"Formulation Characterization and Optimization of Buccal Film of Local Anesthetic"

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MASTER OF PHARMACY

IN

PHARMACEUTICAL TECHNOLOGY &

BIOPHARMACEUTICS

BY

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CERTIFICATE

This is to certify that the dissertation work entitled "Formulation optimization and characterization of buccal film of local anesthetic" submitted by Mr. Jahid Tanwar with Registration No. (13MPH121) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Technology and Biopharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Formulation optimization and characterization of buccal film of local anesthetic" is based on the original work carried out by me under the guidance of Dr. Dhaivat Parikh, Assistant professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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LIST OF ABBREVIATIONS

Abs	Absorbance
AVG	Average
BP	British pharmacopoeia
USP	United States Pharmacopoeia
IP	Indian Pharmacopoeia
PVA	Poly Vinyl Alcohol
% CDR	% Cumulative Drug Release
DT	Disintegration
^{o}C	Degree Centigrade
Cm	Centimeter
Conc.	Concentration
DSC	Differential Scanning Colorimetery
PEG	Poly Ethylene Glycol
FTIR	Fourier Transform Infrared
UV	Ultraviolet
HPMC	Hydroxy Propyl Methyl Cellulose
PVP	Poly Vinyl Pyrrolidone
AAG	Acetic Acid Glacial
HCl	Hydro Chloric Acid
Temp.	Temperature
hrs	Hours
SLS	Sodium lauryl Sulphate
mg	Milligram
K	Dissolution Rate Constant
mins	Minutes
ml	Milliliter
SD	Standard Deviation
SSF	Simulated Saliva Fluid
rpm	Rotations Per Minute
µg	Micro Gram
cps	centipoise
λ_{max}	Absorption Maxima
Conc.	Concentration

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ABSTRACT

Formulation, Characterization and Optimization of Buccal Film of local Anesthetic.

Benzocaine is the most widely used as a local anesthetic as its produce reversible loss of sensation and temporary relive from the local pain. Benzocaine is available in the number of formulation like gels, cream, ointment, suspension, lozenges, tablet and spray. Among all, the semisolid formulations of benzocaine are widely used for the treatment of mouth ulcer, as they provide the comparatively higher retention of drug in oral cavity by slow release of drug and ease of application on specific location. However, longer stay of such formulation cause patient incompliance and difficulty in eating. To overcome the above drawbacks, the aim of the present study was to formulate and characterize the novel sustained release buccal film containing local anesthetic that could be hold for longer period into oral cavity which provides constant release of the drug for the longer period. A number of film forming polymers (HPMC 15cps, HPMC K4M, Pectin, Carbopol 934, HPC, PVP, PVA and Chitosan) were explored alone and in different combinations to obtain the film formulation by solvent casting method; with desired characteristics like tensile strength, % elongation, folding endurance and drug release. Experiments were also performed to optimize casting surface and amount of plasticizer to obtain the better film properties. Based on the preliminary trials, Chitosan and PVA (poly vinyl alcohol) were found to be promising polymers in combination. To further optimize the film formulation, 3^2 full factorial design was applied by selecting three different amounts of both polymers (Chitosan and PVA). All nine batches were evaluated for tensile strength, % elongation, drug diffusion (using Franz diffusion cell) and drug release (in USP apparatus – II) using simulated salivary fluid. The results were analyzed by multi-linear regression to generate polynomial equations for various responses. The optimized batches were identified and formulated by generating the overlay contour plots to obtain the desired region. The optimized formulation were compared with the market formulations (semi-solid) for drug release study. It was concluded that the film formulation of benzocaine is promising formulation with improved retention time and desired drug release.

AIM &

OBJECTIVE

AIM OF AND OBJECTIVE

A mouth ulcer or a mucosal ulcer is an ulcer that occurs on the mucous membrane of the oral cavity. Mouth ulcers are very common, occurring in association with many diseases and by many different mechanisms, but usually there is no serious underlying cause. Mouth ulcers often cause pain and discomfort, and may alter the person's choice of food while healing occurs. For the treatment of the mouth ulcer number of the formulation were used like buccal tablet, gel, spray and film. Among which film drug delivery is best preferable for the fast and sustained release action because of its advantage like ease of administration for person who are mentally disabled, and there is no requirement of water, film either can dissolve fast or it could be give sustained release action accordingly it was designed. Most benefit with the buccal film it is the cost effective because of the manufacturing process is not tedious.

For the treatment of mouth ulcer number of the drugs were used such as Bupivacaine, Lidocaine Prilocaine, Benzocaine, Ropivacaine, Lidocaine, Prilocaine, Chlorhexidineand and Cetalkonium Chloride. Among which Benzocaine is mostly preferable because it's reported as a good anesthetic. It's produce the reversible loss of sensation and relive from the local pain. The number of formulation such as oral gel, spray, and tablet are available in the market except buccal film. The benzocaine drug was selected for buccal formulation because it was not used in the film formulation and its active ingredient to treat the mouth ulcer.

The main objective of the work is development of a novel, sustained release buccal film containing local anesthetic drug by using solvent casting method. To explore the various polymers alone or in the combination that can be hold in mouth above 30 min and gives sustained release action. To obtain the best formulation based on preliminary trial by applying the QbD approaches. PEG 600 will be selected as a plasticizer and optimize the best formulation of benzocaine with the most suitable mucoadhesive polymer. Chitosan and PVA will be selected in the combination as a sustained release film forming polymer. The film of the benzocaine will completely dissolved within one an hour and give the complete release of the drug. So there is no chances of residue remain in the mouth. It will be evaluated for thickness, tensile strength, % elongation, folding endurance, disintegration and % drug release.

INTRODUCTION

2.1 Introduction of the oral film [1] [2] [3] [4]

Pharmaceutical science and technology has progressed enormously in the recent years. These advances in therapeutics and the need to optimize drug delivery in the body have increased the value of dosage form in therapy. This increased awareness has resulted in an increased sophistication and level of expertise in the design development, manufacture, testing and regulation of drugs and dosage forms.

Despite tremendous advancements in drug delivery, the oral route remains the perfect route for the administration of therapeutic agents because of the low cost and the ease of administration which in turn has increased the level of patient compliance.

Many pharmaceutical dosages are administered in the form of pills, granules, powders, and liquids. Generally, a pill is designed to swallow or chew so as to deliver a precise dosage of medication to patients. The pills, which include tablets and capsules, are able to retain their shapes under moderate pressure. However, some patients, particularly pediatric and geriatric patients, have difficulty in swallowing or chewing solid dosage forms. Many pediatric and geriatric patients are unwilling to take these solid preparations due to fear of choking.

Patient convenience and compliance oriented research has resulted in bringing out safer and newer drug delivery systems. Recently mouth dissolving drug delivery systems have started gaining popularity and acceptance due to their rapid onset action, greater bioavailability and ease of administration.

The oral route of administration still continues to be widely used accepted route, contributing to 50-60% of total drug formulations because of ease of administration. Self-medication, and pain avoidance as compared to parenteral. Mainly elderly patients may experience problems in swallowing solid dosage forms. Oral administration of conventional tablets poses problems especially, when the patient is mentally ill, developmentally disabled. In some cases motion sickness, sudden episode of allergic attack or coughing and unavailability of water, poses problem in swallowing. To fulfill these medical needs pharmaceutical technologists developed several mouth dissolving drug delivery systems, like films, patches.

Normally films are formed fast dissolving these soluble in water at room temperature that will break up in 30 sec and disappear in one minute. But recently in trend film was also

formulated for the sustained release action. The faster the drug goes into the solution, quicker its absorption and onset of clinical effect. By altering the condition and formulation factors. It is possible to slow down or speed up dissolving rate in the mouth by doing modification in which polymer is used. The mouth dissolving films-contain active ingredients, flavors, sweeteners and other ingredients, which are released as the film dissolves. These films dissolve instantaneously when placed on the tongue and called as orally Dissolving Films (ODFs). Most people are familiar with ODFs in the form of breath freshening strips or cough suppressants. However, the drug delivery advantages and healthcare benefits of this dosage form extend far beyond these applications. A major claim of the some ODFs is increased bioavailability compared to traditional dosage form. Because of dispersion in saliva while still in the oral cavity, there can be pre-gastric absorption from some formulations in those cases where the drug dissolves quickly. Buccal, pharyngeal and gastric regions of absorption of the many formulations. However, other formulations show nearly identical plasma-concentration profiles. Any pre-gastric absorption avoids first pass metabolism and can be a great advantage in drugs that undergo a great deal of hepatic metabolism. However, if the amount of swallowed drug varies, there is the potential for inconsistent bioavailability. While the claimed increase in bioavailability is disputable, it is clear that the major advantage of these formulations is convenience.

2.1.1 Advantage of buccal drug delivery system

1. Ease of Administration.
2. Permits localization of the drug in the oral cavity for a prolonged period of time.
3. Offer excellent route for systemic delivery of drug with high first pass metabolism, there by offering a greater bioavailability.
4. A significant reduction in dose can be achieved, thereby reducing dose dependent side effects.
1. Drug which are unstable in acidic environment of the stomach or are destroyed by the enzymatic or alkaline environment of the intestine can be administered by this route.
2. The presence of saliva ensures relatively large amount of water for dissolution unlike the rectal and transdermal routes.
3. Its offer the passive system for drug absorption and does not require any activation.

4. It can be made unidirectional to ensure only buccal absorption.
5. The buccal mucosa is highly perfused with blood vessels and offer greater permeability than the skin.
6. Therapeutic serum concentration of the drug can be achieved more rapidly.
7. Better patient compliance than vaginal, rectal and nasal route of administration.
8. Buccal mucosa is less prone to damage or irritation than nasal mucosa nasal mucosa and shows short recovery times after stress or damage.
9. Termination of therapy is easy.
10. Can be administered to unconscious patient.
11. Increased patient compliance.

2.1.2 Disadvantage

1. Once placed at the absorption site, the dosage form should not be disturbed.
2. The drug swallowed in saliva is lost.
3. Properties like unpleasant taste or odour, irritability, to the mucosa, stability at salivary pH poses limitation to the choice of drug.
4. Only drug with small dose can be administered.
5. Eating and drinking may become restricted.

2.1.3 Ideal properties of mouth dissolving films

1. It should have an acceptable taste.
2. Pleasing to mouth.
3. Should have good tensile strength.
4. It should not be harm in environmental condition.
5. All ingredients should be compatible with each other.

2.1.4 Methods of the film formulation [5]

These following method are used to formulate the film.

1. Solventcasting
2. Semisolid casting method
3. Hot-meltextrusion
4. Solid dispersionextrusion
5. Rollingmethod

2.1.4.1 Solvent Casting Technique

The method of solvent casting techniqueinvolves preparation of the film base which involves the mixingof suitable film forming excipients along with drug inasuitable solvent or solvent system. Once the solutionis prepared, the film casting process is performed where inafilm of desired thickness is casted onto a movinginertsubstrate, where suitable rollers are employed for guiding the solution onto the substrate. The clearanceor tolerance between the roller and the substrate determines the required thickness of the film. This process is usedin large scale production wherein glass or Teflon platescan be used as inert support material to cast a film atthelaboratory scale. The formed strip is then subjectedto drying process to remove thesolvent.

The selection of solvent essentially depends on the API to be incorporated into the film. The physicochemical properties of the API like heat sensitivity, shear sensitivity, the polymorphic form of the API employed, compatibility of the API with solvent and filmbased excipients are to be critically studied. Thepredominantfactors to be considered are liquid rheology, desiredmass to be casted and uniformity of drug content.Solventsystems used in the preparation of solution orsuspension should be selected carefully and more preferablyfromICH Class 3 solventlist. Heating processes can be used to assist the complete dissolution of materials. Mixing may cause formation of air bubbles and their entrapment during the solution preparation. Entrapped air tend to produce uneven films. Deaeration step is imperative to get a uniform film which may be achieved by vacuum assisted machines.

Another important aspect is the moisture present in the solution. It is observed that moisture can cause changes in the mechanical properties of the films such as tensile strength, flexibility, folding endurance etc. Hence care should be exercised by using suitable humidity controls in the manufacturing production area. The solution is subjected to continuous mixing process in order to keep the viscosity and concentration unchanged. The solution or suspension may be kept under controlled temperature condition to achieve the desired viscosity of the material.

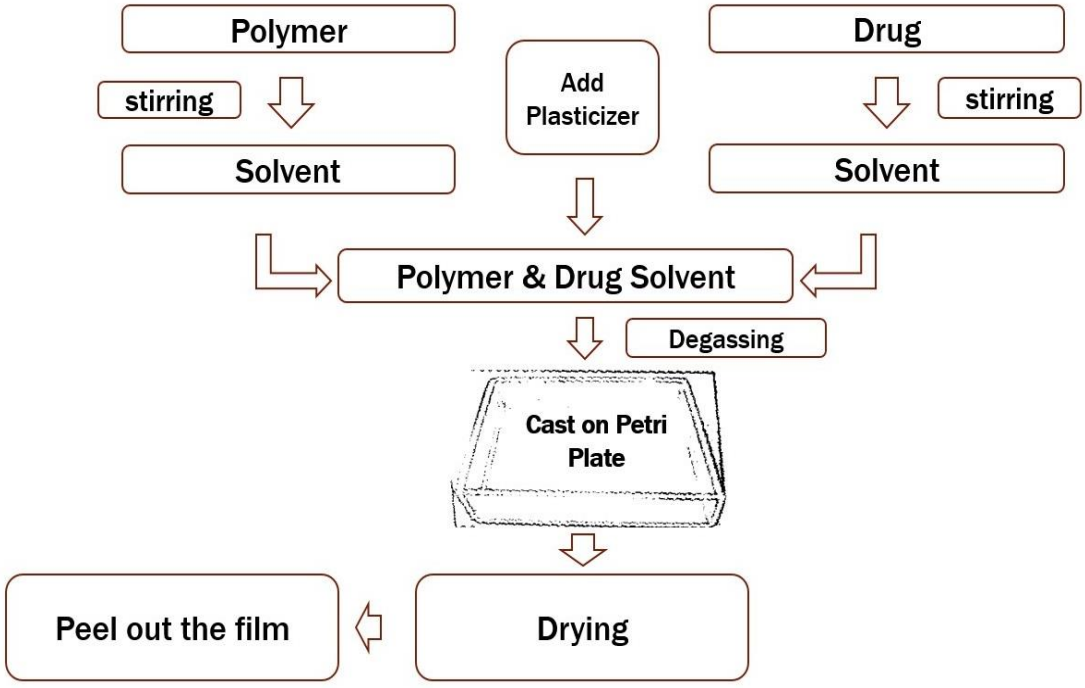

Figure 2.1: Schematic diagram of solvent casting method

2.1.4.2 Semisolid casting:

In this method a gel like formulation is prepared which is then transformed into film. All water soluble film forming polymers are dissolved in water and a solution is prepared. Alongside a solution of acid insoluble polymers in ammonium or sodium hydroxide is prepared. Now aqueous solution is poured into the later prepared solution and a gel like product is obtained this is then casted into film with the help of heat control drums. The ratio of water soluble polymers and acid insoluble polymers should be 4:1.

2.1.4.3 Hot melt Extrusion Process

Hot melt extrusion (HME) is commonly used to prepare granules, sustained-release tablets, and transdermal and transmucosal drug delivery systems. This technique involves shaping a polymer into a film via the heating process rather than through the traditional solvent casting method. In this process API and other ingredients are mixed in dry state which are subjected to heating process and then extruded out in molten state. These processes do not involve use of any solvents systems. The molten mass thus formed is used to cast the film. The films are further cooled and cut to the desired size. The main disadvantage of this process is high temperature used in the process might degrade thermolabile APIs. The critical step is the casting and the drying process. Optimization of speed of casting and drying time are important from the commercial scale output. Hot-melt extrusion include lower temperature and shorter residence time of the drug carrier mix (<2 minutes), absence of organic solvents, continuous operation possibility, minimum product wastage, good control of operating parameters, and possibility to scale up. Repka et al. prepared chlorpheniramine maleate (CPM) topical HPC films by hot melt extrusion technique using hydroxy propyl cellulose as polymer.

2.1.4.4 Solid Dispersion Extrusion

The term "solid dispersions" refers to the dispersion of one or more active ingredients in an inert carrier in a solid state in the presence of amorphous hydrophilic polymers and also using methods such as melt extrusion. This involves a drug which is first dissolved in a suitable liquid solvent and then this solution is incorporated into the melt of suitable polymer, obtainable below 70º C without removing the liquid solvent. The selected solvent or dissolved drug may not be miscible with the melt of the polymer.

2.1.4.5 Rolling Method

In this method, the film is prepared by pre-mixing of an active ingredients and excipients followed by subsequent addition of the solvent. The pre-mix or master batch which includes the film- forming polymer, polar solvent, and any other additives except a drug active is added to the master batch feed tank .Then a pre-determined amount of the master batch is controllably fed via a first metering pump and control valve to either or both of the first and second mixers. The required amount of the drug is added to the desired mixer

through an opening in each of the mixers. After the drug has been blended with the master batch pre-mix for a sufficient time to provide a uniform matrix, a specific amount of the uniform matrix is then fed to the pan through the second metering pumps. The film is finally formed on the inert substrate and carried away via the support roller. Thus the wet film is then dried using controlled bottom drying, desirably in the absence of external air currents or heat on the top (exposed) surface of the film.

2.1.5 Formulation aspects for fast dissolving films.

1. BCS class of drugs/ drug category.
2. Film forming polymer.
3. Plasticizer.
4. Sweetening agents.
5. Saliva stimulating agents.
6. Cooling agent.
7. Flavoring agent.
8. Coloring agent.
9. Surfactants.
10. Stabilizing and thickening agents.

All the ingredients used should be safe and approved for oral use, the details of ingredients used are described as

2.1.5.1 Drug class/ category: A variety of drugs can be formulated in fast dissolving film, they should have appropriate molecular weight and dosage size should be smaller. Different classes of drugs such as antineoplastic, antihistaminic, expectorant NSAIDS etc. can be formulated as fast dissolving films.

2.1.5.2 Film forming polymers: Aqueous soluble polymers are mostly used as they are rapidly disintegrated, have pleasant mouth feel and provide strength to the film. Polymers make the skeleton of the film and their selection plays a key role in deciding the physical character of the film including performance. Polymer used should be nontoxic, tasteless, should not reduce the disintegration of the film, non-irritant, have good wetting

properties, inexpensive, it should not in process of forming or causing secondary infections.

Table 2. 1 List of natural and synthetic mucoadhesive polymers[1]

Sr. No.	Category	Polymers
1.	Synthetic polymer	**1. Cellulose derivatives** • Methylcellulose (MC) • Ethyl cellulose (EC) • Hydroxy ethyl cellulose (HEC) • Hydroxyl propyl cellulose (HPC) • Hydroxy propyl methylcellulose (HPMC) • Sodium carboxy methylcellulose (NaCMC). 2. Poly (Acrylic acid) polymers 3. (Carbomers, Polycarbophil). 4. Poly hydroxyl ethyl methylacrylate. 5. Poly ethylene oxide. 6. Poly vinyl pyrrolidone. **7.** Poly vinyl alcohol
2.	**Natural polymer**	• Tragacanth • Sodium alginate • Guar gum • Xanthan gum • Soluble starch • Gelatin • Chitosan

2.1.5.3 Plasticizers: They should be compatible with the drug and their selection depends on the type of solvent used. They provide flexibility and decide the texture of the film. They are used in concentration between 1 to 20% w/w of dry polymer weight. Examples of film forming agents are Glycerol, PG, PEG (low molecular weight), acetyl citrate, castor oil etc.

2.1.5.4 Sweetening Agents: Sweetener are used to counter the bad taste of the drug or excipient, they are mainly used in paediatric formulations. Many types of natural and synthetic sweetening agents are available which can be used in the formulation of fast dissolving films. For example glucose, sucrose, maltose, stevioside, ribose, saccharine, aspartame etc.

2.1.5.5 Saliva stimulating agents: These agents are used to increase the saliva secretion so that film can disintegrate rapidly and there will be faster absorption of drug hence faster onset of action. Commonly used saliva stimulation agents are lactic acid, malic acid, ascorbic acid, tartaric acid etc.

2.1.5.6 Cooling agents: they are used along with flavoring agent for increasing their strength and provide good mouth feel. They agents used are monomethyl succinate, utracoll II etc.

2.1.5.7 Flavoring agents: These agents are used to mask the unpleasant taste of the formulation. There are many flavoring agents are available which can be used in fast dissolving films for example, different resins, peppermint oil, nutmeg oil, fruity flavors like vanilla, coffee, chocolate etc.

2.1.5.8 Coloring agents: Food, drug and cosmetics approved colors should be used. Their concentration should be less than 1% w/w.

2.1.5.9 Surfactant: These are used in formulation to reduce the surface tension and increase the wetting of the film, they help in rapid disintegration, and dissolution of the film and hence rapid onset of action. Commonly used surfactants are SLS, benzalkonium chloride, tween, span, polaxamer 407 etc.

2.1.5.10 Stabilizing and thickening agents: These agents are used to aid in formulation process by maintaining its viscosity and consistency so that it has a good rheology and can be casted as per need. They should be used in concentration less than 5% w/w. frequently used agents are xanthan gum, locust bean gum, carrageenan and cellulosic derivatives.

2.2 Overview of the oral mucosa [6] [7]

The oral cavity is lined with mucous membranes with a total surface area of 100 cm^2. It is possible to observe several distinct areas; the floor of the mouth (sublingual), the buccal mucosa (cheeks), the gums (gingival), the palatal mucosa and the lining of the lips.

