	40.00	-	-

[#] All formulations contain 13.33%w/w BS and 2% w/w magnesium stearate

SBC: Sodium bicarbonate

CA: Citric acid

CS: Calcium Sulfate

Table 5.2: Weight variation, friability, thickness, assay and bioadhesion data for designed buccal discs

Formulation Code	Mean Weight ± SD (mg)	Friability (% w/w)	Mean Thickness ± SD (mm)	Mean Assay ± SD (%)	Bioadhesion (N)
JB/XG/00	75.16 ± 0.15	0.16	2.51 ± 0.04	100.43 ± 0.67	0.13 ± 0.01
JB/XG/05	75.06 ± 0.11	0.29	2.44 ± 0.03	100.56 ± 0.59	0.10 ± 0.01
JB/XG/10	74.89 ± 0.16	0.33	2.36 ± 0.01	98.65 ± 0.32	0.09 ± 0.03
JB/XG/20	75.21 ± 0.56	0.63	2.30 ± 0.02	101.47 ± 0.81	0.08 ± 0.03
JB/XG/30	75.23 ± 0.19	0.47	2.25 ± 0.01	99.93 ± 0.31	0.07 ± 0.05
JB/XG/40	75.11 ± 0.21	0.59	2.15 ± 0.02	99.40 ± 0.86	0.07 ± 0.01
JB/XG/50	75.09 ± 0.20	0.38	2.04 ± 0.02	98.72 ± 0.77	0.06 ± 0.03
JB/HPMC15/1	74.73 ± 0.18	0.27	2.51 ± 0.01	100.40 ± 0.41	0.96 ± 0.03
JB/HPMC15/2	75.14 ± 0.10	0.53	2.49 ± 0.01	98.06± 0.63	0.95 ± 0.01
JB/HPMC15/3	75.19 ± 0.23	0.13	2.50 ± 0.02	100.14± 0.32	0.96 ± 0.02
JB/HPMC15/4	74.91 ± 0.47	0.60	2.40 ± 0.02	98.80± 0.73	1.04 ± 0.01
JB/HPMC15/5	75.11 ± 0.61	0.33	2.47 ± 0.02	100.06± 0.26	1.01 ± 0.01
JB/HPMC15/6	75.31 ± 0.11	0.73	2.51 ± 0.01	99.67± 0.33	0.99 ± 0.02
JB/HPMC15/7	75.16 ± 0.33	0.27	2.51 ± 0.01	101.23± 0.63	1.20 ± 0.03
JB/HPMC15/8	75.07 ± 0.41	0.27	2.52 ± 0.01	98.80± 0.48	1.21 ± 0.02
JB/HPMC15/9	75.41 ± 0.15	0.40	2.49 ± 0.02	100.40± 0.39	0. 97 ± 0.01

Table 5.2: Weight variation, friability, thickness, assay and bioadhesion data for designed buccal discs (contd.)

Formulation Code	Mean Weight ± SD (mg)	Friability (% w/w)	Mean Thickness ± SD (mm)	Mean Assay ± SD (%)	Bioadhesion (N)
JB/EF/1	75.44 ± 0.13	0.32	2.56 ± 0.01	98.76 ± 0.57	0.87 ± 0.17
JB/EF/2	75.16 ± 0.19	0.35	2.41 ± 0.02	100.56 ± 0.48	0.81 ± 0.36
JB/EF/3	75.27 ± 0.22	0.34	2.36 ± 0.04	99.63 ± 0.74	0.78 ± 0.13
JB/EF/4	75.09 ± 0.13	0.47	2.29 ± 0.04	98.89 ± 0.83	0.69 ± 0.11
JB/EF/5	75.07 ± 0.07	0.51	2.12 ± 0.01	98.16 ± 0.41	0.69 ± 0.08
JB/EF/6	75.14 ± 0.18	0.30	2.24 ± 0.02	99.01 ± 0.29	0.67 ± 0.18
JB/PEO/1	75.33 ± 0.26	0.41	2.36 ± 0.04	99.68 ± 0.68	2.99 ± 0.11
JB/PEO/2	75.18 ± 0.34	0.29	2.52 ± 0.03	100.11 ± 0.24	2.67 ± 0.09
JB/PEO/3	75.29 ± 0.44	0.26	2.44 ± 0.01	99.14 ± 0.37	2.18 ± 0.19
JB/PEO/4	75.11 ± 0.42	0.34	2.45 ± 0.02	99.21 ± 0.63	2.68 ± 0.27
JB/PEO/5	75.42 ± 0.19	0.22	2.39 ± 0.01	98.90 ± 0.47	2.34 ± 0.14
JB/PEO/6	75.17 ± 0.27	0.49	2.50 ± 0.02	99.47 ± 0.18	2.19 ± 0.24
JB/PEO/7	74.59 ± 0.52	0.38	2.46 ± 0.02	100.06 ± 0.15	2.83 ± 0.17
JB/PEO/8	75.22 ± 0.21	0.42	2.51 ± 0.02	98.16 ± 0.39	2.11 ± 0.13
JB/PEO/9	74.84 ± 0.31	0.29	2.46 ± 0.02	101.08 ± 0.67	2.08 ± 0.18

Table 5.2: Weight variation, friability, thickness, assay and bioadhesion data for designed buccal discs (contd.)

Formulation Code	**Mean Weight ± SD (mg)**	**Friability (% w/w)**	**Mean Thickness ± SD (mm)**	**Mean Assay ± SD (%)**	**Bioadhesion (N)**
JB/HPC/1	75.12 ± 0.19	0.42	2.55± 0.02	99.84 ± 0.38	0.54 ± 0.03
JB/HPC/2	75.19 ± 0.24	0.37	2.54 ± 0.01	100.26± 0.26	0.52 ± 0.02
JB/HPC/3	75.31 ± 0.30	0.31	2.47± 0.04	99.18 ± 0.34	0.57 ± 0.04
JB/HPC/4	75.25 ± 0.19	0.49	2.51 ± 0.01	99.41 ± 0.44	0.56 ± 0.01
JB/PC/1	75.14 ± 0.26	0.61	2.49 ± 0.02	99.84 ± 0.38	0.91 ± 0.03
JB/PC/2	75.06 ± 0.17	0.37	2.53± 0.01	100.26± 0.26	1.33 ± 0.06
JB/PC/3	75.15 ± 0.28	0.53	2.51 ± 0.04	99.18 ± 0.34	1.40 ± 0.07

Table 5.3: Data of drug release kinetics study of formulations

	Zero order		First order			Higuchi		Korsmeyer-Peppas		
Formulation Code	**R^2**	**k_0 (mg% h^{-1})**	**R^2**	**k_1 (h^{-1})**	**$t_{50\%}$ (h)**	**R^2**	**k_H ($h^{-0.5}$)**	**R^2**	**k_{KP} (h^{-n})**	**n-value**
JB/XG/00	0.971	12.144	0.996	0.180	3.842	0.929	24.684	0.992	16.810	0.785
JB/XG/05	0.976	12.881	0.996	0.197	3.525	0.934	26.181	0.996	17.781	0.787
JB/XG/10	0.966	13.691	0.995	0.218	3.180	0.926	27.835	0.988	19.063	0.781
JB/XG/20	0.938	16.466	0.995	0.307	2.259	0.942	33.766	0.983	25.500	0.710
JB/XG/30	0.931	16.864	0.997	0.324	2.142	0.956	34.706	0.988	27.246	0.682
JB/XG/40	0.910	17.707	0.997	0.363	1.911	0.959	36.586	0.983	29.892	0.652
JB/XG/50	0.913	19.004	0.989	0.415	1.671	0.965	39.279	0.987	32.310	0.647
JB/HPMC15/1	0.685	20.168	0.978	0.439	1.580	0.954	38.928	0.956	37.856	0.463
JB/HPMC15/2	0.715	20.726	0.988	0.452	1.534	0.958	39.771	0.958	37.260	0.481
JB/HPMC15/3	0.724	22.233	0.997	0.497	1.393	0.971	42.360	0.972	38.167	0.477
JB/HPMC15/4	0.759	19.365	0.992	0.394	1.761	0.981	37.301	0.982	36.093	0.493
JB/HPMC15/5	0.746	20.291	0.994	0.436	1.590	0.978	39.081	0.978	37.727	0.486
JB/HPMC15/6	0.733	22.685	0.995	0.553	1.254	0.975	43.610	0.975	41.686	0.481
JB/HPMC15/7	0.838	16.071	0.984	0.264	2.622	0.991	30.588	0.993	27.386	0.544
JB/HPMC15/8	0.778	17.159	0.974	0.302	2.294	0.987	32.860	0.987	30.733	0.502
JB/HPMC15/9	0.761	17.859	0.968	0.318	2.179	0.986	34.021	0.986	30.791	0.491

Table 5.3: Data of drug release kinetics study of formulations prepared (contd.)

