FORMULATION, CHARACTERIZATION AND IN-VITRO TESTING OF MUCOADHESIVE FILMS OF FLUOXETINE HYDROCHLORIDE.

OMWENGA TITUS ISOE
B Pharm
U53/80976/2015

DEPARTMENT OF PHARMACEUTICS AND PHARMACY PRACTICE
SCHOOL OF PHARMACY
UNIVERSITY OF NAIROBI

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTSFOR THE AWARD OF THE DEGREE OF MASTER OF PHARMACY IN INDUSTRIAL PHARMACY OF THE UNIVERSITY OF NAIROBI.

NOVEMBER, 2017

DECLARATION

Student Declaration

I, Titus Isoe Omwenga declare that the matter embodied in the dissertation titled **FORMULATION, CHARACTERIZATION AND IN-VITRO TESTING OF MUCOADHESIVE FILMS OF FLUOXETINE HYDROCHLORIDE** is bonafide and genuine work carried out by me under the guidance of my supervisors at the University of Nairobi. I also declare that the same has not previously formed the basis for the award of any associateship, fellowship and degree of any other university or institution.

Signature……..Date……

Registration no: U53/80976/2015

Supervisors

This dissertation has been submitted with our approval as university supervisors:

1. Signature……..Date……

   Dr.Shital Mahindra Maru, (Ph.D)

   Department of Pharmaceutics and Pharmacy Practice

   University of Nairobi

2. Signature……..Date……

   Dr. Dennis Ongarora, (Ph.D)

   Department of Pharmaceutical Chemistry

   University of Nairobi

DEDICATION

Dedicated to God, my family and friends.

ACKNOWLEGMENT

This study was conducted at the Department of Pharmaceutics and Pharmacy Practice and Department of Pharmaceutical Chemistry of the University of Nairobi and the National Quality Control Laboratory.

I would like to sincerely thank my supervisors Dr. Shital Mahindra Maru, (PhD) and Dr. Dennis Ongarora, (PhD) for their extremely invaluable guidance, input and support throughout the project.

I would also like to thank the Chairman Department of Pharmaceutical Chemistry for permission to use the facilities at the Drug Analysis and Research Unit (DARU) and the staff in DARU for their support and guidance in the laboratory.

Special thanks to the Director of National Quality Control Laboratory Dr. Hezekiah Chepkwony for permission to use the facilities at the laboratory.

I would like to thank Mrs. Agnes, Mr. Achoki and Mrs. Lily for their technical support throughout the project.

I would also like to appreciate my classmates for their support and encouragement throughout our studies.

My special gratitude goes to my family for their love, understanding, support and encouragement in my entire life.

TABLE OF CONTENTS

ABBREVIATIONS AND ACRONYMS

W.H.O	World Health Organisation
MDD	Major depressive disorder
YLD	Years lived with disability
DALYs	Disability adjusted life years
5HT	5-hydroxytryptamine
SSRIs	Selective serotonin reuptake inhibitors
APA	American Pharmacist Association
FLX	Fluoxetine
HME	Hot melt extraction
SERT	Serotonin transporter
KBr	Potassium bromide
FTIR	Fourier transform infrared spectroscopy
CMC	Carboxymethyl cellulose
HEC	Hydroxyethyl cellulose
HPC	Hydroxypropyl cellulose
HPMC	Hydroxypropyl methylcellulose
MC	Methyl cellulose

MHEC	Methyl hydroxyethylcellulose
CP	Carbopol
PC	Polycarbophil
PAA	Polyacrylic acid
EC	Ethyl cellulose
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
DARU	Drug Analysis and Research Unit
NQCL	National Quality Control Laboratory

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

INTRODUCTION

Recent research in the area of drug delivery has been focused on design and development of innovative and more effective drug delivery systems with enhanced safety, efficacy and patient compliance. Buccal drug delivery has emerged as one of the alternative platforms for drug delivery both locally and systemically. With a relatively large surface area, relative permeability and ease of access, the buccal mucosa offers ideal location for buccal drug delivery. Major depressive disorder affects a significant portion of the world's population. Treatment of depression takes a long period of time and some patient have difficulty in following through treatment. There is a need to develop buccal films as an alternative drug delivery system to the oral route for antidepressants in order to improve patient compliance, bioavailability, ease of administration and faster onset of action. Fluoxetine is a selective serotonin reuptake inhibitor and exerts its action by inhibiting serotonin transporter receptor. The present work provides insight into formulation of mucoadhesive buccal films containing fluoxetine hydrochloride. Pre-formulation, characterization of prepared films and in-vitro release studies were also performed.

METHODOLOGY

Preparation of fluoxetine mucoadhesive films was done by factorial experimental design. Pre-formulation studies were carried out to determine appropriate solvent system for use in formulation and as well as to ensure no incompatibilities between the drug and the polymers in use. Hydroxypropyl methylcellulose (HPMC) and polyvinylpyrrolidone (PVP) in different concentrations were used as mucoadhesive polymer in the fabrication of the films. Polypropylene glycol was used as a plasticizer. The films were prepared by solvent casting technique using

ethanol/dichloromethane mixture in the ratio of 1:1 as solvent system. The films obtained were analyzed for their organoleptic properties as well as physical properties including film weight, film thickness, pH, swelling index, folding endurance, uniformity of drug content and in-vitro release studies. The in-vitro release data was fitted into various kinetic models by using DD solver excel add in program to obtain the release pattern of the drug.

RESULTS

Pre-formulation studies revealed that ethanol/dichloromethane (1:1) was the best solvent for both drug and polymers. No incompatibilities were detected between the drug and polymers after studies using FTIR spectrophotometer. Fluoxetine buccal films were fabricated by using solvent casting technique. The prepared films were evaluated for organoleptic properties. Only formulations F1, F4, F7, F8 and F9 exhibited smooth properly and non-sticky films and were therefore considered for physical characterization. The selected formulations exhibited good physical characteristics including consistency in weight, thickness, folding endurance and pH. The swelling index ranged from 2.69 to 5.7 for the selected formulations. F1 had the lowest average drug content at 84% while F9 had the highest at 117.9%. F1 had the least cumulative drug release after 3hrs of in-vitro release studies. All the other selected formulations had release percentages between 89.9% and 93.3%.

The release data of the selected formulations were subjected to various mathematical models to understand the release pattern with the value of the coefficient of regression (R^2) suggesting the best fit kinetic model. The best fit kinetic model for the formulations was found to be Korsmeyers-Peppas. Formulations F1, F4 and F7 had release exponents values below 4.5 indicating that the drug transport mechanism was mainly Fickian diffusion. Formulations F8 and F9 had the value of

n at above 0.45 thus indicating drug transportation mechanism in this films to be mainly anomalous transport i.e. drug release is governed by both diffusion and erosion of polymer.

CONCLUSION

In the embodied work, buccal films containing fluoxetine were prepared by factorial design. HPMC and PVP were demonstrated to have good film forming and swelling properties for use in formulation of buccal films. Formulation F8 can be optimized to produce controlled release of the drug beyond 3hrs.The concentration of plasticizer used in the formulations was found to be optimum at 2-3% producing elegant non-sticky films. In summary, HPMC and PVP are potentially useful polymers for preparing mucoadhesive films of fluoxetine for buccal drug delivery system.

CHAPTER ONE: INTRODUCTION

1.1 Mental Disorders

There are two main diagnostic categories of mental disorders; depressive disorders and anxiety disorders(World Health Organization 2017). These disorders are widespread and highly prevalent in the world with a report of the WHO on depression and mental disorders estimating that 300 million people suffer from depression (World Health Organization 2017). It was estimated that by 2010, depressive disorders were the second leading cause years lived with disabilities (YLDs). Major depressive disorder account for 8.2% of the global YLDs and about 2.5% of global disability adjusted life years (DALYs) (Ferrari et al. 2013). These disorders have symptoms that range in terms of their severity and duration; from mild to severe, from months to years and are diagnosable health conditions (World Health Organization 2017).

Major depressive disorder is mainly characterised by periods where an individual suffers from depressive mood lasting over two weeks. Some of the other symptoms associated with major depressive disorder (MDD) include disturbed sleep, loss of appetite, lack of concentration, feeling of guilt and suicidal thoughts(Wenthur et al. 2014; Mill & Petronis 2007).

Mental disorders account for about 12% of global health burden of disease (Aillon et al. 2014). It is estimated that globally over 300 million people suffer from depression which is about 4.3% of the total world population (World Health Organization 2017). This number is on the increase especially in lower income countries including African nations. In Kenya, the prevalence of MDD in primary health care setting is estimated to be over 40% (Aillon et al. 2014).

The exact etiology of MDD remains unknown and some of the proposed causes include; psychological, psycho-social, hereditary and biological (Mill & Petronis 2007).

Medication and psychotherapy are some of the most effective methods of management of the MDD. Some of the drugs used in depression including selective serotonin reuptake inhibitors, 5HT receptor inhibitors, serotonin norepinephrine re-uptake inhibitors, monoamine oxidase inhibitors and tricyclic antidepressants (Taurines et al. 2011). Antidepressants generally act by influencing the balance of neurotransmitters in the brain (Richelson 2001).

Selective serotonin reuptake inhibitors (SSRIs) which include drugs such as sertraline, fluoxetine fluvoxamine, paroxetine,citalopram and escitalopram are used as first line drugs in management of MDDs. SSRIs have an advantage over the other classes of drugs because of their relative selectivity, ease of dosing and low toxicity in case of drug over dose. Fluoxetine is the first line antidepressant recommended for the treatment of depression in children and adolescents (Taurines et al. 2011). Fluoxetine is also the first-line drug for late-onset depression and this recommendation is supported by the 2011 APA guideline.

SSRIs have less prominent adverse effects than some of other agents, this promotes compliance with patients. Common adverse effects of SSRIs include; sexual dysfunction, gastrointestinal upset and changes in energy level (i.e. fatigue, restlessness). The SSRIs are safe to use in patients with cardiac disease as they do not affect cardiac conduction or cardiac rhythm, blood pressure and heart rate (Brown 2011).

1.2Buccal drug delivery system

The oral route is the most commonly used route of drug delivery. Over time, research efforts have been focused on developing new and alternate routes of drug delivery to remedy the drawbacks associated with oral drug delivery route (Kaul et al. 2011). One of the alternate drug delivery route

extensively explored is the oral mucosa with both the oral mucosa and sublingual mucosa receiving most attention (Pather et al. 2008).

Buccal drug delivery system has gained popularity over the recent past gaining some advantage over the other systems. One of the most preferred sites of buccal drug administration is the buccal mucosa. Its unique physiological features including large surface area, relative permeability and ease of access confer it with the desired characteristics for buccal drug delivery. Buccal mucosa is also suitable for both local and systemic delivery of drugs (Madhavi B et al. 2013). Buccal drug delivery offers many advantages over the other drug delivery systems especially in by passing first pass metabolism and increasing drug bioavailability (Shojaei 1998; Singh & Deep 2013; Salamat-Miller et al. 2005).