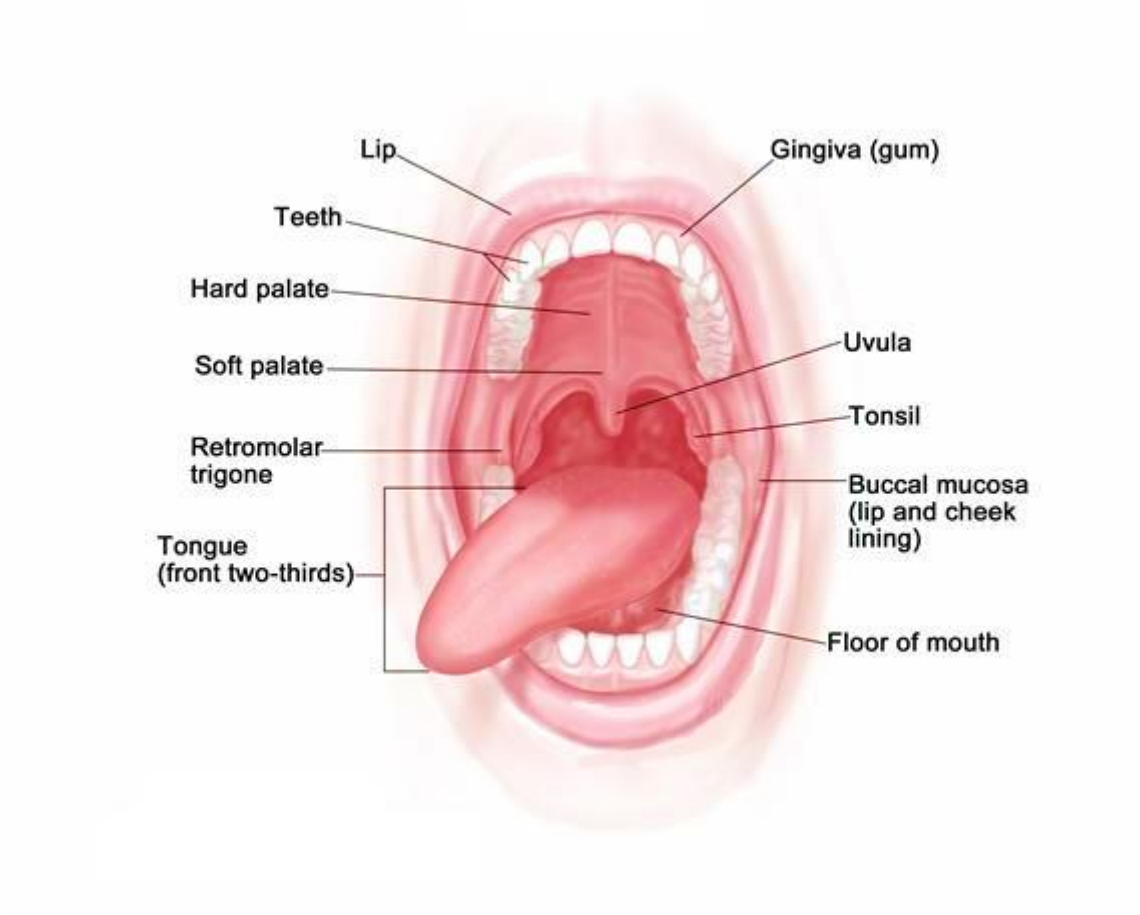

Figure 2. 2 Schematic representation of the open oral cavity

The oral mucosa tissue consists of a multilayered epithelium covered with mucus and consists of a stratum distendum, stratum filamentosum, stratum suprabasale and stratum basale. Below this lies a basal lamina. The basal lamina connects the epithelium to a connective tissue layer, the lamina propria. Below lamina propria lies submucosa.

Epithelium serves as the mechanical barrier that protects underlying tissues whereas lamina propria acts as a mechanical support and also carries blood vessels and nerves.

Table 2. 2 The oral mucosa thickness and turnover time depending on the site

Site	Mean thickness (µg)	Medium turn over time (Days)
Epidermis	120	27
Hard plate	310	24
Buccal mucosa	580	13
Floor of mouth mucosa	190	20

The epithelium of human oral mucosa shows several distinct patterns of maturation, related to the functional demands of the tissue. Some regions of the epithelium are keratinized (dehydrated, mechanically tough and chemically resistant), whereas others are not. Keratinized epithelium is found in less flexible masticatory mucosa of gingival and part of hard palate. Nonkeratinized (flexible) epithelium forms the surface of the distensible lining of mucosa of the soft palate, floor of the mouth, lips and cheek. The nonkeratinized regions, such as buccal mucosa, are more permeable than the keratinized regions. This is due to some extent, to the composition of intercellular lipids comprising the particular region. Whereas keratinized regions contain predominantly neutral lipids (ceramide), nonkeratinized areas are composed of glycosyl ceramide that appears to be derived from membrane coating granules of keratinized tissue.

2.2.1 The membrane coating granules (MCG)

As cells of epithelium mature, small organelles, known as membrane coating granules (MCG), probably derived from the Golgi complex appear in the prickle cell layer. In later stages of differentiation, they migrate towards the superficial part of the cell at the junction of the granular and cornified layers in the keratinized tissues, and in the deeper part of the superficial cell layer in the nonkeratinized tissue. The bounding membrane fuses with the cell membrane, and the contents of the granules ae discharged into the intercellular space. During fusion, the bonding membrane of the granules is introduced

into the plasma membrane of the epithelial cell. The extruded material, composed primarily of lipid is then organized into multiple stacked lipid sheets. MCG of keratinized oral epithelium are ovoid, 0.1-0.3µm in length and have high ratio of lipid to protein. The lipids include phospholipids, cholesterol esters, fatty acids, ceramides and several other natural lipids. In nonkeratinized epithelium, MCG have similar distribution within the epithelium and similar chemical composition to those of keratinized tissue. They are spherical, approximately 0.2 µm in diameter.

A relationship between MCG and permeability has been established, thus, a greater volume of MCG is associated with a lower permeability.

Lipids including small amounts of ceramides, monohexosylceramides, cholesterol esters, cholesterol sulphate and fatty acids and a high proportion of phospholipids, triglycerides and cholesterol fill in the intercellular space of oral keratinized tissue.

In nonkeratinized tissue regions, the chemical nature of the intercellular material is less defined than that in the keratinized epithelium. Since the intercellular spaces of nonkeratinized epithelia appear to contain amorphous material, it is possible that the lipids within them are in a nonlamellar lipid phase, with only occasional short stacks of lipid lamellae. This may result in a barrier that is less efficient than that from, keratinized regions.

2.2.2 Saliva

Saliva is the protective fluid for all the tissues of the buccal cavity and it's necessary for oral health. Saliva protects soft tissues from abrasion by rough materials and from some chemicals. Up to 70 of total mucin found in saliva is contributed by minor salivary glands. Main role of salivary mucin is in the non-immune protection of the oral cavity by acting as a lubricant and as a selective permeability barrier against drying. Saliva is 99% water and contains organic and inorganic materials. The surface of the oral cavity is constantly bathed with a stream of saliva (approximately 1 to 12 lit per day). The pH of whole saliva varies between 6.2-7.5.

2.2.3 Mechanisms involved in drug absorption across the oral mucosa:[8]

The mechanisms by which drugs cross biological lipid membranes are passive diffusion, facilitated diffusion, active transport and pinocytosis. Small water-soluble molecules may pass through, small water filled pores. The main mechanism involved in drug transfer across the oral mucosa, is passive diffusion has also been shown to fake place, primarily with nutrients, Passive diffusion involves the movement of a solute from a region of high concentration in the mouth to a region of low concentration within the buccal tissues. Further diffusion then takes place into the venous capillary system, with the drug eventually reaching the systemic circulation via the jugular vein. The physicochemical characteristics of a drug are very important for this diffusion process.

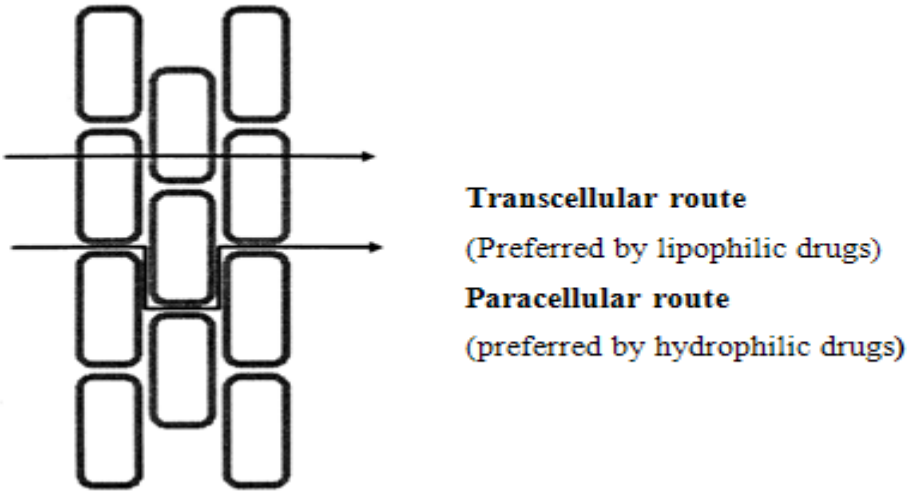

Figure 2. 3 Drug absorption pathway across buccal mucosa

2.3Introduction of the drug [9] [10]

Benzocaine

$C_9H_{11}NO_2$

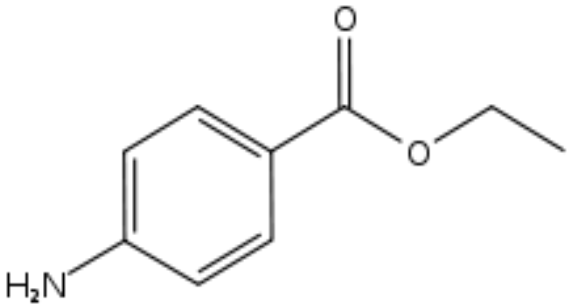

Figure 2. 4 Structure of the benzocaine

An ester type, local anesthetic agent derived from aminobenzoic acid that is most useful when applied topically. It is used in many over-the-counter compounds for pruritus and pain. Benzocaine has a low incidence of toxicity, but sensitization to it may result from prolonged or frequent use. Topical application of benzocaine may cause methemoglobinemia in infants and small children. A minimum of 5% benzocaine is required in a compound to be effective. Benzocaine is used as a local anesthetic, it is used in cough drops and also topical formulations are prepared for relieving pain. It is dispensed as over the counter ointment for treatment of mouth ulcer. It also have ability to treat pain in ear and removing ear wax.

2.3.1 Drug Substance Physicochemical Properties

API Description: .Odorless & Colorless crystals or a white crystalline powder.
IUPAC name: Ethyl 4-aminobenzoate
Therapeutic Category: Local anaesthetic
Molecular Formula: $C_9H_{11}NO_2$
Molecular weight: 165.2 gm/mole
Melting Point: 88-92°
Content: 99.0 percent to 101.0 percent (dried substance)
Dissociation constant pKa: 2.51

Solubility: Freely soluble in ethanol (95 per cent), in Chloroform and in ether, very slightly soluble in water. It is soluble in dilute acids.

Storage: Store protected from light.

2.3.2 Pharmacokinetics

2.3.2.1 Absorption:

Clinically, the site of injection plays an important role in absorption.

Systemic absorption of local anaesthetics is determined by:

- Site of injection
- Dose
- Addition of vasoconstrictor
- Pharmacologic profile of the local anesthetic

Site of injection impacts blood levels of local anesthetic. Areas of high vascularity result in greater uptake and higher blood concentrations. The uptake of local anesthetic from greatest to least is as follows:

IV > Tracheal > Intercostal > Caudal > Para cervical > Epidural > Brachial > Sciatic > Subcutaneous.

2.3.2.2 Mechanism of Action[11]

Benzocaine is a local anesthetic which acts by preventing the generation and transmission of impulses along nerve fibers and at nerve endings. Depolarization and ion-exchange are inhibited. In general, loss of pain occurs before loss of sensory, autonomic and motor functions.

Onset: 1 min

Duration: 15-20 min.

2.3.2.3 Drug interaction

Antagonism with sulfonamide, aminosalicylic acid, anticholinesterases, suxamethonium, antiarrhythmics.

2.3.2.4 Contraindications

Hypersensitivity. Complete heart block. Low plasma-cholinesterase concentrations. Pyrogenic infection at or near the skin. Inj into or application to inflamed, infected tissues, to damaged skin mucosa or on perforated tympanic membrane.

2.3.2.5 Dosage

Adult: As gel, paste, spray or solution up to 20%: Apply to affected area up to 4 times daily.

Elderly: May require lower dose.

2.4 Introduction of excipients

2.4.1 Chitosan[12]

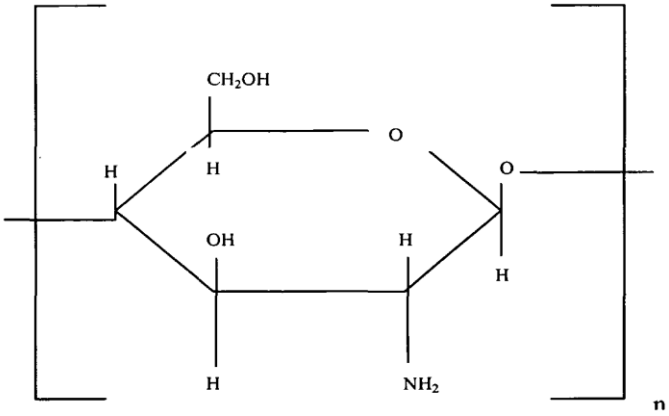

Figure 2. 5 Structure of the Chitosan

Chitosan is a natural mucopolysaccharide (molecular weight 10,000–10, 00,000) of marine origin consists of a linear (1-4)) linked 2- amino-2-deoxy-D glucan, can be chemically prepared from naturally occurring chitin by treatment with alkali at elevated temperature. Chitosan is commercially available in several types and grades that vary in molecular weight and vary in degree of deacetylation and viscosity. Chitosan is nontoxic, biodegradable and biocompatible polymer. Chitosan widely existing in the nature and has antibacterial effect, heavy metal adsorption effect, antioxidation effect and film formability. Chitosan forms viscous solution in various organic acids. These viscous solutions have been used to make functional films. They were readily biodegradable either in sea water or in soil. The cationic properties of chitosan offer the film-maker an additional opportunity to take advantage of electrostatic interactions with other anionic polysaccharides. These films were high modulus, flexible self-supporting and biodegradable, and were advantageous in that all material were derived from petroleum and agricultural products. Chitosan is commercially available in several types and grades that vary in molecular weight by 10,000–10, 00,000 and vary in degree of deacetylation and viscosity.

2.4.1.1 Non-proprietary Names

- BP: Chitosan hydrochloride
- PhEur: Chitosani hydrochloridum

2.4.1.2 Synonyms

2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; deacetylated chitin; deacetylchitin; b-1,4-poly-D-glucosamine; poly-D-glucosamine;poly-(1,4-b-D-glucopyranosamine).

2.4.1.3 Chemical Name

Poly-b-(1, 4)-2-Amino-2-deoxy-D-glucose

2.4.1.4 Functional Category

Coating agent, disintegrant, film-forming agent, mucoadhesive, tablet binder, viscosity increasing agent.

2.4.1.5 Applications in Pharmaceutical Formulation

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications, use as a component of mucoadhesive dosage forms, rapid release dosage forms, improved peptide delivery, colonic drug delivery systems and use for gene delivery. Chitosan has been processed into several pharmaceutical forms including gels, films, bead microspheres, tablets and coatings for liposomes. Furthermore chitosan may be processed into drug delivery systems using several techniques including, spray-drying, coacervation, direct compression and conventional granulation processes.

2.4.1.6 Description

Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the chitosan may look ’cotton like.

2.4.1.7 Moisture content

Chitosan adsorbs moisture from the atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature and relative humidity of the surrounding air.

2.4.1.8 Solubility

Sparingly soluble in water, practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves readily in dilute and concentrated solutions of most organic acids and to some extent in mineral inorganic acids (except phosphoric and sulfuric acids).

2.4.1.9 Viscosity (dynamic)

A wide range of viscosity types is commercially available. Owing to its high molecular weight and linear, unbranched structure, chitosan is an excellent viscosity-enhancing agent in an acidic environment. The viscosity of chitosan solutions increases with increasing chitosan concentration, decreasing temperature, and increasing degree of deacetylation.

2.4.2 Polyvinyl Alcohol [12]

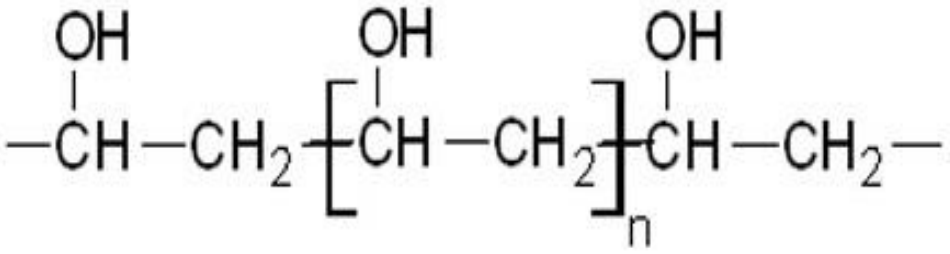

Figure 2. 6 Structure of the PVA

Polyvinyl alcohol is a water-soluble synthetic polymer represented by the formula $(C_2H_4O)_n$. The value of n for commercially available materials lies between 500 and 5000 equivalent to a molecular weight range of approximately 20 000–200 000.

Table 2. 3 different grades of PVA

Sr. No.	Grade	Molecular weight
1	High viscosity	200 000
2	Medium viscosity	130 000
3	Low viscosity	20 00

2.4.2.1 Nonproprietary Names

- PhEur: Poly (vinylis acetas)
- USP: Polyvinyl alcohol

2.4.2.2 Synonyms

Airvol, Alcotex, Elvanol, Gelvatol, Gohsenol, Lemol, Mowiol, Polyvinol; PVA; vinyl alcohol polymer.

2.4.2.3 Chemical Name

Ethenol, Homopolymer

2.4.2.4 Functional Category

Coating agent; lubricant; stabilizing agent; viscosity-increasing agent.

2.4.2.5 Description

PVA occurs as odorless, white granular and nonionic powder free soluble in water, insoluble in organic solvent.

2.4.2.6 Melting point

- 228^{O} C for fully hydrolyzed grades.
- 180–190^{o}C for partially hydrolyzed grades.

2.4.2.7 Applications in Pharmaceutical Formulation or Technology

Polyvinyl alcohol is used primarily in topical pharmaceutical and ophthalmic formulations. It is used as a stabilizing agent for emulsions (0.25–3.0% w/v). Polyvinyl alcohol is also used as a viscosity-increasing agent for viscous formulations such as ophthalmic products. It is used in artificial tears and contact lens solutions for lubrication purposes, in sustained-release formulations for oral administration and in transdermal patches.Polyvinyl alcohol may be made into microspheres when mixed with a glutaraldehyde solution.

2.4.3 Polyethylene glycol

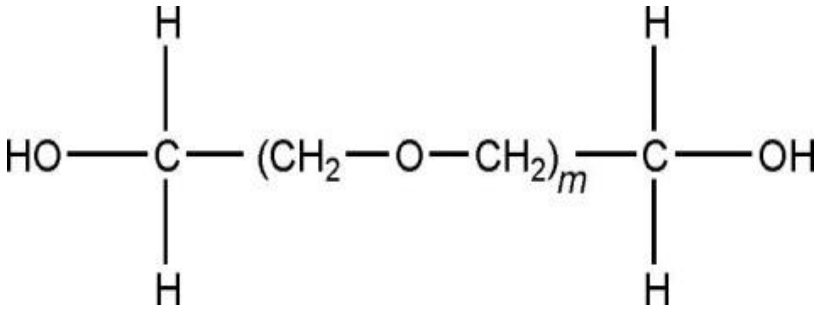

Figure 2. 7 Structure of the Polyethylene glycol

2.4.3.1 Nonproprietary Names

BP: Macrogols

JP: Macrogol 400, Macrogol 1500, Macrogol 4000, Macrogol 6000, Macrogol 20000

PhEur: Macrogola

USPNF: Polyethylene glycol

2.4.3.2 Synonyms

Carbowax, Carbowax Sentry, Lipoxol; Lutrol E, PEG, Pluriol E, Polyoxyethylene Glycol.

2.4.3.3 Chemical Name

α-Hydro-ω-hydroxypoly (oxy-1,2-ethanediyl)

2.4.3.4 Functional Category

Ointment Base, Plasticizer, Solvent, Suppository Base, Tablet and Capsule Lubricant.

2.4.3.5 olubility

All grades of polyethylene glycol are soluble in water and miscible in all proportions with other polyethylene glycols (after melting, if necessary). Liquid polyethylene glycols are soluble in acetone, alcohols, benzene, glycerin, and glycols.

2.4.3.6 Method of Manufacture

Polyethylene glycols are condensation polymers formed by the reaction of ethylene oxideand water under pressure in the presence of a catalyst.

2.5 Preparation of Simulated Saliva Fluid [13]

It is almost impossible to duplicate the properties of human saliva because of its particular characteristics. Saliva is a mixture of fluids secreted by several salivary glands, it is a system with numerous constituents, and it is variable according to the time of day, diet, and so forth. The dilution and rapid elimination of the drugs from the oral cavity are consequences of speech, mastication, and continuous saliva production. Normal, healthy saliva in the oral cavity has a pH between 6.7 and 7.4, but it can temporarily drop below 5 when sweets, carbonated and fruit drinks, and other dietary acids are consumed. Some drugs, such as beta blocking agents, nitrates, and diuretics, as well as tobacco smoking can also reduce salivary pH.

Duffo and Castillo developed artificial saliva composition SS1 for studying the corrosion behaviour of dental alloys. In this solution, all components dissolve completely. It is recommended to add potassium bicarbonate just before use to avoid loss of CO2 and changes in the pH.

Bicarbonate is the major buffering agent of saliva, and is readily lost on standing when exposed to the air. This loss is the principle, if not the sole, cause of rise in pH that is observed on short-term standing. SS1 can be stored at 5°C for at least 8 days without modification of its properties.

Matheka et al used artificial saliva composition SS2 to develop computational models to predict local effects on the mouth from carcinogenic compounds present in tobacco smoke. The interaction of benzethonium-copolymer complex, used in mouth rinse or dentifrices, was evaluated using SS3 by Gaffar et al.

Davis et al. used SS4 in a dynamic dialysis system to investigate the interactions that might occur between drug molecules and the constituents of salivary secretions. Such interactions, if they occur, might influence drug absorption through the oral mucosa SS5 (300ml) was used to monitor the release of salbutamol sulphate from oral fast dissolving films using USP Apparatus 2 (paddles).