	Zero order		**First order**			**Higuchi**		**Korsmeyer-Peppas**		
Formulation Code	**R^2**	**k_0 (mg% h^{-1})**	**R^2**	**k_1 (h^{-1})**	**$t_{50\%}$ (h)**	**R^2**	**k_H ($h^{-0.5}$)**	**R^2**	**k_{KP} (h^{-n})**	**n-value**
JB/EF/1	0.909	13.488	0.981	0.199	3.482	0.974	25.557	0.993	21.964	0.633
JB/EF/2	0.833	15.194	0.957	0.246	2.822	0.984	29.152	0.988	27.578	0.549
JB/EF/3	0.866	23.879	0.994	0.588	1.179	0.976	45.564	0.985	41.239	0.588
JB/EF/4	0.724	19.659	0.956	0.416	1.668	0.989	38.188	0.991	39.712	0.465
JB/EF/5	0.702	30.001	0.980	0.874	0.793	0.991	52.442	0.995	54.714	0.452
JB/EF/6	0.691	39.896	0.995	1.167	0.594	0.989	60.700	0.993	62.298	0.455
JB/PEO/1	0.984	24.151	0.981	0.435	2.070	0.901	40.252	0.984	31.164	0.774
JB/PEO/2	0.982	24.823	0.982	0.460	2.014	0.903	41.416	0.982	32.355	0.764
JB/PEO/3	0.981	26.352	0.990	0.535	1.897	0.934	44.473	0.981	37.609	0.682
JB/PEO/4	0.975	25.583	0.981	0.494	1.954	0.910	42.894	0.975	34.705	0.728
JB/PEO/5	0.988	26.295	0.965	0.502	1.901	0.905	43.831	0.988	33.895	0.774
JB/PEO/6	0.983	28.003	0.986	0.611	1.786	0.946	47.437	0.983	41.128	0.656
JB/PEO/7	0.988	25.267	0.983	0.476	1.979	0.917	42.277	0.988	33.689	0.744
JB/PEO/8	0.985	26.452	0.987	0.534	1.890	0.934	44.576	0.985	37.359	0.692
JB/PEO/9	0.948	30.746	0.991	0.901	1.626	0.948	53.491	0.948	53.560	0.498

Table 5.3: Data of drug release kinetics study of formulations (contd.)

	Zero order		**First order**			**Higuchi**		**Korsmeyer-Peppas**		
Formulation Code	**R^2**	**k_0 (mg% h^{-1})**	**R^2**	**k_1 (h^{-1})**	**$T_{50\%}$ (h)**	**R^2**	**k_H ($h^{-0.5}$)**	**R^2**	**k_{KP} (h^{-n})**	**n-value**
JB/HPC/1	0.922	28.994	0.963	0.631	1.098	0.948	48.927	0.980	41.419	0.681
JB/HPC/2	0.876	26.721	0.986	0.571	1.214	0.978	45.632	0.989	41.831	0.596
JB/HPC/3	0.822	22.068	0.947	0.399	1.737	0.991	37.997	0.992	37.054	0.528
JB/HPC/4	0.764	19.573	0.927	0.332	2.085	0.991	33.987	0.992	34.194	0.493
JB/PC/1	0.9775	16.631	0.9880	0.262	2.641	0.9217	30.831	0.990	21.244	0.817
JB/PC/2	0.9668	18.947	0.9905	0.332	2.089	0.9463	35.374	0.995	26.493	0.749
JB/PC/3	0.9434	22.722	0.9879	0.475	1.458	0.9543	42.664	0.990	33.875	0.700

Table 5.4: First order degradation kinetic parameters of BS in designed formulations

Formulation Code	**CRT: 25 ± 2°C/60 ± 5 % RH**			**AT (40 ± 2°C/75 ± 5 % RH)**		
	K_{deg}x 10^{-4} ($month^{-1}$)	**$t_{90\%}$ (month)**	**R^2**	**K_{deg} x 10^{-4} ($month^{-1}$)**	**$t_{90\%}$ (month)**	**R^2**
JB/XG/00	16.12	65.13	0.9601	52.97	19.82	0.9462
JB/XG/50	20.73	50.66	0.9789	55.27	19.00	0.9514
JB/HPMC15/2	13.82	75.99	0.8677	43.76	24.00	0.9612
JB/HPMC15/3	25.33	41.45	0.9411	46.06	22.80	0.9715
JB/EF/1	36.85	28.50	0.9567	48.36	21.71	0.9873
JB/EF/6	43.76	24.00	0.9816	124.36	8.44	0.9891
JB/PEO/1	27.64	37.99	0.9632	39.15	26.82	0.9912
JB/PEO/9	23.03	45.59	0.9520	34.55	30.40	0.9907
JB/HPC/1	18.42	56.99	0.8904	41.45	25.33	0.9867
JB/HPC/4	20.73	50.66	0.9498	39.15	26.82	0.9839
JB/PC/3	25.33	41.45	0.9811	46.06	22.80	0.9711

References

Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., & Luppi, B. (2012). Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. Carbohydr. Polym., 87(1), 581-588.

Abu-Huwaij, R., Obaidat, R., Sweidan, K., & Al-Hiari, Y. (2011). Formulation and In Vitro Evaluation of Xanthan Gum or Carbopol 934-Based Mucoadhesive Patches, Loaded with Nicotine. AAPS PharmSciTech., 12(1), 21-27.

AlKhatib, H. S., Aiedeh, K. M., Bustanji, Y., Hamed, S., Mohammad, M. K., AlKhalidi, B., & Najjar, S. (2008). Modulation of buspirone HCl release from hypromellose matrices using chitosan succinate: Implications for pH-independent release. Eur. J. Pharm. Biopharm., 70(3), 804-812.

Almeida, A., Brabant, L., Siepmann, F., De Beer, T., Bouquet, W., Van Hoorebeke, L., Siepmann, J., Remon, J. P., & Vervaet, C. (2012). Sustained release from hot-melt extruded matrices based on ethylene vinyl acetate and polyethylene oxide. Eur. J. Pharm. Biopharm., 82(3), 526-533.

Andrews, G. P., Laverty, T. P., & Jones, D. S. (2009). Mucoadhesive polymeric platforms for controlled drug delivery. Eur. J. Pharm. Biopharm., 71(3), 505-518.

Asare-Addo, K., Conway, B. R., Larhrib, H., Levina, M., Rajabi-Siahboomi, A. R., Tetteh, J., Boateng, J., & Nokhodchi, A. (2013). The effect of pH and ionic strength of dissolution media on in-vitro release of two model drugs of different solubilities from HPMC matrices. Colloids Surf., B., 111(0), 384-391.

Ayensu, I., Mitchell, J. C., & Boateng, J. S. (2012a). Effect of membrane dialysis on characteristics of lyophilised chitosan wafers for potential buccal delivery of proteins. Int. J. Biol. Macromol., 50(4), 905-909.

Ayensu, I., Mitchell, J. C., & Boateng, J. S. (2012b). In vitro characterisation of chitosan based xerogels for potential buccal delivery of proteins. Carbohydr. Polym., 89(3), 935-941.

Baumgartner, S., Pavli, M., & Kristl, J. (2008). Effect of calcium ions on the gelling and drug release characteristics of xanthan matrix tablets. Eur. J. Pharm. Biopharm., 69(2), 698-707.

Bergmann, D., Furth, G., & Mayer, C. (2008). Binding of bivalent cations by xanthan in aqueous solution. Int. J. Biol. Macromol., 43(3), 245-251.

Boyapally, H., Nukala, R. K., Bhujbal, P., & Douroumis, D. (2010). Controlled release from directly compressible theophylline buccal tablets. Colloids Surf., B., 77(2), 227-233.

Bueno, V. B., Bentini, R., Catalani, L. H., & Petri, D. F. S. (2013). Synthesis and swelling behavior of xanthan-based hydrogels. Carbohydr. Polym., 92(2), 1091-1099.

