COMPARATIVE EVALUATION OF *IN-SITU* GELS AND FILMS OF HYDROCARTISONE FOR THE TREATMENT OF APHTHOUS ULCER

By

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Under the guidance of,

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I hereby declare that this dissertation entitled **COMPARATIVE EVALUATION OF *IN-SITU* GELS AND FILMS OF HYDROCARTISONE FOR THE TREATMENT OF APHTHOUS ULCER** is a bonafide and genuine research work carried out by me under the guidance of **Dr. MOHAMMED GULZAR AHMED** Professor and Head, Department of Pharmaceutics, SAC College of Pharmacy. B.G.Nagara.

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DEDICATED TO LORD GANESHA MY DAD AND MOM

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ABSTRACT

The present work was aimed to develop a in-situ gels and films of hydrocortisone for the treatment of aphthous ulcer. The in-situ gels and film was developed by using methylcellulose, based on the concept of temperature dependant gelling system. The sol-to-gel transformation occurred during the reduction of temperature. The in-situ gels were evaluated for gelling capacity, drug content, viscosity & in-vitro release were as in the film its evaluated for tensile strength, folding endurance, thickness etc. The experimental part shows that viscosity of the sol was increased by increasing the concentration of polymer. All the results were found to be satisfactory, when compared between the in-situ gels and films. The has shown the best formulation because of their therapeutic efficacy and provided sustained release of the drug over a period of time. These results demonstrate that the developed system is an alternative to conventional drug delivery system, patient compliance, industrially oriented and economical.

Key words: *In situ,* Film, Methylcellulose, Gellation temperature & gellation time.

ABBREVIATIONS

ABBREVIATIONS	EXPANSIONS
%	Percentage
%CDR	Percentage cumulative drug release
°C	Degree Centigrade
Abs	Absorbance
Conc	Concentration
Cm	Centimeter
C_{max}	Maximum concentration
FTIR	Fourier Transform infrared
MC	Methyl cellulose
Hr	Hour
Hrs	hours
Mdf	Mouth dissolving film
mcg/µg	Microgram
Mg	Milligram
Mm	Micro meter
Min	Minute
Ml	Milliliter
pH	Negative logarithm of hydrogen ion concentration
Rpm	revolutions per minute
S.D	Standard Deviation

Sol	solution
Std	Standard
UV	Ultraviolet
Vs	Versus
w/v	Weight by volume
w/w	Weight by weight

LIST OF FIGURES

1. INTRODUCTION.

Over the past 30 years greater attention has been focused on development of sustained release[1], sustained action, prolonged action, controlled release, extended release and depot release dosage form are the terms used to identify drug delivery system that are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose of a drug and become the standard in modern pharmaceutical design[2].An intensive research have been undertaken in achieving much better drug product effectiveness reliability & safety.

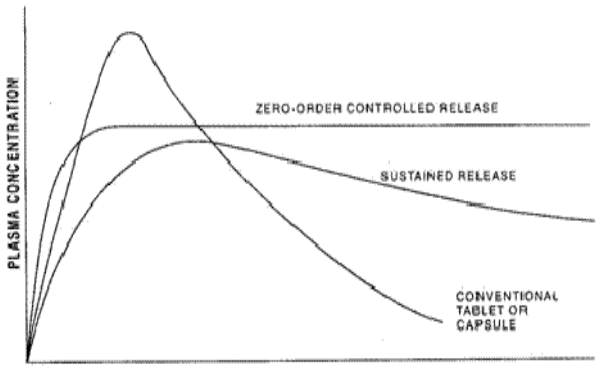

Figure 1: Plasma concentration behaviour.

Sustained release system include any drug delivery system that achieves slow release of the drug over an extended period of time. If the system is successful in maintaining control drug level in the blood or target tissue it is considered as controlled release system. If it is unsuccessful at this but nevertheless extends the duration of action over that achieved by conventional delivery, it is considered a prolonged release system[3]. The oral route of administration for sustained release system has received greater attention because of more flexibility in dosage form design. The design of oral sustained release delivery system are subject to several

inter- related variables of considerable importance such as type of delivery system the disease being treated, the patient, the length of therapy and the properties of drug[4].

Advantages[5]:

1. The frequency of drug administration is reduced
2. Patient compliance can be improved
3. Drug administration can be made more convenient
4. The blood level oscillation characteristics of multiple dosing of conventional dosage form is reduced, because a more even blood level can be maintained
5. Better control of drug absorption can be attained, since the high blood level peak that may be observed after administration in an extended action form
6. The characteristic blood level variations due to multiple dosing of conventional dosage form can be reduced
7. The total amount of drug administration can be reduced, thus
   - Maximizing availability with minimum dose
   - Minimize or eliminate local side effects
   - Minimize or eliminate systemic side effects
   - Minimize drug accumulation with chronic dosing
8. Safety margin of high potency drugs can be increased and the incidence of both local and systemic adverse side effects can be reduced in sensitive patients
9. Improve efficacy in treatment
   - Cure or control condition more promptly
   - Improve/ control i.e. reduces fluctuation in drug level.
   - Improve bioavailability of some drugs
   - Make use of special effect e.g. sustained release aspirin for morning relief of
   - arthritis by dosing before bed time.

