"FORMULATION AND EVALUATION OF SUBLINGUAL FILMS OF APIXABAN"

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ABSTRACT

The concept of sublingual film dosage form has become popular as new delivery system. This system will provide maximum therapeutic efficacy, increased bioavailability and maximum stability by reducing the frequency of dosage. It will also avoid first pass metabolism of the drugs. This system provides more rapid drug absorption from the pre gastric area which may provide quick onset of action. The present research aimed to prepare sublingual films of apixaban reduces the risk of stroke and systemic embolism in patients with nonvalvular atrial fibrillation. The film was prepared using solvent casting method and optimized employing 3^2 factorial design considering two independent variables film forming polymer (HPMC E5) and PEG 400. Disintegration time, drug release and folding endurance were taken as dependent variables. The prepared optimized formulation showed minimum disintegration time (35 s), highest dissolution rate (99 %) and satisfactory physicochemical properties. It is evident from the above results that the developed formulation can be an innovative dosage form to improve the drug delivery, onset of action as well as improve patient compliance.

Key Words: Apixaban, Sublingual films, HPMC

1. INTRODUCTION

1.1 Introduction of Drug Delivery System

1.1.1 Introduction of Sublingual Drug Delivery [1-4]

The term sublingual plainly means 'under the tongue'. It refers to a method of administering drug substances via mouth in such a way that the drug substances are rapidly absorbed in systemic circulation via highly vascularized sublingual route in the oral mucosa rather than digestive tract.

Dysphagia (difficulty in swallowing) is a common problem of all age groups, especially elderly, children, and patients who are mentally retarded, uncooperative, nauseated or on reduced liquid intake diets have difficulties in swallowing these dosage forms. Sublingual administration of the drug means placement of the drug under the tongue and drug reaches directly in to the blood stream.

The main mechanism for the absorption of the drug in to oral mucosa is via passive diffusion into the lipoidal membrane. The absorption of the drug through the sublingual route is 3 to 10 times greater than oral route and is only surpassed by hypodermic injection. For these formulations, the small volume of saliva is usually sufficient to result in tablet disintegration in the oral cavity.

Nitroglycerine, for example, is an effective antianginal drug but is extensively metabolized when taken orally (>90%). It is rapidly absorbed through the sublingual mucosa, and its peak plasma level is reached within 1-2 min. The blood concentration of nitroglycerine declines rapidly to a level below the therapeutic concentration within 10-15 min, because of its short biological half-life (3-5 min).

This overview describes a complete representation of sublingual drug delivery system comprising merits and demerits, various dosage forms and their formulation parameters, commonly used superdisintegrants, evaluation and some commercially available sublingual dosage form.

1.1.2 Types of Drug Delivery of Drugs via the membranes:- [5]

Within the oral cavity, delivery of drugs via the membranes of the oral cavity is classified into three categories.

A. Sublingual Delivery: -

Systemic delivery of drugs through the mucosal membranes lining the floor of the mouth to the systemic circulation.

B. Buccal Delivery: -

Drug administration through the mucosal membranes lining the cheeks and the area between the gums and upper and lower lips to the systemic circulation.

C. Local Delivery: -

Drug delivery to periodontal, gingival, delivery for the local treatment of ulcers, bacterial and fungal infections and periodontal disease. Sublingual administration of the drug means placement of the drug under the tongue and drug reaches directly into the blood stream through ventral surface of the tongue and floor of the mouth. The drug solutes are rapidly absorbed into the reticulated vein which lies underneath the oral mucosa, and transported through the facial veins, internal jugular vein, and brachiocephalic vein and then drained in to systemic circulation. The sublingual route usually produces a faster onset of action than orally ingested tablets and the portion absorbed through the sublingual blood vessels bypasses the hepatic first-pass metabolic processes

1.1.3 Advantages of Sublingual Drug Delivery System: - [6]

- ✓ Ease of administration to patients who refuse to swallow a tablet, such as pediatric, geriatric patients and psychiatric patients.
- ✓ A relatively rapid onset of action can be achieved compared to the oral route, and the formulation can be removed if therapy is required to be discontinued.
- ✓ The large contact surface of the oral cavity contributes to rapid and extensive drug absorption.
- ✓ Liver is bypassed and also drug is protected from degradation due to pH and digestive enzymes of the middle gastrointestinal tract.
- ✓ They also present the advantage of providing fast dissolution or disintegration in the oral cavity, without the need for water or chewing

1.1.4   Disadvantages of Sublingual Drug Delivery System: [7]

- ✓ Although this site is not well suited to sustained drug delivery systems.
- ✓ Sublingual medication cannot be used when a patient is uncooperative or unconscious.
- ✓ The patient should not smoke while taking sublingual medication, because smoking causes vasoconstriction of the blood vessels. This will
Decrease the absorption of the medication.

1.1.5 Criteria of Drugs for Sublingual Administration: - [8]

- ✓ No bitter taste
- ✓ Dose lowers than 20mg, e.g. nifedipine
- ✓ Small to moderate molecular weight
- ✓ Good stability in water and saliva
- ✓ Partially non ionized at the oral cavities pH
- ✓ Undergoing first pass effect e.g. ketotifen fumarate

1.1.6 Formulation of Sublingual Films: - [9, 10]

Mouth dissolving film/Sublingual films is a thin film with an area of 5-20 cm2 containing an active ingredient. The immediate dissolution, in water or saliva respectively, is reached through a special matrix from water-soluble polymers. A typical composition contains the following:

Table 1 Composition of fast dissolving oral film

Sr. No.	Composition of Film	Quantity (%)
1	Active pharmaceutical agent	01-25
2	Film forming polymer	40-50
3	Plasticizer	0.-20
4	Saliva stimulating agent	2-6
5	Sweetening agent	3-6
6	Flavoring agent	10
7	Coloring agent	1

1. **Active pharmaceutical agent**

The drugs selected for oral films should possess good stability in saliva and water with low dose. The film should consist of 1- 25% w/w of the drug. Small dose molecules are the best candidates to be incorporated in Oral fast dissolving film. Multi vitamin sup to 10% w/w of dry film weight was incorporated in the films with dissolution time of less than 60 seconds. It is always useful to have micronized API which will improve the texture of the film and also for better dissolution and uniformity in the Oral fast dissolving film.

2. **Film forming polymer**

A variety of polymers are available for preparation of fast dissolving films. The polymers can be used alone or in combination to obtain the desired films properties. The films obtained should be tough enough so that there won't be any damage while handling or during transportation. The robustness of the strip depends on the type of polymer and the amount in the formulation The polymers can be used alone or in combination to obtain the desired strip properties. Water-soluble polymers are used as film formers. The use of film forming polymers in dissolvable films has attracted considerable attention in medical and nutraceutical application. The water-soluble polymers achieve rapid disintegration, good mouth feel and mechanical properties to the films. The disintegration rate of the polymers is decreased by increasing the molecular weight of polymer film base. Both natural as well as synthetic polymers can be used in the formulation of sublingual films. In order to prepare a film formulation that is water-soluble, excipients or polymer must be water soluble with low molecular weight and excellent film forming capacity. At least 45% w/w of polymer should generally be present based on the total weight of dry film but typically 60-65%w/w of polymer is preferred to obtained desired properties. The polymer employed should be non-toxic, non-irritant and devoid of leachable impurities. It should have good wetting and spread ability property. The polymer should exhibit sufficient peel, shear and tensile strengths.The various natural as well as synthetic polymers to make fast dissolving films include cellulose or cellulose derivatives, pullulan, gelatin, hypromellose, hydroxyethyl cellulose, hydroxypropyl cellulose, polyvinyl pyrrolidone, carboxymethyl cellulose, polyvinyl alcohol, sodium alginate, xanthan gum, tragacanth gum and guar gum. Pullulan is a natural polymer obtained from nonanimal origin and does not require chemical modification.

3. **Plasticizers**

It helps to improve the flexibility of the strip and reduces the brittleness of the strip. Plasticizer significantly improves the strip properties by reducing the glass transition temperature of the polymer. Glycerol, propylene glycol ,low molecular weight propylene glycols, phthalate derivatives like dimethyl, diethyl and dibutyl phthalate ,citrate derivatives such as tributyl ,triethyl, acetyl citrate, triacetin and castor oil are some commonly used plasticizer excipients. Typically the plasticizers are used in the concentration of 0-20% w/w of the dry polymer weight.

4.   Saliva stimulating agent

The purpose of using saliva stimulating agents is to increase the rate of production of saliva that would aid in the faster disintegration of the rapid dissolving strip formulations .These agents are used alone or in combination between 2-6% w/w of the strip. Citric acid, malic acid, lactic acid, ascorbic acid and tartaric acid are the few examples of salivary stimulants.

5.   Sweetening agents

Sweeteners have become the important part of pharmaceutical products intended to be disintegrated or dissolved in the oral cavity. The classical source of sweetener is sucrose, dextrose, fructose, glucose, liquid glucose and isomaltose .Polyhydric alcohols such as sorbitol, mannitol and isomalt can be used in combination as they additionally provide good mouth feel and cooling sensation. The artificial sweeteners like Saccharin, cyclamate and aspartame are the first generation of the artificial sweeteners followed by acesulfame-k, sucralose, alitame and neotame which fall under the second generation artificial sweeteners. Generally sweeteners are used in the concentration of 3 to 6 %w/w either alone or in combination.

6.   Flavouring agents

Preferably up to 10%w/w flavors are added in the Fast dissolving film formulations The acceptance of the oral disintegrating or dissolving formulation by an individual is largely depends on the initial flavor quality which is observed in first few seconds after the product has been consumed and the after taste of the formulation which lasts for at least about 10 min. The geriatric population like mint or orange flavors while younger generation like flavors like fruit punch, raspberry etc. Flavoring agents can be selected from the synthetic flavor oils, oleo resins, extracts derived from various parts of plants like leaves, fruits and flowers. Any flavor can be added such as essential oils or water soluble extracts of menthol, intense mints such as peppermint, sweetmint, spearmint, wintergreen, cinnamon, clove, sour fruit flavor such as lemon, orange or sweet confectionary. Flavors such as vanillin, chocolate or fruit essence like apple, raspberry, cherry, pineapple.

7.   Coloring agents

A full range of colors is available including FD&C colors, EU colors, natural coloring agents and natural juice concentrates, pigments such as titanium dioxide, silicon dioxide and zinc oxide and custom pantone matched colors.

1.1.7 Manufacturing Methods: - [11, 12]

Following processes can be used to manufacture fast dissolving films:

> 1. Solvent casting
>
> 2. Semi solid casting
>
> 3. Hot melt extrusion
>
> 4. Solid dispersion extrusion
>
> 5. Rolling method

1.   Solvent casting method

In solvent casting method water soluble polymers are dissolved in water and the drug along with other excipients is dissolved in suitable solvent then both the solutions are mixed and stirred and finally casted in to the Petri plate, dried and cut in to uniform dimensions.

2.   Semi solid casting method

In semisolid casting method firstly a solution of water-soluble film forming polymer is prepared. The resulting solution is added to a solution of acid insoluble polymer (e.g. cellulose acetate phthalate, cellulose acetate butyrate), which was prepared in ammonium or sodium hydroxide. Then appropriate amount of plasticizer is added so that a gel mass is obtained. Finally the gel mass is casted in to the films or ribbons using heat controlled drums. The thickness of the film is about 0.015-0.05 inches. The ratio of the acid insoluble polymer to film forming polymer should be 1:4.

3.   Hot melt extrusion method

In hot melt extrusion method firstly the drug is mixed with carriers in solid form. Then the extruder having heaters melts the mixture. Finally the melt is shaped in to films by the dies.

There are certain benefits of hot melt extrusion.

➢   Fewer operation units

➢   Better content uniformity

➢   An anhydrous process

4.   Solid dispersion extrusion

In this method immiscible components are extrude with drug and then solid dispersions are prepared. Finally the solid dispersions are shaped in to films by means of dies.
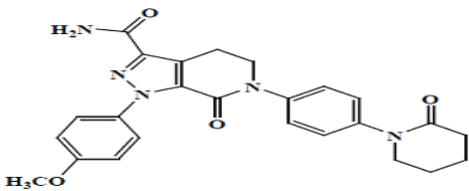
5.   Rolling method

In rolling method a solution or suspension containing drug is rolled on a carrier. The solvent is mainly water and mixture of water and alcohol. The film is dried on the rollers and cut in to desired shapes and sizes..

1.2   Introduction of Drug

• **Apixaban:-**[13-17]

Table 2 Drug Information

General Properties:-	
Name	Apixaban
Description	Apixaban is an oral, direct, and highly selective factor Xa (FXa) inhibitor (of both free and prothrombinase-bound FXa independently of antithrombin III) for the prevention and treatment of thromboembolic diseases. It is marketed under the name Eliquis. FDA approved on December 28, 2012.
Appearance	White to pale-yellow powder
Structure	
CAS number	503612-47-3
Category	Oral anticoagulant
Molecular Weight	459.4971 g/mol

Chemical Formula	$C_{25}H_{25}N_5O_4$
IUPAC Name	1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-1H,4H,5H,6H,7H-pyrazolo[3,4-c]pyridine-3-carboxamide
Solubility	Its aqueous solubility across the physiological pH range is ~0.04 mg/ml.
Water Solubility	1.98 mg/ml
Log P	1.83
pKa	13.12
Melting point (°C)	235-238 °C
Identification	FTIR, UV, HPLC
BCS Class	III
Hygroscopic	Non Hygroscopic
Dose	2.5/5 mg twice daily

Pharmacokinetic Properties:-

Absorption	Apixaban is absorbed in the stomach and small intestine. For doses up to 10 mg, the absolute bioavailability is about 50%.
Bioavailability	50 %
Protein binding	87 %
Metabolism	Hepatic
Half life	If administered orally, the half life is 12 hours (due to prolonged absorption). If administerd by I.V., the half-life is about 5 hours.
Excretion	About 27% of total Apixaban is renally cleared.