Cappello, B., De Rosa, G., Giannini, L., La Rotonda, M. I., Mensitieri, G., Miro, A., Quaglia, F., & Russo, R. (2006). Cyclodextrin-containing poly(ethyleneoxide) tablets for the delivery of poorly soluble drugs: Potential as buccal delivery system. Int. J. Pharm., 319(1–2), 63-70.

Capra, R. H., Baruzzi, A. M., Quinzani, L. M., & Strumia, M. C. (2007). Rheological, dielectric and diffusion analysis of mucin/carbopol matrices used in amperometric biosensors. Sens. Actuators, B., 124(2), 466-476.

Charde, S., Mudgal, M., Kumar, L., & Saha, R. (2008). Development and Evaluation of Buccoadhesive Controlled Release Tablets of Lercanidipine. AAPS PharmSciTech., 9(1), 182-190.

Chen, Y. Y., Hughes, L. P., Gladden, L. F., & Mantle, M. D. (2010). Quantitative ultra-fast MRI of HPMC swelling and dissolution. J. Pharm. Sci., 99(8), 3462-3472.

Choi, H.-G., Jung, J.-H., Yong, C. S., Rhee, C.-D., Lee, M.-K., Han, J.-H., Park, K.-M., & Kim, C.-K. (2000). Formulation and in vivo evaluation of omeprazole buccal adhesive tablet. J. Control. Release., 68(3), 405-412.

Choi, H.-G., & Kim, C.-K. (2000). Development of omeprazole buccal adhesive tablets with stability enhancement in human saliva. J. Control. Release., 68(3), 397-404.

Dário, A. F., Hortêncio, L. M. A., Sierakowski, M. R., Neto, J. C. Q., & Petri, D. F. S. (2011). The effect of calcium salts on the viscosity and adsorption behavior of xanthan. Carbohydr. Polym., 84(1), 669-676.

Darwish, M., Kirby, M., Robertson Jr, P., Tracewell, W., & Jiang, J. G. (2006a). Pharmacokinetic properties of fentanyl effervescent buccal tablets: A phase I,

open-label, crossover study of single-dose 100, 200, 400, and 800 μg in healthy adult volunteers. Clin. Ther., 28(5), 707-714.

Darwish, M., Tempero, K., Kirby, M., & Thompson, J. (2006b). Relative bioavailability of the fentanyl effervescent buccal tablet (FEBT) 1080 pg versus Oral transmucosal fentanyl citrate 1600 pg and dose proportionality of FEBT 270 to 1300 μg: A single-dose, randomized, open-label, three-period study in healthy adult volunteers. Clin. Ther., 28(5), 715-724.

Das, N., Madan, P., & Lin, S. (2012). Statistical optimization of insulin-loaded Pluronic F-127 gels for buccal delivery of basal insulin. Pharm. Dev. Technol., 17(3), 363-374.

Debord, B., Lefebvre, C., Guyot-Hermann, A. M., Hubert, J., Bouché, R., & Cuyot, J. C. (1987). Study of Different Crystalline forms of Mannitol: Comparative Behaviour under Compression. Drug Dev. Ind. Pharm., 13(9-11), 1533-1546.

Dentini, M., Crescenzi, V., & Blasi, D. (1984). Conformational properties of xanthan derivatives in dilute aqueous solution. Int. J. Biol. Macromol., 6(2), 93-98.

Eichman, J. D., & Robinson, J. R. (1998). Mechanistic Studies on Effervescent-Induced Permeability Enhancement. Pharm. Res., 15(6), 925-930.

El-Samaligy, M. S., Yahia, S. A., & Basalious, E. B. (2004). Formulation and evaluation of diclofenac sodium buccoadhesive discs. Int. J. Pharm., 286(1–2), 27-39.

Espinoza, R., Hong, E., & Villafuerte, L. (2000). Influence of admixed citric acid on the release profile of pelanserin hydrochloride from HPMC matrix tablets. Int. J. Pharm., 201(2), 165-173.

Eyjolfsson, R. (2004). Calcium sulphate dihydrate: an useful excipient for tablets containing labile actives. Pharmazie, 59(9), 725-726.

Garcia-Ochoa, F., Santos, V. E., Casas, J. A., & Gómez, E. (2000). Xanthan gum: production, recovery, and properties. Biotechnol. Adv., 18(7), 549-579.

Giovino, C., Ayensu, I., Tetteh, J., & Boateng, J. S. (2012). Development and characterisation of chitosan films impregnated with insulin loaded PEG-b-PLA nanoparticles (NPs): a potential approach for buccal delivery of macromolecules. Int. J. Pharm., 428(1-2), 143-151.

Gohel, M. C., Parikh, R. K., Aghara, P. Y., Nagori, S. A., Delvadia, R. R., & Dabhi, M. R. (2009). Application of Simplex Lattice Design and Desirability Function for the Formulation Development of Mouth Dissolving Film of Salbutamol Sulphate. Curr. Drug Deliv., 6(5), 486-494.

Gong, J., Peng, Y., Bouajila, A., Ourriban, M., Yeung, A., & Liu, Q. (2010). Reducing quartz gangue entrainment in sulphide ore flotation by high molecular weight polyethylene oxide. Int. J. Miner. Process., 97(1–4), 44-51.

Govindasamy, P., Kesavan, B. R., & Narasimha, J. K. (2013). Formulation of unidirectional release buccal patches of carbamazepine and study of permeation through porcine buccal mucosa. Asian Pac. J. Trop. Biomed., 3(12), 995-1002.

Gurny, R., Doelker, E., & Peppas, N. A. (1982). Modelling of sustained release of water-soluble drugs from porous, hydrophobic polymers. Biomaterials., 3(1), 27-32.

Han, R.-Y., Fang, J.-Y., Sung, K. C., & Hu, O. Y. P. (1999). Mucoadhesive buccal disks for novel nalbuphine prodrug controlled delivery: effect of formulation variables on drug release and mucoadhesive performance. Int. J. Pharm., 177(2), 201-209.

Harris, D., & Robinson, J. R. (1992). Drug delivery via the mucous membranes of the oral cavity. J. Pharm. Sci., 81(1), 1-10.

Higiro, J., Herald, T. J., & Alavi, S. (2006). Rheological study of xanthan and locust bean gum interaction in dilute solution. Food Res. Int., 39(2), 165-175.

Holgado, M. A., Caraballo, I., Alvarez-Fuentes, J., Fernández-Hervás, M. J., Fernández-Arévalo, M., & Rabasco, A. M. (1995). Influence of diluents and manufacturing method on the in vitro dissolution of carteolol hydrochloride matrix tablets. Int. J. Pharm., 118(2), 151-160.

Hosny, E. A., & Al-Angary, A. A. (1995). Bioavailability of sustained release indomethacin suppositories containing polycarbophil. Int. J. Pharm., 113(2), 209-213.

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., 111C, 644-650.

Jaipal, A., Pandey, M. M., Charde, S., sadhu, N., Ajjarapu, S., & Reddy, P. G. (2014). Controlled Release Effervescent Buccal Discs of Buspirone Hydrochloride: In-vitro and In-Vivo Evaluation Studies. Drug Delivery, 1-7 (DOI: 10.3109/10717544.10712014.10917388).

Jian, H., Zhu, L., Zhang, W., Sun, D., & Jiang, J. (2012). Galactomannan (from Gleditsia sinensis Lam.) and xanthan gum matrix tablets for controlled delivery of theophylline: In vitro drug release and swelling behavior. Carbohydr. Polym., 87(3), 2176-2182.

Kanjanabat, S., & Pongjanyakul, T. (2011). Preparation and characterization of nicotine-magnesium aluminum silicate complex-loaded sodium alginate matrix tablets for buccal delivery. AAPS PharmSciTech., 12(2), 683-692.

Kaur, A., & Kaur, G. (2012). Mucoadhesive buccal patches based on interpolymer complexes of chitosan-pectin for delivery of carvedilol. Saudi Pharm J, 20(1), 21-27.

Kianfar, F., Antonijevic, M., Chowdhry, B., & Boateng, J. S. (2013). Lyophilized wafers comprising carrageenan and pluronic acid for buccal drug delivery using model soluble and insoluble drugs. Colloids Surf., B., 103, 99-106.