Table 2. 4 Composition of Simulated Saliva Fluid (SS)

Composition	SS 1 (g/L)	SS 2 (g/L)	SS 3 (g/L)	SS 4 (g/L)	SS 5 (g/L)
Potassium chloride	0.720	0.720	-	0.149	-
Calcium chloride dihydrate	0.220	0.220	0.228	-	-
Sodium chloride	0.600	0.600	1.017	1.117	8.00
Potassium phosphate monobasic	0.680	0.680	-	-	0.19
Sodium phosphate dibasic	0.866	0.866	0.204	-	2.38
Potassium bicarbonate	1.500	1.500	-	-	-
Potassium thiocyanate	0.060	0.060	-	-	-
Citric Acid	0.030	0.030	-	-	-
Magnesium chloride hexahydrate	-	-	0.061	-	-
Potassium carbonate hemihydrate	-	-	0.603	-	-
Sodium phosphate monobasic monohydrate	-	-	0.273	-	-
Sodium bicarbonate	-	-	-	2.100	-
Submaxillary mucin	-	-	1.000	-	-
Alpha-amylase	-	-	2.000	2.000	-
Mucin gastric	-	-	-	1.000	-
pH	6.5	7.4	-	-	6.8

2.6 Introduction of QbD [14]

2.6.1 Quality: the suitability of either a drug substance or a drug product for its intended use. This term includes such attributes as the identity, strength and purity.

QBD: A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.

Incremental steps

1. **Q8:** pharmaceutical development
2. **Q9:** Quality risk management
3. **Q10:** Pharmaceutical quality system

2.6.2 Elements of QbD

2.6.2.1 Product and process design development

a) Define desired product performance upfront, identify product CQAs
b) Design preformulation and process to meet product CQAs

2.6.2.2Risk assessment and risk control

a) Understand impact of material attributes and process parameters on product CQA.
b) Identify and control sources of variability in material and process
c) Continually monitor and update process to assure consistent quality.

2.6.2.3 CQA (critical quality attributes)

A CQA s is a physical, chemistry, biological or microbiological property or characteristic that should be with in an appropriate limit, range or distribution to ensure the desired product quality (ICH Q8 R2).

2.6.2.3.1 CQAs are generally associated with the

- Drug substance
- Excipients
- Intermediates (in process material)
- Drug product

2.6.2.3.2 Risk assessment: Link raw material attributes and process parameters to CQAs.

2.6.2.4 Material attributes

2.6.2.4.1 Material: Raw material, starting material, reagents, solvents process aids, intermediates APIs and packaging and labeling materials ICH Q7A

2.6.2.4.2 Attributes: A physical, chemical, biological or microbiological property or characteristic.

2.6.2.4.3 Material Attributes: It can be excipient's CQAs, raw material CQAs, starting material CQAs and drug substance CQAs etc.

- A material attribute is quantified
- Typically fixed
- Can sometimes be changed during further processing.

2.6.2.5 Process Parameters

A process parameter whose variability has an impact on a critical quality attributes & therefore should be monitored or controlled to ensure the process produces the desired quality (Q8R2) CPPs have a direct impact on the CQAs. A process parameter can be measured and controlled.

2.6.2.6 Principle of a quality risk management

1) The evaluation of the risk to quality should be based on scientific knowledge and ultimately link to the protection of the patient.
2) The level of effort, formality and documentation of the quality risk management process should be commensurate with the level of risk.

2.6.2.7 Quality Risk Management

Describes systematic in processes for the assessment, control, communication and review of quality risk. Applies over product life cycle: development, manufacturing and distribution. Includes principles, methodology and examples of quality risk management.

2.7 3^2 Full factorial design[15]

Design of experiments should be such that it helps in finding the optimized batch in minimum number of trials which are to be performed. Out of the various designs, the 3^2 full factorial was found for further studies of various parameters in final formulation. These experimental designs consist of 8 runs. Various series of experimental trials were performed and evaluation was based on the setup of experimental runs taken at different combinations of factor levels. All the values of responses were fitted to a linear equation model and the adequacy of this model was checked by various statistical parameters such as ANOVA, lack of fit, and multiple correlation coefficients R^2 tests.

2.7.1 Important consideration of factorial design

- **2.7.1.1 Factor to be studied:** Factor can be quantitative, i.e. they have numerical values, or they can be qualitative. The latter will often have names rather than numbers. They will choose accordance with the object of the experiment. Though the objectives of the experiment may be define two or more factors, there may be other factors that would influence the outcome of the experiment and these must be kept constant.
- **2.7.1.2 The level of the factors:** this is often a difficult decision, in which the experience of the researcher plays an important role. A commonly used starting point is 25^{th} or 75^{th} percentile levels of the range of possible values of the factors, through this might not be practicable.
- **2.7.1.3 The response to be measured:** The response must be capable of being expressed numerically. Adjective description (big, bigger and biggest) or ordinal numbers.

2.7.2 Merits

In the absence of interaction, factorial design it has maximum efficiency in estimating main effect.

If interaction exist, factorial designs are necessary to revel and identify the interaction.

- Maximum use is made of the data since all main effects and interactions are calculated from all of the data.

- Factorial designs are orthogonal, all estimated effects and interactions are independent of effects of other factors.

2.7.3 The equation constructed from 3 factorial experiments is in the following form

$Y= B_0 + B_1X_1+ B_2X_2+B_{12}X_1X_2+B_{11}X_1^2+B_{22}X_2^2$

Where,

B_0 = intercept

X_1 and X_2 = variables

B_1 and B_2 = Co-efficient of X_1 and X_2 variable

B_{12} = Co- efficient of interaction

B_{11} and B_{22} = Co- efficient of quadratic terms

LITERATURE REVIEW

3.1Literature review (Patents)

3.1.1 Mc Donald, III et al. (2015) reviewed a consumable film adapted to adhere and to dissolve in the oral cavity hat provides a local anesthetic and therapeutic agents for the treatment of oral burns or injuries. The film is designed to instantly release benzocaine, or other types of local anesthetic or therapeutic agent, upon adhesion to the affected areas of the mouth, and will continue to release sufficient quantities for pain relief and for healing over an extended period of time. The film consist of benzocaine, lidocaine and menthol as an active ingredient and Glycerol, polaxamer 188 and HPMC polymers were used in the film that was producing the Bioadhesive buccal film.[1]

3.1.2 Alur et al. (2013) was showed that composition and method for treating a disease, such as infection, pain or inflammation, by using the compositions. Particularly disclosed are composition and method of treating oral pain, wherein the compositions undergo in-situ gelation, optimal adhesion to the oral mucosa, controlled erosion and controlled release of the active ingredient, i.e., benzocaine, which provides a superior degree of pain relief or analgesia for extended period of time.[2]

3.1.3 Myers et al. (2014) reviewed that invention was relates to products and methods for treatment of narcotic dependence in a user. The invention more particularly relates to self-supporting dosage forms which provide an active agent for treating narcotic dependence while providing sufficient buccal adhesion and therapeutically effective dosage, essentially matching with currently marketed tablets containing the same active ingredient, such compositions are particularly useful for treating narcotic dependence while providing sufficient buccal adhesion of the dosage form.[3]

3.1.4 Schiraldi et al. was present invention relates to a controlled- releasing medicament- containing preparation for intra-oral use, and is more especially concerned with such a preparation (and the process of using it) in the form of a very thin extruded thermoplastic film (which can be in single layer or laminated multi-layer form) having at least one Bioadhesive layer containing 40-95% of a thermoplastic cellulose ether and 5-60% of a homopolymer of ethylene oxide which can adhere to the mucosa of the oral cavity. The extruded film drug delivery system of the present invention, which has

incorporated there in the medicament to be dispensed, is so thin and flexible when wet as to be unobtrusive to the patient after it has been properly positioned and placed in the mouth.[4]

3.1.5 Lerner et al. In which patent shows invention is directed to a controlled- release solid composition for the oral cavity or "pharmaceutical oral patch" that adheres to hard dental surfaces, such as teeth and denture, and release an active pharmaceutical agent into the oral cavity. Release of the agent is for a predetermined period of time and at a predetermined sustained concentration. The site of action of the agent is local systemic.[5]

3.1.6 Johnson et al. This patent describe composition and method for treating disordered tissues, such as caused by pathogens and/or by toxins. The treatment composition include an anti-infective active agent, a liquid carrier, and benzocaine in an amount so that the treatment composition penetrates more quickly into disordered tissue compared to the treatment composition in the absence of the benzocaine. The treatment compositions and method may employ the use of an applicator adapted for use in promoting penetration of the treatment composition and/or agitation of the disordered tissue to further enhance penetration.[6]

3.2 Literature review on drug

3.2.1 M. Mohseni et al: This article was describe about the buccoadhesive film of benzocaine were prepared by solvent casting technique using various amount of HPMC and PVP as film forming polymers and propylene glycol plasticizer. Film were evaluated for their for their parameters including thickness, diameter, weight of the films, mucoadhesive strength, folding physiochemical endurance, extend of swelling, drug content and in vitro release studies in pH 6.8 phosphate buffer solution. It was concluded that increasing the amount of HPMC the mucoadhesive strength and folding endurance were increased however the amount of drug release was slow down. Overall the formulation containing 2% HPMC and 1% PVP managed to adhere to the mucosal surface strongly and release 85% of drug content within 12h, formulation could be found suitable as a template for bucoadhesive delivery of benzocaine.[7]

3.2.2 D. Glycol et al: In which article describe about use of benzocaine as pain reliving agent for the animal. Standard therapeutic doses of benzocaine ranged from 150 to 750 mg per animal. Benzocaine is also currently used as surface anaesthetic as ointment (0.5% benzocaine) for wounds and uncerated surface in horses, cattle and sheep applied twice a day until healing. Benzocaine is mainly hydroxylated in the metabolite para-aminobenzoic acid (PABA) that inhibits he action of sulphonamides.[8]

3.2.3 Eslamian et al: In which article studying the effect of benzocaine mucoadhesive patches (20%) on orthodontic pain caused by elastomeric separators. A split-mouth design was used in 30 patients (12 female, 18 male, aged 23 ± 3.75 years). They were instructed to apply benzocaine and placebo patches randomly for right or left first permanent molars of maxillary/mandibular arches for 20 min and repeat this procedure every 6 h with a similar type patch. The benzocaine 20% patches significantly reduced the post-separation orthodontic pain.[9]

3.2.4 So, Tsz Yin et al: In which article reviewed the topical application of benzocaine may induce methemoglobinemia in the pediatric population. Benzocaine is an anesthetic agent that is often used before procedures and clinical tests, such as esophagoscopy,

bronchoscopy, and endotracheal intubation. However, a potential deadly condition known as methemoglobinemia can occur with this agent. It causes the oxidation of hemoglobin to methemoglobinemia to occur more rapidly than the reduction of methemoglobin back to hemoglobin.[10]

3.2.5 De Araujo et al: This article was present development of anesthetic bioadhesive film containing benzocaine and study there in vitro skin permeation and in vivo performance, in comparison with commercial formulations. In vitro permeation assays were performed in vertical diffusion cells using full-thickness pig ear skin as barrier. Intensity and duration of analgesia were evaluated in rats by tail-flick test, and skin histological analysis was carried out. Results from our study indicate that benzocaine-induced analgesia was significantly prolonged with the films compared to commercial creams, in agreement with the higher in vitro permeation.[11]

3.3 Literature review on dosages form

3.3.1 Okamoto HiroKazu and others prepared the polymeric films of hydroxypropylcellulose for sustained delivery of Lidocaine via buccal route. They prepared the films of hydroxypropylcellulose by three different techniques: compression of physical mixture, direct compression of spray dried powder and solvent evaporation method. The prepared films were subjected to dissolution and in vitro permeation studies. They also studied the various factors like compression pressure, preparation method, powder composition and their effect on drug release rate.[12]

3.3.2 Mona s et al. (2008) prepared the mucoadhesive buccal films of Glipizide by solvent casting technique using Hydroxypropyl Methyl Cellulose, Sodium CMC, Carbopol 934P and Eudragit RL- 100. Prepared films were evaluated for weight, thickness, surface pH, swelling index. In-vitro residence time, folding endurance, In- vitro release permeation studies and drug content uniformity. The optimized formulation showed that HPMC with Sodium CMC combination has shown good swelling, a convenient residence time and promising controlled drug release.[13]

3.3.3 Deshmane V Subhash et al. (2005) prepared the Mucoadhesive buccal film of diltiazem hydrochloride by solvent casting technique using sodium CMC, polyvinyl pyrrolidone K30 and polyvinyl alcohol. Prepared films were evaluated for their weight, thickness, surface pH, swelling index, In-vitro residence time, folding endurance, In-vitro release, permeation studies and drug content uniformity. From this study shows that formulation in polyvinyl alcohol and polyvinyl pyrrolidone showed moderate swelling, a convenient residence time and promising drug release.[14]

3.3.4 N. Salamat-Miller: This article was highlights the use of mucoadhesive polymers in buccal drug delivery, characteristics of the desired polymers, cover the theories behind the adhesion of bioadhesive polymers to the mucosal epithelium.[15]

3.3.5 Maren Preis et al: In which discuss about the various film former and various method and formulation development of bilayered film. Different methods for characterizing bilayered films was evaluated and it became obvious that some tests, such

as disintegration, dissolution, and mechanical strength, need to be optimized in terms of oromucosal film formulations.[16]

3.3.6 Javier O. Morales: This article was present the advantage of mucoadhesive film it's having improved patient compliance due to their small size and reduced thickness, compared for example to lozenges and tablets. The development of mucoadhesive buccal films has increased dramatically over the past decade because it is a promising delivery alternative to various therapeutic classes including peptides, vaccines, and nanoparticles. This review will consider the literature that describes the manufacture and characterization of mucoadhesive buccal films.[17]

3.3.7 C. F. Wong et al: Formulate the controlled release buccal patches were fabricated using Eudragit NE-40D. Bioadhesive polymer such as HPMC, sodium carboxy methyl cellulose and Carbopol of different grade were incorporated into the patches to modify their bioadhesive properties as well as drug release. The incorporation of hydrophilic polymers was found to affect the drug release as well as enhance the bioadhesiveness. High viscosity polymers can enhance the bioadhesiveness of the patches.[18]

MATERIALS AND METHODS

4.1 Lists of Materials and Equipment.

Various materials and equipment were used to carry out the experimental work. The list of materials and equipment used are presented in the tables below.

Table 4. 1 List of Equipment

Equipment	Company name
Diffusion cell apparatus	Orchid Scientifics, Mumbai.
Digital Balance	Citiweigh, Tejas exports, Ahmedabad
Digital pH meter	Analab scientific instruments, India.
Digital Tensiometer	EIE Instruments Pvt Ltd., Ahmedabad
Digital Vernier caliper	Insize Co. Ltd. China.
Dissolution test apparatus USP	Electrolab TDT-08L, Mumbai
FTIR	Jasco FTIR 6100 Type-A, Japan
Hot air oven	EIE Instruments Pvt Ltd., Ahmedabad
Magnetic stirrer with hot plate	EIE Instrument Pvt Ltd., Ahmedabad
QTS Texture Analyser	Brookfield Engineering Laboratories, Inc. USA
Sonicator Bath	Trans-o-Sonic D-Compact, Ahmedabad
Thermonik Tablet Tester, DTH – 250	Campbell electronics, Mumbai
UV/VIS Double beam spectrophotometer	Shimdzu UV 1800 corporation, Japan

Table 4. 2 List of materials

Materials	Company name
Acetic Acid Glacial	High purity laboratory chemical Pvt Ltd. Mumbai
Benzocaine	Balagi Drug, Surat
Cellophane Membrane	Hi-media, Mumbai.
Chitosan	Hi-media, Mumbai.
Glycerol	Central Drug House Ltd., New Delhi
Methanol	SD Fine chem Ltd. Mumbai
Poly Ethylene Glycol-20000 (PEG)	Central Drug House Ltd., New Delhi
Poly Vinyl Alcohol (PVA- Medium Viscous)	Central Drug House Ltd., New Delhi
Potassium Dihydrogen Phosphate	Central Drug House Ltd., New Delhi
Sodium Chloride	Central Drug House Ltd., New Delhi
Sodium Dihydrogen Phosphate	Central Drug House Ltd., New Delhi
Sodium Lauryl Sulphate	SD Fine chem Ltd. Mumbai.

4.1 Identification of Benzocaine

4.1.1 Melting Point Determination [32]

Melting point is the temperature at which the pure liquid and solid exist in the equilibrium. In the practice it is taken as equilibrium mixture at an external pressure of 1 atmosphere. The thiel's tube method of melting point determination in liquid paraffin was used in the present study.

4.1.2 IR Spectra [33]

IR spectra of drug in KBR pellets at moderate scanning speed between 2000-400 cm^{-1} was carried out using FTIR (Jasco FTIR 6100 TYPE A). All the powder samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture.

4.1.3 UV absorption maxima benzocaine in methanol

UV scanning was done for 5µg/ml drug solution from 200-400 nm in methanol as a blank using Shimadzu UV 1800 double beam UV/Visible spectrophotometer.

4.1.4 Standard curve of Benzocaine in methanol [34]

4.1.4.1 Preparation of stock solution

10 mg of Benzocaine was accurately weighed and transferred in 10 ml volumetric flask. It was dissolved in Methanol and volume was made up to the mark with Methanol to get 1000 µg/ml solution. Then from the solution of 1000 µg/ml, 1 ml sample is transferred in to 10 ml of volumetric flask and diluted up to the mark to get 100 µg/ml.

4.1.4.2 Preparation of standard curve in Methanol

From the stock solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 ml samples were transferred to 10 ml volumetric flask and diluted with the water up to the mark to obtain Benzocaine concentration of 1-7µg/ml respectively. The standard curve was performed in triplicate.

4.1.5 Standard curve of Benzocaine in Simulated Saliva Fluid (pH 6.8) [34]

4.1.5.1 Preparation of stock solution

10 mg of Benzocaine was accurately weighed and transferred in 100ml volumetric flask. It was dissolved in simulated saliva fluid (pH 6.8) and volume was made up to the mark with simulated saliva fluid to get 100 µg/ml solution. Then from the solution of 100 µg/ml.

4.1.5.2 Preparation of standard curve in Simulated Saliva Fluid (pH 6.8)

From the stock solution 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 ml samples were transferred to 10 ml volumetric flask and diluted with the water up to the mark to obtain Benzocaine concentration of 1-7µg/ml respectively. The wavelength maxima of benzocaine in SSF was found to be 287nm. Absorbance of each solution was measured at 287 nm. The standard curve was performed in triplicate.

4.1.6 Drug Excipient Compatibility Study

4.1.6.1 Differential Scanning Calorimetry (DSC) [33]

DSC Study was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipient compatibility study.

4.1.6.2 Operating Procedure: [35]

Weigh an appropriate quantity of the sample shown in table to be examined and place in the sample crucible. Set the initial temperature 30^0 C and final temperature 350^0 C, and the heating rate10^0 C/min. Begin the analysis and record the thermogram, with the temperature on the x-axis and the energy change on the y-axis. The temperature at which the phenomenon occurs (the onset temperature) corresponds to the intersection of the extension of the baseline with the tangent at the point of greatest slope (inflexion point) of the curve. The peak of the curve indicates the end of the thermal phenomenon.

4.2 Method for film formulation[1] [36] [31]

The film was formulated by the solvent casting method. 1g polymer was weight accurately. 50 ml of solvent was taken in a separate beaker and polymer was added in to the solvent with a constant stirring at 900 RPM on 40^o C until polymer was completely dissolved. Drug was dissolved in a separate beaker in respective. Drug solution was

poured in to the polymer solution with constant stirring. During the stirring process, 200 mg plasticizer was added in to the polymer solution. After the complete mixing of the solution, it was allowed for degasify on sonicator until air bubble were removed. The resultant solution was poured in to 100 cm^2 (10x10), then it was kept for drying for required time period at 50^o C in hot air oven. Film was scraped out and characterized.

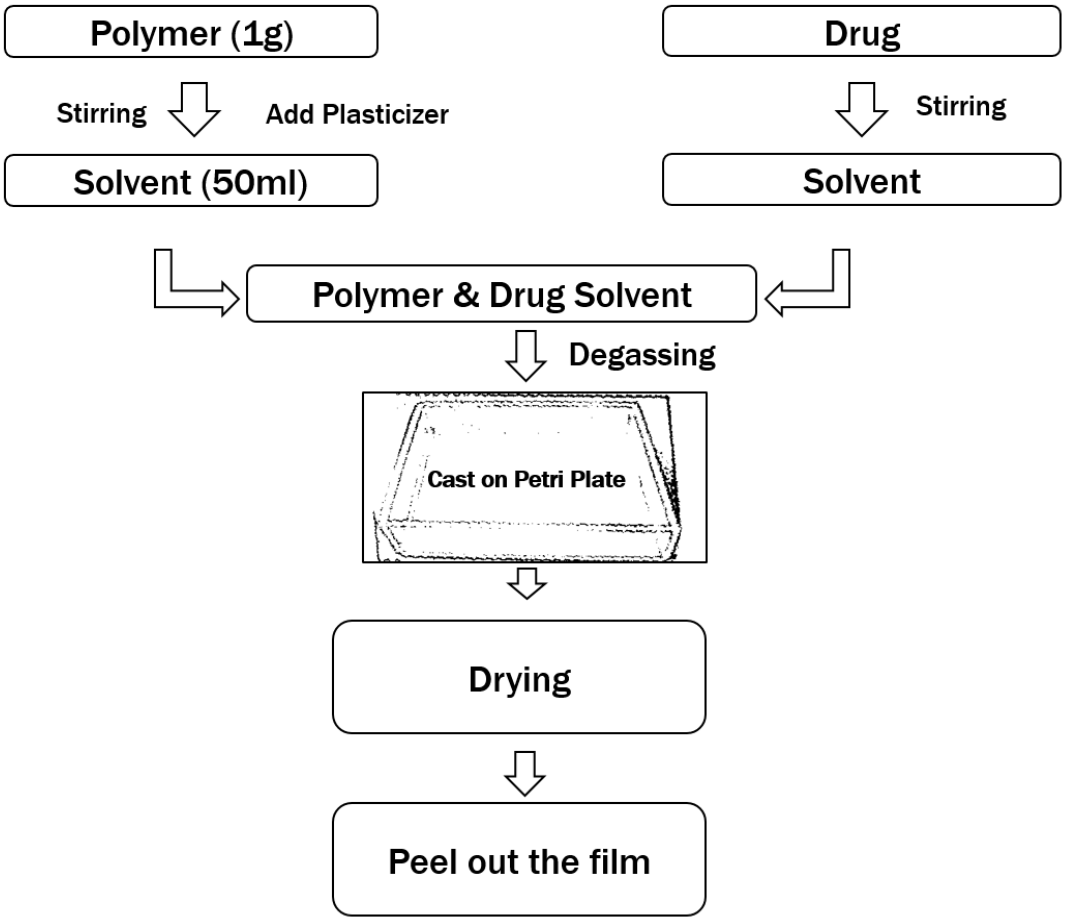

Figure 4. 1Schematic diagram for film formulation

4.3 Evaluation parameters of the film[33] [37] [38]

4.3.1 Weight variation of the film

Film of 2cm x 2cm (4cm^2) was cut from five different places in the casted film. The weight of each film strip was taken, and the average weight and weight variation was calculated.