- Economy

Disadvantages[6]:

1. Administration of sustained release medication does not permit prompt termination of therapy
2. Flexibility in adjustment in dosage regimen is limited
3. Controlled release forms are designed for normal population i.e., on the basis of average drug biological half lives.
4. Economy factors may also be assessed, since most costly process and equipment are involved in manufacturing so many controlled release dosage forms.

Oral *In situ* gel:

In situ gel forming drug delivery is a type of mucoadhesive drug delivery system. The development of *in situ* gel systems has received considerable attention over the past few years[7]. Capable of releasing drug in a sustained manner maintaining relatively constant plasma profiles. These hydrogels are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. These have a characteristic property of temperature dependent, pH dependent and cation induced gelation. Compared to conventional controlled release formulations, *in situ* forming drug delivery systems possess potential advantages like simple manufacturing process, ease of administration, reduced frequency of administration, improved patient compliance and comfort[8]. *In situ* gel can be easily applied in liquid form to the site of drug absorption. At the site of drug absorption they swell to form a strong gel that is capable of prolonging the residence time of the active substance, *In situ* gels are administered by oral, ocular, rectal, vaginal, injectable and intra-peritoneal routes[9].

Approaches for *in situ* gelling system:

The various approaches for *in situ* gelling system are:

1. Stimuli-responsive *in situ* gel system[10].

- Temperature induced *in situ* gel systems
- pH induced *in situ* gel systems

2. Osmotically induced *in situ* gel systems (Ion activated systems)[11,12].

3. Chemically induced *in situ* gel systems[13,14].

- Ionic cross linking
- Enzymatic cross linking
- Photo-polymerization

1. Stimuli-responsive *in situ* gel system:

Stimuli-responsive polymers are defined as polymers that undergo relatively large and abrupt, physical or chemical changes in response to small external changes in the environmental conditions.

Temperature induced *in situ* gel systems:

In this system, gelling of the solution is triggered by change in temperature, thus sustaining the drug release. These hydrogels are liquid at room temperature (20–25 °C) and undergo gelation when in contact with body fluids (35– 37 °C), due to an increase in temperature. The use of biomaterial whose transitions from sol-gel is triggered by increase in temperature is an attractive way to approach in-situ formation. The change of temperature is not only relatively easy to control, but also easily applicable both *in vitro* and *in vivo.* The polymers which show temperature induced gelation are cellulose derivatives.

pH induced *in situ* gel systems:

Gelling of the solution is triggered by a change in pH. Polymers containing acidic or alkaline functional groups that respond to changes in pH are called pH sensitive polymers. The pH is an important signal, which can be addressed through pH-responsive materials At pH 4.4 the formulation is a free-running solution which undergoes coagulation when the pH is raised by the tear fluid to pH 7.4. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic)groups. The polymers which shows pH induced gelation are cellulose acetate phthalate (CAP) latex, carbomer and its derivatives.

2. Osmotically induced *in situ* gel systems (Ion-activated systems):

In this method, gelling of the solution instilled is triggered by change in the ionic strength. It is assumed that the rate of gelation depend on the osmotic gradient across the surface of the gel. The aqueous polymer solution forms a clear gel in the presence of the mono or divalent cations typically found in the tear fluids. The electrolyte of the tear fluid and especially Na^{+}, Ca^{2+} and Mg^{2+} cations are particularly suited to initiate gelation of the polymer when instilled as a liquid solution in the conjunctival . The polymer which shows osmotically induced gelation are gelrite or gellan gum, hyaluronic acid and alginates etc.

3. Chemically induced *in situ* gel systems:

The chemical reaction which forms *in situ* gel systems are Ionic crosslinking, Enzymatic crosslinking and Photo-polymerization.

Ionic crosslinking:-

Certain ion sensitive polysaccharides such as carragenan, Gellan gum (Gelrite), Pectin, Sodium Alginate undergo phase transition in presence of various

ions such as K^+ , $Ca^{2+,}$ $Mg^{2+,}$ Na^+**.** These polysaccharides fall into the class of ion-sensitive ones**.** For example, Alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca^{2+} due to the interaction with guluronic acid block in alginate chains.

Enzymatic crosslinking:

In situ formation catalysed by natural enzymes has not been investigated widely but seems to have some advantages over chemical and photochemical approaches. For example, an enzymatic process operates efficiently under physiologic conditions without need for potentially harmful chemicals such as monomers and initiators. Intelligent stimuli-responsive delivery systems using hydrogels that can release insulin have been investigated. Cationic pH-sensitive polymers containing immobilized insulin and glucose oxidase can swell in response to blood glucose level releasing the entrapped insulin in a pulsatile fashion. Adjusting the amount of enzyme also provides a convenient mechanism for controlling the rate of gel formation, which allows the mixtures to be injected before gel formation**.**

Photo-polymerization:

A solution of monomers or reactive macromer and initiator can be injected into a tissues site and the application of electromagnetic radiation used to form gel. Acrylate or similar polymerizable functional groups are typically used as the polymerizable groups on the individual monomers and macromers because they rapidly undergo photo-polymerisation in the presence of suitable photo initiator. Photopolymerizable systems when introduced to the desired site via injection get photocured *in situ* with the help of fiber optic cables and then release the drug for prolonged period of time.