Kianfar, F., Chowdhry, B. Z., Antonijevic, M. D., & Boateng, J. S. (2012). Novel films for drug delivery via the buccal mucosa using model soluble and insoluble drugs. Drug Dev. Ind. Pharm., 38(10), 1207-1220.

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983a). Mechanisms of potassium chloride release from compressed, hydrophilic, polymeric matrices: Effect of entrapped air. J. Pharm. Sci., 72(10), 1189-1191.

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., & Peppas, N. A. (1983b). Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm., 15(1), 25-35.

Lambert, F., Milas, M., & Rinaudo, M. (1985). Sodium and calcium counterion activity in the presence of xanthan polysaccharide. Int. J. Biol. Macromol., 7(1), 49-52.

Launay, B., Cuvelier, G., & Martinez-Reyes, S. (1997). Viscosity of locust bean, guar and xanthan gum solutions in the Newtonian domain: a critical examination of the log (ηsp)o-log c[η]o master curves. Carbohydr. Polym., 34(4), 385-395.

Lee, J., & Kellaway, I. W. (2000). Combined effect of oleic acid and polyethylene glycol 200 on buccal permeation of [D-Ala2, D-Leu5]enkephalin from a cubic phase of glyceryl monooleate. Int. J. Pharm., 204(1–2), 137-144.

Martínez González, I., & Villafuerte Robles, L. (2003). Influence of enteric citric acid on the release profile of 4-aminopyridine from HPMC matrix tablets. Int. J. Pharm., 251(1–2), 183-193.

Mezdour, S., Cuvelier, G., Cash, M. J., & Michon, C. (2007). Surface rheological properties of hydroxypropyl cellulose at air–water interface. Food Hydrocoll., 21(5–6), 776-781.

Mirhosseini, H., Tan, C. P., Hamid, N. S. A., & Yusof, S. (2008). Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion. Food Chem., 107(3), 1161-1172.

Miro, A., d'Angelo, I., Nappi, A., La Manna, P., Biondi, M., Mayol, L., Musto, P., Russo, R., Rotonda, M. I. L., Ungaro, F., & Quaglia, F. (2013). Engineering poly(ethylene oxide) buccal films with cyclodextrin: A novel role for an old excipient? Int. J. Pharm., 452(1–2), 283-291.

Mohammed, Z. H., Haque, A., Richardson, R. K., & Morris, E. R. (2007). Promotion and inhibition of xanthan 'weak-gel' rheology by calcium ions. Carbohydr. Polym., 70(1), 38-45.

Mumtaz, A. M., & Ch'ng, H.-S. (1995). Evaluation of bioadhesive buccal tablets containing triamcinolone acetonide in healthy volunteers. Int. J. Pharm., 121(2), 249-254.

Nafee, N. A., Ismail, F. A., Boraie, N. A., & Mortada, L. M. (2004). Mucoadhesive Delivery Systems. I. Evaluation of Mucoadhesive Polymers for Buccal Tablet Formulation. Drug Dev. Ind. Pharm., 30(9), 985-993.

Narendra, C., Srinath, M. S., & Prakash Rao, B. (2005). Development of three layered buccal compact containing metoprolol tartrate by statistical optimization technique. Int. J. Pharm., 304(1–2), 102-114.

Nicolazzo, J. A., Reed, B. L., & Finnin, B. C. (2005a). Buccal penetration enhancers—How do they really work? J. Control. Release., 105(1–2), 1-15.

Nicolazzo, J. A., Reed, B. L., & Finnin, B. C. (2005b). Enhancing the buccal mucosal uptake and retention of triamcinolone acetonide. J. Control. Release., 105(3), 240-248.

Oh, D.-H., Chun, K.-H., Jeon, S.-O., Kang, J.-W., & Lee, S. (2011). Enhanced transbuccal salmon calcitonin (sCT) delivery: Effect of chemical enhancers and electrical assistance on in vitro sCT buccal permeation. Eur. J. Pharm. Biopharm., 79(2), 357-363.

Pajander, J., Baldursdottir, S., Rantanen, J., & Ostergaard, J. (2012). Behaviour of HPMC compacts investigated using UV-imaging. Int. J. Pharm., 427(2), 345-353.

Park, C. R., & Munday, D. L. (2004). Evaluation of Selected Polysaccharide Excipients in Buccoadhesive Tablets for Sustained Release of Nicotine. Drug Dev. Ind. Pharm., 30(6), 609-617.

Peppas, N. A., & Sahlin, J. J. (1996). Hydrogels as mucoadhesive and bioadhesive materials: a review. Biomaterials., 17(16), 1553-1561.

Pygall, S. R., Kujawinski, S., Timmins, P., & Melia, C. D. (2009). Mechanisms of drug release in citrate buffered HPMC matrices. Int. J. Pharm., 370(1–2), 110-120.

Repka, M. A., & McGinity, J. W. (2001). Bioadhesive properties of hydroxypropylcellulose topical films produced by hot-melt extrusion. J. Control. Release., 70(3), 341-351.

Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J. Control. Release., 5(1), 37-42.

Rochefort, W. E., & Middleman, S. (1987). Rheology of Xanthan Gum: Salt, Temperature, and Strain Effects in Oscillatory and Steady Shear Experiments. J. Rheol., 31(4), 337-369.

Rossi, S., Sandri, G., & Caramella, C. M. (2005). Buccal drug delivery: A challenge already won? Drug Discov. Today. Technol., 2(1), 59-65.

Sadeghi, A. M. M., Avadi, M. R., Ejtemaimehr, S., Abashzadeh, S., Partoazar, A., Dorkoosh, F., Faghihi, M., Rafiee-Tehrani, M., & Junginger, H. E. (2009). Development of a Gas Empowered Drug Delivery system for peptide delivery in the small intestine. J. Control. Release., 134(1), 11-17.

Salamat-Miller, N., Chittchang, M., & Johnston, T. P. (2005). The use of mucoadhesive polymers in buccal drug delivery. Adv. Drug Deliv. Rev., 57(11), 1666-1691.

Sander, C., Nielsen, H. M., & Jacobsen, J. (2013). Buccal delivery of metformin: TR146 cell culture model evaluating the use of bioadhesive chitosan discs for drug permeability enhancement. Int. J. Pharm., 458(2), 254-261.

Şenel, S., Çapan, Y., Sargon, M. F., İkinci, G., Şolpan, D., Güven, O., Boddé, H. E., & Hincal, A. A. (1997). Enhancement of transbuccal permeation of morphine sulfate by sodium glycodeoxycholate in vitro. J. Control. Release., 45(2), 153-162.

Şenel, S., & Hıncal, A. A. (2001). Drug permeation enhancement via buccal route: possibilities and limitations. J. Control. Release., 72(1–3), 133-144.

Shakya, R., Thapa, P., & Saha, R. N. (2013). In vitro and in vivo evaluation of gastroretentive floating drug delivery system of ofloxacin. Asian. J. Pharm. Sci., 8(3), 191-198.

Shalviri, A., Liu, Q., Abdekhodaie, M. J., & Wu, X. Y. (2010). Novel modified starch–xanthan gum hydrogels for controlled drug delivery: Synthesis and characterization. Carbohydr. Polym., 79(4), 898-907.

Siepmann, J., & Peppas, N. A. (2001). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv. Drug Deliv. Rev., 48(2–3), 139-157.

Smart, J. D. (2005). The basics and underlying mechanisms of mucoadhesion. Adv. Drug Deliv. Rev., 57(11), 1556-1568.

Sudhakar, Y., Kuotsu, K., & Bandyopadhyay, A. K. (2006). Buccal bioadhesive drug delivery — A promising option for orally less efficient drugs. J. Control. Release., 114(1), 15-40.

Tadros, M. I. (2010). Controlled-release effervescent floating matrix tablets of ciprofloxacin hydrochloride: Development, optimization and in vitro–in vivo evaluation in healthy human volunteers. Eur. J. Pharm. Biopharm., 74(2), 332-339.

Tajarobi, F., Abrahmsén-Alami, S., Carlsson, A. S., & Larsson, A. (2009). Simultaneous probing of swelling, erosion and dissolution by NMR-microimaging—Effect of solubility of additives on HPMC matrix tablets. Eur. J. Pharm. Sci., 37(2), 89-97.

Talukdar, M. M., & Kinget, R. (1995). Swelling and drug release behaviour of xanthan gum matrix tablets. Int. J. Pharm., 120(1), 63-72.