Pharmacological Properties:-

Indication	Apixaban is to lessen the danger of stroke and foundational embolism in patients with nonvalvular atrial fibrillation. It has likewise been utilized to bring down the danger of creating venous thrombosis post-orthopedic surgeries.
Mechanism of action	Apixaban acts by directly inhibiting, in a reversible manner, free and clot-bound factor Xa to inhibit coagulation.

Marketed Preparations:-

Brand/Generic Name	Availability	Company Name	
Eliquis	Tablets:- 2.5/5 mg	Pfizer	

1.3 Introduction of Excipients

1.3.1   Hydroxy Propyl Methyl Cellulose (HPMC) [18]

Nonproprietary Names	BP: Hypromellose JP: Hypromellose Ph. Eur: Hypromellose USP: Hypromellose
Synonyms	Benecel MHPC; E464; hydroxypropyl methylcellulose; HPMC; hypromellosum; Methocel; methylcellulose propylene glycol ether; methyl hydroxypropylcellulose; Metolose; MHPC; Pharmacoat; Tylopur.
Chemical Name and CAS Registry Number	Cellulose hydroxypropyl methyl ether [9004-65-3]
Functional Category	Bioadhesive material; coating agent; controlled-release agent; dispersing agent; dissolution enhancer; emulsifying agent; emulsion stabilizer; extended-release agent; film-forming agent; foaming agent; granulation aid; modified-release agent; mucoadhesive; release-modifying agent; solubilizing agent; stabilizing agent; suspending agent; sustained-release agent; tablet binder; thickening agent; viscosity-increasing agent.
Applications in Pharmaceutical Formulation or Technology	Hypromellose is widely used in oral, ophthalmic, nasal, and topical pharmaceutical formulations. In oral products, hypromellose is primarily used as a tablet binder, in film-coating, and as a matrix for use in extended release tablet formulations. Concentrations between 2% and 5% w/w may be used as a binder in either wet-or dry-granulation processes. High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10–80% w/w in tablets and capsules.

1.3.2 Polyethylene Glycol (PEG) [18]

Nonproprietary Names	BP: Macrogols JP: Macrogol 400 Macrogol 1500 Macrogol 4000 Macrogol 6000 Macrogol 20000 Ph. Eur: Macrogols USP-NF: Polyethylene Glycol
Synonyms	Carbowax; Carbowax Sentry; Lipoxol; Lutrol E; macrogola; PEG; Pluriol E; polyoxyethylene glycol.
Chemical Name and CAS Registry Number	A-Hydro-o-hydroxypropyl (oxy-1, 2-ethanediyl) [25322-68-3]

Functional Category	Ointment base; plasticizer; solvent; suppository base; tablet and capsule lubricant.
Applications in Pharmaceutical Formulation or Technology	Polyethylene glycols (PEGs) are widely used in a variety of pharmaceutical formulations, including parenteral, topical, ophthalmic, oral, and rectal preparations. Polyethylene glycol has been used experimentally in biodegradable polymeric matrices used in controlled-release systems.

1.3.3 Citric Acid [18]

Nonproprietary Names	BP: Citric Acid Monohydrate JP: Citric Acid Hydrate Ph Eur: Citric Acid Monohydrate USP: Citric Acid Monohydrate
Synonyms	Acidum citricum monohydricum; E330; 2-hydroxypropane-1,2,3-tricarboxylic acid monohydrate.
Chemical Name and CAS Registry Number	2-Hydroxy-1,2,3-propanetricarboxylic acid monohydrate [5949-29-1]
Functional Category	Acidifying agent; antioxidant; buffering agent; chelating agent; flavor enhancer; preservative.
Applications in Pharmaceutical Formulation or Technology	Citric acid (as either the monohydrate or anhydrous material) is widely used in pharmaceutical formulations and food products, primarily to adjust the pH of solutions. It has also been used experimentally to adjust the pH of tablet matrices in enteric-coated formulations for colon-specific drug delivery. Citric acid monohydrate is used in the preparation of effervescent granules, while anhydrous citric acid is widely used in the preparation of effervescent tablets.(2–4) Citric acid has also been shown to improve the stability of spray-dried insulin powder in inhalation formulations. In food products, citric acid is used as a flavor enhancer for its tart, acidic taste. Citric acid monohydrate is used as a sequestering agent and antioxidant synergist.

1.3.4 Aspartame

Synonyms	BP: Aspartame PhEur: Aspartamum USPNF: Aspartame
Chemical Name and CAS Registry Number	N-α-L-Aspartyl-L-phenylalanine 1-methyl ester [22839-47-0]
Empirical Formula and Molecular Weight	$C_{14}H_{18}N_2O_5$ ‚ 294.31gm/mole
Functional Category	Sweetening agent
Applications in Pharmaceutical Formulation or Technology	Aspartame is used as an intense sweetening agent in beverage products, food products, and table-top sweeteners, and in pharmaceutical preparations including tablets, powder mixes and vitamin preparations. It enhances flavor systems and can be used to mask some unpleasant taste characteristics; the approximate sweetening power is 180–200 times that of sucrose. Unlike some other intense sweeteners, aspartame is metabolized in the body and consequently has some nutritive value: 1 g provides approximately 17 kJ (4 kcal). However, in practice, the small quantity of aspartame

	consumed provides a minimal nutritive effect. Therapeutically, aspartame has also been used in the treatment of sickle cell anemia.

2   LITERATURE REVIEW

2.1 Review of Literature on Drug Delivery System

Rajni B et al [19] prepared fast dissolving films (FDF) of aprepitant used in the prevention and treatment of chemotherapy-induced nausea and vomiting. The FDF was prepared using solvent casting method and optimized employing central composite design considering two independent variables film forming polymer (pullulan) and PEG 400. Disintegration time, wetting time, drug release and folding endurance were taken as dependent variables. The prepared optimized formulation showed minimum disintegration time (20 s), highest dissolution rate (88.87%) and satisfactory physicochemical properties. It is evident from the above results that the developed formulation can be an innovative dosage form to improve the drug delivery, onset of action as well as improve patient compliance.

Soha S et al [20] developed Terbutaline sulfate fast dissolving sublingual films were prepared using seven drug compatible film formers in different combinations and proportions. The film polymers are maltodextrin, Na alginate, Carpabol 430, xanthan gum, HPMC E5, PVP K-25, and Na CMC. Propylene glycol and sorbitol were used as plasticizers and mannitol as filler. The optimum polymer concentrations and the plasticizer amount were selected on the basis of flexibility, tensile strength, and stickiness of the films. The prepared films were evaluated for their tensile strength, thickness uniformity, disintegration time (in vitro and in vivo), in vitro dissolution, and moisture content. Polymer type rather than total polymer concentration or plasticizer amount showed a significant effect on the tested film properties. A randomized, single dose, crossover study was conducted in four healthy volunteers to compare the pharmacokinetic profile of terbutaline sulfate from the prepared films and the conventional oral tablets. The film formula of choice gave a significantly faster drug absorption rate and recorded a relative bioavailability of 204.08%. Sublingual films could be promising as a convenient delivery system for terbutaline sulfate in patients with swallowing problems. The improved extent of absorption (higher AUC (0–24)) indicates success in improving drug bioavailability, and the faster absorption rate could be promising for the management of acute episodes of asthma.

Ramya D et al [21] formulate and evaluate oral thin films for sublingual administration of Amlodipine besylate. Amlodipine besylate is a calcium channel blocker used in the treatment of hypertension, angina and other cardiovascular diseases. Films were formulated using HPMC (15cps, 60cps), PVA, and HPC as different film-forming agents (3 and 4% (w/v)). Poly ethylene glycol 400 was used as plasticizer and SSG as disintegrant. Sodium saccharine was used to mask the bitter taste of the drug. Films were prepared by solvent casting method and evaluated for drug content, disintegration time, thickness, tensile strength, folding endurance and % cumulative drug released. The optimized formulation (HPMC 15 cps) F5 exhibited acceptable folding endurance (95), disintegration time (33 sec) and drug release of 98.5% in 360 sec. It can be concluded from the study that the Amlodipine oral thin films for sub lingual administration can be a potential novel drug dosage form.

Rakesh K et al [22] developed fast dissolving oral film of terbutaline sulphate using HPMC K15 LV as film forming polymer. The films were prepared by solvent casting method. Optimization of HPMC E15 LV, SSG and PEG-400 was carried out using 23 full factorial designs. The formulated films of terbutaline sulphate were evaluated for their physico-mechanical parameters like disintegration time, tensile strength, percent elongation, folding endurance and In-vitro drug release. Estimation of drug content uniformity of terbutaline sulphate films was performed and the results were satisfactory. Optimized batch F6 has

thickness (0.254±0.01mm), disintegration time (19.90±0.42 sec.) tensile strength (2.95±0.09 Mpa), % Elongation (18.95±0.12), Folding endurance (153±7.0), CPR1min 35.16±4.56% and CPR10min 80.29±4.15%.

Ali M et al [23] formulate the Fast dissolving oral films (FDOF) of Diazepam an anti epileptic drug which is normally administered by intramuscular route or as rectal suppository in acute conditions of seizure emergencies. Oral films were prepared by solvent casting method using HPMC E3, E5, and E15 as a film formers and propylene glycol, PEG 400 as plasticizers and evaluated for mechanical properties, disintegration and in vitro dissolution. All formulations showed good mechanical properties and in vitro drug release. The optimized (F4A) Formulation (HPMC E5 and PEG 400) Exhibited drug release of 99.89% in 15 minutes which was significantly high when compared to marketed tablet valium (68.81%).

Maryam M et al [24] worked on Sumatriptan succinate which is a 5-HT1 receptor agonist which is used in the treatment of migraine. It shows low bioavailability (15%) due to high hepatic first pass metabolism. The present work intended to formulate mucoadhesive sublingual films of sumatriptan combined with metoclopramide and sumatriptan alone with the objective of improving the therapeutic efficacy, patient compliance, and bioavailability. The sublingual films were formulated by solvent casting technique using mucoadhesive polymer of hydroxypropyl methylcellulose and propylene glycol as plasticizers. This study was also designed to evaluate the physicochemical and mucoadhesive characteristics of the films. The films were evaluated for their mechanical strength, folding endurance, drug content uniformity, swelling, in vitro residence time, in vitro release, in vitro bioadhesion, and in vivo mucoadhesion. They showed good appearance and elasticity. The best drugs of polymer ratio were S3 (1:2) and SM2 (2.7:1:8). The film of S3 and SM2 showed 10.6 and 11.01 mg weight, 2.2 and 22.5 μm thickness, 300 folding endurance, 55.9 and 100% content uniformity, respectively. The Differential Scanning Calorimetry (DSC) showed no stable sample of sumatriptan and metoclopramide in the drug loaded films and revealed amorphous form and transition of hydrate to anhydrous form for metoclopramide. The results showed that the films prepared were fast dissolving. The films (sumatriptan combined with metoclopramide and sumatriptan alone) exhibited very good mucoadhesive properties and shorter retention time (15-30 s). The formulations were found to be suitable candidates for the development of sublingual films for therapeutic uses.

Viviana D et a [25] Administered by an oral route, Furosemide (FUR), a diuretic used in several edematous states and hypertension, presents bioavailability problems, reported as a consequence of an erratic gastrointestinal absorption due to various existing polymorphic forms and low and pH-dependent solubility. A mucoadhesive sublingual fast-dissolving FUR based film has been developed and evaluated in order to optimize the bioavailability of FUR by increasing solubility and guaranteeing good dissolution reproducibility. The Differential Scanning Calorimetry (DSC) analyses confirmed that the film prepared using the solvent casting method entrapped FUR in the amorphous state. As a solid dispersion, FUR increases its solubility up to 28.36 mg/mL. Drug content, thickness, and weight uniformity of film were also evaluated. The measured Young's Modulus, yield strength, and relative elongation of break percentage (EB%) allowed for the classification of the drug-loaded film as an elastomer. Mucoadhesive strength tests showed that the force to detach film from mucosa grew exponentially with increasing contact time up to 7667 N/m^2. FUR was quickly discharged from the film following a trend well fitted with the Weibull kinetic model. When applied on sublingual mucosa, the new formulation produced a massive drug flux in the systemic compartment. Overall, the proposed sublingual film enhances drug solubility and absorption, allowing for the prediction of a rapid onset of action and reproducible bioavailability in its clinical application.

2.2 Review of Literature on Drug

Gambhire M et al [26] prepared and evaluated immediate release Apixaban tablets which contains super disintigrants via direct compression, to improve disintegration, dissolution and to get faster onset of action. Super disintigrants used in this formulation are microcrystalline cellulose and cross carmellose sodium, sodium starch glycolate.