Bio-adhesion is a new phenomenon in drug delivery system whereby drugs are formulated in polymers that can adhere to biological membranes over a given period of time. Bio-adhesion occurs by different interaction means between the biological surface and the synthetic or natural polymers. Buccal adhesive films are made to adhere to the buccal mucosa and release the drug over a given period (Smart 2005).

1.3 Problem statement

Fluoxetine is one of the most preferred first line drugs in the management of depression. However, first pass hepatic metabolism, high protein binding and delay in absorption have resulted in low bioavailability and delayed onset of action of the drug. A drug delivery system which can ensure a combination of a faster onset of action and ease of administration and compliance by patients to alleviate the depressive mood is highly desired.

1.4 Objectives

1.4.1 General objectives:

To formulate buccal adhesive films containing fluoxetine hydrochloride.

1.4.2 Specific Objectives

- To perform pre-formulation studies to aid in the fabrication of fluoxetine buccal film.
- To develop the buccal film of fluoxetine using two mucoadhesive polymers blends.
- To characterise the prepared buccal films and evaluate their qualities and release kinetics.

1.5 Significance and anticipated outcomes

The prepared buccal films of Fluoxetine are expected to be within the limits of the critical quality attributes set for films. The film should be bioadhesive, have adequate swelling capacity and release the drug in a sustained release manner.

1.6 Delimitations

- Compatibility studies of the active pharmaceutical ingredient and excipients are determined to avoid drug excipient incompatibility.
- The different formulae for compounding were set up for a buccal film of Fluoxetine and were evaluated for their quality.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

The oral route is one of the most common and preferred routes of drug administration (Kaul et al. 2011). However, this route is associated with some drawbacks which hinder effective drug administration and bioavailability. Some of the problems associated with the oral route of drug administration includes first pass metabolism, gastric irritation and enzymatic degradation of the drug in GI tract (Kaul et al. 2011; Singh & Deep 2013; Shojaei 1998).

Alternative routes of administration are being sought and used to deliver drugs systemically to avoid the above problems. Such routes include intranasal (IN), buccal, pulmonary and transdermal delivery routes. The buccal drug delivery route is a new, attractive and feasible route for systemic drug delivery and offers several advantages over the per oral route (Kaul et al. 2011; Singh & Deep 2013).

Drugs administered by the buccal route is absorbed either sublingually, through the buccal mucosa or through the gums (gingival). The buccal mucosa is the preferred region for buccal drug administration compared to sublingual mucosa because of accessibility and ability to offer sustained release of a drug because of the relative permeability of buccal mucosa (Kaul et al. 2011).

To achieve localised drug delivery, formulations are increasingly incorporating mucoadhesive polymers along with the active ingredients. Mucoadhesive polymers increase the contact and interaction between the drug polymer combination and the mucus membrane thus increase the residence time for the absorption of the drug in the body. Ultimately less drug concentration and dosing frequency are needed to achieve the desired therapeutic outcomes (Kaul et al. 2011).

2.2. Anatomy of the buccal cavity

2.2.1 Anatomy of the oral mucosa

The oral mucosal region is generally adhesive in nature and acts as a lubricant reducing friction and allowing cells to move easily relative to each other. Drug delivery in this region is mainly through the following sites: buccal cavity, sublingual area, the palate and the gingival region (Madhavi B et al. 2013). The oral mucosa is composed of three distinct layers; the oral epithelium, basement membrane and connective tissues (fig 2.1) (Salamat-Miller et al. 2005; Madhavi B et al. 2013).

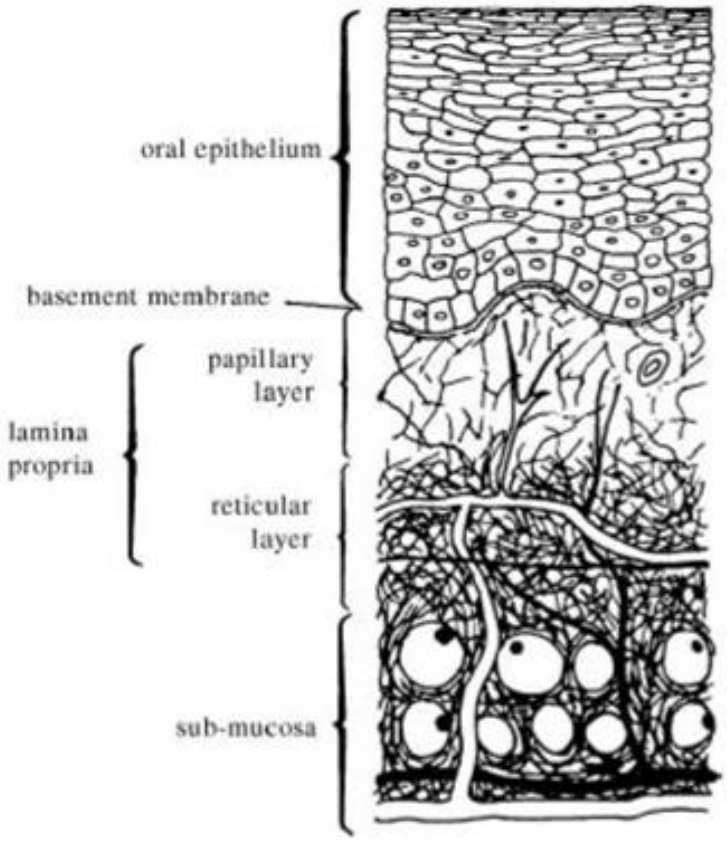


Figure 2.1: Anatomy of buccal mucosa (Salamat-Miller et al. 2005)

The epithelium lines the oral cavity providing protection for the tissues and cells beneath it. It is classified into keratinized and non-keratinized epithelium. The keratinized epithelium mostly lines the hard palate while the non-keratinized epithelium lines the soft palate floor of the mouth, lips vestibules and the other supple parts of the oral cavity. Epithelial cells develop from the basal cells and as they mature they move towards the surface, changing their size and shape. It is approximated that the buccal epithelium is about 500-800µm in thickness (Salamat-Miller et al. 2005). The buccal epithelium consists of non-keratinized cells penetrated by connective tissues known as lamina propria which consists of collagen fibres, blood vessels and smooth muscles. Blood supply for the buccal cavity is drawn from various arteries including buccal artery, some of the facial arteries and infraorbital artery (Kaul et al. 2011).

The basement membrane is a layer between the epithelium tissue above and connective tissue below. It provides support for the epithelium and adheres the two layers together. The basement layer also plays a role controlling the passage of materials across the epithelium into the connective tissue (Kaul et al. 2011).

2.2.2 The mucus layer

The mucus layer is a gel like translucent and viscid secretion of the oral mucosa that usually covers the entire oral cavity. The mucus layer is about 50-540um in thickness in human beings and its pH ranges between 6.2 and 7.4. The mucus acts as a protective barrier for the cells below and apart from that provides mucoadhesion and lubrication to the mucosal membrane. The mucus is produced by major and minor salivary glands in the oral cavity. It consists mostly of water soluble glycoproteins (0.5-5%), water (95%), enzymes, proteins and electrolytes. It is the glycoprotein part

(also known as mucin) that provides the viscoelastic properties of the mucus layer (Ramineni 2014).

2.3 Mucoadhesive drug delivery system

Mucoadhesive drug delivery system consists of water soluble polymers that hydrate upon contact with mucus tissue. The polymer and mucus tissue interact to form adhesive bonds. This intimate contact between the mucoadhesive system and mucosa increases the residence time of the system at the site, therefore, increasing the bioavailability of the drug (Ramineni 2014).

2.3.1 Penetration through the buccal mucosa

Drug penetration through the squamous epithelium lining of the mucosa occurs by two main routes:

- Transcellular (compounds pass through the cell) and
- Paracellular (compounds pass around the cell through intercellular spaces)

The physicochemical properties of the compounds will determine the route of permeation through the oral mucosa. Intercellular space which is hydrophilic in nature act as a barrier to lipophilic drugs while the lipophilic cell membranes act as barriers to hydrophilic drugs (Ramineni 2014). The paracellular route is the main route of drug permeation across buccal mucosa (Kaul et al. 2011; Morales & Mcconville 2011). Although it has been demonstrated that most compounds permeate the buccal mucosa by simple passive diffusion (i.e. Fickian diffusion), some are also transported by the aid of a carrier mediated process (Salamat-Miller et al. 2005).

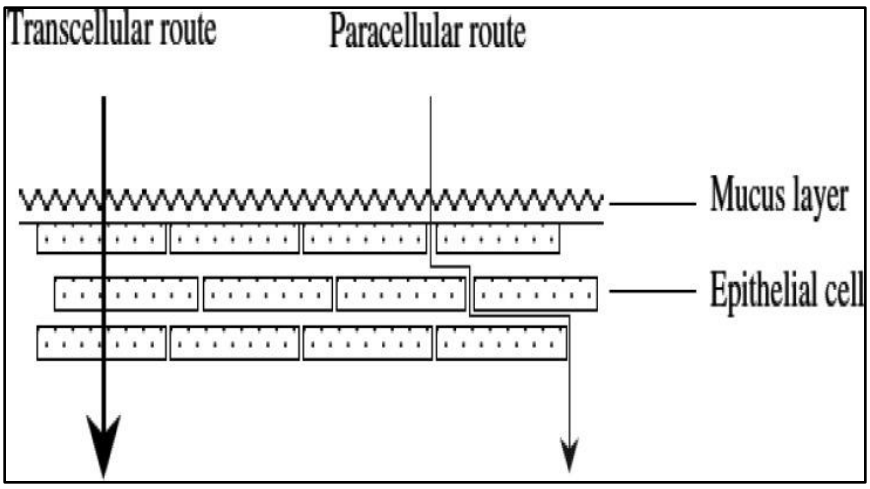


Figure 2.2: Paracellular and transcellular penetration routes in buccal drug delivery(Pather et al. 2008)

2.4 Properties of buccal film

2.4.1 Buccal adhesion

Salamat-Miller et al. (2005) describe bio adhesion as "the adherence of polymeric material to the biological surface or to mucosal lining." Buccal adhesion would then refer to attachment of polymeric material to the buccal mucosa.

2.4.2 Theories of mucoadhesion

From literature, various theories have been put forward to explain the mechanism of mucoadhesion (Donnelly et al. 2011; Ramineni 2014). It is believed that the polymers and mucin which is found in the mucus lining the oral cavity form bonds and adhere to each other by the following mechanisms;

I. Electronic theory

The difference in electronic properties between the polymer and mucin leads to electron transfer between the two surfaces resulting in their attraction (Salamat-Miller et al. 2005). The polymer being positively charged is attracted to the negatively charged mucin thus promoting adhesion (Ramineni 2014; Woertz et al. 2013).