4.3.3 Thickness of the film

The thickness of the film was determined using 'Digital Vernier caliper' (Insize model-1108) at different positions by placing the film between the nobe of vernier caliper, and the average thickness was calculated.

4.3.4 Folding endurance

The folding endurance is expressed as the number of folds (number of times a film is folded at the same plane) required to break the film or to develop visible cracks. This gives an indication of brittleness of the film. A strip of 2cm x 3cm ($6cm^{2)}$ was subjected to this test by folding the film at the same place repeatedly several times until a visible crack was observed.

4.3.5 Tensile strength & % Elongation

The mechanical properties of films 2cm x 3cm (6 cm^2) were evaluated by Brookfield QTS Texture Analyzer using dual grip jig assembly. The Texture expert software recorded the data when the probe started withdrawing from the film. The peak load for break and length at break obtained from the texture profile were used to assess the tensile strength and % elongation respective of the films. Each measurement was repeated three times.

Table 4. 3 Detailed information of parameters using texture analyzer for tensile strength study

TEST INFORMATION	
Test type	Tension
Trigger point	5 gm.
Target value	100 mm
No. of cycles	1
Test speed	15mm/min
Probe type	Dual Grip Jig

4.3.6 Percentage elongation

Percentage elongation was calculated by measuring the increase in length of the film after tensile measurement by using the following formula.

Percent Elongation = [L - L_0] X 100/ L_0

L = final Length

L_0 = Initial Length

4.3.7 Disintegration time

2cm x 2cm (4cm2) film was kept in glass petriplate containing 20 ml of SSF (pH 6.8). The petriplate was manually shaken horizontally, and the time required to initiate disintegration & time required for complete disintegration were recorded.

4.3.8 Drug Content

Film 2cm x 2cm ($4cm^2$) film was cut and placed in 50 ml volumetric flask containing 50 ml SSF (pH 6.8), and shaken vigorously to ensure complete disintegration & dissolution of film. 1 ml of filtered solution was diluted to 10 ml with SSF. The absorbance of the solution was measured at 287.

4.3.9 Content uniformity

Film 2cm x 2cm ($4cm^2$) were cut from three different places from the casted film. Each film was placed in 50 ml volumetric flask containing 50 ml SSF (pH 6.8), and shaken vigorously to ensure complete disintegration & dissolution of film. 1 ml of filtered solution was diluted to 10 ml with SSF. The absorbance of the solution was measured at 287.

4.3.10 Surface pH

Surface pH of the formulated films was measured by wetting the surface of the film with distilled water and checking with pH strip, average of six readings was taken.

4.3.11 Diffusion

The amount of drug diffused from the film at different time interval was determined by diffusion study using the Franz diffusion cell apparatus. The receiver compartment of

diffusion apparatus was filled with of SSF containing 1% SLS (pH 6.8). Temperature of the diffusion assembly was maintained at 37^0 C through entire study. The cellophane membrane was kept on receiver compartment cover the mouth. The square shape film containing 20 mg drug was mounted on cellophane membrane. The donor compartment was also filled with 20 ml of SSF (pH6.8). The SSF in receiver compartment was stirred at 500 RPM to initiate the diffusion. The samples were collected at different time interval (5, 10, 15, 20, 25, 30 and 60 min.) using 1 ml syringe and equal amount of fresh SSF (blank) was replaced immediately. The Samples were diluted with 10 ml of SSF solution and analyzed by UV spectrophotometer to determine drug diffusion and take the absorbance. Calculate the release of the drug.

4.3.12 Dissolution

The amount of drug released from the film at different time interval was determined by dissolution study using the dissolution apparatus type II. The receiver compartment of dissolution apparatus was filled with 900 ml SSF containing 1% SLS (pH 6.8). Temperature of the diffusion assembly was maintained at 370 C through entire study. The square shape film containing 20 mg drug enclosed in cellophane membrane was tied on glass slide placed in to the receiver compartment. The SSF in receiver compartment was stirred at 50 RPM to initiate the release of drug. The samples were collected at different time interval (5, 10, 15, 20, 25, 30 and 60 min.) using 1 ml syringe and equal amount of fresh SSF (blank) was replaced immediately. The Samples were diluted with 10 ml of SSF solution and analyzed by UV spectrophotometer take the absorbance and calculate the release of the drug.

EXPERIMENTAL WORK

5.1Identification of Benzocaine

5.1.1 Melting Point Determination

Actual melting point	**88°C-92°C**
Observed melting point	**90°C**

5.1.1.1 Result: The melting point of Benzocaine was found to be 90°C.

5.1.1.2 Conclusion: The melting point determined is within the range of standard value (88-92^{0}C), hence, it is concluded that the drug sample having intimate physical property as standard drug.

5.1.2 IR Spectra

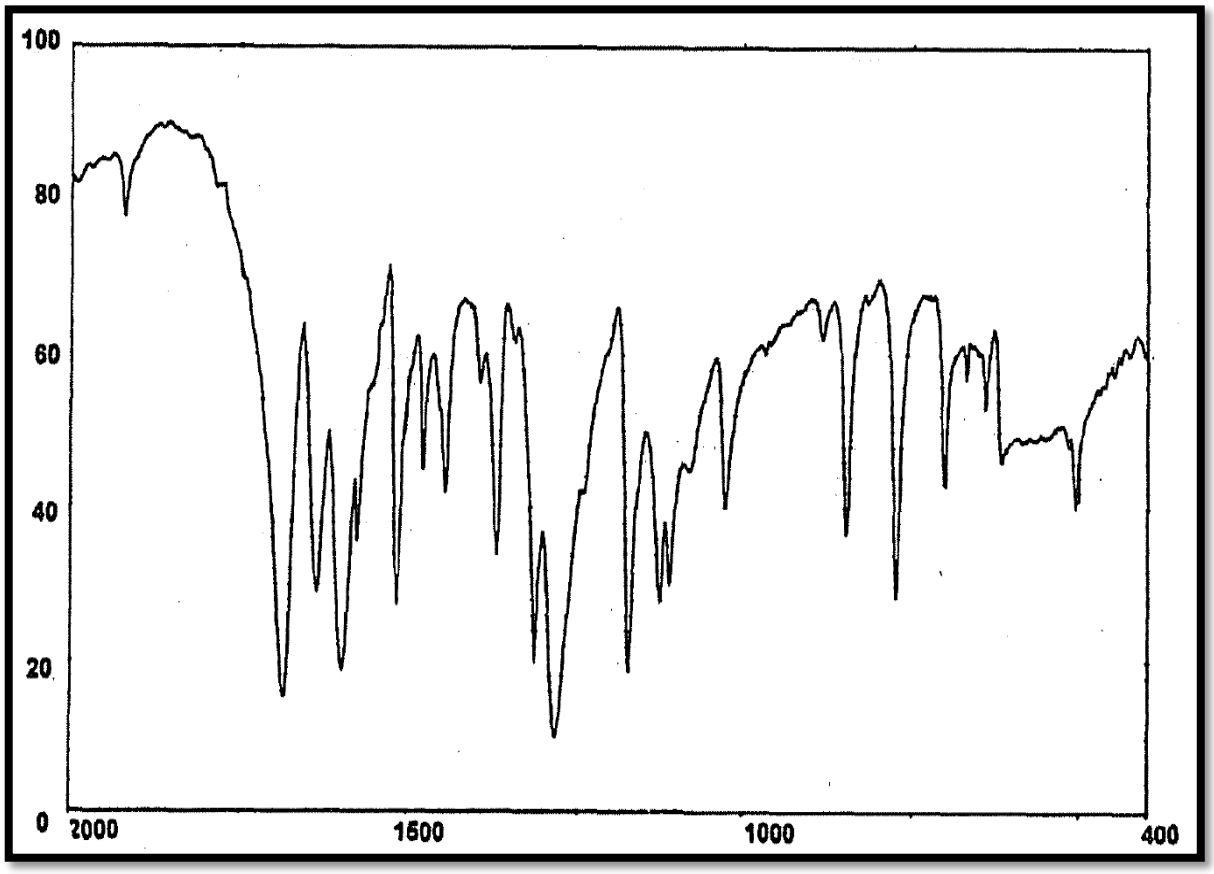

Figure 5. 1 Reference FTIR Spectra of Benzocaine

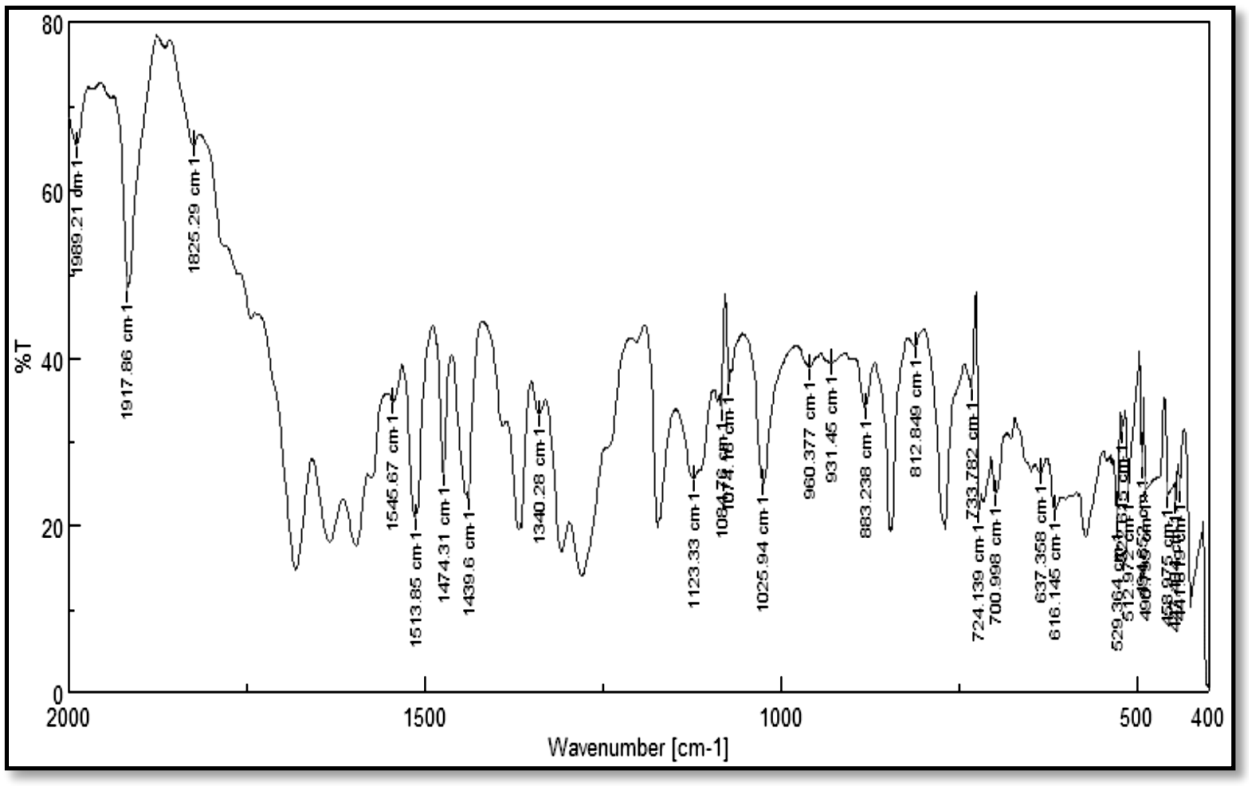

Figure 5. 2: Observed FTIR Spectra of Benzocaine

5.1.2.1 Discussion: The sample spectrum of Benzocaine was compared with standard and the spectra were similar in peak values representing wave numbers. Thus, it can be concluded that procured Benzocaine sample was a pure drug.

5.1.3 UV Spectra

5.1.3.1 UV absorption maxima benzocaine in methanol

UV scanning was done for 5µg/ml drug solution from 200-400 nm in methanol as a blank using Shimadzu UV 1800 double beam UV/Visible spectrophotometer. The absorption maxima was found be at 292 nm.[1]

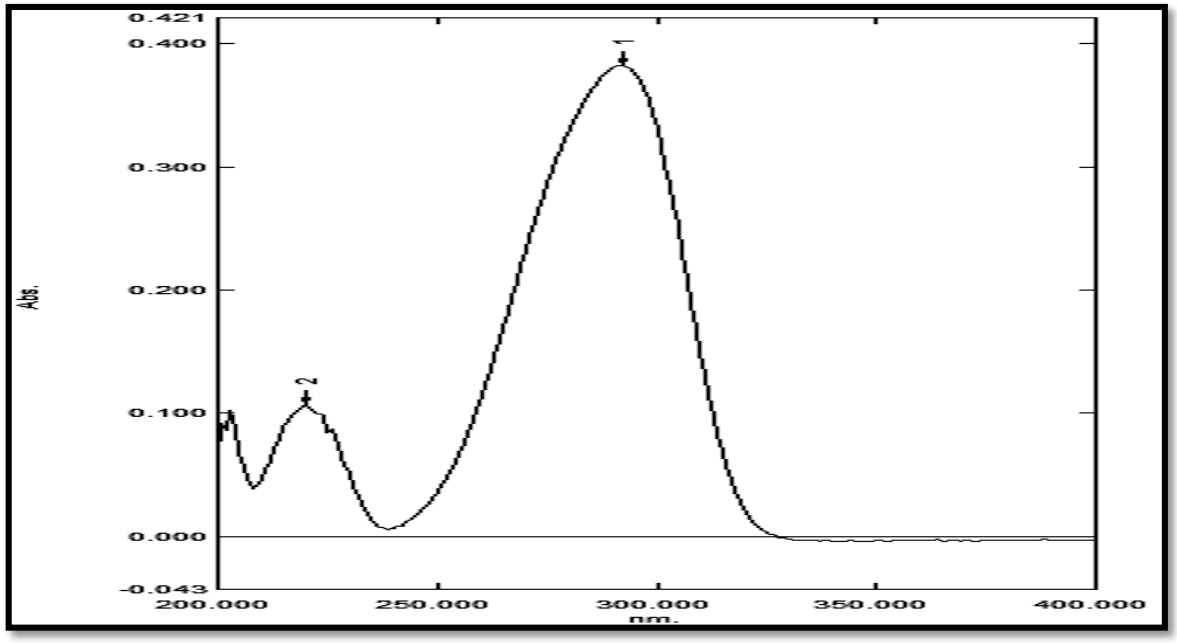

Figure 5. 3: UVAbsorbance Spectra of Benzocaine in Methanol

Table 5. 1: Benzocaine Maximum Absorbance and Concentration

Concentration	Peak 1		Peak 2	
	λ (nm)	**Absorbance**	**λ (nm)**	**Absorbance**
5 µg/ml	**292**	**0.382**	**220**	**0.007**

5.1.3.1.1 Discussion

The UV spectra of Benzocaine shows λmax at 292 nm, which remains constant after dilution and is similar to the reported standard value 292 nm. This also indicates identity and purity of the drug sample.

5.1.3.2 Standard curve of Benzocaine in methanol

Table 5. 2: UV Absorbance Average mean in Methanol

Concentration µg/ml	Absorbance			Mean	S.D.
	I	II	III		
1	0.19	0.193	0.19	0.191	0.001732
2	0.294	0.297	0.297	0.296	0.001732
3	0.421	0.422	0.42	0.421	0.001
4	0.584	0.581	0.584	0.583	0.001732
5	0.708	0.705	0.708	0.707	0.001732
6	0.863	0.861	0.862	0.862	0.001
7	1.013	1.011	1.012	1.012	0.001

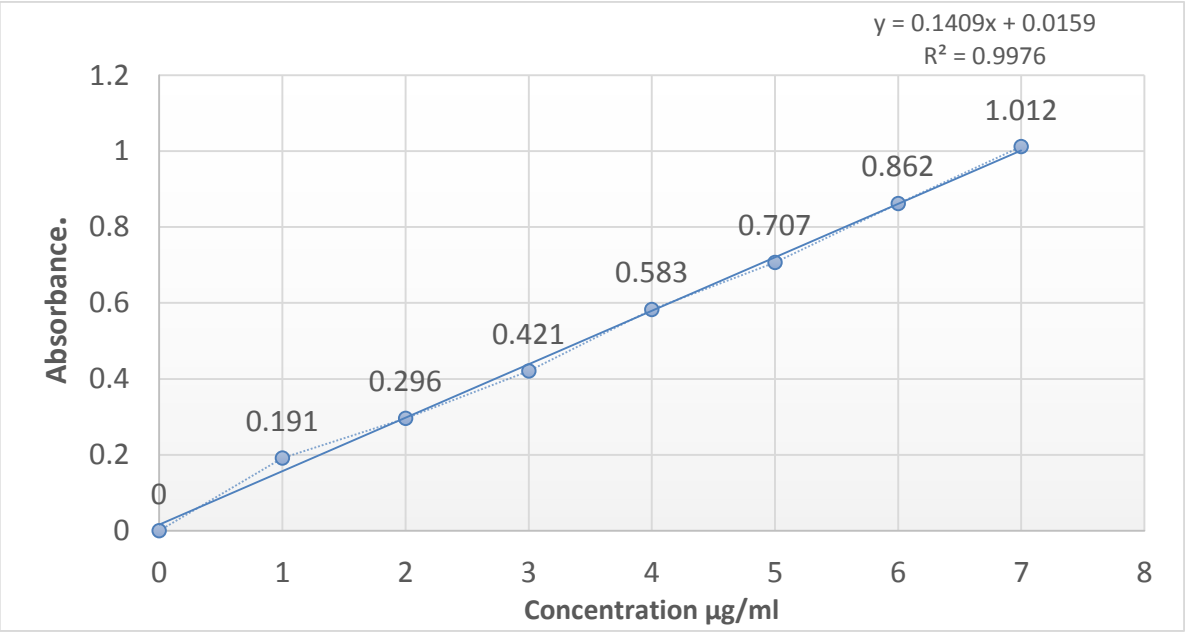

Figure 5. 4: Benzocaine calibration curve with methanol

Table 5. 3 Regression Analysis for Standard Curve Methanol

Regression parameter	Value
Correlation coefficient	0.9976
Slope	0.1409
Intercept	0.0159

5.1.3.3 Standard curve of Benzocaine in Simulated Saliva Fluid (pH 6.8)

Table 5. 4: UV Absorbance average mean in Methanol

Concentration µg/ml	Absorbance			Mean	S.D.
	I	II	III		
1	0.1	0.13	0.1	0.11	0.01732
2	0.19	0.23	0.21	0.21	0.02
3	0.302	0.306	0.304	0.304	0.002
4	0.411	0.415	0.416	0.414	0.002646
5	0.524	0.524	0.524	0.524	0
6	0.623	0.622	0.627	0.624	0.001
7	0.74	0.746	0.74	0.742	0.001

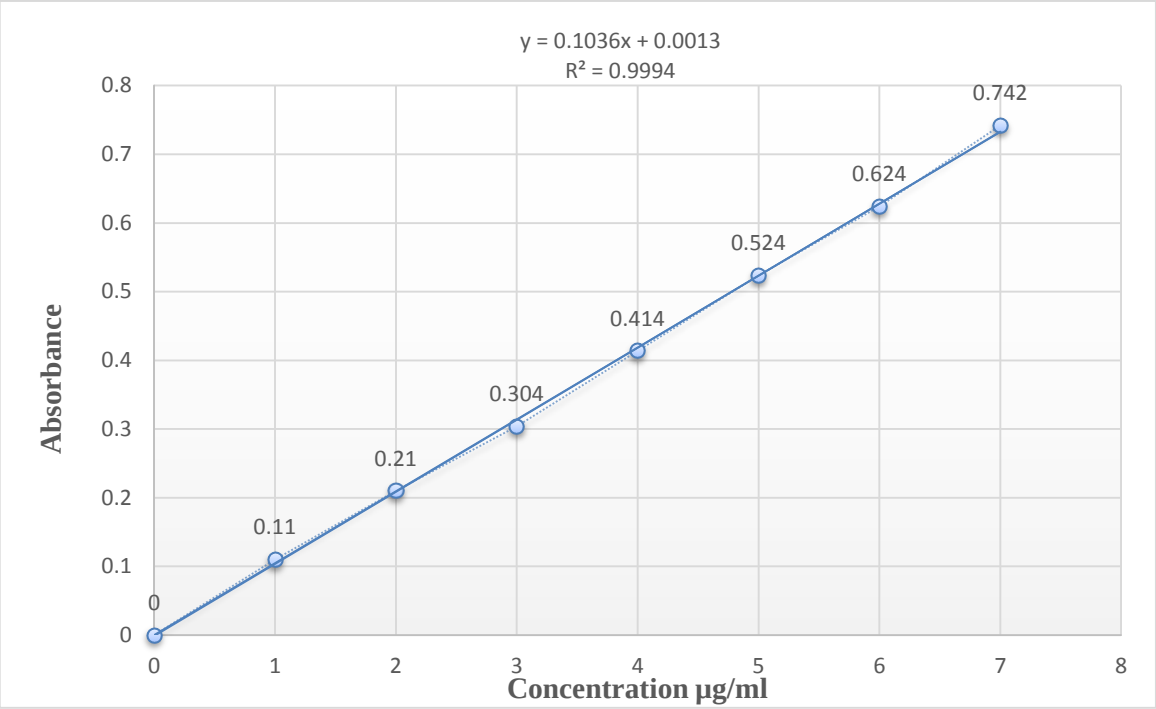

Figure 5. 5: Benzocaine calibration curve with Simulated Saliva Fluid (pH 6.8)

Table 5. 5: Regression Analysis for Standard Curve Methanol

Regression parameter	Value
Correlation coefficient	0.9976
Slope	0.1409
Intercept	0.0159

5.1.3.6 Drug Excipient Compatibility Study

5.1.3.6.1 Differential Scanning Calorimetry (DSC)

DSC Study of pre-formulation Sample was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipient compatibility study.