Talukdar, M. M., Van den Mooter, G., Augustijns, P., Tjandra-Maga, T., Verbeke, N., & Kinget, R. (1998). In vivo evaluation of xanthan gum as a potential excipient for oral controlled-release matrix tablet formulation. Int. J. Pharm., 169(1), 105-113.

Taylan, B., Capan, Y., Güven, O., Kes, S., & Atilla Hincal, A. (1996). Design and evaluation of sustained-release and buccal adhesive propranolol hydrochloride tablets. J. Control. Release., 38(1), 11-20.

Tsutsumi, K., Obata, Y., Takayama, K., Loftsson, T., & Nagai, T. (1998). Effect of the cod-liver oil extract on the buccal permeation of ionized and nonionized forms of ergotamine using the keratinized epithelial-free membrane of hamster cheek pouch mucosa. Int. J. Pharm., 174(1–2), 151-156.

van de Ven, T. G. M., Qasaimeh, M. A., Pigeon, C., & Paris, J. (2007). The effect of calcium ions on the efficiency of polyethylene oxide–cofactor retention aid systems. Colloids Surf., A, 297(1–3), 79-83.

Wang, L., & Tang, X. (2008). A novel ketoconazole bioadhesive effervescent tablet for vaginal delivery: Design, in vitro and 'in vivo' evaluation. Int. J. Pharm., 350(1–2), 181-187.

Wong, C. F., Yuen, K. H., & Peh, K. K. (1999). An in-vitro method for buccal adhesion studies: importance of instrument variables. Int. J. Pharm., 180(1), 47-57.

Yehia, S., El-Gazayerly, O., & Basalious, E. (2008). Design and In Vitro/In Vivo Evaluation of Novel Mucoadhesive Buccal Discs of an Antifungal Drug: Relationship Between Swelling, Erosion, and Drug Release. AAPS PharmSciTech., 9(4), 1207-1217.

Zeng, W. M. (2004). Oral Controlled Release Formulation for Highly Water-Soluble Drugs: Drug–Sodium Alginate–Xanthan Gum–Zinc Acetate Matrix. Drug Dev. Ind. Pharm., 30(5), 491-495.

Zhong, L., Oostrom, M., Truex, M. J., Vermeul, V. R., & Szecsody, J. E. (2013). Rheological behavior of xanthan gum solution related to shear thinning fluid delivery for subsurface remediation. J. Hazard. Mater., 244–245(0), 160-170.

Zhu, Z., Zhai, Y., Zhang, N., Leng, D., & Ding, P. (2013). The development of polycarbophil as a bioadhesive material in pharmacy. Asian. J. Pharm. Sci., 8(4), 218-227.

6.1 Introduction

In-vivo buccal absorption studies usually provide an insight in to the ability of formulation to deliver a drug in to the systemic blood circulation. Appropriate animal models and /or human subjects (Artusi et al., 2003; Junginger et al., 1999; Patel et al., 2012) are to be selected to establish or to understand the complete pharmacokinetic profile for the selected drug in the designed dosage form. In-vivo pharmacokinetic studies for the developed bioadhesive buccal formulations are often evaluated in rabbits (Martin et al., 2003; Ravi Kumar Reddy et al., 2013; Shin et al., 2000; Xu et al., 2002), rats (Christrup et al., 1997; Onishi et al., 2014; Tsagogiorgas et al., 2013), guinea pigs (Kiptoo et al., 2008; Tsutsumi et al., 2002), pigs (Campisi et al., 2010; Hoogstraate et al., 1996; Tsagogiorgas et al., 2013) and dogs (Jain et al., 2002; Zhang et al., 1994). The main intention for designing bioadhesive buccal dosage forms is to improve bioavailability by preventing drug degradation in GIT and avoiding hepatic first pass metabolism. So, buccal availability of drug from the developed formulation need to be compared with bioavailability obtained by oral or intra venous (IV) administered drug to prove the clinical relevance of the developed formulations.

In the present chapter, In-vivo studies were performed for the developed controlled release bioadhesive buccal formulations of BS on New Zealand white rabbits. Buccal dosage forms with optimum drug release and bioadhesion were selected for in-vivo study to understand the pharmacokinetic profile of BS from the designed buccal discs. The data obtained from this study was used to propose the buccal bioavailability and pharmacokinetics of BS from the designed buccal formulations. In the present study, buccal bioavailability was compared with bioavailability of BS after intra venous bolus administration.

6.2 Materials

Buspirone hydrochloride (BS) was provided as a gift sample by Astron Research limited, Gujarat, India. Other materials and reagents used were same as mentioned in chapter 3, 4 and 5.

6.3 Animal Model

Male New Zealand white rabbits were provided by Central Animal Facility of Birla Institute of Technology and Science, Pilani with mean weight of 1.65 ± 0.15 kg. The study was conducted with an approval (Protocol approval number:

IAEC/RES/16/04) and as per guidelines prescribed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Institutional Animal Ethics Committee and under the supervision of registered veterinarian. Animals were issued 10 days prior to experimentation for acclimatization and were kept on standard pellet diet and water ad libitum. Animals were fasted 4–6 h prior to experimentation.

6.4 Preparation of Formulation

Bioavailability of BS given intravenously was compared with bioavailability of designed buccal discs. For in vivo studies representative batches showing desired in-vitro profile were selected. It was decided to select one formulation to prove the hypothesis that the buccal delivery of BS improves bioavailability. For this reason buccal disc prepared using combination of various viscosity grades of PEO (JB/PEO/9) was selected. Further to understand the role of permeation enhancer and to establish role of carbon dioxide in enhancement of buccal permeation of BS, buccal discs designed using HPMC with and without agents responsible for effervescence were selected for in-vivo studies.

BS (100 mg) was accurately weighed and transferred to 10 mL standard volumetric flask. To this 2.5 mL of sterile water was added and vortex mixed to dissolve the drug, finally volume was made up to 10 mL using sterile water. An aliquot of resultant solution was appropriately diluted and assayed using analytical method 2 of chapter 3.

For in vivo studies, fresh batches of JB/EF/1, JB/EF/5 and JB/PEO/9 polymer were prepared prior to animal experimentation containing 10 mg of BS. The complete composition of these formulations is given in Table 5.1 of chapter 5. Quality of the prepared formulations was evaluated by checking content uniformity, friability, and thickness of prepared formulations. In-vitro bioadhesion and drug release studies were also carried out prior to using the formulations for animal studies. The methodology adopted for all these tests has been mentioned at length in chapter 5.

6.5 Dosing

Rabbits were divided into four groups of three rabbits each. Prior to dosing, animals were anesthetized by an I.M injection of 1:5 mixtures of xylazine (1.5 mg/kg) and ketamine (9.0 mg/kg). The light plane of anesthesia was maintained by an

intramuscular (I.M) injection of one third of initial dose of xyalzine and ketamine as needed.

The mouth of rabbit was opened using specially designed mouth restrainers and the pre-moistened buccal disc was placed in the buccal cavity using forceps. The disc was pressed gently against mucosal lining of cheek for 1 min to ensure adhesion.

To the first group 1 mL solution of BS in sterile water (10 mg/mL) was administered intravenously (I.V) through marginal ear vein as bolus dose. Controlled release bioadhesive buccal discs of batches JB/EF/1, JB/EF/5 and JB/PEO/9 were administered to second, third and fourth groups respectively. The entire study was carried out in triplicates. Each rabbit was dosed with specific dose (10 mg) of BS without taking weight of the rabbit into consideration.

6.6 Blood Sample Collection

For each study, blood samples were withdrawn from marginal ear vein before dosing and 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0, 24.0 h post dosing. Blood samples were collected in 1.5 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 Processing and Analysis

Stored plasma samples were thawed at ambient temperature (25 ± 2 °C) for at least 60 min and processed using solid phase extraction (SPE) cartridges. SPE cartridges were conditioned (1 mL methanol, 2500 rpm) and equilibrated (25mM potassium dihydrogen orthophosphate, 2500 rpm) before loading the sample. Sample was loaded and centrifuged at 2000 rpm to remove interfering components and matrix, washing step was performed using 3 % v/v methanol (1 mL, 2500 rpm) to remove the remaining interfering components and finally elution was carried out using 0.025 M phosphate buffer pH 3.0 and acetonitrile (75:25, 1 mL, 3000 rpm) solvent system. The separation was performed using a centrifuge (Remi) and the elutions were collected in 5 mL disposable tubes. The processed plasma 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 BS 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 was carried out for BS administered intravenously and for bioadhesive buccal discs. The plasma concentration versus time profiles of BS following administration of 10 mg single dose by I.V and buccal routes are given in Figure 6.1. Summary of pharmacokinetic parameters obtained using non-compartmental data analysis are listed in Table 6.1. Absolute bioavailability for the designed formulations was calculated using equation 1.

$$F = \frac{(Dose_{i.v}) \times (AUC_{0-t_{buccal}})}{(Dose_{buccal}) \times (AUC_{0-t_{i.v}})} \qquad \text{(Eq. 1)}$$

The drug was detectable in blood within 15 min of buccal administration for all the formulations indicating rapid absorption of released drug. In-vivo pharmacokinetic parameters obtained during the study are tabulated in Table 6.1.