II. Adsorption theory

In this theory, covalent and non-covalent bonds are formed upon initial contact between the polymer and mucus. The non-covalent bonds that may be formed include electrostatic, van der-waal forces, hydrogen bonds and hydrophobic bonds (Salamat-Miller et al. 2005; Ramineni 2014; Woertz et al. 2013).

III. Wetting theory

The bioadhesive polymer spreads on the mucosal surface. The theory is mainly used to explain liquid bioadhesive systems (Salamat-Miller et al. 2005).

IV. Diffusion theory

This theory suggests entanglement between mucin glycoproteins and the mucoadhesive polymer to form a network. The diffusion process is dependent on the concentration gradient and diffusion coefficient of polymer involved (Woertz et al. 2013).

V. Mechanical theory

The roughness of the buccal delivery system promotes adhesion due to increased contact area (Ramineni 2014).

2.5 Buccal films and film preparation methods

2.5.1 Buccal adhesive polymers

Shojaei et al. (1998) describe bioadhesive polymers as “polymers that can adhere to a biological surface” Buccal adhesive polymers then apply to mean the surface is buccal mucosa.

Many polymers have been studied for use as mucoadhesives. These include synthetic polymers such as cyanoacrylate, polyacrylic acid, hydroxypropyl methylcellulose and polymethacrylate derivatives as well as natural polymers like chitosan and hyaluronic acid (Shojaei 1998).

Mucoadhesive polymers generally contain numerous hydrogen bond forming groups which make the polymer hydrate and swell when in contact with aqueous solution. Mucoadhesive polymers need to be adequately hydrated to interact with the mucosa but not to be overhydrated as this could lead to loss of adhesive properties (Mortazavi & Aboofazeli 2000).

The two general characteristics of mucoadhesive polymers are that they should be non-irritant as well as be small and flexible (Shojaei 1998).

Mucoadhesive polymers usually hydrate upon contact with mucosal lining forming interactions with it leading to adhesion of the two surfaces.

2.5.2 Classification of mucoadhesive polymers

From literature, mucoadhesive polymers have been classified in different ways based on their source, solubility, charge and new generation of specific polymers (Ramineni 2014) as shown in Table 1 below (Salamat-Miller et al. 2005).

Table 2.1: Mucoadhesive polymers used in buccal drug delivery(Salamat-Miller et al. 2005; Singh & Deep 2013)

Criteria	Categories	Examples
Source	Natural/semi-synthetic	Agarose, chitosan, gelatin Hyaluronic acid Various gums (hakea, guar, xanthan, gellan, carragean, pectin and sodium alginate
	Synthetic	**Cellulose derivatives** [CMC thiolated CMC, sodium CMC, HEC, HPC, HPMC, MC,MHEC] **Polyacrylic acid based polymers** [CP, PC, PAA, polyacrylates, poly(methylvinylether-co-methacrylic acid), poly(2-hydroxyethyl methacrylate), poly(acrylic acid-co-ethylhexylacrylate), poly(methacrylate), poly(alkylcyanoacrylate), poly(isobutylcyanoacrylate), copolymer of acrylic acid and PEG] **Others** Poly(N-2-hydroxypropyl methacrylamide) PHPMAm, polyoxyethylene, PVA, PVP, thiolated polymers
Water solubility	Water soluble	CP, HEC, HPC, HPMC, PAA, sodium CMC, sodium alginate
	Water insoluble	Chitosan (soluble in aqueous acids) EC, PC

Charge	Cationic	Aminodextran, chitosan, dimethylamino ethyl (DEAE)-dextrose, trimethylated chitosan
	Anionic	Chitosan-EDTA, CP, CMC, pectin, PC, sodium alginate, sodium CMC, xanthan gum
Potential bioadhesive forces	Non ionic	Hydroxyethyl starch, HPC, poly(ethlenoxide), PVA, PVP, scleroglucan
	Covalent	Cyanoacrylate
	Hydrogen bond	Acrylates, PC, CP, PVA
	Electrostatic interaction	Chitosan

Natural polymers are synthesised naturally and harnessed from plant or animal origin. Examples of natural polymers used in mucoadhesion include; agarose, chitosan, hyaluronic acid, gelatin, xathan gum, gellan, pectin, sodium alginate, guar and hekea gum. These polymers are generally linear, hydrophilic in nature and contain negatively charged groups (Ramineni 2014; Salamat-Miller et al. 2005).

Synthetic polymers are derived from natural polymers through modification of some of the properties. The modifications made include; increase in molecular weight, addition of functional groups and introduction of charge to the polymer. Cellulose derivatives e.g. hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), hydroxyethyl cellulose (HEC), methyl cellulose (MC), acrylic acid polymers e.g. carbopol (CP), poly acylic acid (PAA) and poly

methylacrylate (PMA) and vinyl polymers eg. polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA) are some examples of synthetic polymers used in mucoadhesive films.

Depending on the charge, mucoadhesive polymers can be either cationic e.g. chitosan or can be anionic e.g. polycarbophil. New generation polymers have been developed by addition of thiol functional group to the polymer side chains. This provides specific interaction of the polymer with the mucin thus greatly increasing the mucoadhesion of the polymer. An example of such modified polymer includes chitosan/N-acetylcysteine, polyacrylic acid/ cysteine, chitosan/ thioglycolic acid. Lectin mediated polymers have also been developed. These polymers bind directly to the epithelial cell surface thus increasing retention of the system at the site even with wash out of mucus and saliva. Lectin is conjugated with the original polymer to develop this kind of polymer. Lectins, which are found in bacteria and plants interact with the sugars on the cell membrane to produce the adhesive interaction (Ramineni 2014; Andrews et al. 2009).

2.5.3. Methods of film preparation

The two main processes involved in the manufacture of buccal films are film casting and hot melt extrusion.

2.5.3.1. Film casting method:

Film casting is one of the most widely used method of preparation of films, mostly because of the ease of preparation and low cost of operation (Morales & Mcconville 2011). Morales and McConville (2011) describe film casting as a six step process with the following steps: fabrication of casting solution; deaeration of the solution; transfer of solution into a mould; drying of the casted solution; cutting the dosage form containing the desired amount of drug. Deaeration is an

important step to remove air that is in advertently introduced into the solution during the solution casting stage. This ensures uniformity and homogeneity in the system. The use of organic solvents in film casting is also an issue of concern. The problems associated with organic solvents include solvent collection, residual solvent and hazard to theenvironment and human health (Karki et al. 2016). Only ICH class 3 solvent list should be used in formulations whose drug and excipient properties depend on the organic solvent system (EMEA 2003).

2.5.3.3.2 Hot melt extrusion of films

The hot melt extrusion technology is not as widely used as the solvent casting method. This method produces the drug in the form of a solid dispersion and is therefore, mostly used to increase the solubility of poorly soluble drugs (Repka et al. 2005). In HME, the drug excipient mixture is blended, dried, melted in an extruder device and then passed through an orifice to form the films (Morales & Mcconville 2011). HME method has several advantages including; prepared films being non-brittle on storage, solvent free process, continuous processing and less time and energy consumption (Repka et al. 2005).

2.6. Active Pharmaceutical Ingredient

Fluoxetine hydrochloride

2.6.1. Chemistry and physicochemical properties:

The IUPAC name of fluoxetine (FLX) is N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine**.** It is a white to off white crystalline odorless powder with the chemical structure shown in figure 2.3 below. It has the molecular formula $C_{17}H_{19}ClF_3NO$ corresponding to a molecular weight of 345.79.

Fluoxetine melts in the range of 158.4 – 158.8℃. The molecule is freely soluble in methanol and ethanol. It is soluble in chloroform and acetonitrile but only slightly soluble in dichloromethane, ethyl acetate and water (maximum 14mg/ml). FLX is insoluble in toluene, cyclohexane and hexane (Risley & Bopp 1990).

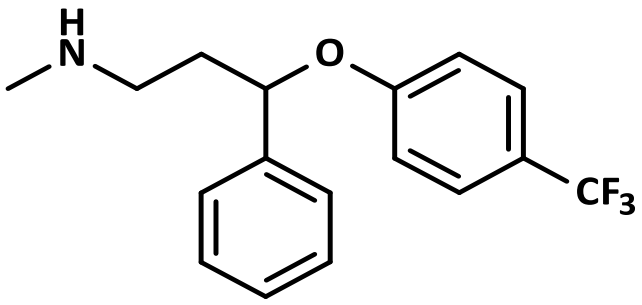


Figure 2.3: Chemical structure of fluoxetine

2.6.2. Pharmacodynamic profile

2.6.2.1. Mechanism of action

Fluoxetine exhibits its antidepressant activity by inhibition of serotonin (5-hydroxytryptamine, 5HT) uptake in a selective manner. FLX selectively inhibits 5HT transport by allosterically binding the serotonin transporter receptor (SERT) on a different site other than the serotonin binding site. The SERT is a glycoprotein embedded in the axon terminal and cell membrane of serotonergic neurones. When extracellular serotonin binds to receptors on the transporter, conformational changes occur in the transporter and serotonin. Na+, Cl- is moved into the cell. Binding of intracellular K+ the results in the release of serotonin inside the cell (Katzung & Trevor 2015; Wong et al. 2005; Benfield & Ward 1986).

2.6.3 Pharmacokinetic Profile

2.6.3.1. Absorption

Fluoxetine is rapidly absorbed after oral administration with 40mg dose giving peak plasma concentration of 15-55 ng/ml after 6-8hrs of administration. Systemic bioavailability of fluoxetine is about 60-70% (Altamura et al. 1994).

Systemic bioavailability of fluoxetine is not affected by presence of food, however, food may cause delay its absorption inconsequentially (Benfield & Ward 1986).

2.6.5.2. Distribution

Fluoxetine has a volume of distribution estimated at 30 to 40 l/kg and it's 94% protein bound (Altamura et al. 1994; Benfield & Ward 1986).

2.6.5.3. Metabolism and Excretion

Fluoxetine undergoes hepatic metabolism in which norfluoxetine and a number of other unidentified metabolites are produced. Norfluoxetine is an active metabolite and is formed by demethylation of fluoxetine (Altamura et al. 1994). Norfluoxetine exhibits equal potency and selectivity as of a serotonin uptake blocker and essentially its activity is equivalent to that of fluoxetine (Wong et al. 2005; Benfield & Ward 1986; Altamura et al. 1994).

Multiple cytochrome P450 isoenzymes, including CYP2D6, are responsible for the conversion of fluoxetine to norfluoxetine. Fluoxetine is primarily eliminated by hepatic metabolism to inactive metabolites excreted by the kidney with only 2-5% excreted unchanged (Altamura et al. 1994).