Table 5. 6 Amount of Sample for DSC

Sr.No.	Sample Name	Sample Quantity
1	Benzocaine	1.5 mg
2	Chitosan	1.5 mg
3	PVA	1.5 mg
4	Mixture (Drug & excipient)	2 mg

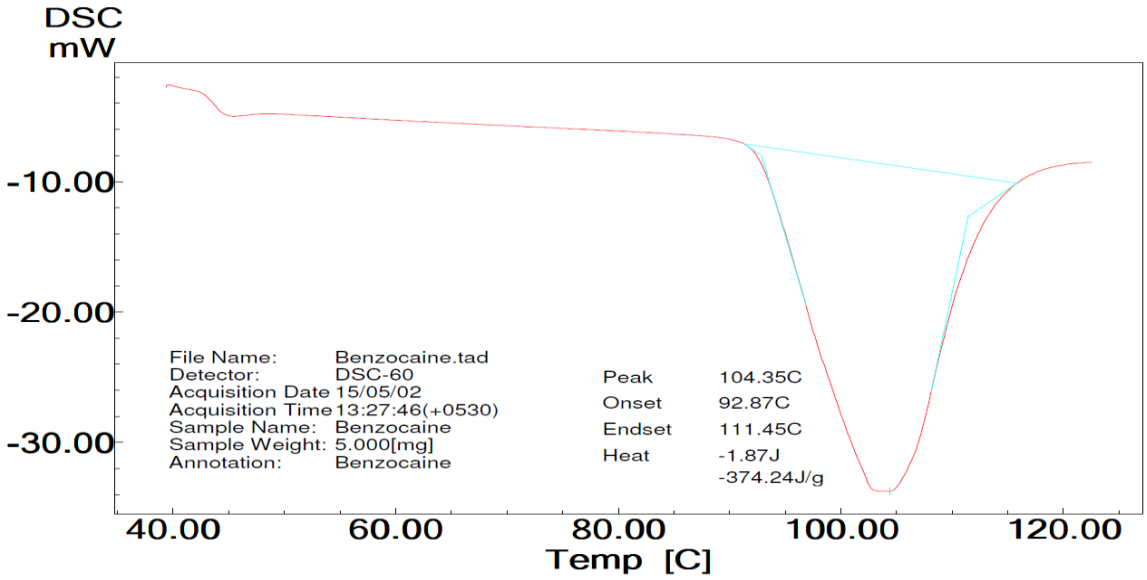

Figure 5. 6: Thermograph of Benzocaine

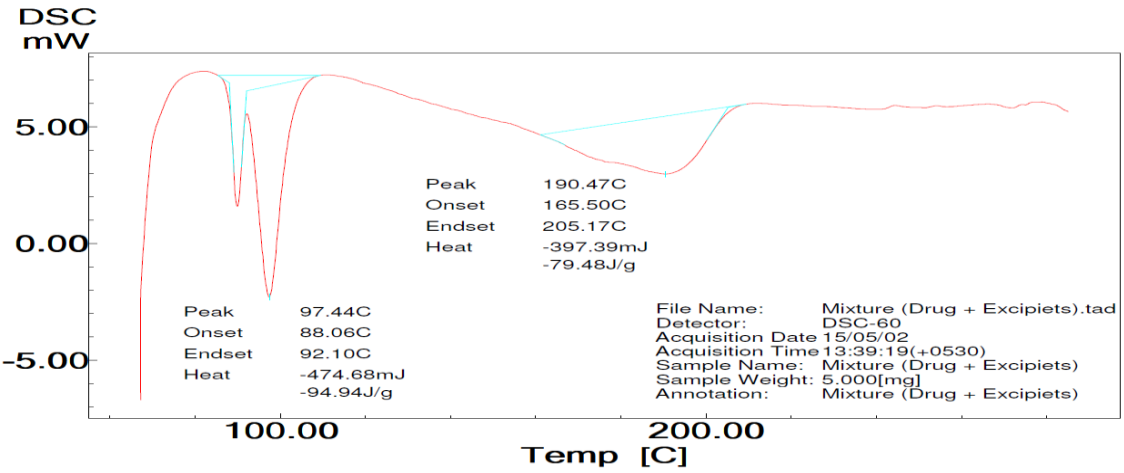

Figure 5. 7: Thermograph of mixture drug& excipients (Chitosan, PVA & Benzocaine)

5.1.3.6.2 FTIR for drug compatibility

FTIR Study of pre-formulation Sample was performed using a Jasco FTIR 6100 Type-A, to determine the drug excipient compatibility study.

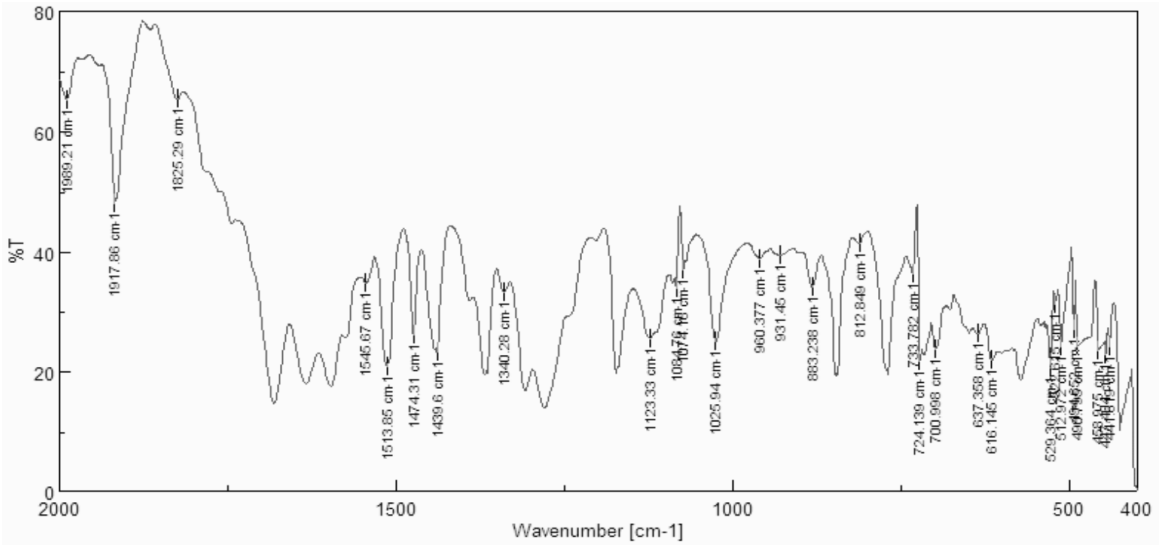

Figure 5. 8: FTIR spectra of Benzocaine

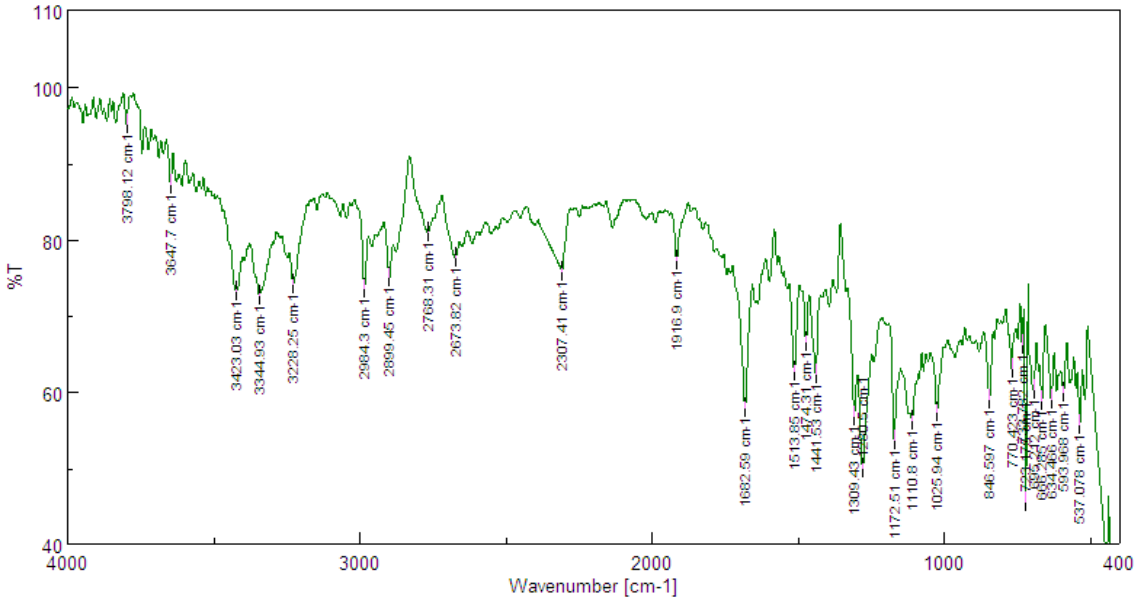

Figure 5. 9 FTIR spectra of Drug & excipients (Chitosan, PVA & Benzocaine)

5.2 Preliminary Trials

5.2.1 Selection of casting surface

These are the following polymer are tested to formulate the films without drug.

Preliminary trials were conducted to check the film forming property of various polymer on different casting surface.

Table 5. 7: List of polymer used in single polymer film

Code No.	Polymer
S1	HPMC 15cps
S2	HPMC K4M
S3	Pectin
S4	Carbopol 934
S5	HPC
S6	PVP
S7	PVA
S8	Chitosan

5.2.1.1 Parameter for the film formulation

Amount solvent used: (50 ml)

Name of the solvent

- Glacial acetic acid (for chitosan)
- Water (Except chitosan all above was dissolved in water)

Method: Solvent casting method

Area of the Petri plate: 100 cm^2(10x10)

Casting surface: Glass, Plastic, Acrylic

Stirring: 900 RPM at 40˚C

Drying temperature: 50˚C.

Table 5. 8: Evaluation of casting surface optimization

Batch	polymer	Solvent	Conc. % w/v	Film separation		
				Glass	Plastic	Acrylic
S1	HPMC 15cps	Water	1%	+	++	+++
			2%			
S2	HPMC K4M	Water	1%	+	++	+++
			2%			
S3	pectin	Water	2%	+	++	+++
			4%			
S4	Carbopol 934	Water	1%	-	-	-
			2%			
			3%			
S5	HPC	Water	1%	++	+++	+++
			2%			
S6	PVP	Water	1%	-	-	-
			2%			
S7	PVA Medium grade	Water	1%	+	++	+++
			2%			
S8	Chitosan	1% Acetic acid glacial	0.5%	-	++	+++
			1%			
			2%			

Film was not formed (-) Poor (+) Good (++)Excellent(+++)

5.2.1.2 Discussion:

The initial trials for film formation were taken using different polymers in different concentration (0.5%, 1%, 1.5%, and 2%) on Glass, Plastic and Acrylic surface. It has been reported that different casting surface showed different scrap out property (poor, partially good, and good) for various polymer. The films casted on glass and surface could not be scraped out easily. Where film casted on plastic surface it's showed good peeling than glass surface but its poor than acrylic surface. Acrylic is showed better scraped out for all polymers that are tested in preliminary trial So Acrylic plate is selected for the further process as a film casting surface.

It was concluded that acrylic surface gives the good scraped out of the film and alone Carbopol and PVP does not have film forming capacity hence addition of some other film forming polymer was necessary.

5.2.2 Selection of plasticizer

Literature review suggests that PEG (polyethylene glycol) is exhibiting good plasticizer property impart the plasticity and flexibility in the film. Three different of PEG were explored to check their comparative effect in the film property % Elongation, Folding endurance and Tensile strength.

A- PEG 200
B- PEG 400
C- PEG 600

Table 5. 9: Evaluation PEG grade optimization

Code No.	Polymer (w/v)	% Elongation			Folding Endurance			Tensile strength (kg)		
		A	B	C	A	B	C	A	B	C
S2	HPMC K4M (2%)	160	170	200	>300	>300	>300	0.29	0.36	0.40
S4	Chitosan (2%)	110	110	130	65	70	74	0.57	0.56	0.54
S5	Pectin (2%)	110	110	120	120	130	144	0.09	0.09	0.10
S6	PVA (2%)	280	300	340	>300	>300	>300	4.0	4.2	4.5

NOTE: 20% concentration of plasticizer was used of polymer concentration.

5.2.2.1 Discussion:

The result in above table indicates that the grade of PEG as plasticizer does not significantly affect the film properties like (% elongation, tensile strength and folding endurance); however, the type of film forming polymer (HPMC K4M, Chitosan, Pectin, PVA) has significant effect on film properties. Over all, film formed with PEG 600 (higher grade) excluded better properties compared to higher grade of PEG, hence PEG 600 was finalized as plasticizer for all further studies.

5.2.2.2 Polymer tried with plasticizer (PEG 600)

Parameter for the film formulation

Same as previous phase

Plasticizer: PEG 600 (20% of polymer)

Polymers: (S1, S2, S3, S4, S5, S6, S7, S8)

Table 5. 10: Evaluation of the single polymer film

Batch	Polymer	Amount in (gm)	Amount in (%)	Thickness	Tensile strength	% Elongation	Folding endurance	Disintegration Start (sec.)	Adhesion
S1	**HPMC 15cps**	**0.75**	1.5 %	0.20	0.18	124	>300	30s	+
		1	2 %	0.22	0.2	160	>300	34s	+
S2	**HPMC K4M**	**1**	2%	0.12	0.4	200	>300	44s	+
S3	**Pectin**	**1**	2%	0.16	0.10	130	144	8s	+
		2	4%	0.25	0.13	140	126	10s	+
S4	**Carbopol 934**	**0.5**	1%	**Film was not formed**					
		1	2%						
		1.5	3%						
S5	**HPC**	**1**	2%	0.30	0.037	100	Zero	120s	+
S6	**PVP**	**1**	2%	**Film was not formed**					
S7	**PVA**	**1**	2%	0.11	4.5	340	>300	10s	+
S8	**Chitosan**	**0.5**	1%	0.12	0.38	100	80	145	+
		1	2%	0.19	0.54	110	74	180	+

5.2.2.3 Discussion

- Film formulated with HPMC 15cps & HPMC K4M polymer showed good appearance, flexibility, transparency.
- Carbopol alone was not able to form the film with acceptable properties. However the film formulated using Carbopol alone exhibited stickiness, which may be considered as prominent mucoadhesive property.
- PVP alone also does not able to form the film with acceptable properties.
- Film formulated with Pectin showed same appearance, flexibility and transparency like HPMC 15cps & HPMCK4M but thickness obtained lower.
- Film formulated with PVA showed smooth, good appearance, excellent elasticity property, than rather polymers that are used in preliminary trial.
- When the film was prepared with alone HPC obtained opaque it gives zero folding endurance so it was found unstable.
- Chitosan film was prepared in acidic solution, 1% acetic acid glacial was prepared to dissolve the chitosan for film formulation. Film formulated with chitosan showing yellowish appearance, semitransparent and thick film, Chitosan reported as a good Bioadhesive polymer and it mostly used in the sustained release drug delivery formulation.

From the film evaluation data it was concluded film formulated with alone polymer that did not match with desired prospective of the project objective. PVA was gives better elasticity but does not hold in the mouth above 30 min. PVA and Pectin shows fast dissolving property. Where HPMC K4M and HPMC 15 cps has been shown the slowly disintegration but they cannot be hold in the mouth above 30 min with a proper adhesion. Literature review suggests that chitosan is a sustained release polymer it could be hold in the mouth more than 1 hour because it does not dissolved in the water freely. But Chitosan did not shows proper flexibility and folding endurance.

So it was concluded that film formed using alone polymers were not shows acceptable properties or characteristics. So then polymers were checked in the combination.

5.3 Selection of the polymer combination

Table 5. 11 Polymer checked in the combination

Sr. No.	Polymer
S1	HPMC 15cps
S2	HPMC K4M
S3	Pectin
S4	Carbopol 934
S5	HPC
S6	PVP
S7	PVA
S8	Chitosan

5.3.1 Parameter for the film formulation

Amount of solvent used: (50 ml)

- Glacial acetic acid (for chitosan)
- Water (Except chitosan)

Plasticizer: PEG 600

Polymers: (S1, S2, S3, S4, S5, S6, S7, S8)

Method: Solvent casting method

Area of the Petri plate: 100 cm^2 (10x10)

Casting surface: Acrylic plate.

Stirring: 900 RPM at 40˚C

Drying temperature: 50˚C.

5.3.2 Film was formed in the combination

There are the different combination of the polymers were tried to make the film it's would be meet with the prospective object of the project.

Table 5. 12: Film formed in combination

Polymer	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
HPMC 15cps	-	-	-	-	-	750	-	-	-	-	-	-	-	-	-
HPMC K4M	750	250	800	600	667	-	-	-	-	-	-	-	-	-	-
Pectin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbopol 934	-	-	200	-	-	250	250	-	-	-	-	-	-	-	-
HPC	-	-	-	400	-	-	750	-	-	-	-	-	-	-	-
PVP	-	-	-	-	333	-	-	500	333	250	750	-	-	-	-
PVA	-	-	-	-	-	-	-	-	-	-	-	375	500	250	750
Chitosan	250	750	-	-	-	-	-	500	667	750	250	375	500	750	250
PEG	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
Water	25	25	50	50	50	50	50	25	25	25	25	25	25	25	25
GAA	25	25	-	-	-	-	-	25	25	25	25	25	25	25	25

Table 5. 13 Evaluation of the film was formed in the combination

Polymer	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
Thickness	0.13	0.12	0.13	0.24	0.10	0.11	0.32	0.12	0.14	0.15	0.13	0.11	0.13	0.16	0.11
Tensile Strength	1.5	2.2	1.3	1.4	0.46	1.2	0.41	1.12	1.36	1.43	0.533	1.85	0.89	1.30	1.10
% Elongation	140	110	120	140	135	100	110	114	110	108	180	133	130	120	260
Folding Endurance	> 300	> 235	> 300	> 300	> 300	> 300	> 235	> 300	> 300	> 300	> 300	> 300	> 300	> 300	> 300
Disintegration Start (sec.)	30	100	30	20	21	28	35	85	130	145	60	70	105	130	60
Adhesion	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

5.3.2.1 Discussion

After the evaluation of the film that was prepared in the combination of different type of polymer it was concluded that the various combination of the film was showed different nature from each other. For example batch no. C1 and C2 showed most parameter same but disintegration start time showed much different. So its mean when I was increased the concentration of the chitosan it was directly affect the disintegration and increase the time of disintegration where disintegration start. Also it was found that Adhesion in batch no.C1 and C2 was not good. In this study chitosan was mostly tried with other polymer because it was reported as a sustained release and mucoadhesive polymer. From the observed data it was find out the any of the film that was prepared without the chitosan cannot be adhere or retained above 30min in the mouth and they was not showing the

adhesion up to 30 min. but only chitosan cannot be give all specification and results in direction of project objective. So here must be require to set the amount of two polymer they could give the area of desired specification. When the chitosan was tried with PVP batch no. C13 the film was formed good appearing and it was showed folding endurance is also good where the tensile strength was tolerable but main problem with this combination it was there disintegration. When the film was dissolved in water then polymer of film was separated from each other because the PVP is the water soluble so it was quickly dissolved in the water but chitosan was still undissolved and film becomes in network like structure because PVP was reduced from it. So it was concluded that when film was formed in combination of Chitosan and PVP so these polymers was not properly interact to each other. So disintegration of the film was not in a constant order.

When film was formed in combination of Chitosan and PVA it was showing the also different results. There are the 4 batches were formulated in these combination in different ratio of the polymer. Different ratios of the polymer was showing the different outputs. Among the all batches of the chitosan and PVA batch no. C13 was observed good batch. In this batch the Chitosan and PVA were taken in (1:1) ratio. This batch was give the desirable outputs. % Elongation, tensile strength, thickness and disintegration time all are reported in a desired requirement. But the selection of the polymer was mainly based on the proper adhesion and retention time of the film into the mouth. So this batch was also reported its gives good adhesion and film can be hold above 30 min in the mouth.

These two polymer having their different property and they were play the individual role in the film. When we are talking about the PVA so it was mainly responsible for elasticity and it gives the flexibility to the film, PVA is also a mucoadhesive polymer so it was helped in to increase the adhesion of the film when it was used with Chitosan. The initial disintegration of the film was mainly depended on amount of the PVA that was present in the film. Where Chitosan was also played an important role in the property of the film it was affect physical property of the film. Chitosan was mainly affect tensile strength of the film. When the concentration of the chitosan was increased the tensile strength was also increased with the concentration. The main purpose of usingthe chitosan it was its sustained drug release action. Chitosan could be held or retain in the mouth above 30 to 1

hr. when it was used in combination with PVA in the (1:1) ratio that was used in the batch no. C13.

On base of all observed data that was comes from the preliminary trial of different polymer they were used single and in the combination. It was concluded that PVA and Chitosan was selected to carry the process further. Because these polymer was showed good property as desired, Chitosan was showed better Adhesion and PVA shows better elasticity but PVA did not show good adhesion where Chitosan did not showing elasticity, but aim of the project is film should be hold in the mouth above 30 min with proper adhesion and gives continuous release of drug. So on the base polymer properties and project desired it was concluded that PVA and Chitosan combined film was best fitted in model of desired project. So these two polymer were taken to do the study forward.

After screening of the Polymer, Casting surface and Plasticizer there is an idea was comes to prepare the film that could be more desirable to give the desired outcomes.

5.3.2.2 Content that was screened out in preliminary trial.

Polymer: PVA (MW: 125000), Chitosan

Solvent: Water, 1% AAG solution

Plasticizer: PEG 600(200mg)

5.3.2.3 Process parameters

Method: Solvent casting method

Area of the Petri plate: 100 cm^2 (10x10)

Area of film: 4 cm^2

Total number of strips in a one batch: 25

Casting surface: Acrylic plate.

Stirring speed: 900 RPM at 40˚C

Drying temperature: 50˚C for 20 hrs.

5.4 Selection of Design

The selection of experimental design among available options depends upon the relationship between independent factor and dependent factors (responses). When variables and results of experiments having linear relationship normal full factorial design with two level is sufficient. Whereas when variables and results of experiment having non-linear relationship one should take higher model or RSM design for understanding quadratic effects.

Based on the preliminary trials results and selection guide for DoE designs, it was decided to apply higher model to study the relationship between independent variable and dependent variables. So 3^2 full factorial design was selected for further study.