Pravalika P et al [27] developed a simple, accurate and precise UV Spectrophotometric method for determination of Apixaban in bulk and pharmaceutical formulations. The optimum conditions for the analysis of drug are established and Apixaban is found to exhibit maximum absorption at 282 nm with DMSO as a solvent. The present method is validated as per guidelines of the International Conference on

Hormonization (ICH) guidelines including parameters like linearity, accuracy, and precision, limit of detection and limit of quantification.

Dudhe P et al [28] developed a simple, new, precise and reproducible two UV - spectrophotometric methods for the estimation of Apixaban in bulk and tablet dosage form. Methanol was used as a solvent to prepare standard and sample solutions.

2.3 Summary of PSAR Report

Sr. No.	Patent number Application	Title of Patent
1.	25/2017	Pharmaceutical Composition Of Apixaban
2.	47/2017	Apixaban Mouth Dissolving Formulations
3.	32/2015	Apixaban Liquid Formulations
4.	27/2015	Novel Crystalline Forms of Apixaban
5.	52/2017	Pharmaceutical Formulations of Apixaban

- **Looking at above 05 patents, your Dissertation project is novel up to what extent?**
  Novelty grade: 50 to 90%

- **RATIONAL OF PATENT**

Above five patents describes different formulation of Apixaban in different dosage form. No any patented work done on Apixaban sublingual films of. The principle of Apixaban sublingual films preparation offers a simple and practical approach to achieve quick onset of action for the dosage form and bypass the hepatic first pass effect of drug which ultimately improves bioavailability. Hence the selected title is novel and appropriate.

3 AIM & OBJECTIVES

3.1 Aim of Work

"Formulation and Evaluation of Sublingual Films of Apixaban"

3.2 Rationale

- ✓ Apixaban is an oral, direct, and highly selective factor Xa (FXa) inhibitor for the prevention and treatment of ***thromboembolic diseases***.
- ✓ Apixaban is to ***reduce the risk of stroke*** and ***systemic embolism*** in patients with nonvalvular ***atrial fibrillation***. It has also been used to lower the risk of developing venous thrombosis post-orthopedic surgical procedures.
- ✓ Its recommended oral dose is **2.5/5 mg/day**.
- ✓ Apixaban is belongs from **BCS class III** drug which has high solubility and low permeability.
- ✓ Half life is 12 hours.
- ✓ Its **Log P is 1.83** and Molecular weight is **459.49 g/mol.**
- ✓ **Bioavailability** of Apixaban is approximately **50%** due to **Hepatic first pass metabolism**.
- ✓ So its low molecular weight, high solubility and hepatic first pass effect makes it a good candidate for immediate release type of sublingual dosage form.
- ✓ By giving sublingual route, ***quick onset of action*** will achieved which requires in stroke condition and also ***by pass the hepatic first pass metabolism***.

3.3 Objectives of Work

- ✓ To check Drug: Excipients compatibility study by FTIR.
- ✓ To formulate Sublingual films of Apixaban having size of 2x2 cm^2 using film casting polymers by solvent casting method.
- ✓ To optimize the amount of polymer and Plasticizer in Films.
- ✓ To achieve disintegration time within 60 sec.
- ✓ To achieve more than 90 % drug release within 10 min in drug release study.

To perform accelerated stability study of films.

4   MATERIALS AND EQUIPMENTS

4.1 List of Materials

Table 3 List of materials

Sr. No.	Material	Function	Sources of Material
1.	Apixaban	API	Astra Life care, Ahmedabad
2.	Polyethylene glycol 400	Plasticizer	ACS Chemicals, Ahmedabad.
3.	HPMC E3, HPMC E5, HPMC E15,	Polymer	ACS Chemicals, Ahmedabad.
4.	Aspartame,	Sweetener	ACS Chemicals, Ahmedabad.
5.	Citric Acid	Saliva Stimulating Agent	ACS Chemicals, Ahmedabad.

4.2 List of Equipments

Table 4 List of equipments

Sr. No.	Equipments	Manufacturers
1.	Digital weighing balance	Reptech weighing balance ltd., Ahmadabad
2.	Vernier Caliper	Mitutoyo, Japan.
3.	Dissolution apparatus	Electrolab , Mumbai
4.	U.V.Visible spectrophotometer	Shimadzu-1601, Kroyoto, Japan.
5.	FTIR	FTIR8400S, Shimadzu, Kroyoto, Japan.
6.	Magnetic stirrer.	Janki Impex Pvt. Ltd, Ahmedabad
7.	pH Meter	Janki Impex Pvt. Ltd, Ahmedabad

8.	Stability Chamber	EIE Instrument Pvt. Ltd. Ahmedabad.
9.	Differential scanning calorimeter	DSC TA-60, M/s Shimadzu

5   EXPERIMENTAL WORK

5.1 Preformulation Study

5.1.1 Characterization of drug

By checking its visual Description, odour and solubility's in various solvents according to described in Indian Pharmacopeia monograph.

5.1.2 Drug-Excipients compatibility

5.1.2.1 FTIR Study

The FTIR of pure drug and optimized film was measured using Fourier transform infrared spectrophotometer. The pure drug and optimized film were separately mixed with IR grade KBr. This sample was scanned over a wavelength range of 4000 to 400 cm^{-1}.

5.1.2.2 DSC Study

The DSC of pure drug and optimized film was measured using Differential scanning calorimeter. Melting point of drug should be check and compared.

5.2 Determination of $λ_{max}$ and Standard Curve of Apixaban

Standard calibration curve of Apixaban was prepared using 6.8 phosphate buffer solutions. Weighed amount of drug 10 mg was added to 6.8 phosphate buffer. Further dilution was done with the help of same solvent. 5, 10, 15, 20, 25 and 30 μg/ml concentration solutions were prepared and absorption was taken at 279 nm in UV spectroscopy. Standard curve was plotted by taking concentration on X-axis and absorbance on Y-axis.

5.3 Calculation of the dose of Apixaban

Diameter of the Petridish = 9 cm

Radius = Diameter /2 = 9/2 = 4.5 cm.

Area of petridish = $πr^2$ = 3.14 X 4.5 X 4.5 = 63.585 cm^2

Now, Dose is 2.5 mg and Cut the pieces in 2 cm X 2 cm = 4 cm^2

4 cm^2 contain = 2.5 mg Drug

So, 63.585 cm^2 contain (?) Drug = ***39.74 mg ~ 40 mg Apixaban***

5.4 Preparation of Sublingual Films of Apixaban

5.4.1 Method of Preparation

Solvent Casting Method:

→   The solvent casting method was used for the preparation of the film.
→   Take required amount Jof drug and then dissolve it in Water.
→   Take required amount of film forming polymer and dissolve in water.
→   Mix both the solution with continuous stirring and then uniformly dispersed to get clear solution of film forming polymer.

→ After that required amount of plasticizer to be added to film forming solution.
→ Other ingredients & sweetener were dissolved one by one in previously prepared film forming solution with constant stirring to form clear solution.
→ The Prepared solution was kept in undisturbed condition till the entrapped air bubbles were removed.
→ The aqueous solution was casted in a glass petridish having area of 63.58 cm^2 and was dried at room temperature.
→ The petridish were put on the leveled surface during drying to avoid variation in the thickness.
→ The film took approximately 24 hours to dry at room temperature.
→ The dried film was carefully removed from the mould and was cut into size required for testing. The films were stored in airtight plastic bags till further use.

5.4.2 Formulation development of Apixaban sublingual films

Table 5 Trial Batches of Apixaban sublingual films

Ingredients	F1	F2	F3	F4	F5	F6
Apixaban	2.5	2.5	2.5	2.5	2.5	2.5
HPMC E3	100	200	-	-	-	-
HPMC E5	-	-	100	200	-	-
HPMC E15	-	-	-	-	100	200
PEG 400 (ml)	5	5	5	5	5	5
Citric acid	5	5	5	5	5	5
Aspartame	5	5	5	5	5	5
Water (ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Trial batches of Apixaban sublingual films were initiated with different grade of HPMC polymer. Three grade of HPMC polymer namely E3, E5 and E15 selected as they were widely used polymer in film formulations for fast drug release. These grades were different with respect to their viscosity grade. PEG 400 used as a plastsizer and citric acid used for saliva stimulation. Aspartame was used for sweetening purpose. Sufficient amount of water used for film casting as it was not present in final formulation. Approximately 30.0 ml water was used in all formulation.

5.4.3 Application of 3^2 Full Factorial Design for formula optimization

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man-hours and raw materials. Traditionally pharmaceutical formulations are developed by changing one variable at a time approach. The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interactions.

The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Yi) is measured for each trial

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{12} + b_{22}X_{22} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(1)$$

Where Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs and bi is the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed.

The polynomial equation can be used to draw conclusion after considering the magnitude of coefficient and the mathematical sign it carries, i.e., positive or negative. The high values of correlation coefficient for the dependent variables indicate a good fit. The equation 1 may be used to obtain estimate of the response because small error of variance was noticed in the replicates.

Table 6 3^2 Full Factorial Designs

Batch No.	X1 Amount of PEG 400	X2 Amount of HPMC E5
A1	-1	-1
A2	-1	0
A3	-1	+1
A4	0	-1
A5	0	0
A6	0	+1
A7	+1	-1
A8	+1	0
A9	+1	+1
Translation of coded level in actual limit		

Independent variables	Real Value		
	Low(-1)	Medium(0)	High(+1)
Amount of PEG 400 (X$_1$) ml	5	10	15
Amount of HPMC E5 (X$_2$) mg	150	200	250

All 9 batches were evaluated for the Disintegration time, folding endurance and Q $_{2\ min}$ % Release. (Y$_1$, Y$_2$ and Y3) to find out effect of the both parameters (X$_1$, X$_2$) on the film.
Independent variables
X$_1$-Amount of PEG 400
X$_2$-Amount of HPMC E5
Dependent variables
Y$_1$- Disintegration time
Y$_2$- Folding Endurance
Y3- Q $_{2\ min}$ % Release

Table 7 Factorial Batches of Apixaban sublingual films

Ingredients	A1	A2	A3	A4	A5	A6	A7	A8	A9
Apixaban (mg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
HPMC E5 (mg)	150	200	250	150	200	250	150	200	250
PEG 400 (ml)	5	5	5	10	10	10	15	15	15
Citric acid (mg)	5	5	5	5	5	5	5	5	5
Aspartame (mg)	2	2	2	2	2	2	2	2	2

Water (ml)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

5.5 Evaluation of Apixaban sublingual films

Films should be stiff, flat and should not curl on the edges. The Sublingual film strip must be robust enough to be removed from the unit-dose packaging and to be handled by the consumer without breaking. The film must also dissolve readily in order to deliver the active agent rapidly when placed in the oral cavity. Mechanical property of fast dissolving film plays an important role in deciding all these things. Therefore, the mechanical property of fast dissolving film is as important as its solubility rate. So the prepared sublingual films were evaluated for the following parameters:

5.5.1 Weight Variation

Four centimeter square (2 X 2 cm) of the film was cut at three different places from the casted film. The weight of each film was taken and weight variation was calculated.

5.5.2 Thickness

Randomly 3 films were selected and thickness was measured using a digital vernier caliper (Mitutoyo, Japan).

5.5.3 Measurement of *in vitro* disintegrating

The disintegration time was measured (n=3) for film of each batch in 10 ml of simulated saliva (pH 6.8) in glass petri dish. Film sample (2 cm x 2 cm) was placed in 10 ml of simulated saliva. The time for complete solubilization of the film was recorded as disintegration time. The average of three measurements was taken into consideration.

5.5.4 Measurement of Mechanical Properties

The mechanical property of the film gives idea about to what extent the film can withstand the force or stress during processing, packaging, transport and handling. The measurement of mechanical properties gives an indication of the strength and elasticity of the film, reflected by the parameters, i.e. tensile strength, % elongation and folding endurance. A suitable film should have a relatively moderate tensile strength, high % elongation at break but a low elastic modulus.

Mechanical properties of films like Tensile Strength measured by texture analyzer. A small film strip (2 cm X 2 cm) was cut and fixed to assembly. Each test strip was placed in tensile grips on the texture analyzer. Speed 1mm/s. The force in Newton required to break the film was noted and simultaneously film elongation was measured with the help of pointer mounted on the assembly. Measurements were done in triplicate for each batch. Three mechanical properties namely tensile strength, % elongation and folding endurance of films were evaluated.

- **Tensile Strength**

Tensile strength is the maximum stress applied to a point at which the film specimen breaks. The tensile strength (TS) can be calculated by dividing the maximum load by the original cross-sectional area of the specimen and it is expressed in force per unit area (N/mm^2).

$$\text{Tensile strength} = \frac{\text{Load at failure}}{\text{Strip thickness} \times \text{Strip width}}$$

- **Percent Elongation at break (%E)**

When stress is applied, a film sample stretches, and this stress is referred to as strain. Strain is basically the deformation of the film divided by the original dimension of the sample. As the plasticizer content increases, the elongation of film is observed.

$$\text{Percentage elongation} = \frac{\text{Increase in length of strip}}{\text{Initial length of strip}} \times 100$$

- **Folding Endurance**

Folding endurance was determined by repeatedly folding the film at the same place until it break. The numbers of times the film can be folded at the same place without breaking give the value of folding endurance.