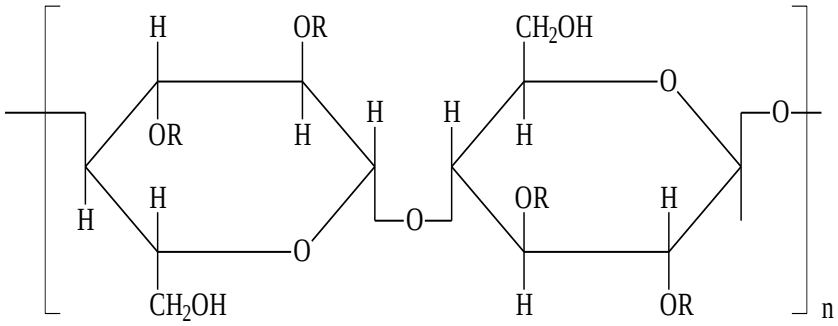
2.6.6. Dosage and administration

- For major depression, initial dose of 20mg administered daily then increased after 3-4 weeks if necessary and at appropriate intervals thereafter. Maximum dose is 60mg daily. For children 8-18 years, starting dose is 10mg daily, increased after 1-2 weeks to a maximum of 20mg daily.
- For management of bulimia nervosa, adults over 18 years administered daily dosage of 60mg as a single or divided dose.
- In obsessive compulsive disorder, adults over 18 years administered 20mg daily gradually increased to a maximum of 60mg (Katzung & Trevor 2015).

2.7 Polymers

2.7.1 Hydroxypropyl methylcellulose (HPMC)

HPMC is a water soluble ether derivative of cellulose with enhanced water retention capacity, pseudoplastic behavior and film forming property. HPMC is used in formulation of controlled release systems because of its good hydration and gel forming capabilities (Kadajji & Betageri 2011). Its structure is shown in figure 2.4 below.



n = polymer degree, R = —H, —CH_3, —$(CH_2CH(CH_3)O)_xH$

$(CH_2CH(CH_3)O)_xCH_3$

Figure 1.4: Structure of HPMC (Uslu & Aytimur 2012)

2.7.2 Polyvinylpyrrolidone (PVP)

PVP is a water soluble polymer commonly used as binder in tablets. It has a molecular weight range from 40.000 to 360.000 (Kadajji & Betageri 2011) and its structure is given in figure 2.5.

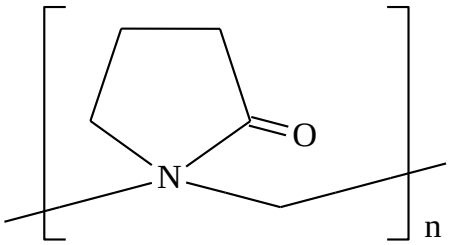


Figure 2.5: Structure of PVP

CHAPTER THREE: METHODS AND MATERIALS

3.1 Study design

This is a factorial experimental study.

3.2 Study location

The study was carried out in the laboratories of the Departments of Pharmaceutical Chemistry and Pharmaceutics at the University of Nairobi - School of Pharmacy and at the National Quality Control Laboratory located in Kenyatta National Hospital compound.

3.3 Active Pharmaceutical Ingredient and Excipients

Fluoxetine hydrochloride (Zhejiang Regen Chemical Co., LTD, China) was used as an active pharmaceutical ingredient (API) in this study. The excipients used include Hydroxypropyl methylcellulose E15 (Oxford Lab Chem, India), Polyvinylpyrrolidone K90 (BASF SE, Germany), ethanol AR grade (solvent), dichloromethane AR grade (solvent) and propylene glycol lab grade (Oxford Lab Chem, India).

3.4 Pre-Formulation Studies

3.4.1. Identification of active pharmaceutical ingredient

The identity of fluoxetine was confirmed by Fourier transform infrared spectroscopy. A potassium bromide (KBr) disk of fluoxetine raw material was prepared and scanned to obtain an IR spectrum of fluoxetine in the range of 4000 – 600 cm^{-1} using a Shimadzu FTIR spectrophotometer (Shimadzu IR prestige- 21). The spectrum was matched with those obtained from the literature reference standard.

3.4.2. Drug excipient compatibility study

Drug excipient compatibility study was performed using FTIR spectroscopy. The FTIR spectra of the pure drug and physical mixture of drug and polymers (1:1 ratios) were obtained from 4000 to 600cm-^{1}using KBr disc.

3.4.3 Solubility of drug and polymers

The solubility of 0.5 g Fluoxetine in various solvents was evaluated by dissolving the given amount of the drug in 20ml of distilled water, ethanol, methanol, dichloromethane, a mixture of ethanol and dichloromethane and a mixture of methanol and distilled water at room temperature and pressure.

3.5 Fabrication of fluoxetine hydrochloride buccal films

The films was prepared by solvent casting method. The calculated quantities of hydroxypropyl methylcellulose and polyvinylpyrrolidone (PVP) of each batch were dispersed in ethanol and dichloromethane according to table 3.1 below. An accurately weighed quantity of fluoxetine hydrochloride was mixed with 30% propylene glycol and sonicated for 10min. This solution was then mixed with the polymeric solution with continuous stirring to form a clear and consistent solution. This solution was poured into a glass Petri dish of 10cm diameter placed on a flat surface. An inverted funnel was placed over the dish to control the rate of evaporation. The mould was then left to dry overnight at room temperature to obtain cast films. The amount of drug per plate was 0.27g. Upon drying, the obtained films were checked manually looking out for any imperfections. The films were covered in wax paper and stored in desiccators for further analysis.

Table 3.1: Composition of fluoxetine films

Batch code	Polymer in % w/v		Plasticizer in % v/v
	HPMC	PVP	Propylene glycol
F1	2	0.5	2
F2	2	1	3
F3	2	1.5	4
F4	3	0.5	2
F5	3	1	3
F6	3	1.5	4
F7	4	0.5	2
F8	4	1	3
F9	4	1.5	4

3.6 Characterization of fluoxetine hydrochloride buccal films

3.6.1 Film weight and thickness

Three films of each formulation were weighed individually on a digital weighing balance. The mean weights were determined and standard deviations calculated. The film thickness was determined using a digital Vernier calliper, at least six different points in the film were measured and the average thickness calculated (Semalty et al. 2010).

3.6.2 Measurement of film pH

The buccal pH ranges from 5.5-7.4. All the formulations were tested for surface pH to ensure they do not produce any irritation in the buccal mucosa. One film from each batch was selected for determination of pH. The film was dissolved in 3ml of distilled water and the pH was measured using a Jenway pH meter (Jenway 3510 pH meter). The pH electrode was brought into contact with the film solution and after 5mins of stabilizing, the pH readings were recorded (Zaman et al. 2016).

3.6.3 Swelling index

Sample (n=3) of each formulation were pre-weighed on a cover slip. The films on the cover slips were then be placed in a Petri dish and simulated saliva solution pH 6.75 was added. The films were re-weighed at pre-set time intervals of 0.25, 0.5, 1, 2, 3, 4 and 5 hours to determine the increase in weight (Semalty et al. 2010). The swelling index was calculated using the following equation:

$\%S = [(W_t - Wo)/ Wo]$

Where Wt is the weight of the swollen film after time t, Wo is the initial film weight at time zero (Semalty et al. 2010).

The composition of simulated saliva includes 2.38gm of sodium hydrogen phosphate (Na_2HPO_4), 0.19g potassium hydrogen phosphate (KH_2PO_4) and 8.00g sodium chloride (NaCl) per litre of distilled water adjusted to pH 6.75 with phosphoric acid (Kshirasagar et al. 2012).

3.6.4 Folding endurance

This test is conducted to find out the flexibility of the film. The folding endurance of each of the formulated films was measured by repeatedly folding the film at the same point till it breaks or is folded up to 300 times. The number of folds a film can withstand without breaking is given as folding endurance. The mean and standard deviation of the values was calculated described in literature (Bala et al. 2014).

3.6.5 Drug content uniformity

Three cut films from the different formulations were each placed in separate volumetric flasks containing 25ml of simulated saliva solution. The flasks were shaken to dissolve the films. The final volume was adjusted to 100ml with simulated saliva solution pH 6.75. The solutions were filtered and analysed by UV spectrometer at λ max of 226nm. The average drug content of three films was determined using a calibration curve and taken as final reading (Chevala et al. 2015).

3.6.6 In-vitro drug release study

In-vitro drug release from fluoxetine films was studied using USP type II dissolution apparatus (Labindia DS 8000). Simulated saliva pH 6.75 was used as dissolution medium at 37±2ºC and the apparatus was stirred at 50 rpm. Each film containing 10mg of drug was cut and mounted on glass slide and held in position using paperclips then placed at the bottom of a dissolution beaker containing the simulated saliva. Twenty ml aliquots were withdrawn at predetermined time intervals of 15mins, 30min, 1hr, 2hr and 3hr and replaced with fresh simulated saliva solution to maintain sink conditions. The samples collected were assayed by UV spectrophotometer (Shimadzu U-1800 UV spectrophotometer) at λ max 226nm by comparing against a standard. Drug

release mechanism from the film was determined by fitting the release data to different kinetic models, zero order, first order and Higuchi (V et al. 2012) by using DD solver excel add-in program (Zhang et al. 2010).

CHAPTER 4: RESULTS

4.1 Pre-formulation studies

4.1.1Identification of active pharmaceutical ingredient

Identification of fluoxetine hydrochloride was carried out by using FTIR (Shimadzu IR prestige-21). From the FTIR spectrum obtained, the identity of fluoxetine was confirmed as shown in fig 4.1 below. The FTIR spectrum of fluoxetine showed major peaks corresponding to the major bonds and functional groups present in the molecule as shown in table 4.1.