Following intravenous administration of BS, maximum plasma concentration (C_{max}) observed was 1384.68 ± 467.21 ng/mL. Plasma concentrations of BS were detectable up to 10 h post dosing of I.V. AUC $_{(0-\infty)}$ was found to be 5188.27 ± 430.81 ng. h/mL. Half life ($t_{1/2}$) and mean residence time (MRT) was found to be 2.53 ± 0.12 h and 3.66 h respectively (Table 6.1). Buccal discs designed using blend of PEO 1L and PEO 40L (JB/PEO/9) when administered via buccal route, the bioavailability of BS was considerably improved (24%) nearly 6 folds compared to the reported values of orally administered BS (4%). This proves the hypothesis of improved bioavailability of drugs delivered through buccal route

When non-effervescent controlled release HPMC buccal disc (JB/EF/1) administered via buccal route the bioavailability of BS observed was high compared to that of reported values of orally administered BS. The plasma concentrations of BS

detectable up to 12 h post dosing. AUC $_{(0 - \infty)}$ was found to be 1662.27 ± 745.26 ng h/mL. Half life ($t_{1/2}$) and mean residence time (MRT) for the HPMC buccal disc (JB/EF/1) was found to be 4.78 ± 0.26 h and 7.47 h respectively (Table 6.1).

Controlled release effervescent buccal discs (JB/EF/5) resulted in higher AUC $_{0-\infty}$ of 2687.07 ± 912.32 when compared to non-effervescent HPMC buccal discs (JB/EF/1) (1662.27 ± 745.26) Absolute bioavailability of effervescent HPMC buccal discs (JB/EF/5) observed was 0.55 ± 0.06, and is significantly more ($P<0.05$) when compared to that bioavailability (0.32 ± 0.11) observed with non-effervescent HPMC buccal discs (JB/EF/1). Enhanced permeation of BS might have also prevented the drug loss caused by salivary swallowing.

Buccal discs designed using blend of PEO 1L and PEO 40L has shown increased t_{max} (2.00 h) compared to HPMC buccal discs (2.50 h). This difference might be due to increased solubility of PEO compared to HPMC polymer. However polymer type, amount and excipients used are also an important factor for drug release behavior.

Moreover, the maximum plasma concentration was achieved at an earlier time (t_{max} 2.5 h) in case of effervescent buccal discs compared to non-effervescent buccal discs, further indicating increase in rate of permeation due to release of carbon dioxide. These results clearly indicate usefulness of effervescent systems for permeation enhancement (Figure 6.1).

The possible mechanisms for enhanced permeation is structural alteration of buccal epithelial membrane by creation of new or widening of pre-existing pores leading to paracellular transport or by solvent drag due to increased pressure gradient by carbon dioxide (Eichman & Robinson, 1998). The unionized species of BS are likely to be transported by transcellular pathways due to their hydrophobic nature. Carbon dioxide increases hydrophobicity of mucosal membrane due to higher partitioning in mucosal membrane resulting in better flux for absorption of unionized drug. Reports suggests that carbon dioxide has direct effect on structural integrity of mucosal membrane with no indication of cell membrane damage for prolonged time, the damage to the epithelial barrier properties was reestablished within 20 min, relatively a very short period of time (Eichman & Robinson, 1998).

Plasma concentration of BS was also detected within 15 min of buccal administration of buccal discs designed using PEO polymer (JB/PEO/9) (Figure 6.1). $AUC_{0-\infty}$ values (1219.17 ± 811.38 ng h/mL) obtained for PEO buccal discs was relatively less compared to HPMC buccal discs, this effect was due to faster drug release, which might have not completely absorbed from the buccal epithelium due to loss in salivary fluids resulting absorption. Moreover, all the designed buccal discs have demonstrated controlled release in in-vivo for at least 8 h.

Designed buccal discs have demonstrated improved bioavailability compared to reported oral bioavailability (4%). Effervescent buccal discs have significantly improved buccal absorption compared to non effervescent buccal discs.

Effervescent buccal discs showed maximum bioavailability of all the formulations with C_{max}, $AUC_{(0-\infty)}$ and time to reach C_{max} (t_{max}) values of 329.31 ± 31.78 ng/ml, 2687.07 ± 912.32 ng h/ml and 2.50 h respectively. The values obtained are significantly higher compared to non effervescent formulations. From the above obtained results it can be confirmed that carbon dioxide gas can be used as a potential permeation enhancer for delivery of drugs via buccal route.

The plasma concentration versus time profiles of BS following administration of 10 mg single dose by I.V and buccal routes are given in Figure 6.1. Summary of pharmacokinetic parameters obtained using non-compartmental data analysis are listed in Table 6.1.

6.10 Conclusions

In-vivo studies performed for the selected formulations of HPMC (JB/EF/01), HPMC effervescent (JB/EF/05) and PEO (JB/PEO/9) have shown significant increase in bioavailability compared to reported oral bioavailability. This effect is due to bypass in first pass metabolism. Polymers and effervescent agents used in the buccal formulations played a significant role in enhancement of drug permeability and bioavailability. Hence designed buccal dosage forms are promising and may lead to substantial dose reduction, more predictable plasma drug concentration profile and longer duration of action of BS as compared to oral conventional marketed preparations.

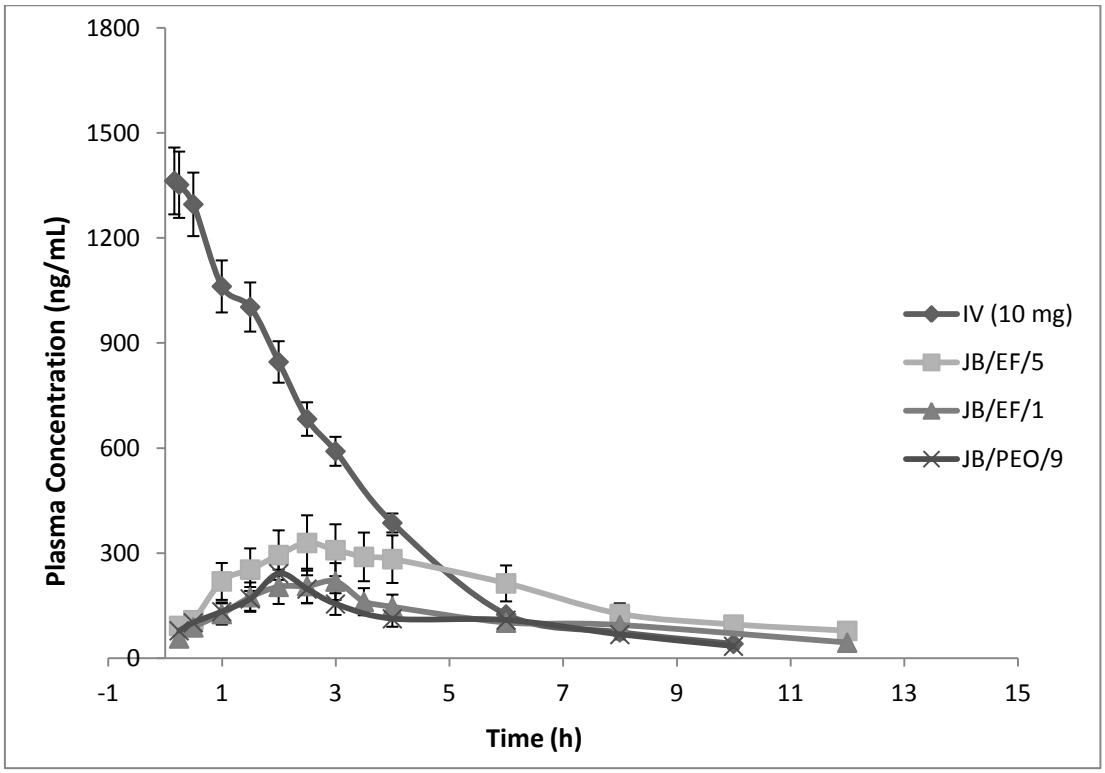


Figure 6.1: In-vivo profiles following administration of single dose of BS (10 mg) in rabbits by IV and buccal route