5.5.5 Drug content

The film unit (n=3) of the dimensions 2 cm× 2 cm was placed in 100 ml of simulated salivary fluid. After complete solubilization, the solution was diluted appropriately, filtered and analyzed by UV method. The average of three films was taken as the content of drug in one film unit.

5.5.6 *In Vitro* Dissolution Study

Dissolution study of sublingual films was carried out using USP type I (Basket apparatus) with 300 ml of simulated salivary fluid (pH 6.8) as dissolution medium maintained at 37 ± 0.5°C. Medium was stirred at 50 rpm. Samples were withdrawn at every 2 min interval, replacing the same amount with the fresh medium. Amount of drug in the withdrawn samples was determined by UV spectrophotometer. The percent drug released was plotted against time.

5.5.7 Surface pH

The surface pH of film was determined in order to investigate the possibility of any side effect in vivo. As an acidic or alkaline pH may cause irritation of the oral mucosa, it was determined to keep the surface pH as close to neutral as possible. A combined pH electrode was used for this purpose. Oral film was slightly wet with the help of water. The pH was measured by bringing the electrode in contact with the surface of the oral film. The procedure was performed in triplicate and average with standard deviation was reported.

5.5.8 Swelling Index

Simulated saliva solution is used to check the swelling studies of films. Initial weight of film is determined and is placed in petri dish containing 10 ml simulated saliva. Increase in film weight is noted at predetermined time interval until no further increase in weight.

$$\text{Degree of swelling} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial Weight}}$$

5.5.9 Stability Study

Stability study was carried out at 45°C/ 75% RH conditions. Each piece of the films of formulation was packed in butter paper followed by aluminum foil and plastic tape. After 1 month, the films were evaluated for the physical appearance, disintegration time, drug content and in vitro drug release.

6   RESULTS AND DISCUSSION

6.1 Preformulation Study

6.1.1 Characterization of drug

First of all after receiving a sample of drug we have to check out the basic characteristic of API which is given in below table.

Table 8 Characterization data of Apixaban	
Test	**Results of Analysis**
Description	It is a white crystalline solid powder
Solubility	Soluble in methanol, soluble in water
Odour	Odorless

Results of Preformulation study for Apixaban are shown in table 8. Drug was found to be freely soluble in methanol and in water, so solvent casting method can be used for Film Preparation.

6.1.2 Compatibility Study by FTIR

The spectra of the selected formula with the pure drug had shown all the characteristic peaks of the drug, from which we conclude the stable nature of the drug during the process. Characteristics peaks obtained for the pure drug correlated well with that of the formulation peaks. This indicated that the drug was compatible with the formulation components.
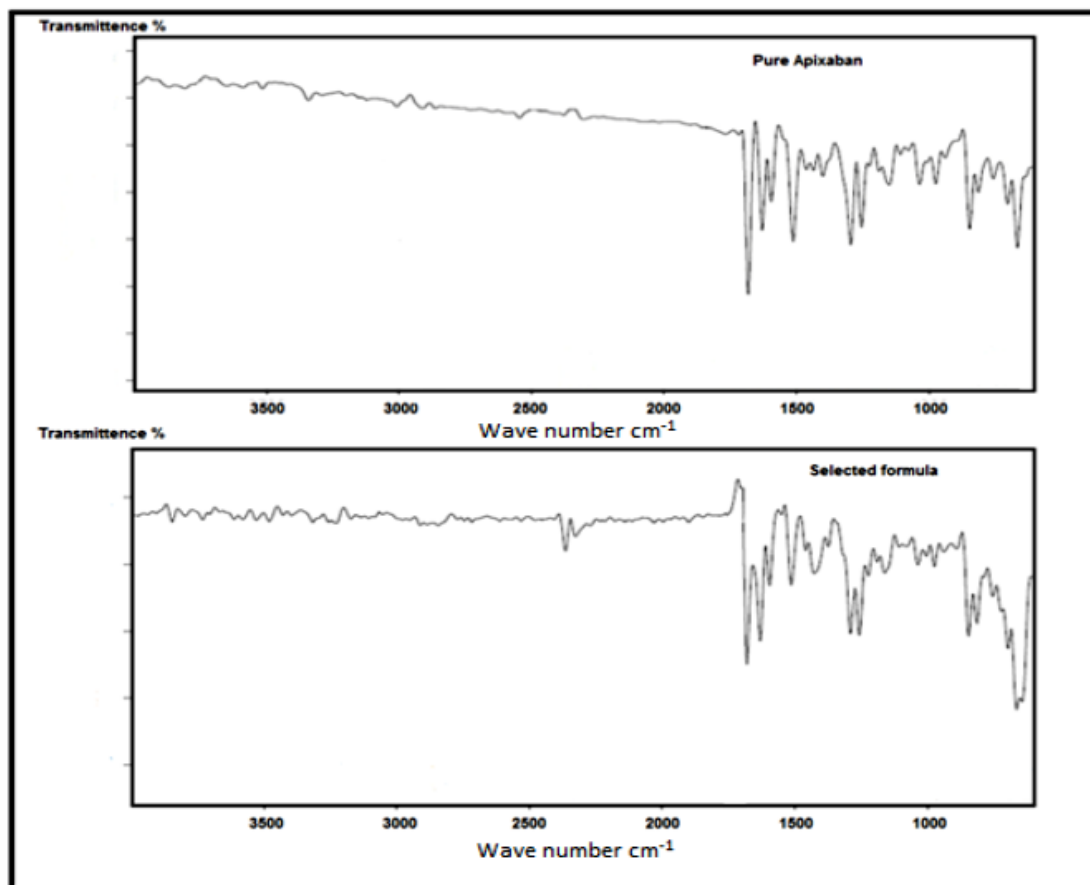
Figure 1 FTIR spectra of Pure API Apixaban and Formulation

Table 9 FTIR Data of Apixaban and Final Formulation

tretching	ure Drug Peak (cm^{-1})	ormulation Peak (cm^{-1})
C-H stretch	325.10	329.18
=O- NH stretch	688.00	686.72
I-H stretch	325.87	328.50

6.1.3 Compatibility Study by DSC

DSC study of pure drug and the final formulation performed to check the compatibility between drug and final formulation excipients. The pure drug peak match with the final formulation peak and hence the drug was found compatible with the selected excipients. Pure drug melting point found 237.39 °C and in formulation it was 237.54 °C. Both values were within the reference data 235-238 °C.
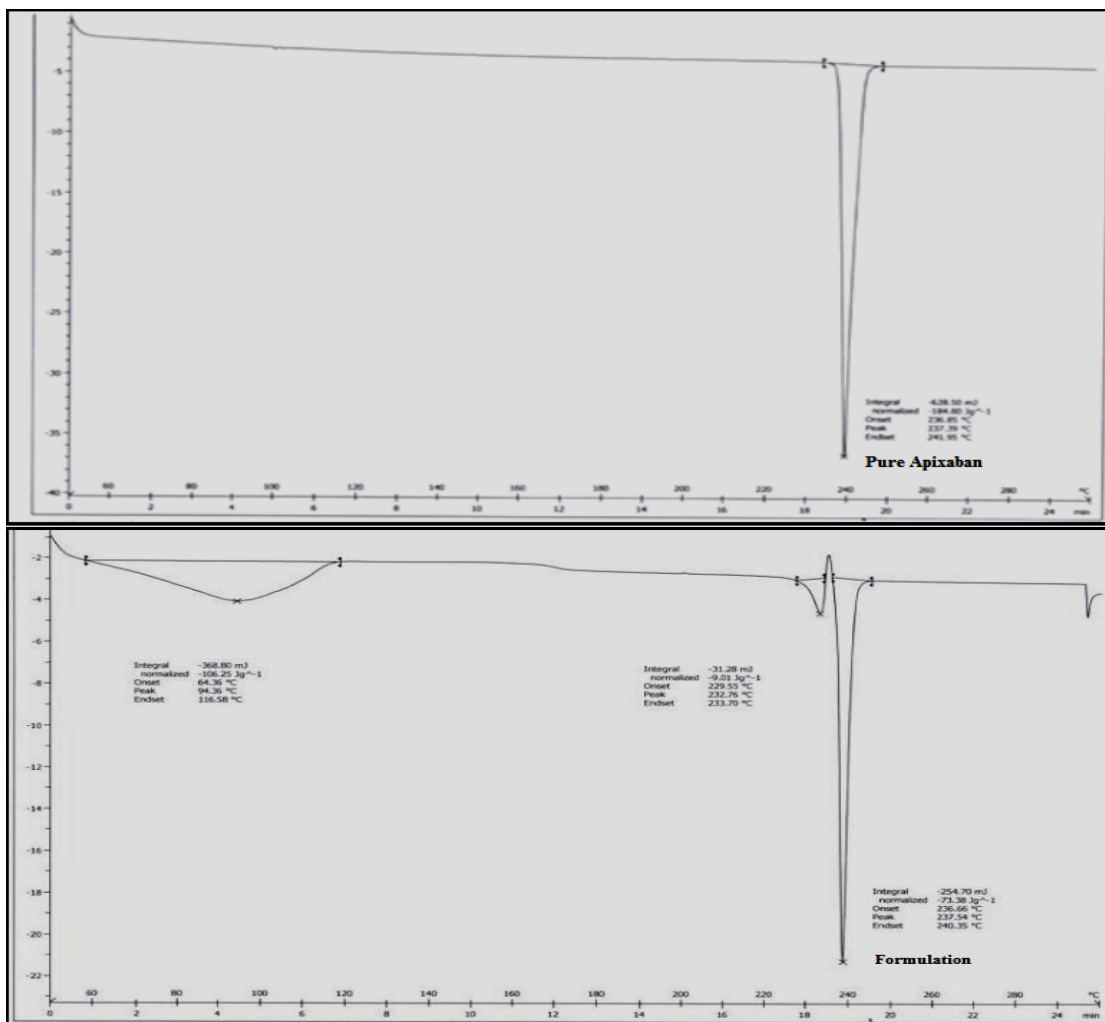
Figure 2 DSC spectra of Pure API Apixaban and Formulation

Table 10 DSC Data of Apixaban and Final Formulation

Reference Data	Pure Drug Melting point	Formulation Melting point	Conclusion
35-238 ° C	37.39 °C	37.54 °C	No Interaction

6.2 Scanning for λmax of Apixaban

Scanning for λmax of Apixaban done using 6.8 phosphate buffers. After scanning for over a range of 400-200 nm on a double beam UV Spectrophotometer, solution of Apixaban of showed maximum absorbance at 279.
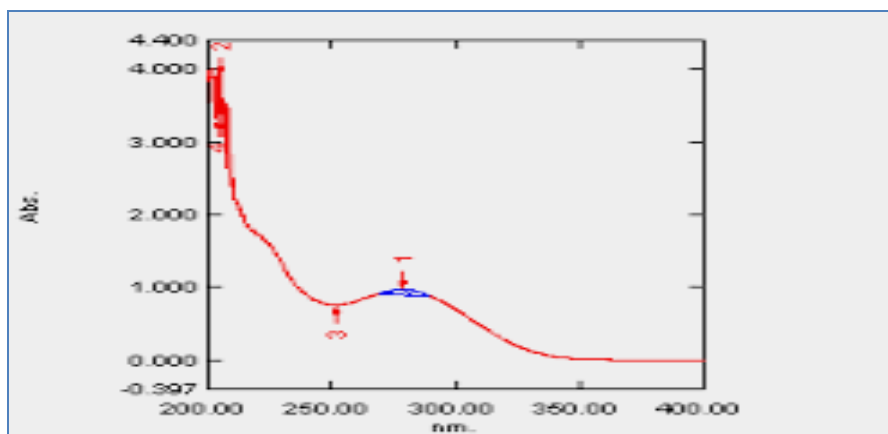
Figure 3 Scanning for λmax of Apixaban

6.3 Calibration Curve of Apixaban

Standard calibration curve of Apixaban was prepared using 6.8 phosphate buffer solution. Standard curve was plotted by taking concentration on X-axis and absorbance on Y-axis.