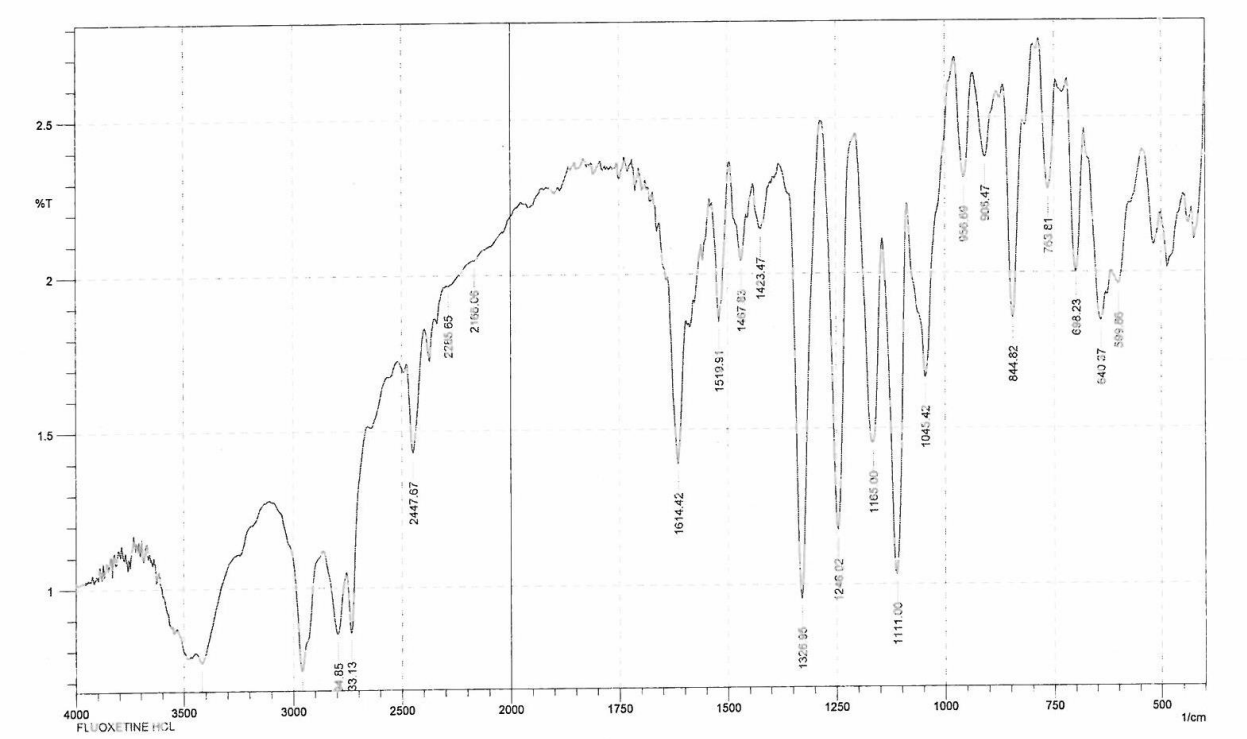


Figure 4.1: IR spectrum of fluoxetine

Table 4.1: Fluoxetine FT-IR peaks

Peaks cm^{-1}	**Bonds/ groups**
3417	N-H stretching of secondary amine
3100	Aromatic C-H stretching
2958	Csp^3-H stretching
1600-1400	Aromatic C=C stretching
1328	Stretching C-O bonds
1400-1000	Halide stretching vibration bonds C-F
1045	N-C stretching

4.1.2 Drug excipient compatibility studies

Drug excipient compatibility studies were conducted using FTIR. Samples of drug and excipients as well as physical mixtures of the drugs and excipients were evaluated by FTIR. From the IR spectra, it was observed that there were no significant shifts in the peaks corresponding to drug or the polymer. This showed that there is no drug excipient interaction. The IR spectra of drug and excipient blends are shown below in figures 4.2, 4.3, 4.4 and 4.5.