Table 6.1: Pharmacokinetic parameters of BS (10 mg) when administered intravenously and via buccal route (Mean ± SD for 3 rabbits)

Pharmacokinetic Parameters	**10 mg BS Solution via I.V**	**JB/EF/1***	**JB/EF/5***	**JB/PEO/9***
C_{max} (ng /mL) [a]	1384.68 ± 467.21	219.22 ± 36.47	329.31 ± 31.78	241.67 ± 45.33
t_{max} (h) [b]	--	3.00	2.50	2.00
$AUC_{(0-\infty)}$ [c] (ng h/mL)	5188.27 ± 430.81	1662.27 ± 745.26	2687.07 ± 912.32	1984.8 ± 811.38
$AUMC_{(0-\infty)}$ [d] (ng h^2/ml)	19004.80	12411.01	24082.99	18863.85
$t_{1/2}$ (h)	2.53 ± 0.12	4.78 ± 1.26	5.76 ± 1.23	2.40 ± 0.39
MRT [e] (h)	3.66	7.47	8.40	9.50
F[f]	--	0.32 ± 0.11	0. 55 ± 0.06	0.24 ± 0.09

[a] C_{max}: Maximum plasma concentration
[b] t_{max}: Time to reach C_{max}
[c] $AUC_{(0-\infty)}$: Area under the plasma concentration-time curve
[d] $AUMC_{(0-\infty)}$: Area under the first moment curve
[e] MRT: Mean residence time
[f] F: Absolute bioavailability with respect to I.V
* $p < 0.05$

References

Artusi, M., Santi, P., Colombo, P., & Junginger, H. E. (2003). Buccal delivery of thiocolchicoside: in vitro and in vivo permeation studies. Int. J. Pharm., 250(1), 203-213.

Campisi, G., Giannola, L. I., Florena, A. M., De Caro, V., Schumacher, A., Göttsche, T., Paderni, C., & Wolff, A. (2010). Bioavailability in vivo of naltrexone following transbuccal administration by an electronically-controlled intraoral device: A trial on pigs. J. Control. Release., 145(3), 214-220.

Christrup, L. L., Christensen, C. B., Friis, G. J., & Jorgensen, A. (1997). Improvement of buccal delivery of morphine using the prodrug approach. Int. J. Pharm., 154(2), 157-165.

Eichman, J. D., & Robinson, J. R. (1998). Mechanistic Studies on Effervescent-Induced Permeability Enhancement. Pharm. Res., 15(6), 925-930.

Hoogstraate, A. J., Verhoef, J. C., Tuk, B., Pijpers, A., van Leengoed, L. A. M. G., Verheijden, J. H. M., Junginger, H. E., & Boddé, H. E. (1996). Buccal delivery of fluorescein isothiocyanate-dextran 4400 and the peptide drug buserelin with glycodeoxycholate as an absorption enhancer in pigs. J. Control. Release., 41(1–2), 77-84.

Jain, A. C., Aungst, B. J., & Adeyeye, M. C. (2002). Development and in vivo evaluation of buccal tablets prepared using danazol–sulfobutylether 7 β-cyclodextrin (SBE 7) complexes. J. Pharm. Sci., 91(7), 1659-1668.

Junginger, H. E., Hoogstraate, J. A., & Verhoef, J. C. (1999). Recent advances in buccal drug delivery and absorption — in vitro and in vivo studies. J. Control. Release., 62(1–2), 149-159.

Kiptoo, P. K., Paudel, K. S., Hammell, D. C., Hamad, M. O., Crooks, P. A., & Stinchcomb, A. L. (2008). In vivo evaluation of a transdermal codrug of 6-β-naltrexol linked to hydroxybupropion in hairless guinea pigs. Eur. J. Pharm. Sci., 33(4–5), 371-379.

Martin, L., Wilson, C. G., Koosha, F., & Uchegbu, I. F. (2003). Sustained buccal delivery of the hydrophobic drug denbufylline using physically cross-linked palmitoyl glycol chitosan hydrogels. Eur. J. Pharm. Biopharm., 55(1), 35-45.

Onishi, H., Yumoto, K., & Sakata, O. (2014). Preparation and evaluation of ritodrine buccal tablets for rational therapeutic use. Int. J. Pharm., 468(1–2), 207-213.

Patel, V. F., Liu, F., & Brown, M. B. (2012). Modeling the oral cavity: In vitro and in vivo evaluations of buccal drug delivery systems. J. Control. Release., 161(3), 746-756.

Ravi Kumar Reddy, J., Indira Muzib, Y., & Chowdary, K. P. R. (2013). Development and in-vivo characterization of novel trans buccal formulations of Amiloride hydrochloride. J. Pharm. Res.,, 6(6), 647-652.

Shin, S.-C., Bum, J.-P., & Choi, J.-S. (2000). Enhanced bioavailability by buccal administration of triamcinolone acetonide from the bioadhesive gels in rabbits. Int. J. Pharm., 209(1–2), 37-43.

Tsagogiorgas, C., Theisinger, S., Holm, P., Thiel, M., Quintel, M., & Holm, R. (2013). Buccal absorption of propofol when dosed in 1-perfluorobutylpentane to anaesthetised and conscious Wistar rats and Göttingen mini-pigs. Eur. J. Pharm. Biopharm., 85(3, Part B), 1310-1316.

Tsutsumi, K., Obata, Y., Nagai, T., Loftsson, T., & Takayama, K. (2002). Buccal absorption of ergotamine tartrate using the bioadhesive tablet system in guinea-pigs. Int. J. Pharm., 238(1–2), 161-170.

Xu, H.-B., Huang, K.-X., Zhu, Y.-S., Gao, Q.-H., Wu, Q.-Z., Tian, W.-Q., Sheng, X.-Q., Chen, Z.-X., & Gao, Z.-H. (2002). Hypoglycaemic effect of a novel insulin buccal formulation on rabbits. Pharmacol. Res., 46(5), 459-467.

Zhang, J., Niu, S., Ebert, C., & Stanley, T. H. (1994). An in vivo dog model for studying recovery kinetics of the buccal mucosa permeation barrier after exposure to permeation enhancers: apparent evidence of effective enhancement without tissue damage. Int. J. Pharm., 101(1–2), 15-22.

7.1 Conclusions

Drug delivery via buccal route has long been advocated as a potential route for delivery of many therapeutic drug molecules. It serves as a promising option for delivery of drugs having biopharmaceutical characteristics that are less suitable for oral administration. Parenteral drug delivery systems are expensive and needs paramedic assistance in almost majority of the cases. Parenteral delivery is an invasive painful process and also sometimes leads to hazardous effects. Buccal delivery of drugs offers several advantages such as non invasive techniques, improved bioavailability, ease of application and removal, economical and high patient compliance.

Buspirone hydrochloride (BS) is an anxiolytic agent belonging to azaspirodecanediones class. BS is a partial agonist for the serotonin 5-HT1A receptors and is an antagonist for the dopamine D2 auto receptors and also has weak affinity for 5-HT2 receptors. The major disadvantages of currently marketed conventional oral tablets are poor oral bioavailability (approx 4%) and erratic drug absorption in presence and absence of food leading to fluctuation in plasma drug concentrations. These problems associated with the oral BS formulations drive us to design and develop a buccal bioadhesive dosageform.

Developed in house validated spectrophotometric analytical method for estimation of BS in bulk and formulations was found to be sensitive and accurate for precise estimation of BS in variety of samples. Developed chromatographic methods were used for estimating BS in formulation, stability and plasma samples. Moreover, lack of interference from excipients and biological matrix in the proposed methods indicated specificity of the developed methods.

Preformulation studies revealed Form 1 polymorph of BS was used during entire research work when analyzed using DSC and FTIR techniques. Log P values obtained for BS indicated relatively higher partitioning towards lipophilic phase. BS demonstrated good stability in solution state at varying pH with $t_{90\%}$ values ranging from 1.14×10^{-4} to 2.28×10^{-4} days. Solid state stability studies indicated BS to be compatible and stable with process excipients used in the design of buccal dosage forms.