Table 11 Calibration curve of Apixaban

Sr. No.	Concentration (µg/ml)	Average Absorbance ± SD
1	0	0.0
2	5	0.121 ± 0.05
3	10	0.245 ± 0.03
4	15	0.356 ± 0.04
5	20	0.482 ± 0.05
6	25	0.602 ± 0.07
7	30	0.723 ± 0.05
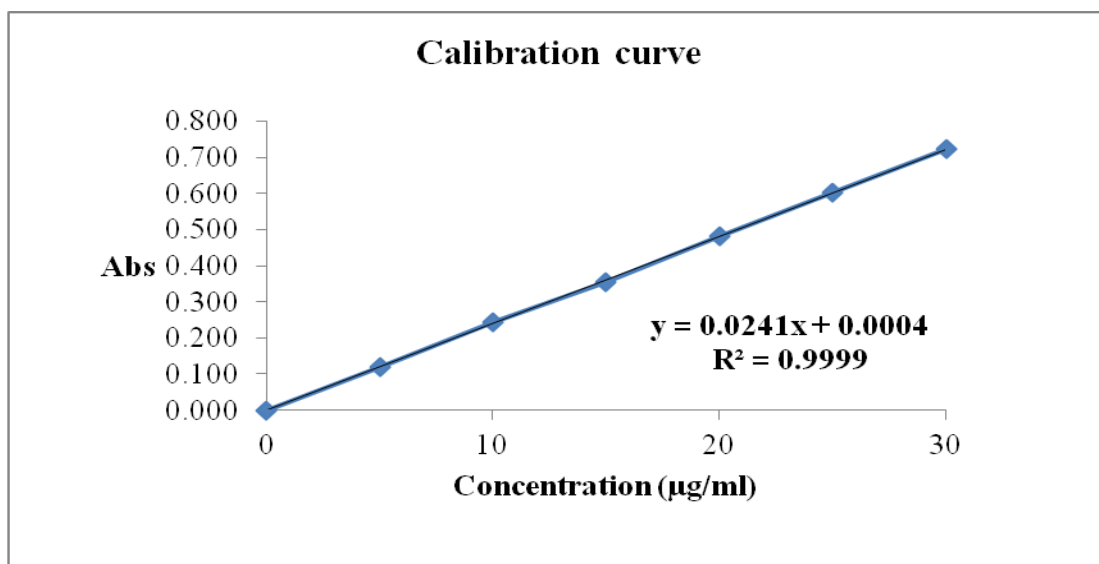
Figure 4 Calibration curve of Apixaban

6.4 Evaluation of Prepared Films of trial batches

6.4.1 Trial Batches of Blank Film

Initial trials were taken for finalization of polymers. The prepared films of trial batches were evaluated for basic evaluation parameters. The results were recorded below in tabulated form.

Table 12 Evaluation of trials batches of Apixaban sublingual films

Formulation Code	Stickiness	Surface appearance	Film clarity	Quality
F1	Sticky	Film did not form	-	Poor
F2	Sticky	Film did not form	-	Poor
F3	Non sticky	Uniform	Clear	Good
F4	**Non sticky**	**Uniform**	**Clear**	**Good**
F5	Non sticky	Non– uniform	Turbid	Average
F6	Non sticky	Non– uniform	Turbid	Average

From the above results it can be concluded that the HPMC E3 polymer did not form a good film. The films were sticky in nature and poor quality. HPMC E5 and E15 make good and average films respectively.

Further some physical and chemical parameters were checked for polymer selection point of view. Weight variation was well within acceptable range. Folding endurance of all formulation checked and recorded. Folding endurance was depending on amount of polymer and flexibility of films. Thickness found uniform in all formulations.

Table 13 Evaluation of trials batches of Apixaban sublingual films

Formulation Code	Weight variation (mg)	Thickness (mm)	Disintegration time	Folding Endurance
F1	112 ± 2.6	0.24 ± 0.12	-	-
F2	210 ± 6.7	0.35 ± 0.19	-	-
F3	115 ± 5.7	0.29 ± 0.15	32 ± 3	85 ± 10
F4	**213 ± 3.8**	**0.37 ± 0.10**	**43 ± 7**	**280 ± 8**
F5	110 ± 1.9	0.31 ± 0.13	51 ± 6	192 ± 13
F6	209 ± 8.5	0.42 ± 0.14	59 ± 4	296 ± 9

For F1 and F2 batches, some of tests were not performed as the films were not form properly. Hence these two batches were omitted from evaluation. F3 and F4 batches found good with respect to disintegration time and folding endurance. F4 batch have a high value of folding endurance. F5 and F6 batch have higher DT time than the HPMC E5 polymer batches.

Hence F4 batch was selected for further screening and factorial design purpose. F4 have a low DT value and good folding endurance. Also the films of F4 batches were non sticky and flexible which was the actual requirement of the formulation.

6.5 Evaluation of Prepared sublingual Films factorial batches

Factorial batches A1-A9 was evaluated for various parameters like weight variation, thickness, Surface pH, Swelling Index, Drug Content, Disintegration time, Folding endurance, % Elongation, Drug release and Tensile strength which were given in below table.

Table 14 Evaluation of A1-A9 Batch for various parameters

Formulation	Stickiness	Surface appearance	Film clarity	Quality
A1	Non sticky	Uniform	Clear	Good
A2	Non sticky	Uniform	Clear	Good
A3	Non sticky	Uniform	Clear	Good
A4	Non sticky	Uniform	Clear	Good
A5	**Non sticky**	**Uniform**	**Clear**	**Good**
A6	Non sticky	Uniform	Clear	Good
A7	Non sticky	Uniform	Clear	Good
A8	Non sticky	Uniform	Clear	Good
A9	Non sticky	Uniform	Clear	Good

Table 15 Evaluation of A1-A9 Batch for various parameters

Formulation	Weight variation (mg)	Thickness (mm)	Surface pH
A1	152 ± 3.5	0.32 ± 0.02	6.7 ± 0.2
A2	205 ± 4.3	0.36 ± 0.03	6.7 ± 0.1
A3	256 ± 3.8	0.34 ± 0.03	6.8 ± 0.4
A4	157 ± 2.9	0.38 ± 0.04	6.7 ± 0.1
A5	**199 ± 1.6**	**0.37 ± 0.03**	**7.0 ± 0.3**
A6	264 ± 2.7	0.30 ± 0.04	6.9 ± 0.1
A7	151 ± 2.9	0.38 ± 0.05	6.9 ± 0.3
A8	204 ± 3.5	0.37 ± 0.04	6.8 ± 0.2
A9	253 ± 2.2	0.39 ± 0.04	6.7 ± 0.2

Table 16 Evaluation of A1-A9 Batch for various parameters

Formulation	Swelling Index (%)	Drug Content (%)	Disintegration Time (Sec)
A1	9.2 ± 0.8	98.7 ± 0.2	32 ± 6

A2	11.5 ± 4.2	97.8 ± 0.4	43 ± 4
A3	13.4 ± 2.6	99.5 ± 0.2	49 ± 6
A4	10.9 ± 1.9	98.7 ± 0.6	39 ± 7
A5	**15.8 ± 3.8**	**97.8 ± 0.2**	**48 ± 6**
A6	17.2 ± 4.6	98.4 ± 0.3	50 ± 5
A7	12.5 ± 2.7	96.7 ± 0.2	51 ± 4
A8	15.9 ± 3.6	98.5 ± 0.2	56 ± 4
A9	25.5 ± 2.8	99.6 ± 0.1	55 ± 2

Table 17 Evaluation of A1-A9 Batch for various parameters

Formulation	Folding Endurance	Tensile Strength (N/mm^2)	% Elongation
A1	284 ± 12	3.45 ± 0.04	25.12 ± 5.12
A2	264 ± 18	2.98 ± 0.05	34.15 ± 4.12
A3	268 ± 20	2.97 ± 0.06	48.25 ± 3.14
A4	272 ± 21	3.65 ± 0.04	30.50 ± 5.15
A5	**284 ± 19**	**3.95 ± 0.02**	**38.54 ± 6.45**
A6	257 ± 14	3.48 ± 0.01	46.12 ± 5.15
A7	265 ± 20	4.65 ± 0.06	40.18 ± 9.15
A8	298 ± 17	4.12 ± 0.08	42.12 ± 6.48
A9	287 ± 20	4.16 ± 0.04	45.25 ± 6.45

Table 18 Evaluation of A1-A9 Batch for various parameters

Formulation	% Drug Release in min					
	2	4	6	8	10	15
A1	42 ± 5.1	54 ± 5.6	63 ± 2.9	74 ± 1.2	86 ± 0.9	95 ± 0.5
A2	36 ± 3.5	48 ± 4.9	58 ± 3.1	78 ± 2.0	89 ± 1.2	96 ± 0.9
A3	33 ± 2.9	46 ± 6.8	57 ± 2.8	68 ± 1.6	79 ± 1.3	84 ± 1.2
A4	40 ± 3.8	52 ± 3.7	61 ± 3.7	71 ± 2.3	85 ± 0.7	93 ± 0.8
A5	**35 ± 2.8**	**50 ± 4.2**	**65 ± 2.8**	**76 ± 1.8**	**88 ± 1.0**	**99 ± 0.5**

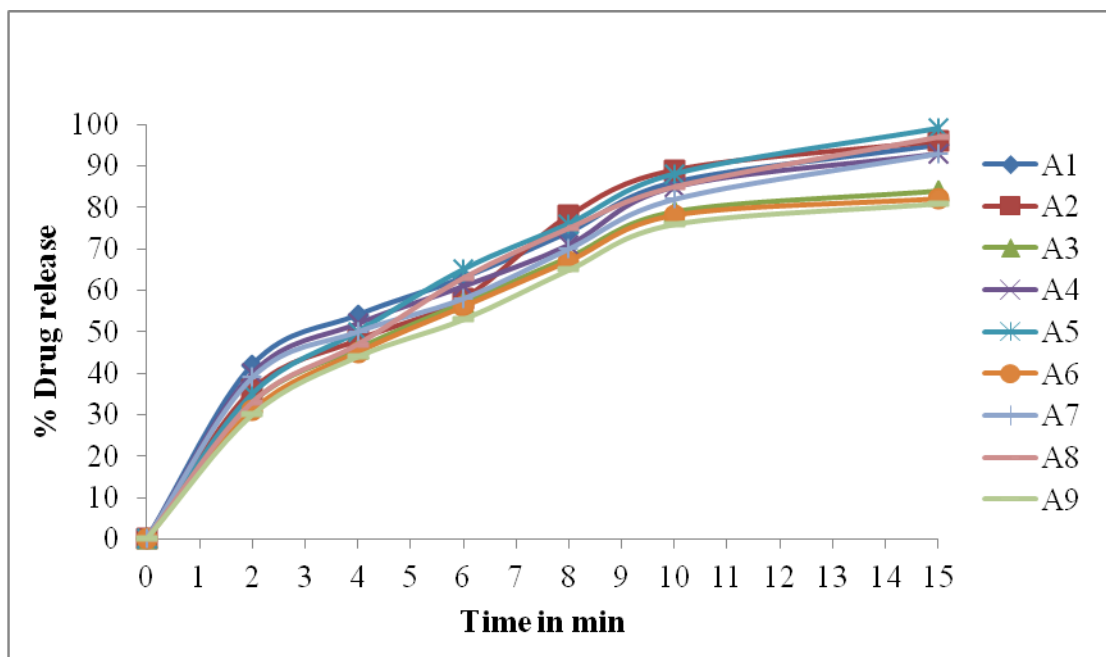|						
A6	31 ± 1.7	45 ± 3.6	56 ± 1.9	67 ± 3.1	78 ± 2.3	82 ± 0.8
A7	39 ± 3.8	50 ± 2.9	58 ± 2.0	70 ± 2.4	82 ± 1.8	93 ± 1.8
A8	33 ± 4.9	47 ± 4.5	63 ± 3.1	75 ± 1.9	85 ± 2.1	97 ± 1.1
A9	30 ± 2.9	44 ± 3.8	53 ± 1.8	65 ± 1.7	76 ± 1.9	81 ± 2.3

Figure 5 Drug release study of factorial batches

In preliminary phase, attention was given to select the proper concentration of film forming agent to develop successful sublingual films. These selections were used to impart suitable ductility, mechanical strength and flexibility to the films under different type of mechanical stress.

HPMC E5 was used as a film forming agent due to its excellent film forming properties. PEG 400 was selected as plasticizer for HPMC E5 based sublingual films as it developed clear homogenous preparation.

The films prepared by using combination of HPMC E5 and PEG 400 found to be flexible with good mechanical strength. Preliminary trials indicated 100-200 mg of HPMC E5 and 5 ml plasticizer level in the film casting solution showed good results for mechanical properties and disintegration time. Levels of other excipients were fixed for all formulations. The selected levels of both factors were optimized using design expert software. 3^2 central composite design enabled prediction of interaction among independent variables and its effect on the dependent variables. It was helpful to investigate that how the response changes when two factors are changed simultaneously.

Therefore 3^2 central composite design was used to prepare apixaban loaded films. The in vitro release of the drug was found to be 99% in 15 min indicating that the films dissolve within 15 minutes and the formulation excipients did not retain the drug. The results of drug content indicated that drug has been uniformly distributed in the film. The drug content of optimized formulation was found to be 97.8%. As no significant difference in drug content was found which indicated good content uniformity among the films.

6.6 Statistical Analysis of factorial design

To generate optimized formulation, different equations and regression behaviors were analyzed and fitted into the Minitab 16 software.

Table 19 Design table

Batch	Coded Factors		Actual factors		Response		
	X1	X2	X1	X2	Y1	Y2	Y3
A1	-1	-1	5	150	32	207	42
A2	-1	0	5	200	43	280	36
A3	-1	1	5	250	49	298	33
A4	0	-1	10	150	39	200	40
A5	**0**	**0**	**10**	**200**	**48**	**271**	**35**
A6	0	1	10	250	50	279	31
A7	1	-1	15	150	41	195	39
A8	1	0	15	200	56	261	33
A9	1	1	15	250	55	269	30

levels of 3^2 Full Factorial Designs

Independent Factors	Levels		
	Low (-1)	Medium (0)	High (1)
X1= Amount of PEG 400 (ml)	5	10	15
X2= Amount of HPMC E5 (mg)	150	200	250
Dependent Factors			
Y1= Disintegration time			
Y2= Folding Endurance			
Y3= Q $_{2\ min}$ % Release			

After fitting of data in Minitab 16, regression analysis was done and the outcome of this analysis showed below.