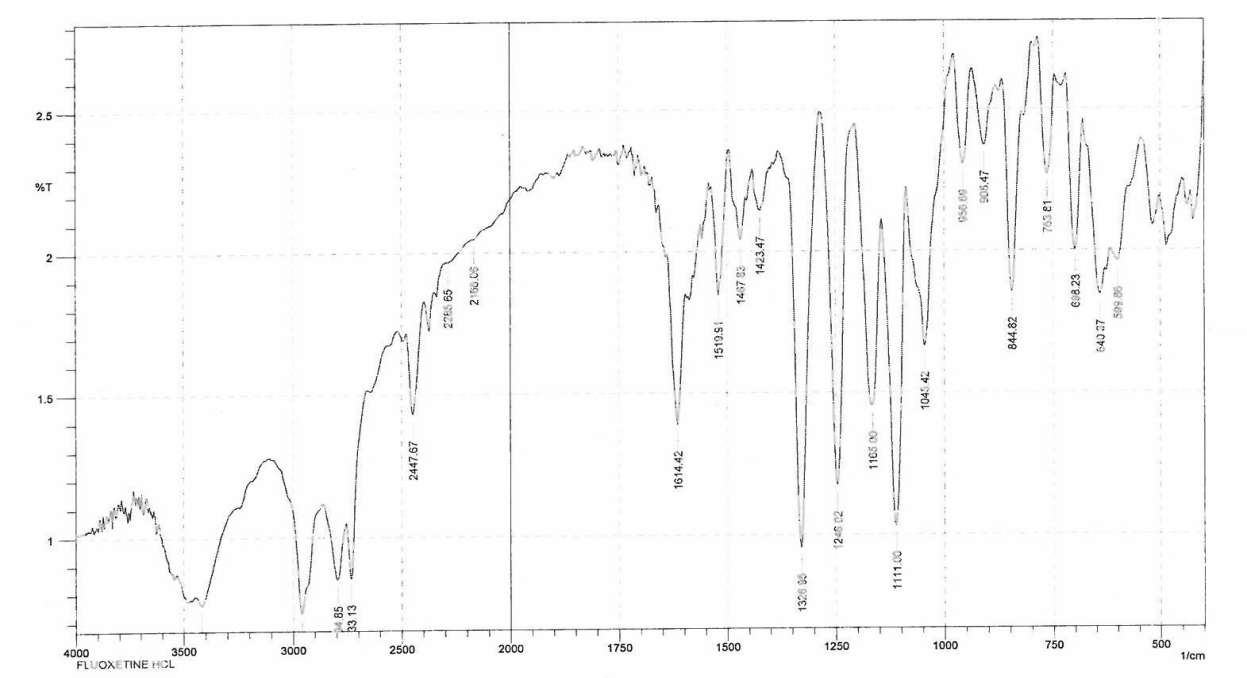


Figure 4.2: IR spectrum of fluoxetine hydrochloride

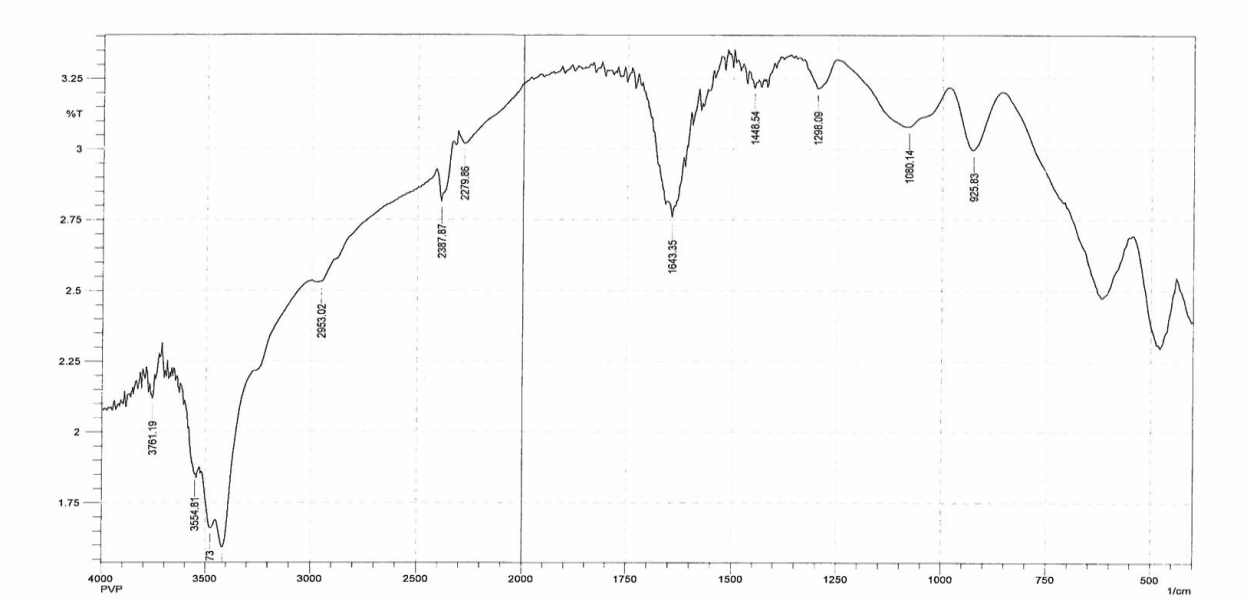


Figure 4.3: IR spectrum of PVP

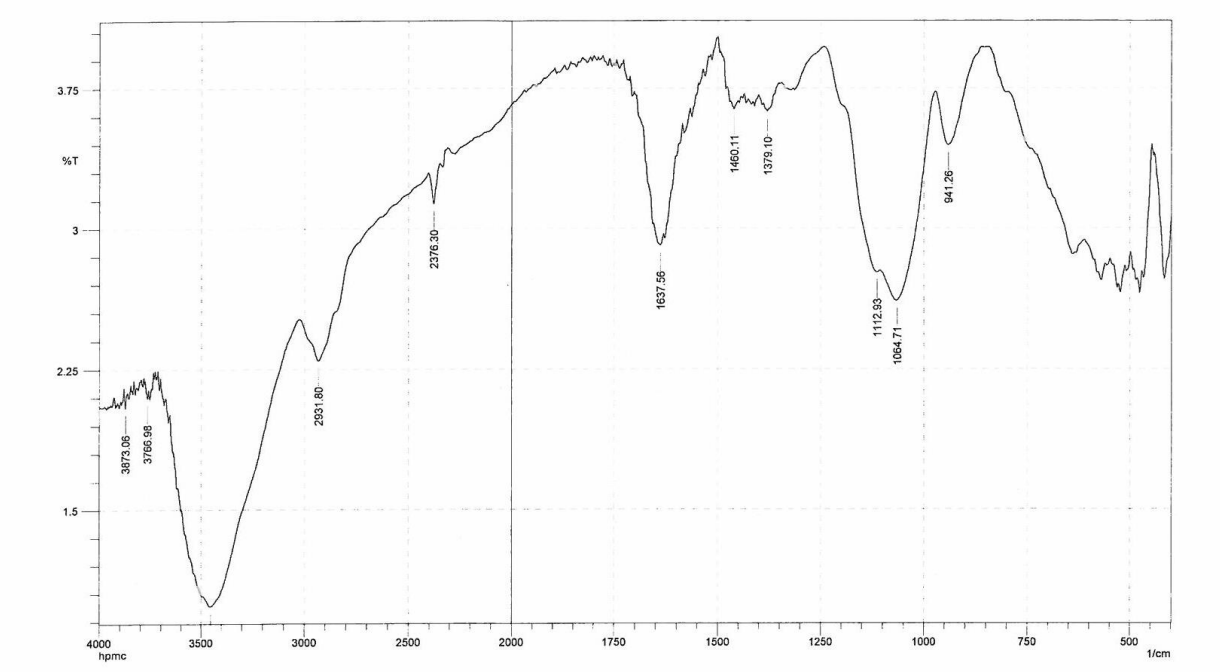


Figure 4.4: IR spectrum of HPMC

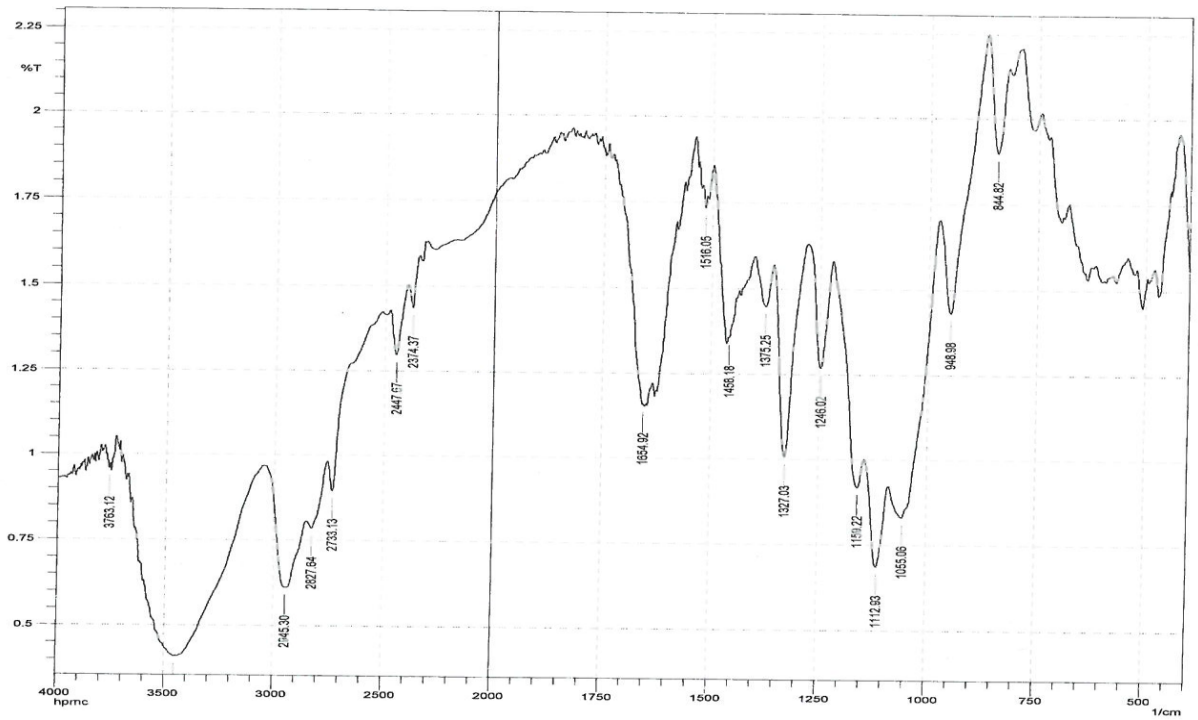


Figure 4.5: IR spectrum of drug polymer blend

4.2.3 Solubility of drug and polymers.

Solubility of the API and the polymers is very critical in the formation of buccal films and delivery of the drug. The solubility of fluoxetine and polymers was determined by mixing the powder in the different solvent systems at room temperature and pressure. Fluoxetine was found to be soluble in all the chosen solvents. HPMC and PVP showed complete solubility in ethanol/dichloromethane system. This was chosen as the solvent system of choice for fabrication of fluoxetine buccal films. Table 4.2

Table 4.2: Solubiltiy of drug and polymer

Solvent system	**Distilled water**	**Ethanol**	**Methanol**	**Dichloro methane**	**Ethanol/dichl oromethane**	**Methanol/ Dichlorome thane**
Fluoxetine	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
HPMC/PVP physical mixture(1:1)	Slightly soluble	Sparingly soluble	Slightly soluble	Slightly soluble	Soluble	Slightly soluble

4.2 Fabrication of fluoxetine buccal films

Buccal films containing fluoxetine hydrochloride were prepared using solvent casting method. Only formulations F1, F4, F7, F8 and F9 produced clear smooth films that were easily removed from the Petri dish without rupturing. Therefore, only films from the above five formulations were considered for further physical characterization.

4.2.1 Film weight and thickness

The average thickness of the prepared fluoxetine buccal films ranged from 0.117 to 0.533mm. Weight variation of the formulations F1, F4, F7, F8 and F9 was found to be between 31mg and 88mg. A proportional gain in weight of the films was observed as the film thickness increased. The increase in weight is due to the increase in polymer concentration across the formulations.

However, the values of weights within the respective groups remained uniform depicting uniformity in the casting of the film. The results of uniformity of weight and thickness are summarized in table 4.3 and 4.4 below.

Table 4.3: Weight of selected films

	Formulation				
Weight in g	**F1**	**F4**	**F7**	**F8**	**F9**
Weight 1 (g)	0.035	0.070	0.060	0.080	0.080
Weight 2 (g)	0.025	0.055	0.070	0.075	0.085
Weight 3 (g)	0.035	0.055	0.065	0.080	0.090
Weight 4 (g)	0.030	0.050	0.070	0.095	0.100
Weight 5 (g)	0.030	0.055	0.080	0.080	0.090
Weight 6 (g)	0.030	0.060	0.075	0.070	0.080
Average weight	**0.031**	**0.058**	**0.070**	**0.080**	**0.088**
Std deviation ±	0.003	0.006	0.006	0.008	0.007

Table 4.4: Thickness of selected films

	Formulation				
Thickness in mm	**F1**	**F4**	**F7**	**F8**	**F9**
Thickness 1 (mm)	0.200	0.200	0.200	0.600	0.600
Thickness 2 (mm)	0.100	0.100	0.200	0.500	0.600
Thickness 3 (mm)	0.100	0.100	0.200	0.500	0.500
Thickness 4 (mm)	0.100	0.100	0.200	0.500	0.500
Thickness 5 (mm)	0.100	0.100	0.200	0.500	0.500
Thickness 6 (mm)	0.100	0.100	0.200	0.500	0.500
Average thickness (mm)	**0.117**	**0.117**	**0.200**	**0.517**	**0.533**
Std deviation ±	0.037	0.037	0.000	0.037	0.047

4.2.2 Measurement of film pH

Alkaline or acidic pH in buccal formulations may cause irritation of buccal mucosa and affect hydration of polymers. The pH of the prepared films was determined by digital pH meter. Formulations F7, F8 and F9 had pH of 6.0. Formulations F1 and F4 had pH of 6.5 and 6.2 respectively. Results of pH of the buccal films are shown in table 4.5 below

Table 4.5: pH of selected films

Formulation/ pH reading	**F1**	**F4**	**F7**	**F8**	**F9**
Reading1	6.667	6.326	5.933	6.223	5.717
Reading2	6.559	6.183	6.221	5.835	6.169
Reading 3	6.448	6.358	5.856	5.957	6.142
Average pH	**6.558**	**6.289**	**6.003**	**6.005**	**6.009**
Std dev ±	0.089	0.076	0.157	0.162	0.207

4.2.3 Swelling index

The swelling behavior of the films over time is illustrated in table 4.6. F1 and F7 had the highest swelling index respectively followed by F4 then F9 and finally F8. There is a considerable drop in swelling index in high polymer concentration films F8 and F9. Swelling property of films has direct influence on the release of the drug.

Table 4.6: Swelling index of selected films

Formulation/ swelling index	**F1**	**F4**	**F7**	**F8**	**F9**
S.I 1	6	5.2	5.8	3	3
S.I 2	6.6	3.9	5	2.42	2.67
S.I 3	4.50	4.17	5.40	2.65	2.85
Average S.I	**5.70**	**4.42**	**5.40**	**2.69**	**2.84**
Std dev ±	0.883	0.560	0.327	0.238	0.135

4.2.4 Folding endurance

All the formulations passed the folding endurance test. Each film tested was folded more than 300 times at the same position without breaking or cracking. This is an indication of adequate amount of plasticizer used in the formulation. The values show the films are mechanically strong to avoid dislocation from site of administration or breaking during administration of the film.

4.2.5 Drug content uniformity

Uniformity of content is important ensure uniform distribution of the drug in film. Drug content uniformity was determined by UV spectroscopy. Results obtained are summarized in table 4.7 below.

Table 4.7: Drug content uniformity of selected films

Formulation/ UV absorbance	**F1**	**F4**	**F7**	**F8**	**F9**
Absorbance 1	0.298	0.382	0.33	0.393	0.446
Absorbance 1	0.321	0.383	0.33	0.392	0.435
Absorbance 1	0.321	0.386	0.36	0.393	0.439
Avg Absorbance	**0.3133**	**0.384**	**0.34**	**0.393**	**0.44**
Std dev ±	0.0108	0.0017	0.0141	0.0004	0.0045
Standard	0.373	0.373	0.373	0.373	0.373
% label claim	**84.00357**	**102.8597**	**91.15282**	**105.2726**	**117.9625**

4.2.6 In-vitro release studies

In-vitro release studies were performed by using simulated saliva solution in USP type II dissolution apparatus. Quantification of amount of fluoxetine released was done by use of UV spectrophotometer at 226nm. The studies were performed up to 3h. The results of in-vitro release studies are summarized below in table 4.8. Data from the in-vitro dissolution studies was fitted

into different kinetic models by using DD solver excel add-in program to investigate the release pattern of the drug. Release constants obtained and the values of coefficient of regression (R^2) are displayed in table 4.9. Graphs illustrating the in vitro release profile of the formulations as well as Higuchi's plot of the data and Korsmeyers-Peppas plot are display in figures 4.6, 4.7 and 4.8 respectively.