- 3^2 full factorial design was selected because among all other design it has minimum runs.
- 3^2 full factorial is also helpful to study factor influence study and response surface mapping.
- 3^2 full factorial design is more important when variables and responses of experiment having Non-linear relationship.
- Useful to find main effect, interaction and non-linear effect efficiently.

5.4.1 3^2 Full Factorial Design:

3^2 experimental design with coded levels and actual values of process inputs with CQAs with their desired values.

Table 5. 14: Coded and actual amount of the factor

Factor	Coded level			Actual level		
	Low	Medium	high	Low	Medium	High
Chitosan	-1	0	1	200	600	1000
PVA	-1	0	1	200	600	1000

Based on the screening study results, two variables (chitosan and PVA content) were selected for the optimization of the film formulation study, using 3^2 full factorial design. In this two factor factorial design, chitosan and PVA content were selected as two formulation factor. As shown in Table 14, each of the two factors was tested at 3 different levels. Total 9 trail of the factorial design were included. Design Expert 9.0 software was used for the design and analysis, and to plot the various 3D and contour graphs.

5.4.2 Full model Equation for 3^2 Factorial design

$Y=$	β_0	Constant
	$+ \beta_1 X_1$	Main effect
	$+ \beta_2 X_2$	Main effect
	$+ \beta_{12} X_1 X_2$	Interaction Between two variable

Where, β0 Intercept=constant; β1 and β2 Co-efficient of X1 and X2; β12 Co-efficient of interaction; β11 and β22 Co-efficient of quadratic terms.

Based on p value and Co-efficient of variable one can determine significant and insignificant variable from mathematical equation. For any formulation if p value is < 5% then formulation batch should be accepted or variable having significant effect. If p value is > 5 % then we should reject the formulation or variable having no significant effect. More the coefficient value in mathematical equation, more the significant variable. The coded equation obtain from software is more reliable to correlate the impact of particular variable on response.

Table 5. 15 Formulation Batches as per 3^2full factorial design

RUN	Coded			Actual		
	Chitosan	**PVA**	**PEG(mg)**	**Chitosan**	**PVA**	**PEG(mg)**
FF-1	-1	-1	200	200	200	200
FF-2	0	-1	200	600	200	200
FF-3	1	-1	200	1000	200	200
FF-4	-1	0	200	200	600	200
FF-5	0	0	200	600	600	200
FF-6	1	0	200	1000	600	200
FF-7	-1	1	200	200	1000	200
FF-8	0	1	200	600	1000	200
FF-9	1	1	200	1000	1000	200

Table 5. 16 Evaluation results of design batches

Batch	Chitosan	PVA	Tensile Load	Thickness	Elongation	Disintegration	Cum Drug diffused in 30 min	Cum Drug diffused in 1 hr	Cum Drug dissolved in 1 hr
FF-1	-1	-1	763	0.17	43.33	70	7.095	12.22	80.66
FF-2	0	-1	3700	0.2	26.67	120	4.096	8.146	52.86
FF-3	1	-1	4265	0.28	87.67	170	2.477	5.65	39.83
FF-4	-1	0	1453	0.26	413.33	60	4.75	9.93	65.02
FF-5	0	0	4690	0.28	219	100	4.47	10.94	72.84
FF-6	1	0	5300	0.3	301.67	150	4.956	8.56	55.03
FF-7	-1	1	3350	0.22	750	40	7.144	11.97	77.19
FF-8	0	1	3441	0.29	270.33	80	6.109	11.47	75.01
FF-9	1	1	7150	0.35	116.33	185	6.492	12.41	81.96

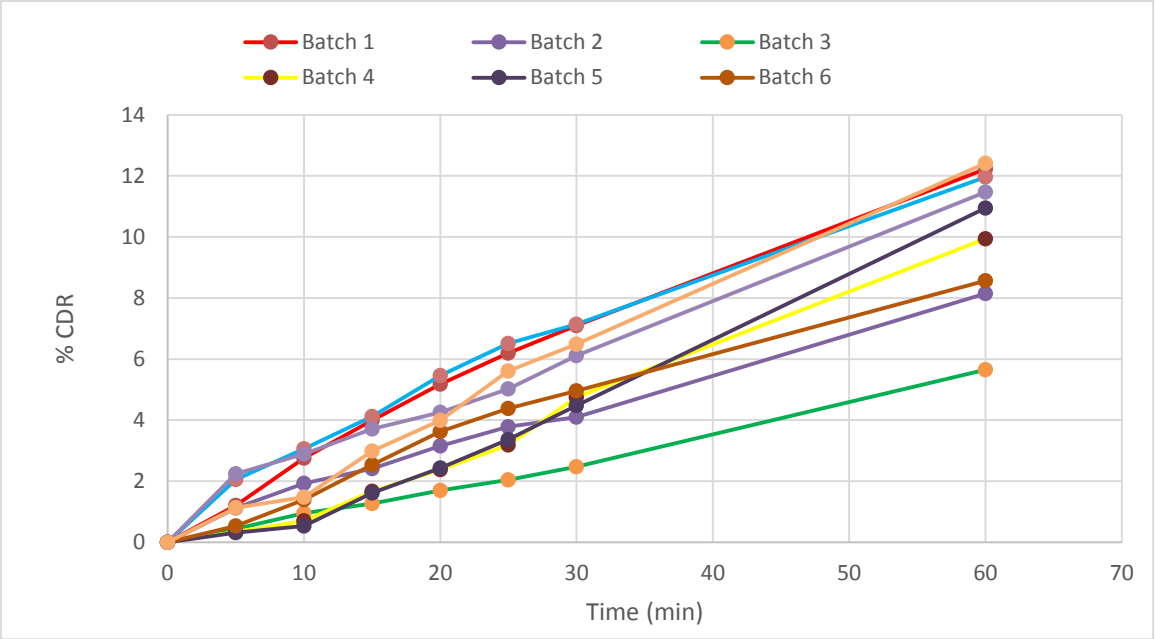

Figure 5. 10: Diffusion of designed batches

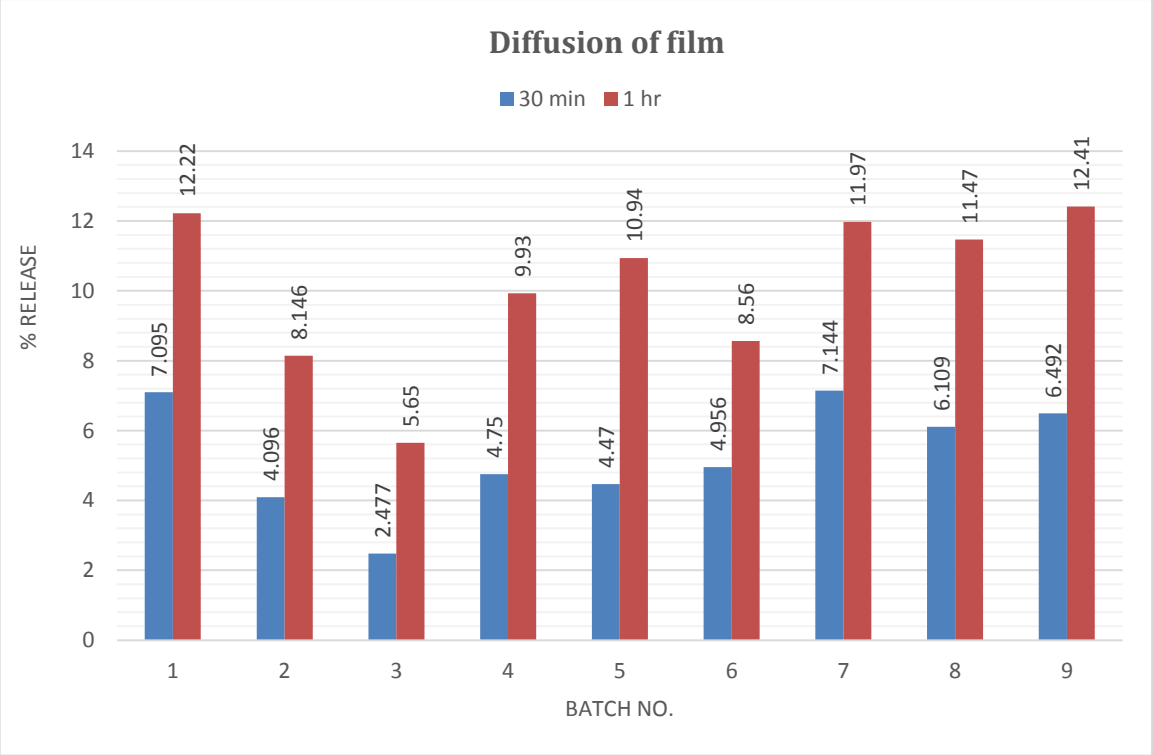

Figure 5. 11: Diffusion of designed batches at 30 and 60 min

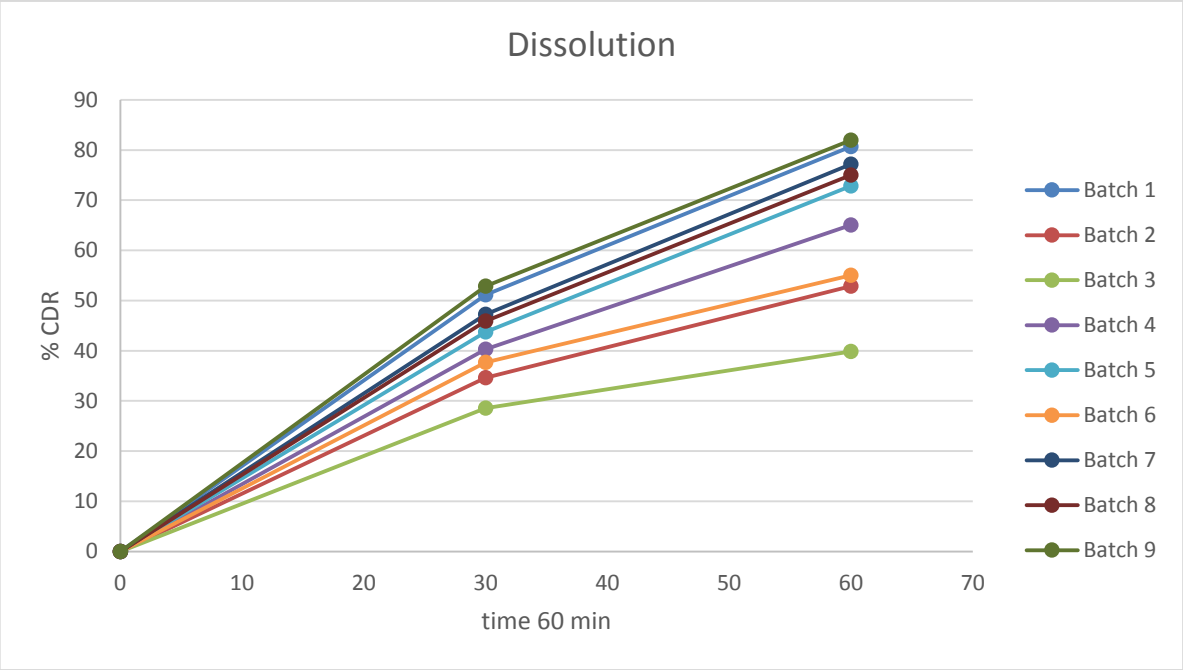

Figure 5. 12: Dissolution of designed batches

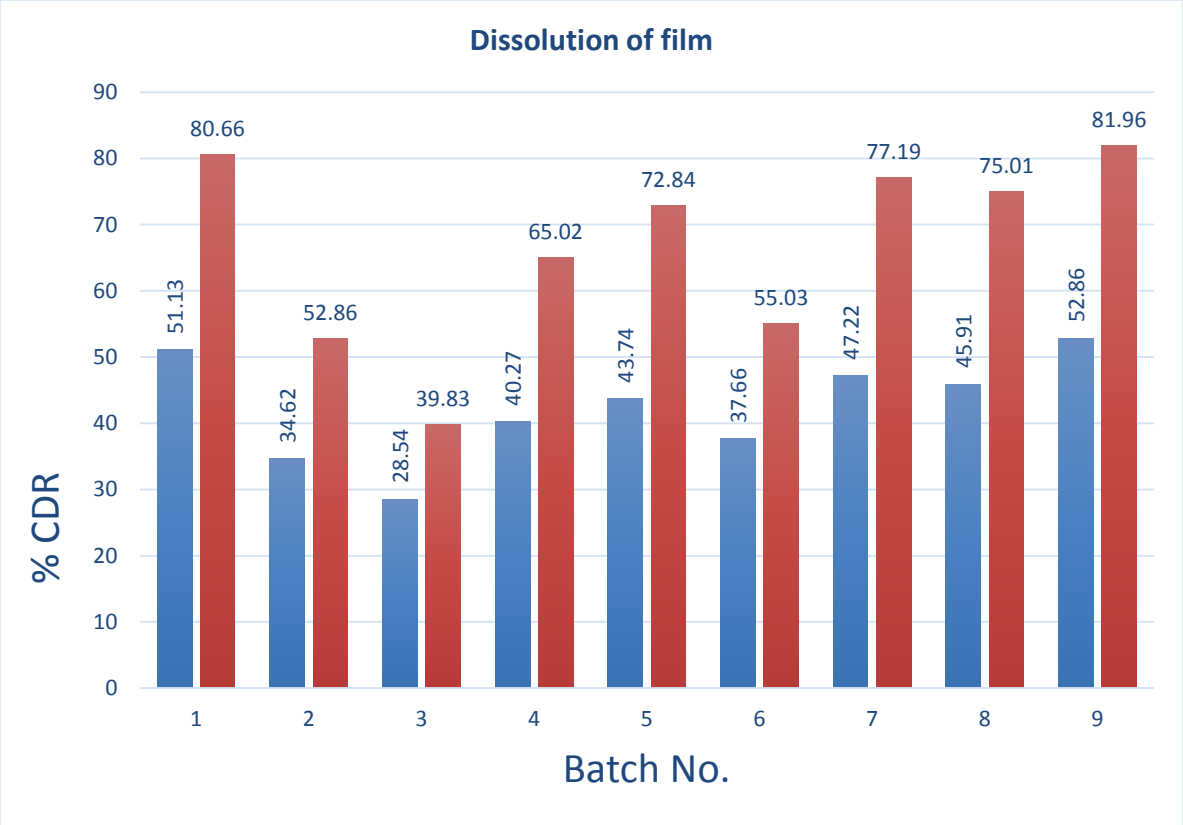

Figure 5. 13: Dissolution of designed batches at 30 and 60 min

5.4.3 Interaction of the factors

5.4.3.1 Response 1 (Tensile load)

5.4.3.1.1 ANOVA for response 1

Table 5. 17: ANOVA for Response 1

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	2.527E+007	3	8.423E+006	9.83	0.0154	significant
A-Chitosan	2.072E+007	1	2.072E+007	24.17	0.0044	
B-PVA	4.529E+006	1	4.529E+006	5.28	0.0699	
AB	22201.00	1	22201.00	0.026	0.8784	
Residual	4.285E+006	5	8.570E+005			
Cor Total	2.955E+007	8				

5.4.3.1.2 Polynomial Equation in Terms of Coded Factors

Tensile load = +3790.22 +1858.17*A +868.83*B +74.50*AB……. (1)

Equation (a) represent relationship between the quantitative effect of independent variables [X1 Chitosan), X2 PVA)] upon the responses. The equation indicates that Chitosan (A) having significant positive effect on tensile load, Whereas PVA (B) having significant positive effect on tensile load. Co-efficient of interaction with positive sign represents an positive effect of the two factor combination on the tensile load.

5.4.3.1.3 Response surface mapping

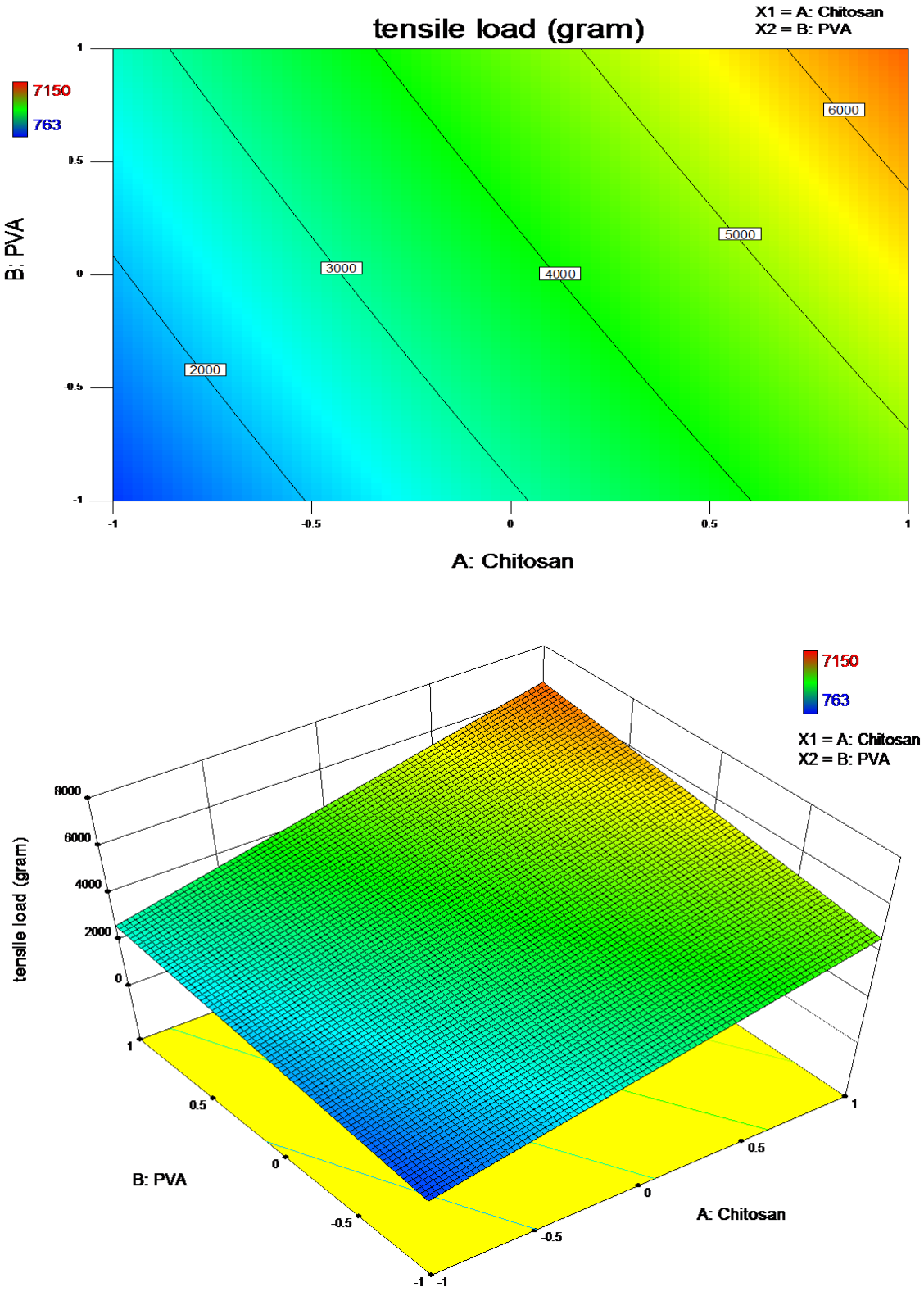

Figure 5. 14 : 3D and Contour plot of Tensile load

5.4.3.2 Response 2 (Thickness)

5.4.3.2.1 ANOVA for Response 2

Table 5. 18: ANOVA for Response 2

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	0.021	3	6.839E-003	8.20	0.0224	significant
A-Chitosan	0.013	1	0.013	15.66	0.0108	
B-PVA	7.350E-003	1	7.350E-003	8.81	0.0312	
AB	1.000E-004	1	1.000E-004	0.12	0.7433	
Residual	4.172E-003	5	8.344E-004			
Cor total	0.025	8				

5.4.3.2.2 Polynomial Equation in Terms of Coded Factors

$$\text{Thickness} = +0.26 +0.047*A +0.035*B +5.000E\text{-}003*AB \ldots\ldots (2)$$

Equation (a) represent relationship between the quantitative effect of independent variables [X1 Chitosan), X2 PVA)] upon the responses. The equation indicates that Chitosan (A) having significant positive effect on thickness, Whereas PVA (B) having significant positive effect on thickness. Co-efficient of interaction with negative sign represents an negative effect or inverse effect of the two factor combination on the thickness.