Table 20 Analysis of Variance for Disintegration time (sec) (coded units)

Source	DF	Seq SS	Adj SS	F value	P value	Remarks
Main Effects	2	424.667	424.667	17.13	0.006	***Significant***
Amount of PEG 400	1	130.667	130.667	10.54	0.023	***Significant***
Amount of HPMC E5	1	294.000	294.000	23.72	0.005	***Significant***
2-Way Interactions	1	2.250	2.250	0.18	0.688	*Non-Significant*
Amount of PEG 400*Amount of HPMC E5	1	2.250	2.250	0.18	0.688	*Non-Significant*
Residual Error	5	61.972	61.972	-	-	-
Total	8	488.889	-	-	-	-

ANOVA table for Disintegration time shows that the HPMC E5 and PEG 400 both have a significant impact on Disintegration time.

Table 21 Estimated Coefficients for Disintegration time

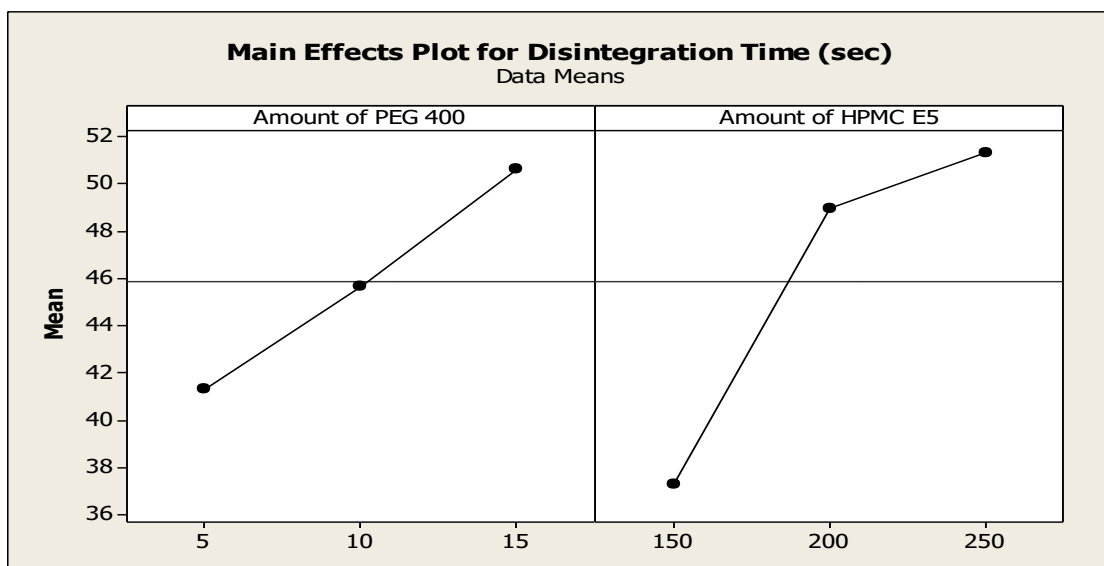| Term	Co efficient
Constant	2.5556
Amount of PEG 400	1.53333
Amount of HPMC E5	0.170000
Amount of PEG 400*Amount of HPMC E5	-0.00300000
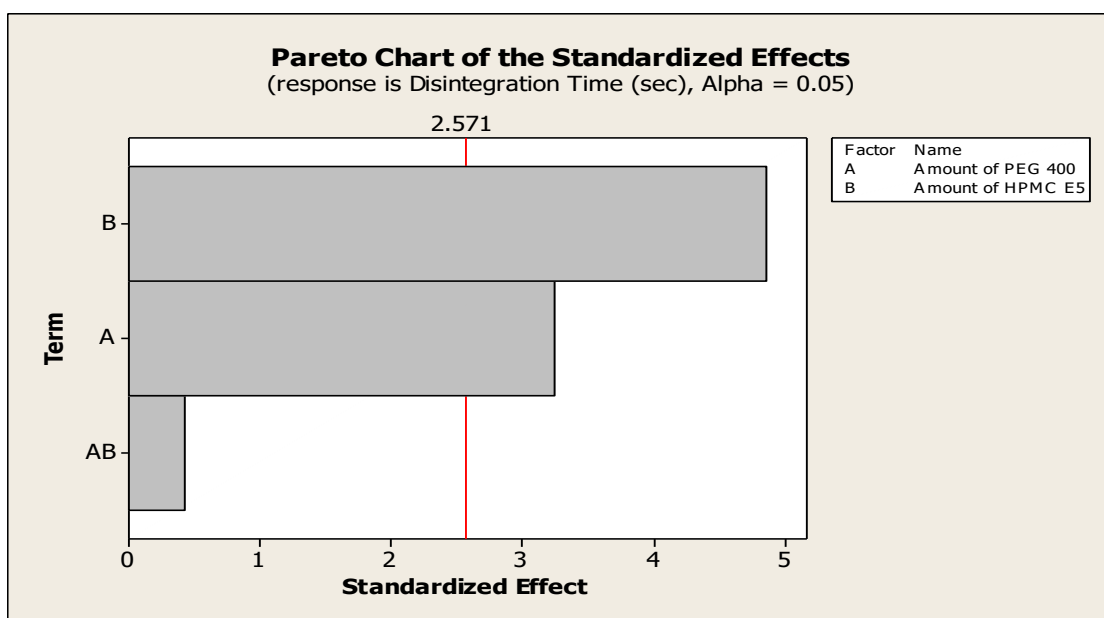
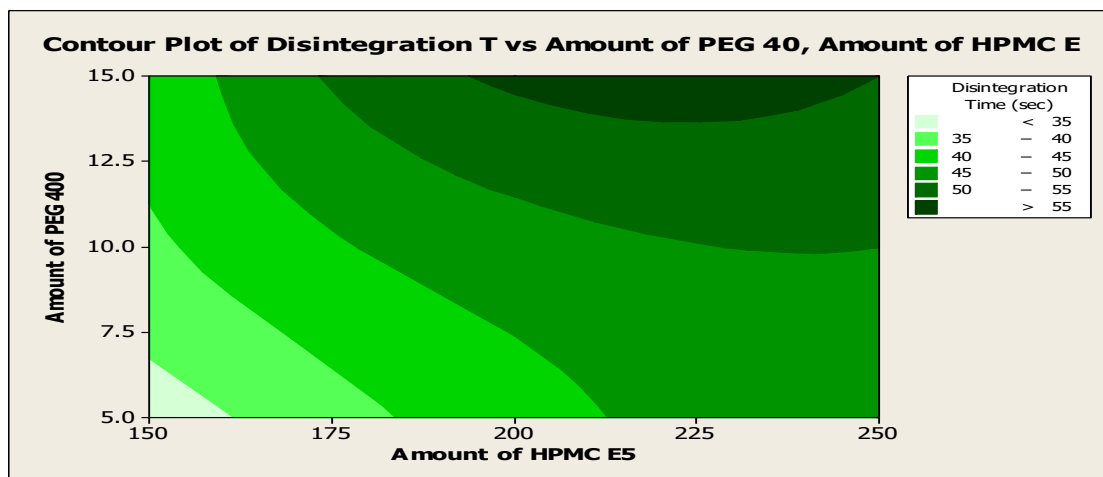**Figure 6 Pareto chart of Disintegration time**

Figure 7 Main effect plot for Disintegration time

Figure 8 Counter plot for Disintegration time
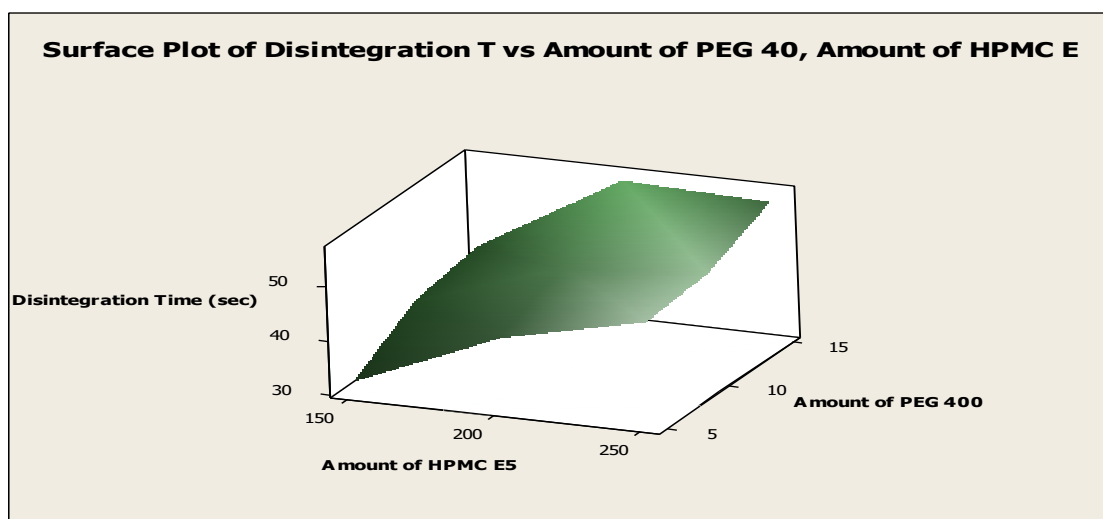
Figure 9 Surface plot for Disintegration time

Table 22 Analysis of Variance for Drug release at 2 min (coded units)

Source	DF	Seq SS	Adj SS	F value	P value	Remarks
Main Effects	2	135.000	135.000	104.74	0.000	*Significant*
Amount of PEG 400	1	13.500	13.500	20.95	0.006	*Significant*
Amount of HPMC E5	1	121.500	121.500	188.53	0.000	*Significant*
2-Way Interactions	1	0.000	0.000	-	-	*Non-Significant*
Amount of PEG 400*Amount of HPMC E5	1	0.000	0.000	-	-	*Non-Significant*
Residual Error	5	3.222	3.222	-	-	-
Total	8	138.222	-	-	-	-

ANOVA table for drug release at 2 min shows that the HPMC E5 and PEG 400 both have a significant impact.