Table 4.8: In-vitro release studies of selected formulations

	Cumulative percentage drug released				
Time in mins	**F1**	**F4**	**F7**	**F8**	**F9**
15 mins	51.135	55.286	33.354	30.775	28.974
30 mins	54.659	68.990	41.112	37.588	38.058
60 mins	57.635	83.242	64.370	55.286	65.309
120 mins	63.900	87.001	78.387	87.940	87.471
180 mins	65.309	91.621	92.169	89.898	93.344

Table 4.9: Selected formulations fitted to various kinetic models

Formulation Codes	Zero order		First order		Higuchi		Korsemeyers-Peppas		
	Release constant	**R^2**	**Release constant**	**R^2**	**Release constant**	**R^2**	**Release constant**	**R^2**	**n**
F1	0.491	-32.46	0.014	-15.97	6.222	-9.958	38.549	0.987	0.102
F4	0.676	-7.074	0.041	0.575	8.442	-1.077	36.251	0.925	0.184
F7	0.613	-0.093	0.017	0.9168	7.280	0.9492	11.090	0.984	0.410
F8	0.614	0.41	0.016	0.9489	7.217	0.9545	8.372	0.958	0.468
F9	0.636	0.381	0.018	0.9854	7.499	0.9569	8.969	0.962	0.462

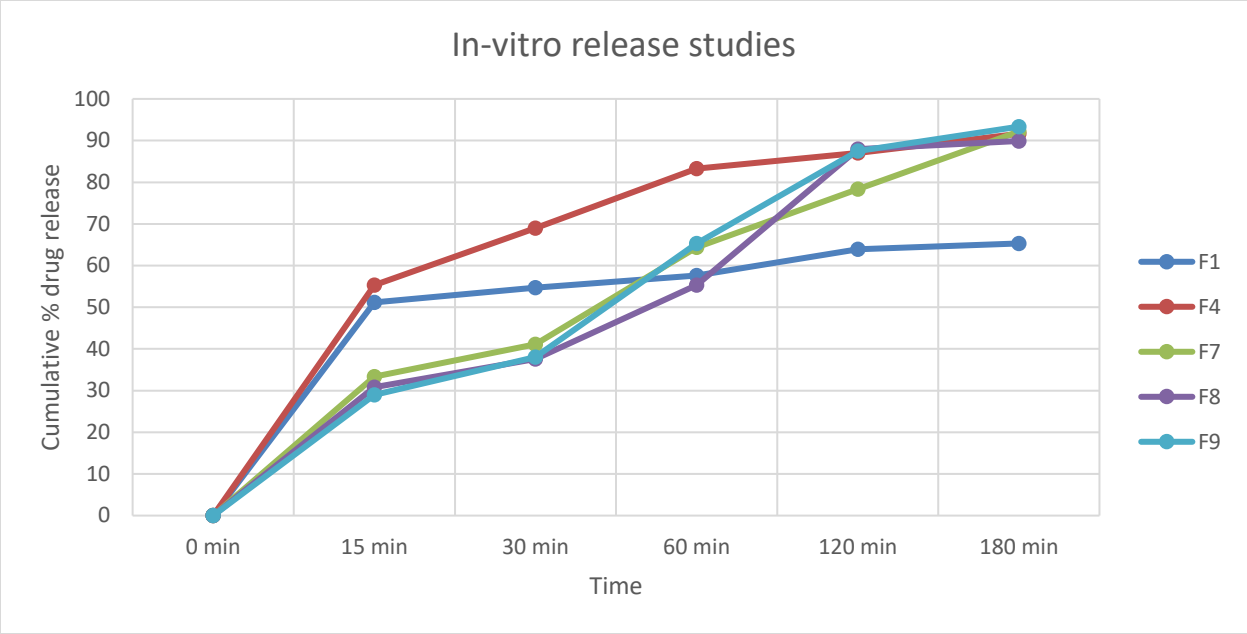


Figure 4.6: Graph of in-vitro drug release studies for selected formulations

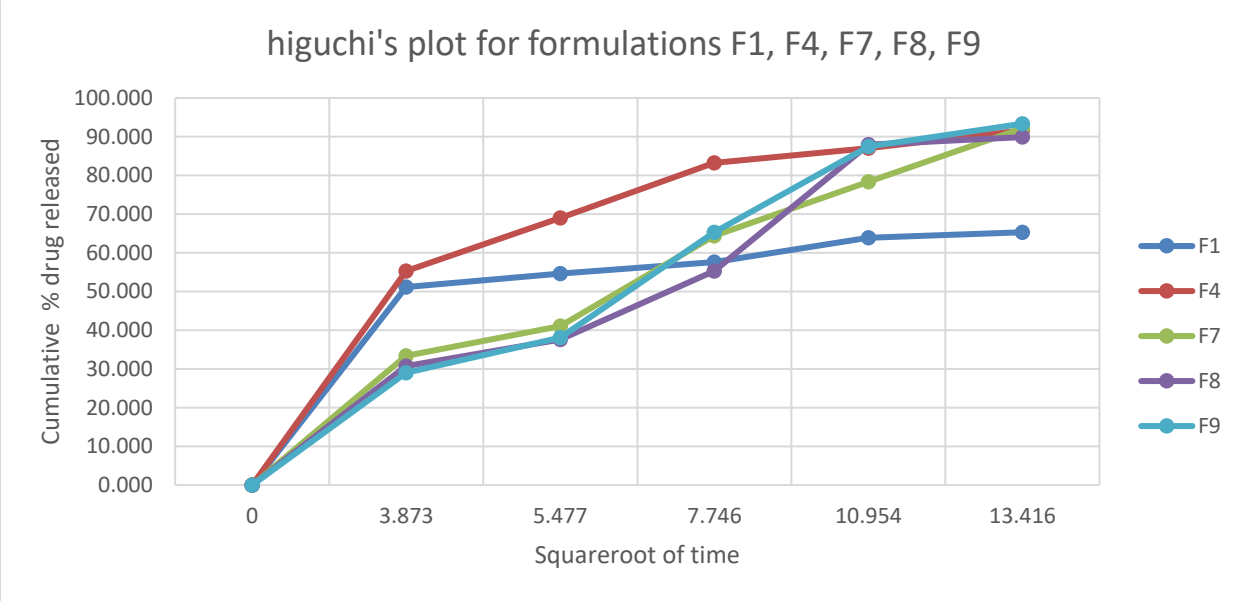


Figure 4.7: Higuchi's plot for selected formulations

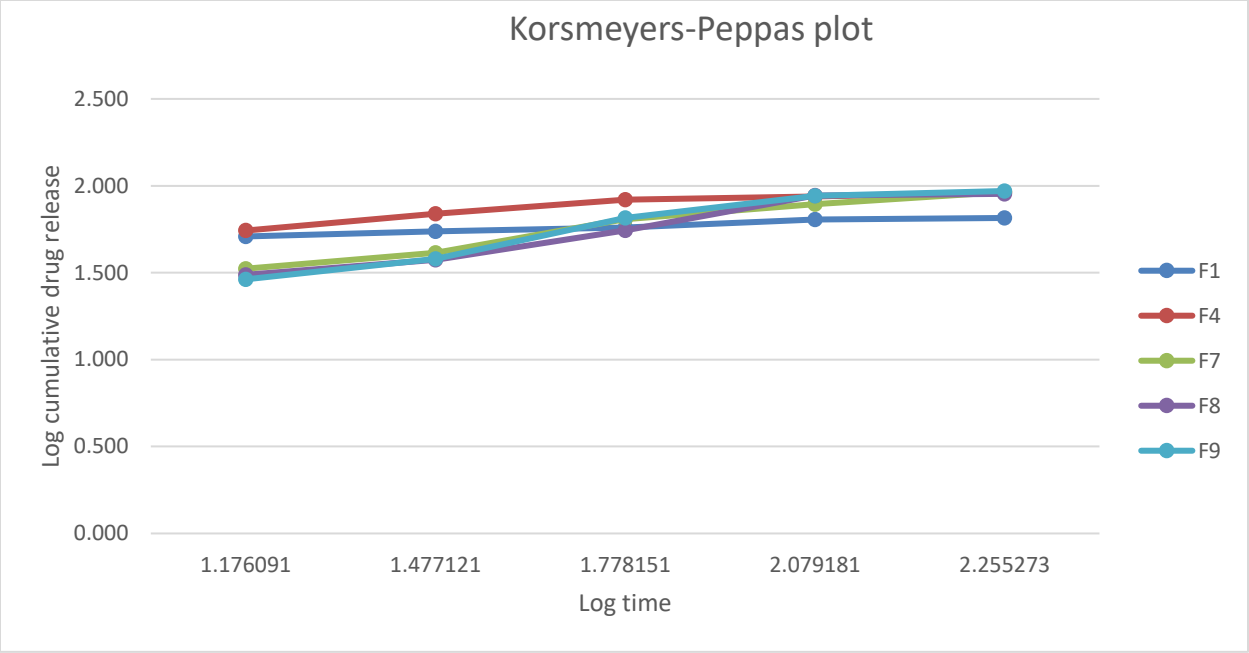


Figure 4.8: Korsmeyers-Peppas plot for selected formulations

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

Mucoadhesive buccal films consist of hydrophilic polymers which show adhesion to the mucosa after wetting with saliva. The mucoadhesive buccal films can be applied on several target sites in the oral cavity including buccal, palatal, lingual and gingival. They are also applicable for local of systemic action. In the presented study, fluoxetine buccal films were formulated by solvent casting technique using the polymers HPMC and PVP intended for systemic action. The polymers HPMC and PVP are both processes properties desired for formulation of buccal films.

In the formulation of the films, the concentration of polymers and plasticizer was varied; HPMC at (2%, 3% and 4%), PVP at (0.5%, 1% and 1.5%) and propylene glycol at (2%, 3% and 4%). The films were then analyzed on their organoleptic properties then on their physical properties.

5.2 Pre-formulation studies

Pre formulation studies were conducted to identify fluoxetine, to eliminate chances of drug excipient incompatibility and to determine suitable solvents for the drug and polymer combination. Identification of fluoxetine and drug excipient compatibility testing was done by FTIR spectroscopy. Fluoxetine was positively identified by the peaks of different functional groups and bonds seen in its IR spectrum as seen in figure 4.1. No drug excipient incompatibility was observed as the IR spectrum of the drug excipients blend maintained peaks characterized in fluoxetine, HPMC and PVP IR spectra. This is shown in figure 4.5. Solubility of the drug and excipients in different solvents was also conducted. The mixture of dichloromethane and ethanol (1:1) showed maximum solubility for the drug excipients blend and was chosen as the solvent system for formulation of the fluoxetine buccal films.

5.3 Fabrication of fluoxetine buccal films

Fluoxetine buccal films were formulated using HPMC and PVP as mucoadhesive and release polymers. Propylene glycol was used as plasticizer. Concentration of the polymers and plasticizers were varied to study their influence on the formulations as shown in table 3.1. Preliminary formulation studies showed recrystallization of fluoxetine in the films one week after formulation. The formulations mostly affected by this problem were those with low polymer concentrations. After further formulation trials and literature studies and the concentration on drug in the films was reduced to less than 20% of polymer weight. This yielded films that did not recrystallize on storage. Analysis was conducted on the organoleptic properties of the all the films and formulations F2, F3, F5 and F6 did not meet the standards of a good film. The formulations above did not have smooth surfaces and were sticky making their removal from the Petri dish very difficult. These imperfections were noted to occur due to the higher amount of plasticizer used in these formulations 2% and 3%. Increase in plasticizer concentration caused sticky and imperfect films. Only formulations F1, F4, F7, F8 and F9 showed good organoleptic properties and were considered for further physical characterization.

5.4 Characterization of prepared fluoxetine buccal films

The average thickness of the prepared fluoxetine buccal films ranged from 0.117 to 0.533mm. Weight variation values of the formulations F1, F4, F7, F8 and F9 were found to be between 31mg and 88mg. A proportional gain in weight of the films was observed as the film thickness increased. The increase in weight and thickness is due to the increase in polymer concentration across the formulations. However, the values of weights within the respective groups remained uniform depicting uniformity in the casting of the film.