5.4.3.2.3 Response surface mapping

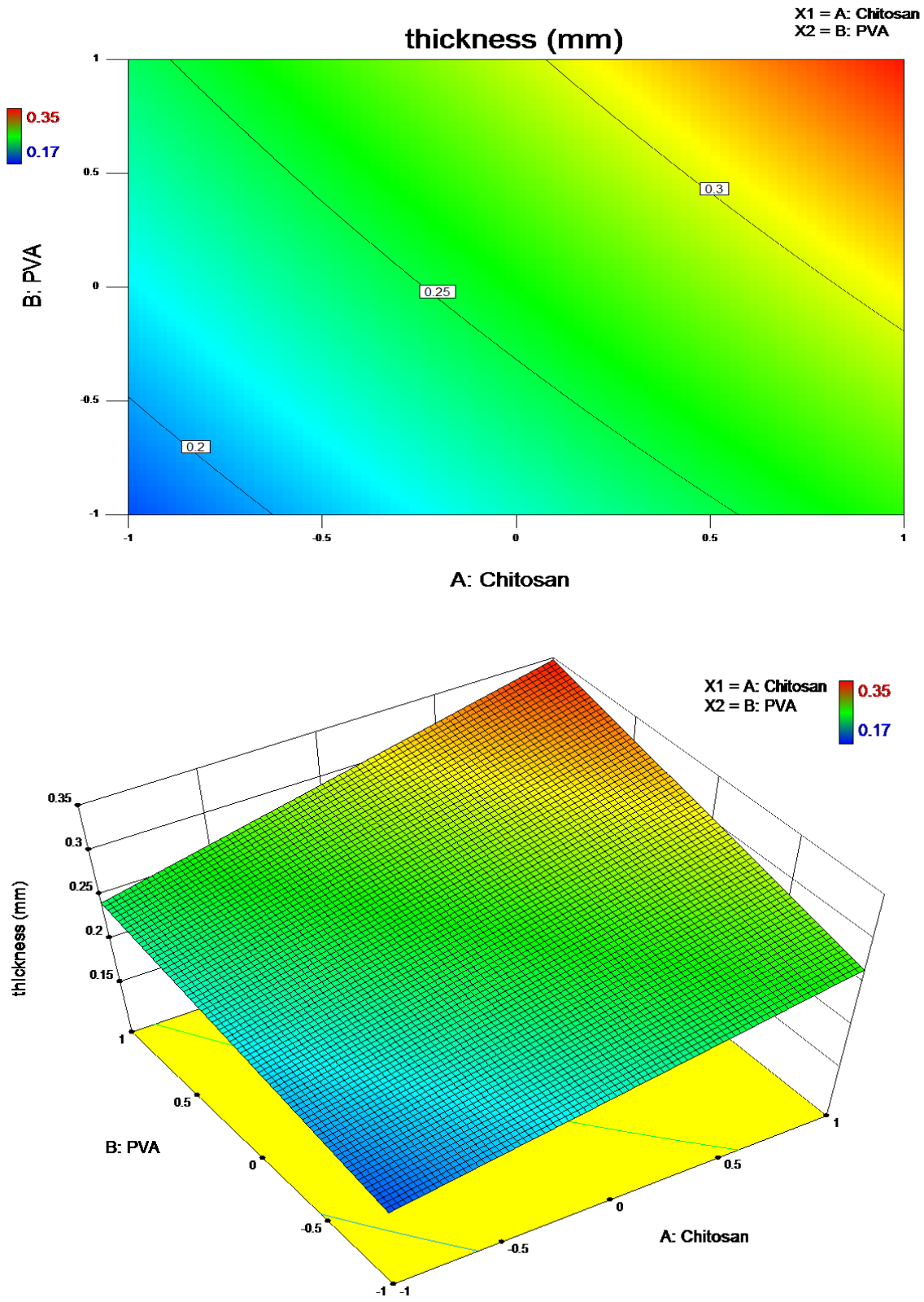

Figure 5. 15: 3D and Contour plot of Thickness

5.4.3.3 Response 3 (% Elongation)

5.4.3.3.1 ANOVA for Response 3

Table 5. 19 ANOVA for Response 3

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	3.566E+005	3	1.189E+005	9.76	0.0157	significant
A-Chitosan	81897.83	1	81897.83	6.72	0.0487	
B-PVA	1.597E+005	1	1.597E+005	13.11	0.0152	
AB	1.149E+005	1	1.149E+005	9.43	0.0277	
Residual	60916.96	5	12183.39			
Cor Total	4.175E+005	8				

5.4.3.3.2 Polynomial Equation in Terms of Coded Factors

$$\text{Elongation} = +247.59 - 116.83*A + 163.16*B - 169.50*AB \quad \ldots\ldots (3)$$

Equation (a) represent relationship between the quantitative effect of independent variables [X1 Chitosan), X2 PVA)] upon the responses. The equation indicates that Chitosan (A) having significant negative effect on elongation, Whereas PVA (B) having significant positive effect on elongation. Co-efficient of interaction with negative sign represents an negative effect or inverse effect of the two factor combination on the elongation.

5.4.3.3.3 Response surface mapping

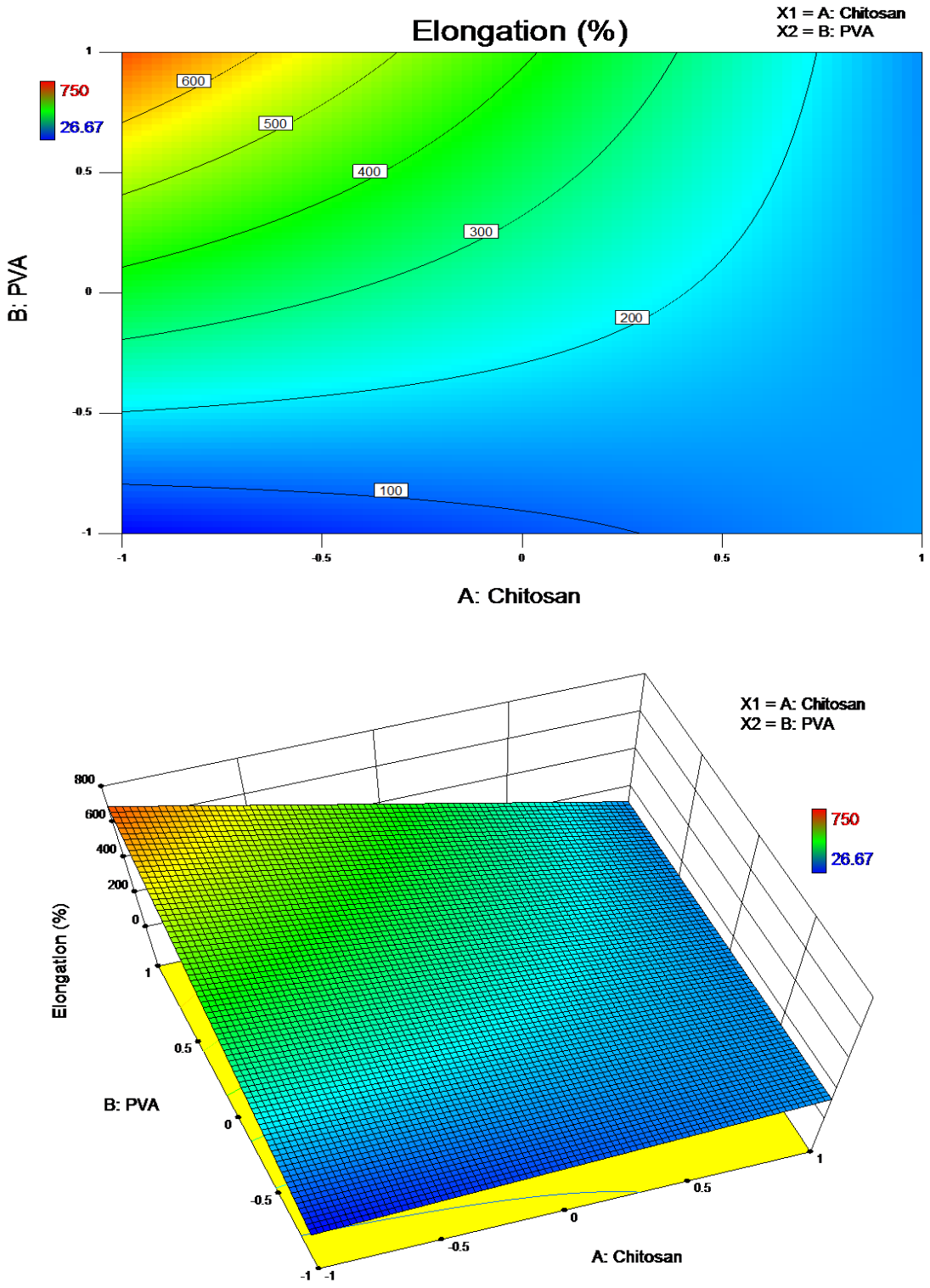

Figure 5. 16: 3D and Contour plot of Elongation

5.4.3.4 Response 4 (Disintegration Initial time Point)

5.4.3.4.1 ANOVA for Response 4

Table 5. 20: ANOVA for Response 4

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	19714.58	3	6571.53	27.72	0.0015	significant
A-Chitosan	18704.17	1	18704.17	78.89	0.0003	
B-PVA	504.17	1	504.17	2.13	0.2046	
AB	506.25	1	506.25	2.14	0.2038	
Residual	1185.42	5	237.08			
Cor Total	20900.00	8				

5.4.3.4.2 Polynomial Equation in Terms of Coded Factors

Disintegration Initial time point = +108.33 +55.83*A -9.17*B +11.25*AB….. (4)

Equation (a) represent relationship between the quantitative effect of independent variables [X1 Chitosan), X2 PVA)] upon the responses. The equation indicates that Chitosan (A) having significant positive effect on disintegration initial time point Whereas PVA (B) having significant negative effect on disintegration initial time point. Co-efficient of interaction with positive sign represents an positive effect of the two factor combination on the disintegration initial time point.

5.4.3.4.3 Response surface mapping

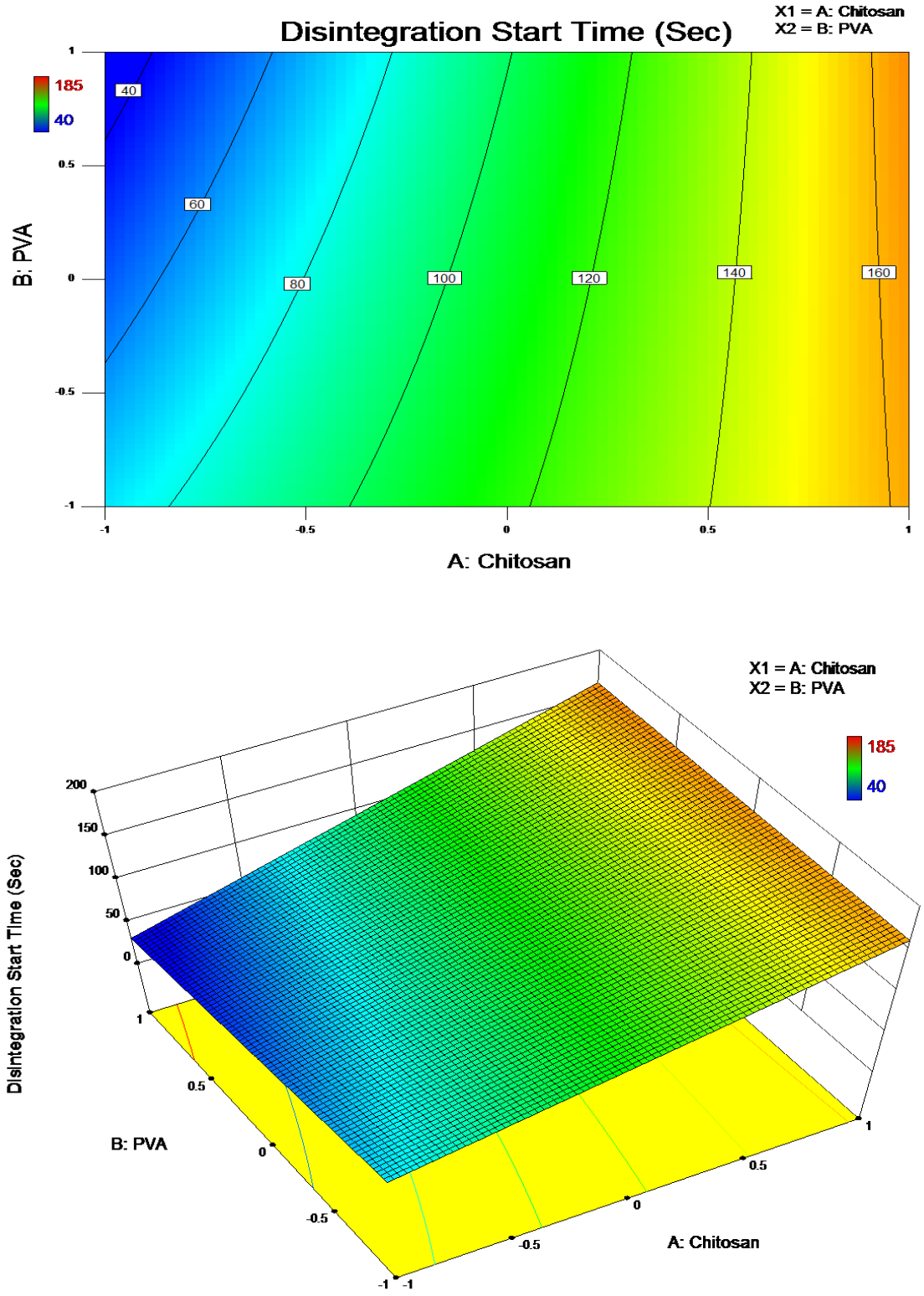

Figure 5. 17 3D and Contour plot of Disintegration

Response 5 (Cum Drug diffused in 30 min)

5.4.3.4.4 ANOVA for Response 5

Table 5. 21: ANOVA for Response 5

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	14.37	3	4.79	4.94	0.0589	not significant
A-Chitosan	4.28	1	4.28	4.42	0.0896	
B-PVA	6.16	1	6.16	6.35	0.0532	
AB	3.93	1	3.93	4.06	0.1001	
Residual	4.85	5	0.97			
Cor Total	19.22	8				

5.4.3.4.5 Polynomial Equation in Terms of Coded Factors

$$\text{Cum drug diffused in 30 min.} = +5.29-0.84*A+1.01*B+0.99*AB\ldots\ldots \quad (5)$$

Equation (a) represent relationship between the quantitative effect of independent variables [X1 Chitosan), X2 PVA)] upon the responses. The equation indicates that Chitosan (A) having significant negative effect on cumulative drug diffused in 30 min., Whereas PVA (B) having significant positive effect on cumulative drug diffused in 30 min. Co-efficient of interaction with positive sign represents an positive effect of the two factor combination on the cumulative drug diffused in 30 min.

5.4.3.4.6 Response surface mapping

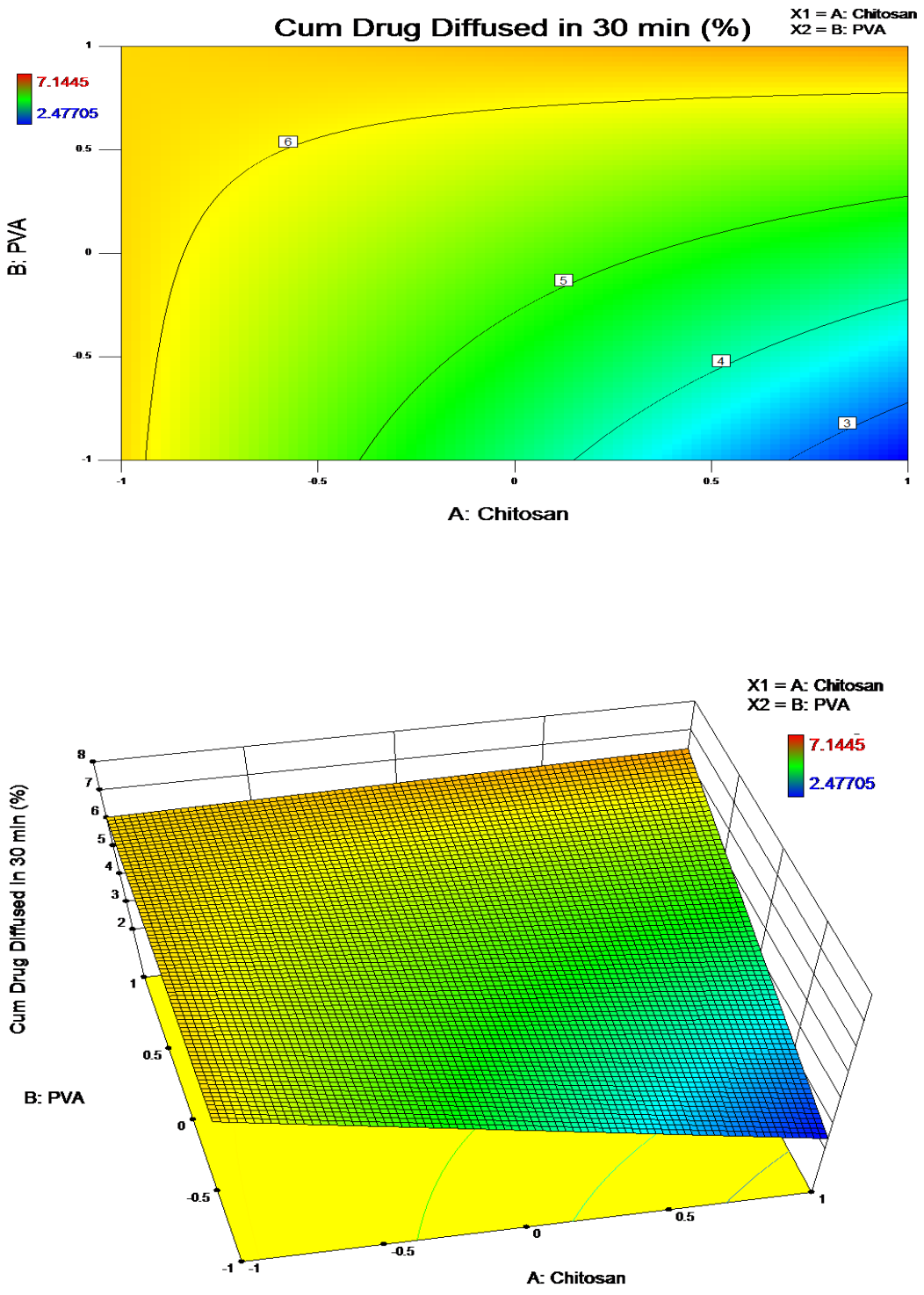

Figure 5. 18 3D and Contour plot for Drug diffused in 30 min

5.4.3.6 Response 6 (Cum Drug diffused in 1 hr)

5.4.3.6.1 ANOVA for Response 6

Table 5. 22: ANOVA for Response 6

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	37.75	3	12.58	15.27	0.0060	Significant
A-Chitosan	9.36	1	9.36	11.35	0.0199	
B-PVA	16.09	1	16.09	19.52	0.0069	
AB	12.31	1	12.31	14.93	0.0118	
Residual	4.12	5	0.82			
Cor Total	41.87	8				

5.4.3.6.2 Polynomial Equation in Terms of Coded Factors

$$\text{Cum drug diffused in 1 hr.} = +10.15 - 1.25*A + 1.64*B + 1.75*AB \ldots\ldots. \quad (6)$$

Equation (a) represent relationship between the quantitative effect of independent variables [X1 Chitosan), X2 PVA)] upon the responses. The equation indicates that Chitosan (A) having significant negative effect on cumulative drug diffused in 1 hr, Whereas PVA (B) having significant positive effect on cumulative drug diffused in 1 hr. Co-efficient of interaction with positive sign represents an positive effect of the two factor combination on the tensile load.

5.4.3.6.3 Response surface mapping

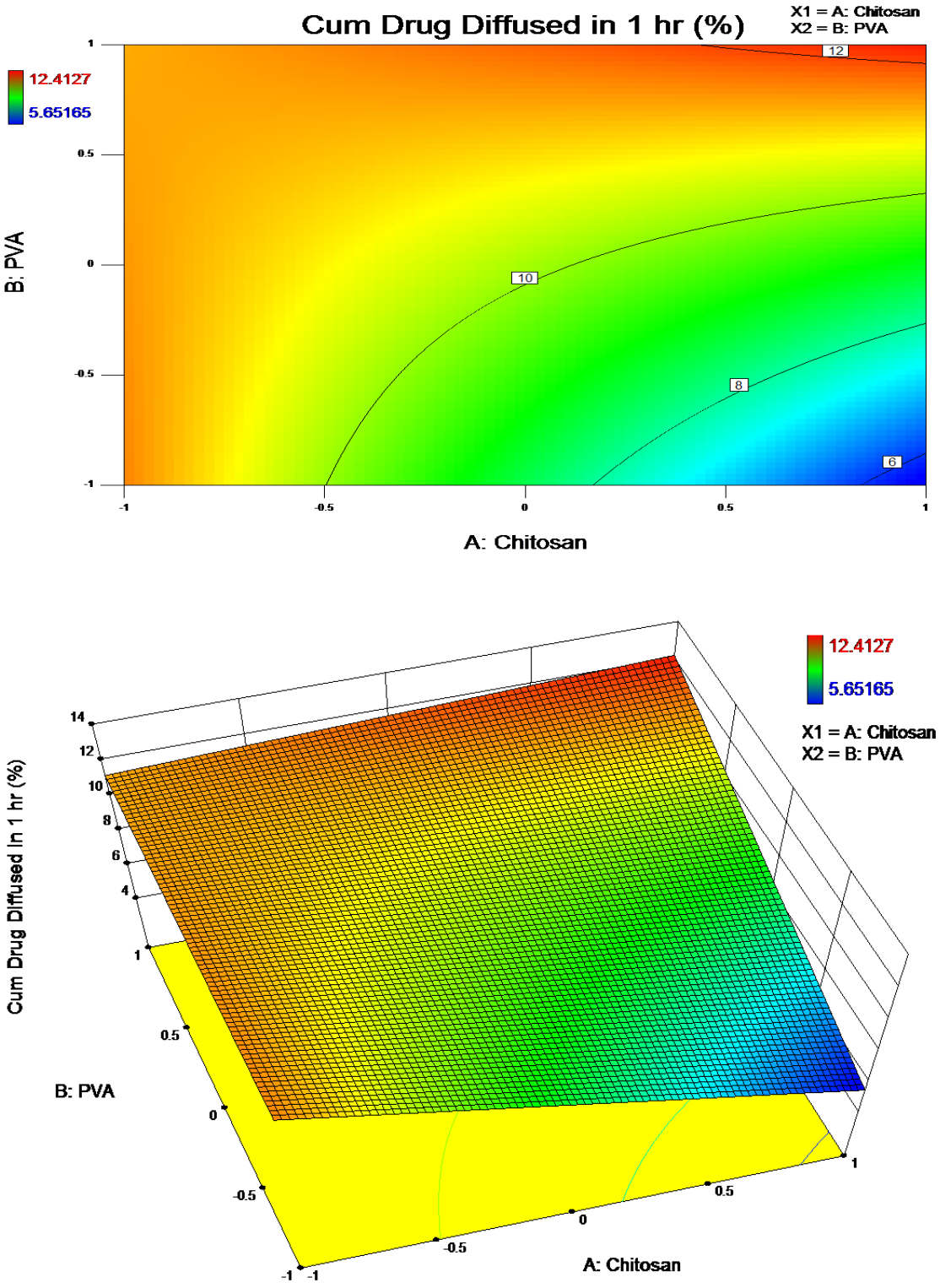

Figure 5. 193D and Contour plot Drug diffused in 1 hr.