The designed bioadhesive buccal formulations of BS were found to possess good physical characteristics indicating suitability of excipients selected and the direct compression process employed for the design of bioadhesive buccal formulations. Drug release and bioadhesive behavior of the designed buccal discs was dependent on polymer proportion, molecular weight of polymer, excipients used and swelling behavior of polymer. In the present study, bioadhesive buccal discs were designed using various polymers and process excipients retarded drug release for 3 to 6 h. The release mechanism was found to be non-fickian anomalous type for all the batches. Bioadhesive behavior for all the formulations was good. In-vivo studies performed using selected bioadhesive buccal formulations of BS demonstrated substantial increase in bioavailability probably due to reduced first pass metabolism. Effervescent controlled release buccal formulations demonstrated significant increase in bioavailability compared to non effervescent buccal formulations due to enhanced drug permeation caused by release of carbon dioxide gas. The possible mechanisms for enhanced permeation is structural alteration of buccal epithelial membrane by creation of new or widening of pre-existing pores leading to paracellular transport or by solvent drag due to increased pressure gradient by carbon dioxide. Carbon dioxide increases hydrophobicity of mucosal membrane due to higher partitioning in mucosal membrane resulting in better flux for absorption of unionized drug. Carbon dioxide has direct effect on structural integrity of mucosal membrane and it has been reported that the structural integrity was reestablished relatively fast.

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

7.2 Future Scope of 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 discs with an optimum dose should be optimized to obtain a desirable controlled drug release and plasma drug concentration in humans. However, all the polymers and excipients used in the study were GRAS (Generally

recognized as safe) listed, other bioadhesive polymers either alone or in combination and excipients should be explored to understand the BS release and bioadhesion, and any interactions of excipients with BS should be established.

The optimized buccal formulations need to be studied clinically in humans for acceptability on the grounds of irritation caused by polymers and excipients and swelling behavior in the oral cavity. For the designed buccal discs the promising nature of the clinical efficacy, safety and confirmatory studies need to be verified for clinical benefit in humans.

Designed controlled release effervescent formulations have shown improved plasma concentration due to increased flux caused by membrane hydrophobicity and solvent drag. Moreover, other reported permeation enhancers in combination with effervescence producing agents need to be investigated for improved drug delivery systems and better understanding of permeation mechanisms.

Apart from this study, BS can be alternatively delivered via transdermal, sublingual, nasal and pulmonary routes to avoid first pass metabolism. Other delivery systems such as microparticles and nanoparticles can as well be explored for improvement of bioavailability.

List of Publications and Presentations from Thesis

1. **Jaipal, A**., Pandey, M. M., Abhishek, A., Vinay, S and Charde, S. Y. 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 and Surfaces B: Biointerfaces *2013*, 111, 644-650.
2. **Jaipal, A**., Pandey, M. M., Charde, S. Y., Nilanjan, S., Srinivas, A., and Prasad, R. G. Controlled Release Effervescent Buccal Discs of Buspirone Hydrochloride: In-vitro and In-Vivo Evaluation Studies. Drug Delivery 2014 (Early Online, DOI: 10.3109/10717544.2014.917388).
3. **Jaipal, A.,** Pandey, M. M., Charde, S. Y., Prasad R. G and Prasanth K. V. Effect of HPMC and Mannitol on Drug Release and Bioadhesion Behavior on Buccal Discs of Buspirone Hydrochloride: In-vitro and In-vivo Pharmacokinetic Studies. Communicated to Saudi Pharmaceutical Journal.
4. **Jaipal, A.,** Pandey, M. M., Charde S.Y, Soorina S and Divya S. Design of PEO Buccal discs of Buspirone: In-Vitro and In-Vivo Evaluation. Communicated to International Journal of Pharmaceutical Investigation.

Poster Presentations at National and International conference from Thesis

1. **Jaipal, A.**, Pandey, M M., Shailaja, P and 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, U.S.A (Abstract number R6233).
2. **Jaipal, A.,** Pandey, M. M., Charde, S. Y and Vinay, S. Design and Evaluation of Buccal Tablets of Buspirone Hcl : Effect of HPC and Mannitol on Drug Release. APTI 17th Annual National Convention, Manipal 2012.
3. **Jaipal, A.,** Pandey, M. M., Tarun, B., Shreya, C and Charde, S. Y. Design and Evaluation of Buccal Tablets of HPMC by Direct Compression Method**.** APTI 17th Annual National Convention, Manipal 2012.
4. **Jaipal, A.,** Pandey, M. M., Abhishek, A., Praveen, L and Charde, S. Y. Design and Evaluation of Buccal Tablets of Buspirone HCl : Effect of Polycarbophil and

Mannitol on Drug Release. APTI 17th Annual National Convention, Manipal 2012.

List of Other Publications

1. Pandey, M. M., **Jaipal, A.,** Kumar, A., Malik, R and Charde, S.Y. Determination of pKa of Felodipine using UV-Visible Spectroscopy. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2013, 115, 887-890.

List of Other Poster Presentations at National and International conference

1. 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.
2. **Jaipal, A.,** Shailaja, P, Mirza, K., and Mukesh, K. Design and evaluation of controlled release mucoadhesive microparticles of Clonazepam. 3rd World Congress on Bioavailability & Bioequivalence, Pharmaceutical R & D Summit, Hyderabad 2012.
3. Pandey, M. M., **Jaipal, A.,** Malik, R., Kumar, A and Charde, S. Y. Dissolution Rate Enhancement of Pioglitazone Hydrochloride by Solid Dispersions using Poloxamer 188. APTI 17th Annual National Convention, Manipal 2012.
4. Pandey, M. M., **Jaipal, A.,** Kumar, A., Malik, R., Parwani, M and Charde, S.Y. Formulation and in vitro Evaluation of Buccal Tablets of Felodipine using Polycarbophil, a Mucoadhesive Polymer. APTI 17th Annual National Convention, Manipal 2012.
5. Pandey, M. M., Raut, P., **Jaipal, A** and 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, Mumbai. 2011.
6. Pandey, M. M., Krishna Kumar, G., Khandelwal, R., **Jaipal, A** and Charde, S. Y. Solubility Enhancement of Felodipine by Solid Dispersion Technique. APTI - 16th Annual National Convection, Moga. 2011.(233).
7. Pandey, M. M., Raut, P., **Jaipal, A** and 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, Developement & Beyond, Mumbai. 2011.

8. **Jaipal, A.,** Shailaja, P., Pandey, M.M and Pinki, S. Design and Evaluation of Fast Disintegration Oral Films of Ondansetron Hcl. 63rd Indian Pharmaceutical Congress, Bengaluru 2011.
9. Aneesh K., **Jaipal A**., Praveena B and Pandey M M. Simple and Validated UV-Spectrophotometric Method for the Estimation of Clonazepam in Bulk and Formulations. Indo-American Pharmaceutical Regulatory Symposium. Ahmedabad 2011.
10. Radhakrishanan R., Raut P.P., **Jaipal A**., Pandey M.M and Charde S.Y. Development and Validation of UV-Spectrophotometric Methods for the Estimation of Ondansetron Hydrochloride in Bulk and Formulations. National Conference on Contemporary Trends in Biological and Pharmaceutical Research, BITS, Pilani. 2011.
11. Tyagi V., **Jaipal A.**, Pandey M.M., Raut P. P and Charde S.Y. Formulation and Evaluation of Directly Compressed Modified Release Tablets of Ondansetron Hydrochloride. National Conference on Contemporary Trends in Biological and Pharmaceutical Research, BITS, Pilani. 2011.
12. Pandey M.M., Raut P., **Jaipal A** and Charde S.Y. Spectrophotometric and Spectrofluorometric Study for the Estimation of Felodipine in Bulk and Formulation. 62nd Indian Pharmaceutical Congress, Manipal, 2010.
13. Raut P.P., **Jaipal A.,** Manish K and Charde S.Y. Formulation and Evaluation of Ondansetron pH Independent Controlled Release Tablet. 10th CRSIC International Symposium, Mumbai 2010.
14. **Jaipal A.,** Pandey M.M and Charde S.Y. Development and evaluation of buccal mucoadhesive drug delivery systems of amlodipine besylate. 10th CRSIC International Symposium, Mumbai 2010.

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