As buccal formulations to be applied of oral mucosa, it is important to ensure the fluoxetine films meet set limits of pH to avoid irritation on use. The pH of the formulations was determined using digital pH meter (n=3). The pH of the formulations F1, F4, F7, F8 and F9 ranged between 6.0 and 6.5 [Table 4.5] and were within the set limits.

The swelling index of mucoadhesive polymer is vital factor in controlling rate of drug release and adhesion property. Upon hydration, the entangled polymer relaxes to expose bioadhesive sites for bond formation and release the drug. All the tested formulations showed gradual swelling during the testing period (data not presented). All the formulations showed sufficient swelling index ranging from 2.69 to 5.70. The degree of swelling is significantly lower in formulations F8 and F9 as compared to F1, F4 and F7 as seen in table 4.6. This decrease can be attributed to increased polymer concentration hence increased entanglement and slow relaxation of the polymers.

All the tested formulations showed adequate folding fortitude. The films were tested by repeatedly folding them at the same position and they were able to sustain more than 300 folds without breaking. The values demonstrate the films are mechanically strong to be handled and administered without breaking.

Uniformity of content test is used to validate dose uniformity in manufactured batch of formulation. It is important to ensure homogeneous distribution of the drug substance in polymer matrix during formulation. The drug content uniformity test for the tested formulations ranged from 84% to 117% [Table 4.7]. Formulations F1 and F9 had 84% and 117% respectively and were out of the target limits of 90-110%. Formulations F4, F7 and F9 had percentage label claim within limits.

All the tested formulations exhibited an initial rapid in-vitro release of the drug within 15min [Table 4.8] with F9 having the lowest percentage release of 28.97% at that time. Formulation F9 also showed the highest release (93.34%) in 3hrs. The release data from the selected formulations was subjected to various mathematical models including zero order kinetics, first order kinetics, Higuchi and Korsmeyers-Peppas models to understand the release pattern. (R^2) which is the value of the coefficient of regression was used to determine the best fit kinetic model. The best fit kinetic model for the formulations was found to be Korsmeyers-Peppas. Formulations F1, F4 and F7 had release exponents values below 4.5 [Table 4.9] indicating that the drug transport mechanism was mainly Fickian diffusion. Formulations F8 and F9 had the value of n at above 0.45 thus indicating anomalous transport was the main mechanism of drug transportation in the films. That means release of the drug from the films was governed by both diffusion and erosion of polymer.

5.5 Conclusion

In the present study, fluoxetine mucoadhesive buccal film based on HPMC and PVP was designed and fabricated and released the drug over the period of 3 hours. This formulation would help to by-pass first pass metabolism to a great extent and improve bioavailability. Buccal films containing fluoxetine were prepared by factorial design and the effect of formulation variables on physical characteristics and drug release were analyzed. On the basis of the results obtained, formulation F8 was found to be optimal formulation with the kinetic model. The formulation F8 can be optimized to produce controlled release of the drug beyond 3hrs after an initial burst release of the drug.

It was demonstrated that HPMC/PVP blend films had good film forming and swelling properties. These properties ensure the film sticks and releases the drug at site of application. The concentration of plasticizer used in formulations was found to be optimum at 2-3% producing

elegant non-sticky films. In summary, HPMC and PVP are potentially useful polymers for preparing mucoadhesive films of fluoxetine for buccal drug delivery system.

5.6 Recommendations

Due to limited resources and time, some detailed tests were not performed. More research needs to be done to establish the mechanical as well as mucoadhesive properties of the films by use of a texture analyzer machine. In-vitro permeation studies can also be performed using Franz diffusion cell to determine effectiveness of the drug delivery system.

It is recommended that further studies be undertaken to establish the stability of the films on storage under different climatic conditions.

REFERENCES

Aillon, J.-L. et al., 2014. Prevalence, types and comorbidity of mental disorders in a Kenyan primary health centre. *Social Psychiatry and Psychiatric Epidemiology*, 49(8), pp.1257–1268.

Altamura, A.C., Moro, A.R. & Percudani, M., 1994. Clinical Pharmacokinetics of Fluoxetine. *Clinical Pharmacokinetics*, 26(3), pp.201–214.

Andrews, G.P., Laverty, T.P. & Jones, D.S., 2009. Mucoadhesive polymeric platforms for controlled drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 71(3), pp.505–518.

Bala, R., Khanna, S. & Pawar, P., 2014. Design optimization and in vitro-in vivo evaluation of orally dissolving strips of clobazam. *Journal of drug delivery*, (Article ID 392783 15 pages).

Benfield, P. & Ward, A., 1986. Fluoxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. *Drugs*, 32(4), pp.313–334.

Brown, C.H., 2011. Pharmacotherapy of major depressive disorder. *U.S. Pharmacist*, 36(11).

Chevala, N.T. et al., 2015. Design and development of fluoxetine hydrochloride oro flash films to alleviate major depressive disorder. *Journal of Pharmaceutical Investigation*, 45(6), pp.493–501.

Donnelly, R. et al., 2011. Mucoadhesive drug delivery systems. *Journal of Pharmacy and Bioallied Sciences*, 3(1), p.89.

EMEA, 2003. ICH guideline Q3C (R6) on impurities: guideline for residual solvents.

International conference of harmonisation, 68(December 2016), p.35.

Ferrari, A.J. et al., 2013. Burden of Depressive Disorders by Country, Sex, Age, and Year: Findings from the Global Burden of Disease Study 2010. *PLoS Medicine*, 10(11).

Kadajji, V.G. & Betageri, G. V, 2011. Water soluble polymers for pharmaceutical applications. *Polymers*, 3(4), pp.1972–2009.

Karki, S. et al., 2016. Thin films as an emerging platform for drug delivery. *Asian Journal of Pharmaceutical Sciences*, 11(5), pp.559–574.

Katzung, B.G. & Trevor, A.J., 2015. *Basic and Clinical Pharmacology*,

Kaul, M. et al., 2011. An overview on buccal drug delivery system. *International journal of pharmaceutical sciences and research*, 2(6), pp.1303–1321.

Kshirasagar, N. et al., 2012. Design and Evaluation of Chitosan containing mucoadhesive buccal patch of Fluxotine Hcl. *International Journal of Scientific and Research Publications*, 2(6), pp.1–5.

Madhavi B, R. et al., 2013. Buccal Film Drug Delivery System-An Innovative and Emerging Technology. *Molecular Pharmaceutics & Organic Process Research*, 1(3), pp.1–6.

Mill, J. & Petronis, A., 2007. Molecular studies of major depressive disorder: the epigenetic perspective. *Molecular psychiatry*, 12(9), pp.799–814.

Morales, J.O. & Mcconville, J.T., 2011. Manufacture and characterization of mucoadhesive buccal films. *European Journal of Pharmaceutics and Biopharmaceutics*, 77(2), pp.187–199.

Mortazavi, S.-A. & Aboofazeli, R., 2000. Preparation and invitro assessment of various mucosa adhesive films for buccal delivery. *DARU*, 8(1&2), pp.9–18.

Pather, S.I., Rathbone, M.J. & Şenel, S., 2008. Current status and the future of buccal drug delivery systems. *Expert Opinion on Drug Delivery*, 5(5), pp.531–542.

Ramineni, S.K., 2014. *Mucoadhesive Films for Treatment of Local Oral Disorders : Development , Characterization and in Vivo Testing*.

Repka, M.A. et al., 2005. Characterization of cellulosic hot-melt extruded films containing lidocaine. *European Journal of Pharmaceutics and Biopharmaceutics*, 59(1), pp.189–196.

Richelson, E., 2001. Pharmacology of antidepressants. *mayo clinic proceedings*, (76), pp.511–527.

Risley, D.S. & Bopp, R.J., 1990. Fluoxetine. *Analytical Profiles of Drug Substances*, 19, pp.193–219.

Salamat-Miller, N., Chittchang, M. & Johnston, T.P., 2005. The use of mucoadhesive polymers in buccal drug delivery. *Advanced Drug Delivery Reviews*, 57(11), pp.1666–1691.

Semalty, A., Semalty, M. & Nautiyal, U., 2010. Formulation and Evaluation of Mucoadhesive Buccal Films of Enalapril Maleate. *Indian journal of pharmaceutical sciences*, 72(5), pp.571–575.

Shojaei, A.H., 1998. Buccal Mucosa As A Route For Systemic Drug Delivery : A Review. *Journal of Pharmacy & Pharmaceutical Science*, 1(1), pp.15–30.

Singh, J. & Deep, P., 2013. A Review Article on Mucoadhesive Buccal Drug Delivery System. *International journal of pharmaceutical science and research*, 4(3), pp.916–927.

Smart, J.D., 2005. The basics and underlying mechanisms of mucoadhesion. *Advanced Drug Delivery Reviews*, 57(11), pp.1556–1568.

Taurines, R. et al., 2011. Pharmacotherapy in depressed children and adolescents. *The World*

Journal of Biological Psychiatry, 12(sup1), pp.11–15.

Uslu, I. & Aytimur, A., 2012. Production and characterization of poly(vinyl alcohol)/ poly(vinylpyrrolidone) iodine/poly(ethylene glycol) electrospun fibers with (hydroxypropyl)methyl cellulose and aloe vera as promising material for wound dressing. *Journal of Applied Polymer Science*, 124(4), pp.3520–3524.

V, P. et al., 2012. Formulation and evaluation of mucoadhesive buccal patches of Aceclofenac. *Der Pharmacia Lettre*, 4(1), pp.297–306.

Wenthur, C.J., Bennett, megan R. & Lindsley, C.W., 2014. Classics in Chemical Neuroscience: Fluoxetine (Prozac). *ACS Chemical Neuroscience*, 5(1), pp.14–23.

Woertz, C. et al., 2013. Assessment of test methods evaluating mucoadhesive polymers and dosage forms: An overview. *European Journal of Pharmaceutics and Biopharmaceutics*, 85(3 PART B), pp.843–853.

Wong, D.T., Perry, K.W. & Bymaster, F.P., 2005. The discovery of fluoxetine hydrochloride (Prozac). *Nature Reviews Drug Discovery*, 4(9), pp.764–774.

World Health Organization, 2017. *Depression and other common mental disorders Global: health estimates*,

Zaman, M., Hanif, M. & Qaiser, A.A., 2016. Effect of polymer and plasticizer on thin polymeric buccal films of meloxicam designed by using central composite rotatable design. *Acta Poloniae Pharmaceutica - Drug Research*, 73(5), pp.1351–1360.

Zhang, Y. et al., 2010. DDSolver: An Add-In Program for Modeling and Comparison of Drug Dissolution Profiles. *The AAPS Journal*, 12(3), pp.263–271.