5.4.3.7 Response 7 (Cum Drug dissolved in 1 hr)

5.4.3.7.1 ANOVA for Response 7

Table 5. 23: ANOVA for Response 7

ANOVA for Response Surface 2FI model						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	37.75	3	12.58	15.27	0.0060	significant
A-Chitosan	9.36	1	9.36	11.35	0.0199	
B-PVA	16.09	1	16.09	19.52	0.0069	
AB	12.31	1	12.31	14.93	0.0118	
Residual	4.12	5	0.82			
Cor Total	41.87	8				

5.4.3.7.2 Polynomial Equation in Terms of Coded Factors

$$\text{Cum drug dissolved in 1 h.} = +66.71 - 7.67*A + 10.14*B + 11.40*AB \ldots (7)$$

Equation (a) represent relationship between the quantitative effect of independent variables [X1 Chitosan), X2 PVA)] upon the responses. The equation indicates that Chitosan (A) having significant negative effect on cumulative drug dissolved in 1 hr, Whereas PVA (B) having significant positive effect cumulative drug dissolved in 1 hr. Co-efficient of interaction with positive sign represents an positive effect of the two factor combination on the cumulative drug dissolved in 1 hr.

5.4.3.7.3 Response surface mapping

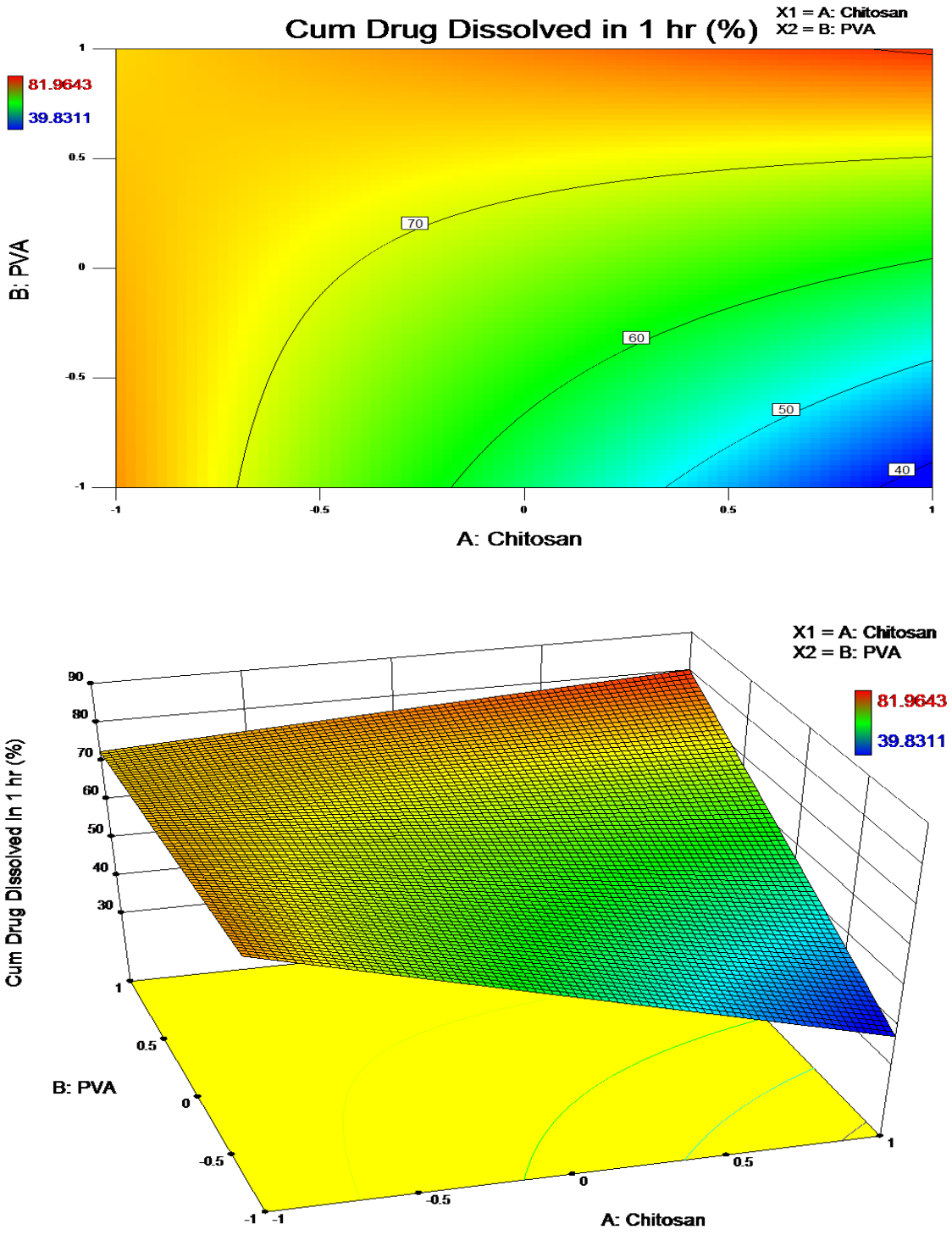

Figure 5. 20 3D and Contour plot for Drug dissolved in 1 hr

5.4.4 Discussion

3^2full factorial design was used to optimize the formulation characteristics where 2FI model was shows interaction between the both factor A (Chitosan) and B (PVA). P vale for all responses was significant at various interaction. Its represent the quantitative relationship or interaction between the factors at various level. Graph for tensile load represent a linear relationship between the factor A (Chitosan) and factor B (PVA) it means both polymers were effect the tensile load in same proportion. When increase the concentration of polymer A and B it was concluded that showing the linear effect on tensile load. For the response 2 (thickness) the model (2FI) was shows same interaction like response 1 (tensile load) it was shows a linear relationship between both factor for the thickness.

For the response 3 (Elongation) graph was not shows linear relation between both factor here factor B (PVA) shows increasing effect on elongation but factor A (Chitosan) was not shows any positive effect hence when increase the concentration of Chitosan it was decreased the elongation. So it was concluded that elongation was mostly depended on PVA. Higher concentration of PVA shows maximum elongation.

For the response 4 (Initial disintegration time point) interaction of polymers was showed in contour plot. Where chitosan shows main role in the disintegration time point hence PVA was not much affect the disintegration. Higher concentration of the chitosan with any concentration PVA shows maximum Initial disintegration time point.

Contour plot for response 5 (drug diffusion) was curvilinear it was shows that when concentration of chitosan was lower shows better diffusion with any concentration of PVA. When increase the concentration of the chitosan it's reduce the diffusion rate. But when both polymer were taken in the higher concentration it's was gives positive effect and produce higher drug release.Response 6 (drug dissolved in 1 hour) are similarly affected like diffusion. Interactionfor the response 6 was similar with response 5.

Check Point analysis and Validation of Design space

5.4.5 Design space (graphical optimization of DoE batches)

Overlay plot of different response result was plotted through which specific design space obtained, which provide working space to get desired dissolution profile.

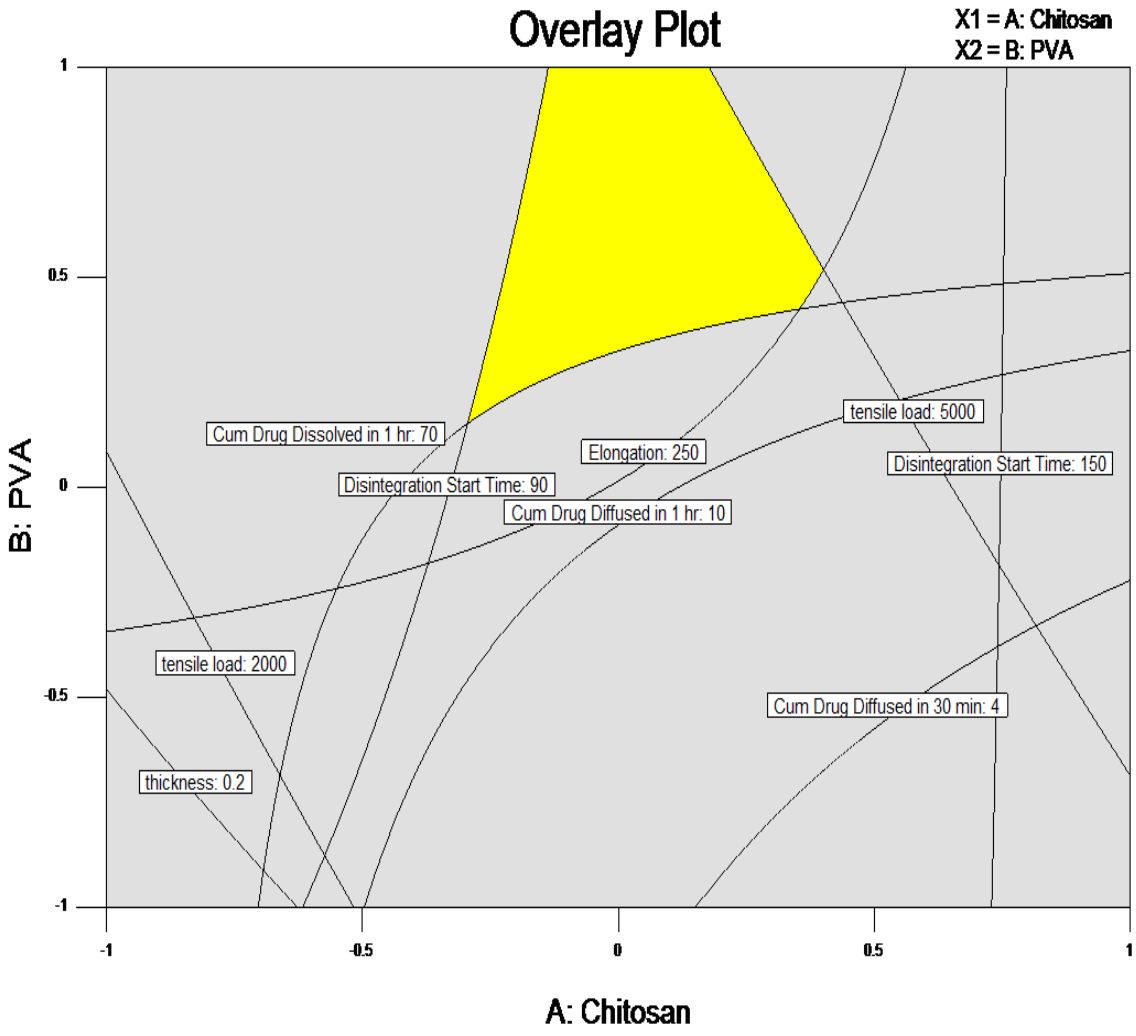

Figure 5. 21 Overlay plot (graphical optimization of DoE batches)

5.4.5.1 Formulations for check point analysis

From the obtained design space several points were selected for validation purpose. CP 2 batch was selected it's having best desirability as per design. CP2, CP3, were selected from design space

Table 5. 24 Content of check point batches

Ingredeints	Concentration (mg)		
	CP1	CP2	CP3
Benzocaine	500	500	500
Chitosan	600	680	600
PVA (medium grade)	800	900	920
PEG 20000	300	300	300
1% Glacacial acetic acid (ml)	25	25	25
Water (ml)	25	25	25
Methanol (ml)	5	5	5

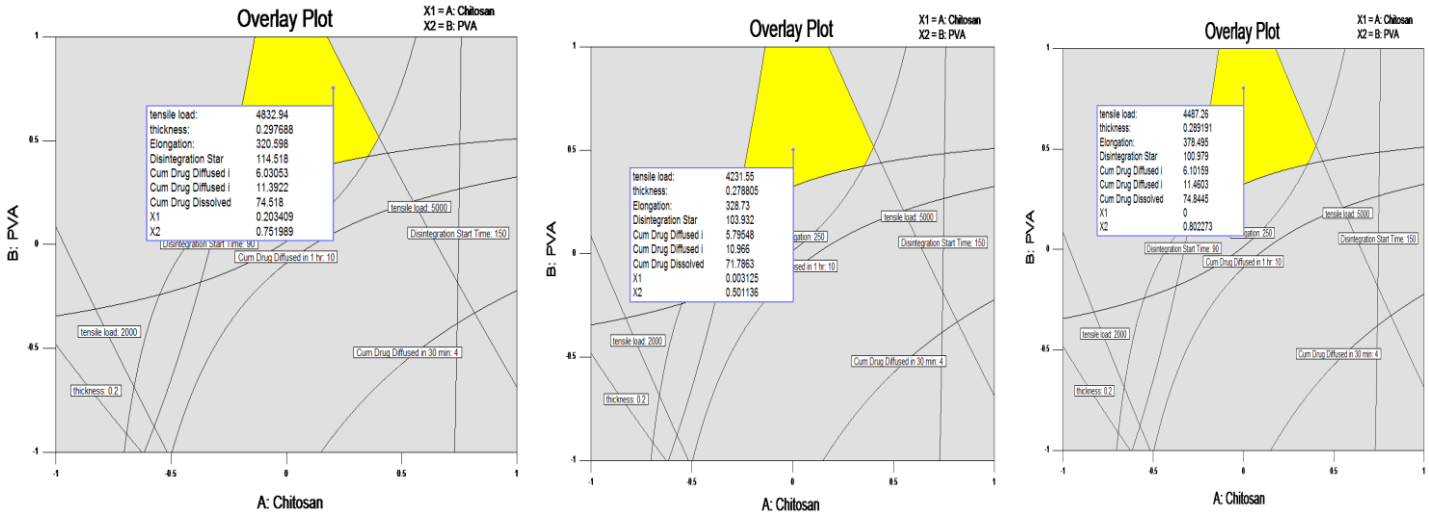

Figure 5. 22: Predicted values gives in designed space

Table 5. 25 comparision of predected and actual value

Sr. No.	Parameter	C1		C2		C3	
		Predicted	Actual	Predicted	Actual	Predicted	Actual
1	Elongation	328	260	320	250	378	300
2	Drug diffused in 30 min	5.78	4.55	6.03	4.83	6.10	4.86
3	Drug diffused in 1 hr.	10.97	10.06	11.39	10.61	11.46	10.75
4	Drug dissolved in 1 hr.	71.79	69.37	74.52	72.41	74.58	71.54
5	Tensile Load (gm)	4231	4120	4833	4880	4487	4300
6	Thickness	0.28	0.32	0.3	0.27	0.29	0.3

5.4.6 Comparison with marketed gel formulation

Description of marketed product

Product name: Mucopain

Company: IPCA Health Products Ltd.

Composition: Benzocaine IP 20% w/w in water –miscible base.

Net weight: 15g

Table 5. 26 Drug release at different time point

Time point (min)	Marketed preparation	Test preparation (C2)
0	0	0
5	4.21	3.34
10	9.86	8.56
15	17.24	15.94
20	24.19	25.06
25	31.58	34.18
30	40.27	44.61
60	67.20	74.58

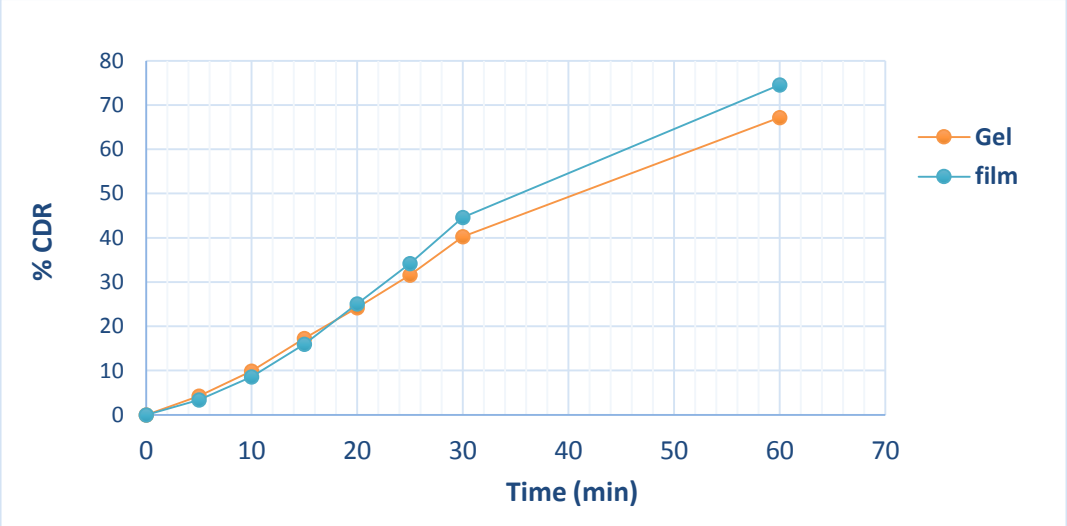

Figure 5. 23 comparative drug release of marketed formulation and C2 batch

SUMMARY

& DISCUSSION

A mouth ulcer is the loss of part of the delicate tissue lining inside the mouth (mucous membrane). The most common cause is injury such as biting your cheek. Other causes include certain drugs, chemicals, systemic disorder and infectious diseases such as herpes or thrush. Mouth ulcer can cure by treatment of the cause, avoiding of irritating food, topical treatment. Generally topical anesthetic was used to treat the mouth ulcer in various formulation such as cream, gel, spray, lozenges. But these conventional formulation cannot maintain the drug concentration for prolonged period of time inside the mouth (buccal cavity) due to short residence time and self-cleansing action of oral mucosa. Buccal film have the number of advantage over the conventional such as easy to apply, no need of water, improve patient compliance, have high stability, no risk of chocking. Benzocaine is local anaesthetic produce reversible loss of sensation and relive from the pain. The aim of the current work is, to development of the sustained release mucoadhesive buccal film of benzocaine that can be hold in the mouth at least 1 hr. using solvent casting method. Chitosan and PVA were selected as a polymer which provide sustained release of drug and keep maintain the effect for loner period. Mucoadhesive buccal film were evaluated for the thickness, tensile strength, % elongation, surface pH, folding endurance, disintegration and % drug release.

Preliminary trials were carried out for optimization of casting surface, plasticizer, and selection of polymer combination. Preformulation study of drug and excipient were done by using UV Spectroscopy, FT-IR Spectroscopy and Differential Scanning Calorimetry. Drug and Excipients were compatible with each other. To optimize the polymer concentration and evaluate the role of polymers applying the 3^2 full factorial design. Responses were measured for tensile load, thickness, % Elongation, initial disintegration time point, % Diffusion and % Dissolution. It was concluded that when increase the concentration of PVA it was increase the elongation decrease initial disintegrating time point where chitosan was increases the tensile load and initial disintegrating time point and decreased the % elongation. Thickness was effected in same manner for the both polymer. Drug release in diffusion and dissolution were higher with any concentration of the PVA hence chitosan increased, it was decreased drug release. But when it was taken with high concentration of PVA its gives maximum drug release. Optimized batch (C2) showed 72% of drug release in 1 hr,

0.27 mm thickness, 4880 gm tensile load, 250% elongation and up 1 hr it was stable in saliva. This batch was further compare with marketed available conventional gel of 20% benzocaine for drug release parameter. Better drug release was obtained than marketed formulation. So it was concluded that sustained release mucoadhesive buccal film of Benzocaine is a stable film and produce desired characteristics of formulation and produce sustained release effect and reduced the dosing frequency.

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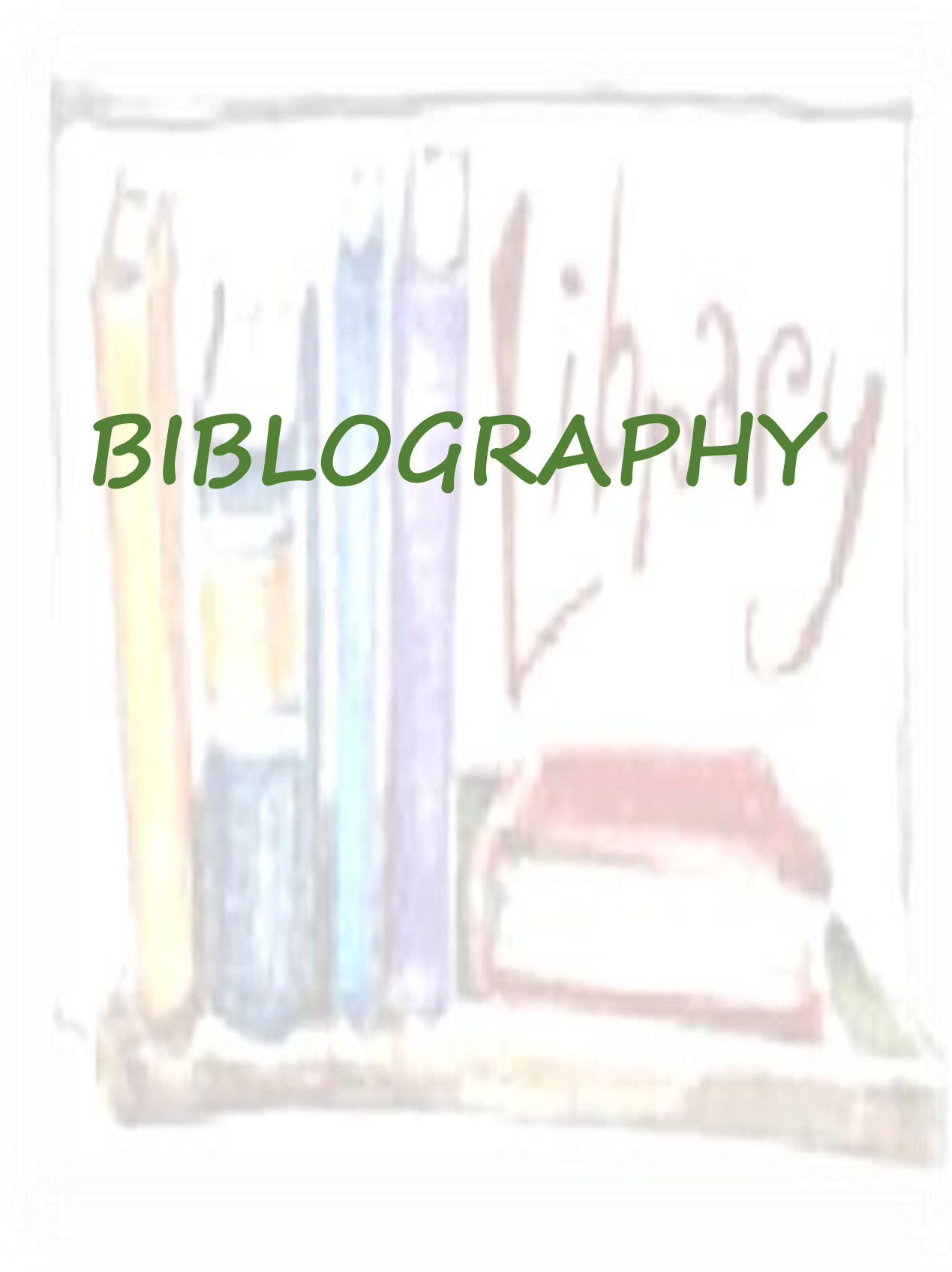

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