Table 23 Estimated Coefficients for Drug release at 2 min

Term	Co efficient
Constant	56.4444
Amount of PEG 400	-0.300000
Amount of HPMC E5	-0.0900000
Amount of PEG 400*Amount of HPMC E5	-0.00000000
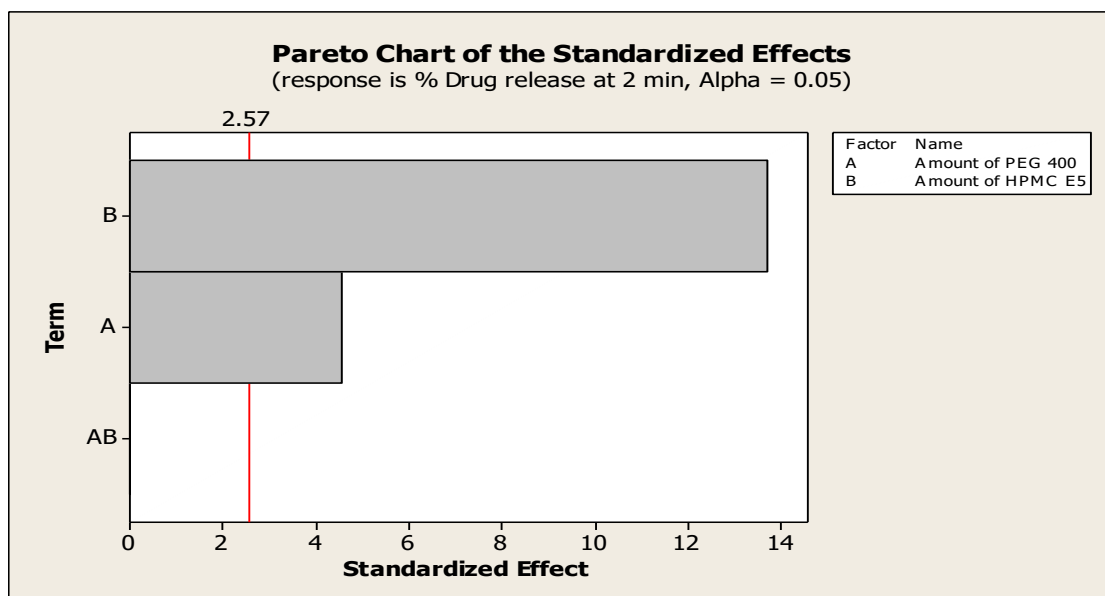
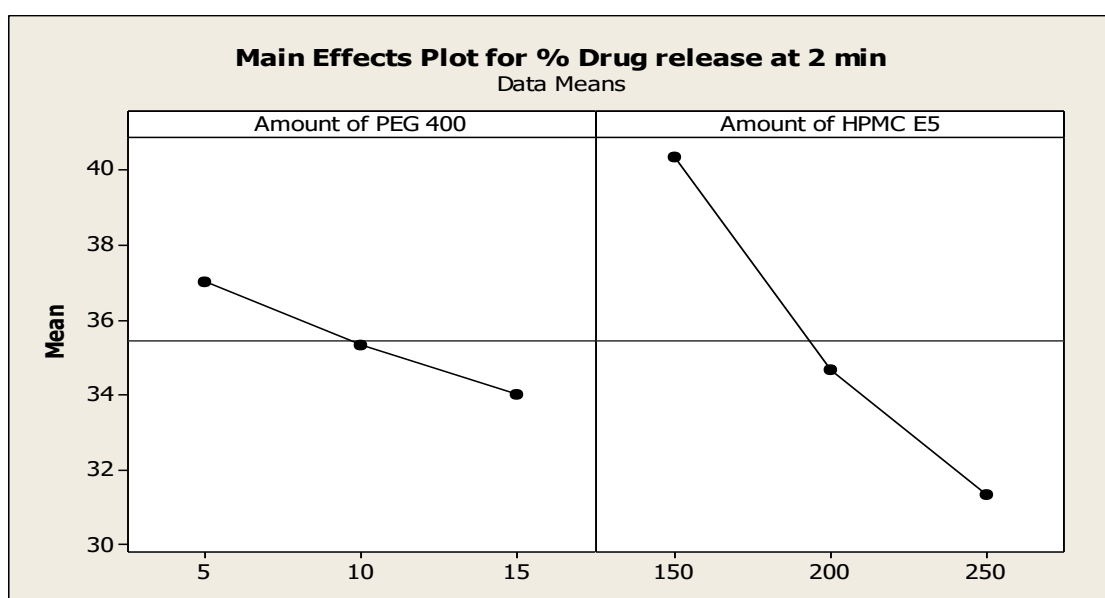**Figure 10 Pareto chart of Drug release at 2 min**
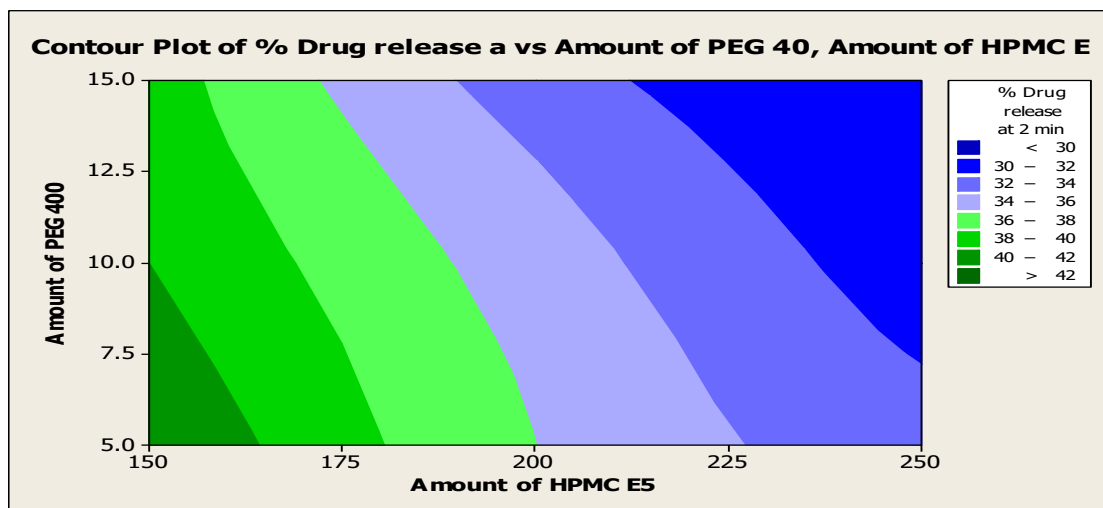

Figure 11 Main effect plot for Drug release at 2 min

Figure 12 Counter plot for Drug release at 2 min
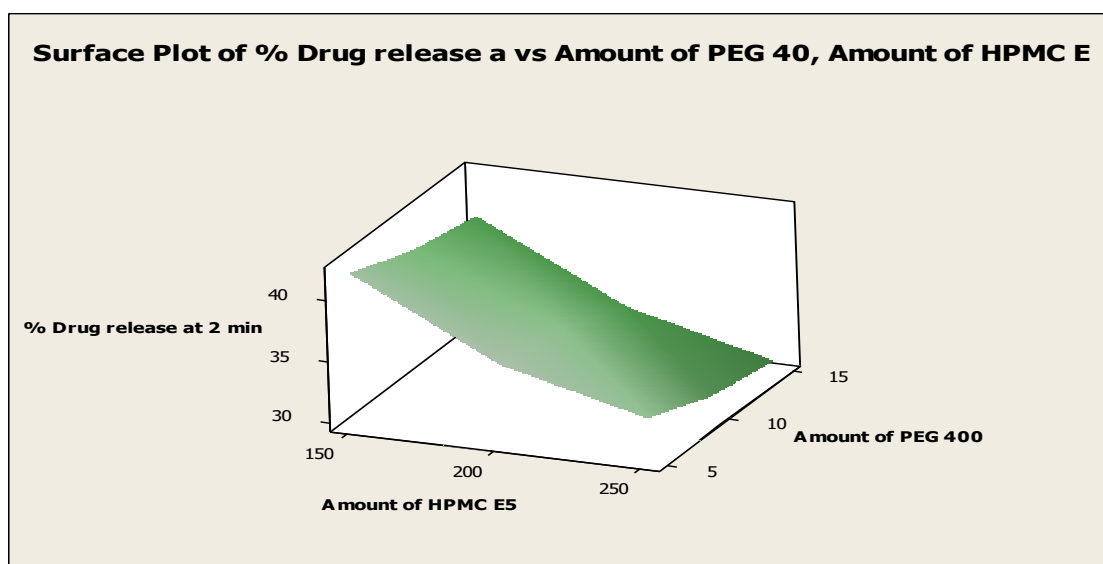
Figure 13 Surface plot for Drug release at 2 min

Table 24 Analysis of Variance for folding endurance (coded units)

Source	DF	Seq SS	Adj SS	F value	P value	Remarks
Main Effects	2	10522.7	10522.7	15.15	0.008	***Significant***
Amount of PEG 400	1	600.0	600.0	1.73	0.246	*Non-Significant*
Amount of HPMC E5	1	9922.7	9922.7	28.58	0.003	***Significant***
2-Way Interactions	1	72.3	72.3	0.21	0.667	*Non-Significant*
Amount of PEG 400*Amount of HPMC E5	1	72.3	72.3	0.21	0.667	*Non-Significant*
Residual Error	5	1736.0	1736.0	-	-	-
Total	8	12330.9	-	-	-	-

ANOVA table for folding endurance shows that the HPMC E5 has significant impact and PEG 400 have non significant impact on films.

Table 25 Estimated Coefficients for folding endurance

Term	Co efficient
Constant	74.4444
Amount of PEG 400	1.40000
Amount of HPMC E5	0.983333
Amount of PEG 400*Amount of HPMC E5	-0.0170000
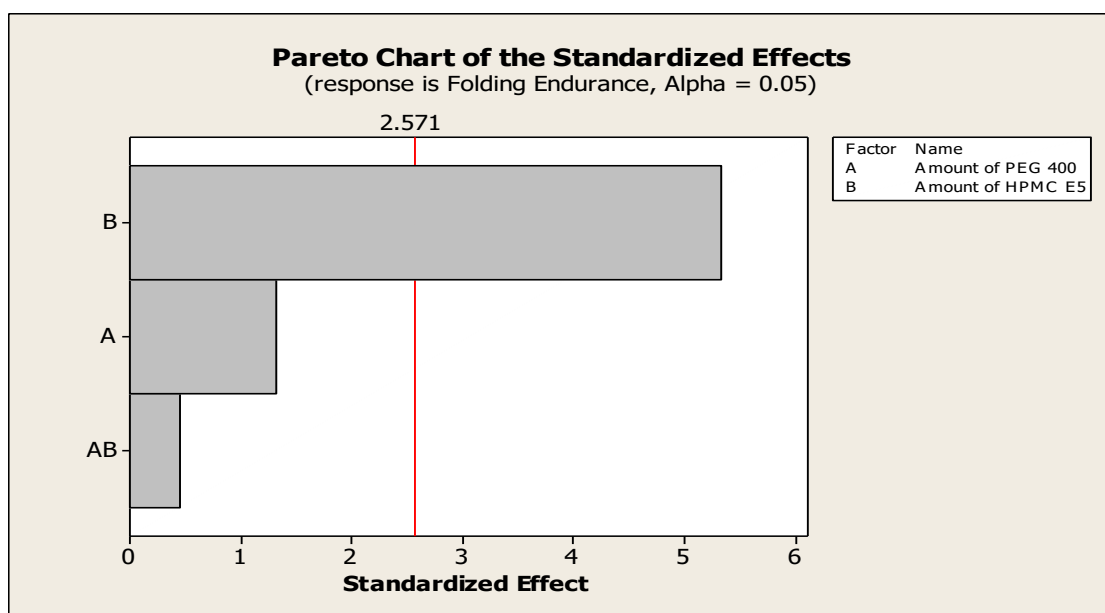
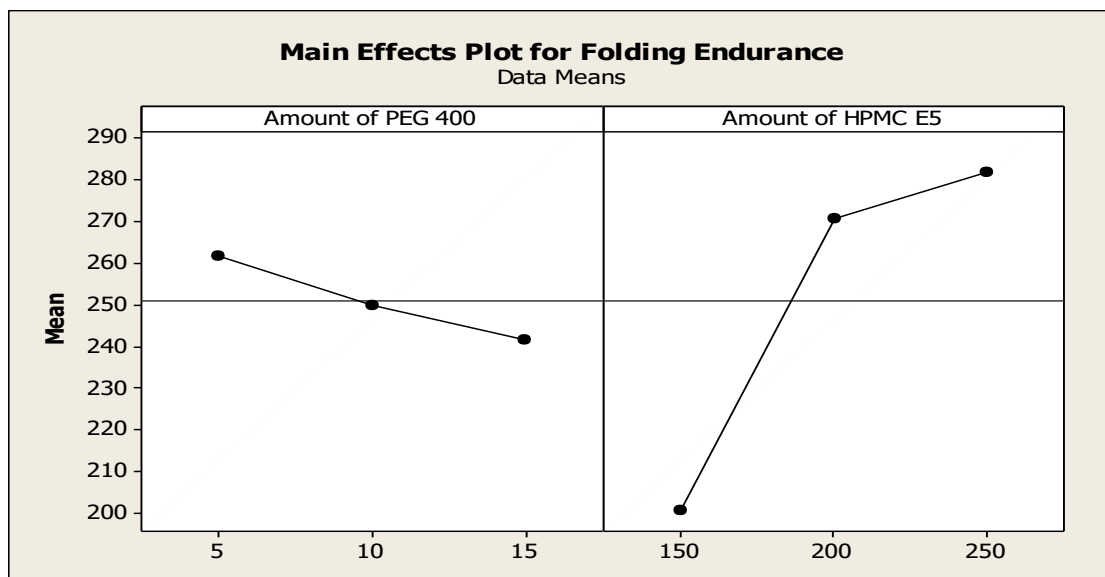**Figure 14 Pareto chart of folding endurance**

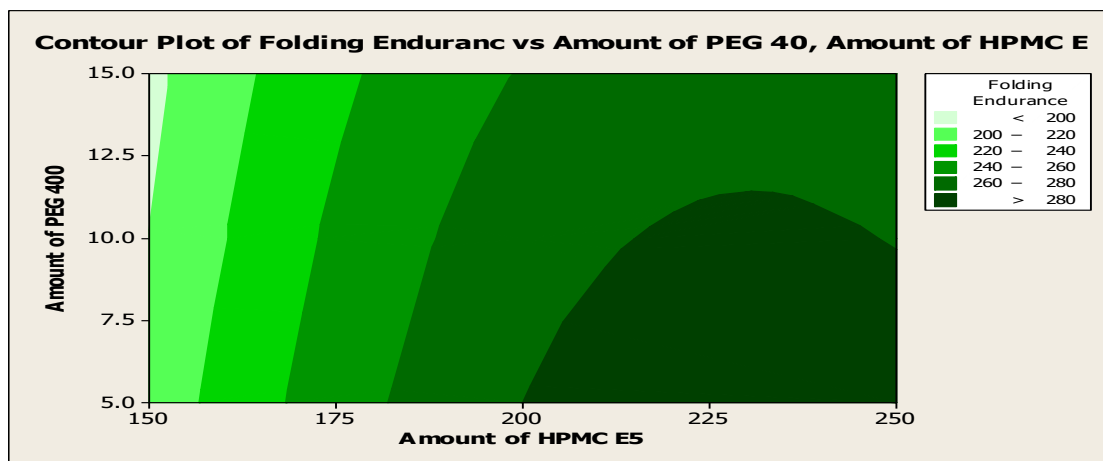**Figure 15 Main effect plot for folding endurance**

Figure 16 Counter plot for folding endurance
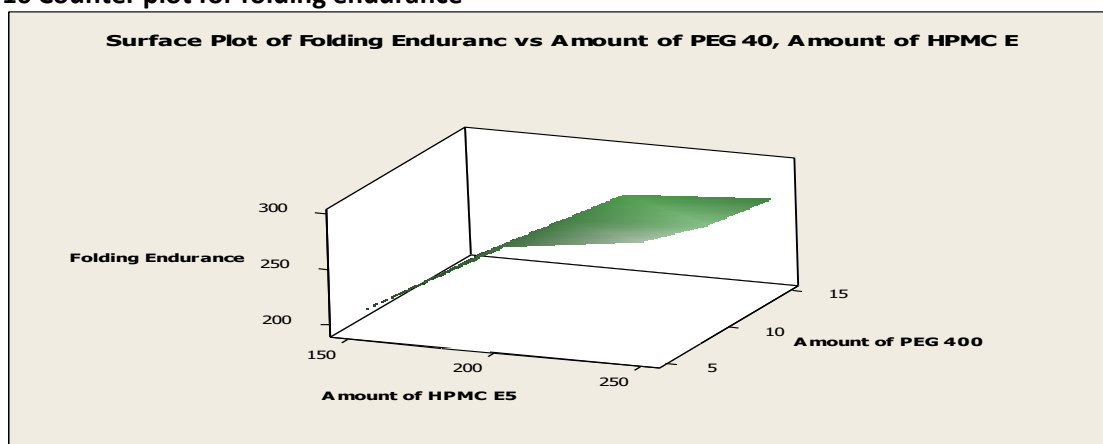

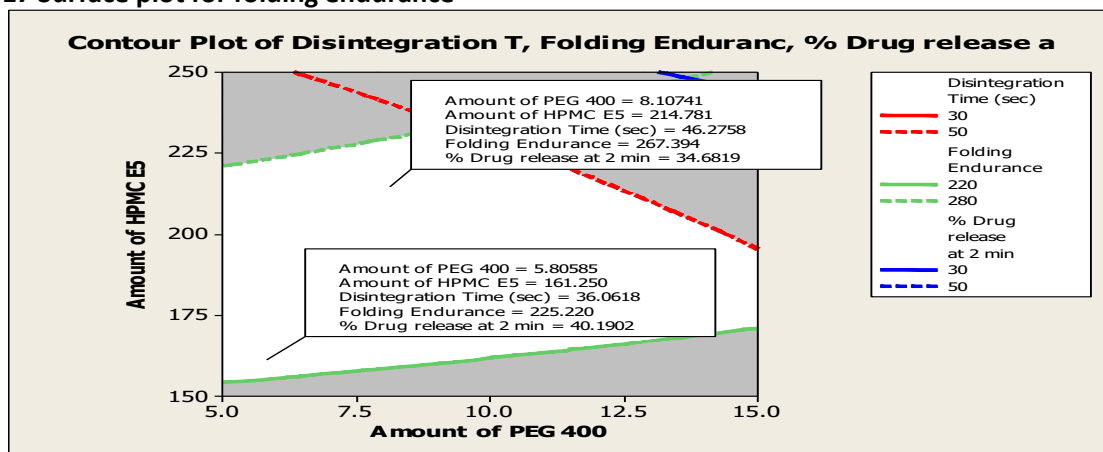
Figure 17 Surface plot for folding endurance

Figure 18 Overlay counter plot

- **Validation of optimized formulation**

A checkpoint batch was designed accordance to the desirability function. To assess the validity of prediction, a checkpoint batch A10 and A11 was prepared and evaluated under the same conditions as outlined for the other batches. The response data was compared with that of required data.

Table 26 Comparison of predicted and obtained responses

Parameter	Predicted	Observed	% Bias
Disintegration time	46	47	0.978
Drug release at 2 min	34	35	1.029
Folding Endurance	267	265	1.007

Discussion: The obtained response variables of check point batch compared with target response parameters. The bias for predicted versus observed responses was acceptable. The results are shown in Table 26.

- **Optimized Batch**

Based on Factorial Design data final optimized batch select from the counter plot to achieve desired drug release, disintegration time and folding endurance. Complete analysis of this batch done and recorded below.

Table 27 Composition of Optimized batch formulation (A12)

Ingredient	A12
Apixaban (mg)	2.5
HPMC E5 (mg)	161.25
PEG 400 (ml)	5.80
Aspartame (mg)	2
Citric acid (mg)	5
Water (ml)	30

Table 28 Results of Optimized batch A12

Parameter	Results	
Weight variation (mg)	175 ± 5.4	
Thickness (mm)	0.34 ± 0.02	
Surface pH	6.9 ± 0.2	
Drug content %	98.8 ± 0.6	
Tensile Strength (N/mm^2)	2.9 ± 0.1	
Percent Elongation	27.21 ± 2.06	
In vitro disintegration time (Sec)	35 ± 3	
Folding endurance	224 ± 8	
% Drug Release	Time (min)	% Drug Release
	0	0
	2	41.5 ± 2.5
	4	52.3 ± 3.4
	6	67.9 ± 1.9
	8	78.5 ± 3.9
	10	90.4 ± 0.6
	12	98.9 ± 0.5

Table 29 Release kinetic study

Kinetic Model	Parameters	Value
Zero Order	R^2	0.925
	K_0	16.28
First Order	R^2	0.842
	K_1	2.164
Higuchi	R^2	0.961
	K_H	0.023
Korsmeyer-Peppas	R^2	0.732
	K_P	0.551
Hixon Crowell	R^2	**0.996**
	K_{HC}	0.649

Based on above drug release kinetic data, the formulation follows Hixon crowell model. The R^2 value for the model was 0.996 which was the best fitted model.
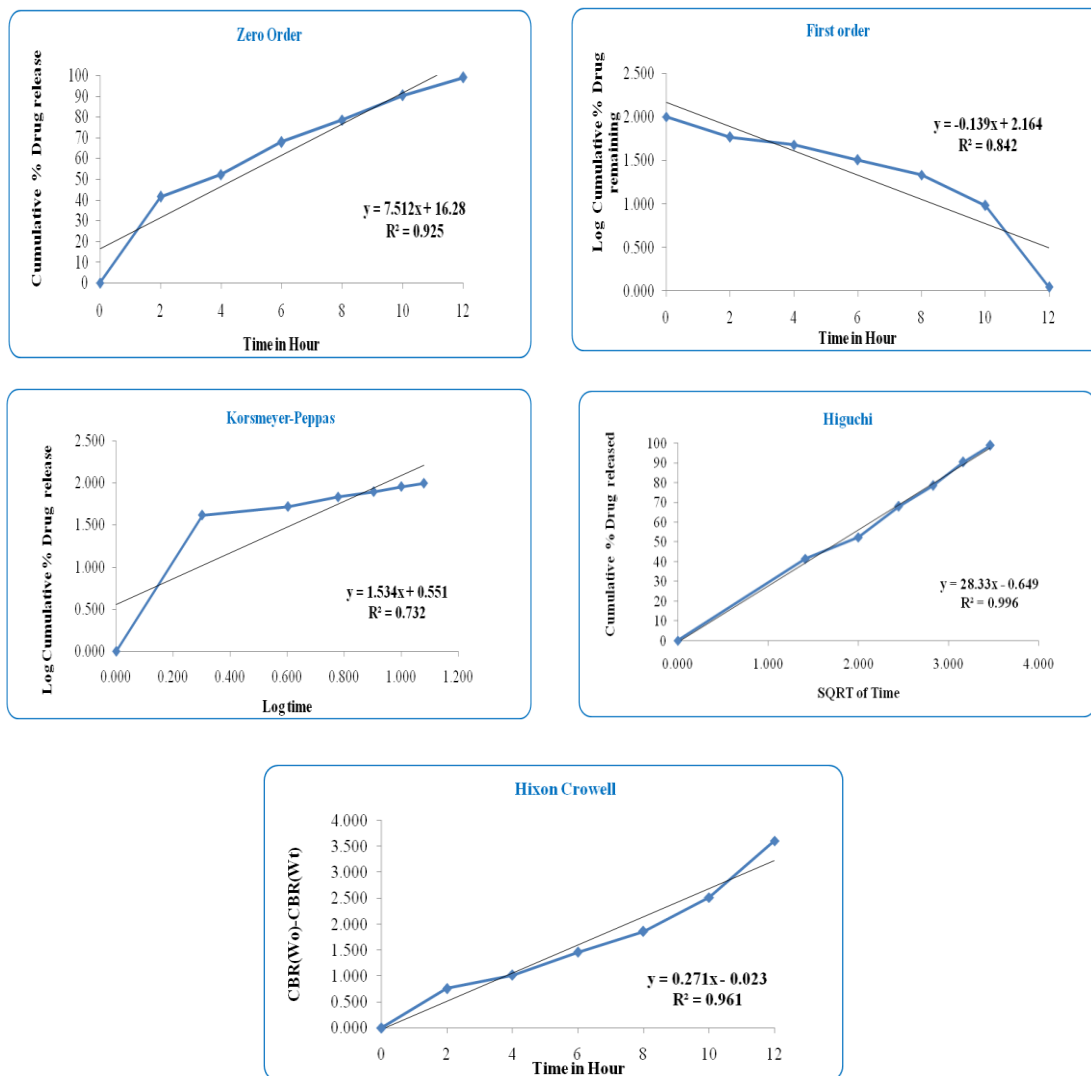
Figure 19 Release kinetic study

- **Comparison with marketed product**

Dissolution profile of final formulation A12 compared with marketed formulation Eliquis 2.5 mg Tablet and results recorded in below table 5.21.

USP Apparatus: I (Basket)

Speed: 50 rpm

Medium: 6.8 pH phosphate buffer

Volume: 300 ml

Table 30 Comparison of A12 formulation with Market Formulation

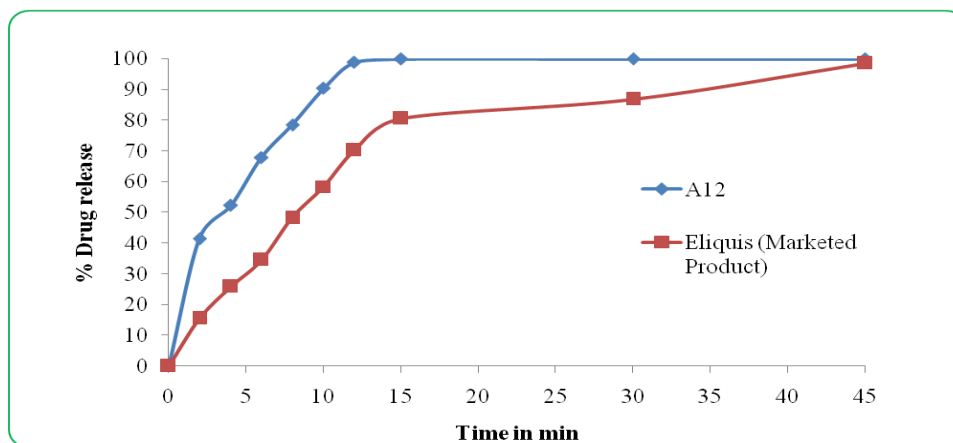| Time in min	A12	Eliquis
2	41.5 ± 2.5	15.6 ± 3.8
4	52.3 ± 3.4	25.9 ± 2.6
6	67.9 ± 1.9	34.6 ± 3.9
8	78.5 ± 3.9	48.3 ± 5.6
10	90.4 ± 0.6	58.4 ± 3.4
12	98.9 ± 0.5	70.3 ± 2.8
15	99.9 ± 0.3	80.6 ± 6.5
30	99.9 ± 0.2	86.9 ± 1.6
45	99.9 ± 0.2	98.6 ± 3.5

Figure 20 Comparison with marketed product

Based on above data it concluded that the formulation of sublingual films were faster as compared to marketed product.

6.7 Stability Study

Stability study of final optimized batch A12 performed for 1 month at 40°C and 75% RH. Initial results and after 1 month results compared and found satisfactory. The batch was found stable during stability. The results were recorded in below table.

Table 31 Stability study data of batch A12

Parameter	Initial	After 1 Month
Appearance	Transparent, Non sticky and flexible	Transparent, Non sticky and flexible
Average Weight (mg)	175 ± 5.4	173 ± 6.1
Folding Endurance	224 ± 8	221 ± 5
% Drug Content	98.8 ± 0.6	98.3 ± 2.5
Disintegration time (Sec)	35 ± 3	39 ± 2
% Drug release after 2 min	41.5 ± 2.5	39.5 ± 4.6

7   CONCLUSION

The aim of present research work was to formulate fast dissolving sublingual films of Apixaban for quick onset of action. In preliminary work various grades of HPMC polymers like HPMC E3, HPMC E5 and HPMC E15 were used to prepare the sublingual films of Apixaban. It has been found that HPMC E5 grade gave good results as compared to other grades. Based on that, 3^2 factorial design was applied to optimized the formulation. HPMC E5 and PEG 400 were taken as independent variable. The formulated films of Apixaban were evaluated for their physico-mechanical parameters like tensile strength, folding endurance, thickness, and disintegration time and *in-vitro* drug release. Estimation of drug content of Apixaban films was performed and the results were found satisfactory. Analysis of factorial design done for dependent variables folding endurance, disintegration time and *in-vitro* drug release at 2 min. Final formulation A12 selected based on design space obtained by factorial design. Stability study of A12 formulation found satisfactory. Also the A12 formulation gives faster drug release as compared to marketed product. Hence, the A12 formulation was optimized batch.

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