IMPORTANCE OF IN SITU GELLING SYSTEM[15]:

- The major importance is the possibilities of administrating accurate and reproducible quantities compared to already formed gel.
- *In-situ* forming polymeric delivery system having advantages like ease of administration and reduced frequency of administration improved patient compliance and comfort.
- Poor bioavailability and therapeutic response exhibited by conventional ophthalmic solution due to rapid precorneal elimination of drug may be overcome by use of gel system that are instilled as drops into eye and undergoes a sol-gel transition from instilled dose.
- Liquid dosage form that can sustain drug release & remain in contact with cornea of eye for extended period of time is ideal.
- Reduced systemic absorption of drug drained through the nasolacrimal duct may result in some undesirable side effects**.**

Oral Films:

A Film can be defined as a dosage form that employs a water-dissolving polymer (generally a hydrocolloid, which may be bio adhesive polymer), which allows the dosage form to quickly hydrate, adhere and dissolve when placed on the tongue or in that oral cavity (i.e., buccal, palatal, gingival, lingual or sublingual, etc.) to provide rapid local or systemic drug delivery[16].

Peroral dosage forms can be distinguished as solid or liquid oral dosage form in which the prior fall in category of pills, capsules, granules and powders. While the later includes solution/ suspensions or emulsions offering more advantages over monolithic solid dosage forms. However they also posses certain disadvantages such as finding non-toxic excipients and need preservatives, which might cause adverse

effects in children, microbiological stability, and also shows problems with the taste masking and dose accuracy. To overcome these problems associated with the liquids dosage forms, oral dissolving tablets were designed in early centaury which slowly led to their further development and thus came the existence of oral disintegrating films[17].

Characteristics of an ideal orally soluble film drug delivery system[18]:

- Do not require water to swallow and should dissolve or diintigrate in the mouth within few seconds
- Compatible with taste masking and other excipients
- They posses pleasant mouth feel.
- Leave minimal or no residue in the mouth after oral administration.
- They can withstand the rigors of the manufacturing process and post manufacturing handling.
- Resistant to environmental condition such as humidity and temperature.
- They are adaptable and amendable to the excisting processing and packaging machinery.
- Processing and packaging of films can be done at low costs prices.

Advantages of oral films [19]:

- The oral film administered sublingually and buccally deliver the drug with high potential to improve the onset of action, lower the dose, and enhance the efficacy and safety profile of the medicament.
- All single unit dosage forms, soft gels and liquid formulations primarily enter the blood stream via the gastrointestinal tract, which subjects the drug to degradation form stomach acid, bile, digestive enzymes and other first pass effects. As a result, such formulations often require higher doses and generally have a delayed

onset of action, which can be overcome using current oral film drug delivery system that avoid these issues and yield quicker onset of action at lower doses.

- Oral film is more stable, durable and quicker dissolving than other conventional dosage forms.
- Oral film enables improved dosing accuracy relative to liquid formulations since every film is manufactured to contain a precise amount of the drug.
- Oral film ensures more accurate administration of drugs.
- Oral film can improve compliance due to the intuitive nature of the dosage form and its inherent ease of administration. These properties are especially beneficial for paediatric, geriatric and neurodegenerative disease patients where proper and compete dosing can be difficult.
- Oral film's ability to dissolve rapidly without the need for water provides an alternative to patients suffering from nausea, such as those patients receiving chemotherapy.
- Oral film drug delivery has the potential to allow the development of sensitive drug targets that may otherwise not be possible in tablet or liquid formulations.
- From a commercial perspective oral film drug delivery technology offers an opportunity to extend revenue lifecycles for pharmaceutical companies whose drug patent is expiring and will soon be vulnerable to generic competition.
- Sublingual film delivers a convenient, quick-dissolving therapeutic dose contained wioralan abuse-deterrent film matrix that cannot be crushed or injected by patients, and rapidly absorbs under the tongue to ensure compliance.

Disadvantages of oral film:

Oral disintegrating films have limitations in terms of the amount of drug that can be incorporated in each unit dose. For lyophilized dosage forms, the drug dose must generally be less than 400mg for insoluble drugs and less than 60mg for soluble drugs.

Now the tremendous research work was carried out in this current field to delivery active ingredients through oral cavity, using soluble film technology.

Manifacturing Process of films [20,21,22,23,24]:

One (or a combination) of the fallowing processes may be used to manufacture the oral films.

- Solvent casting
- Hot-melt extrusion
- Solid dispersion extrusion
- Rolling method

Solvent casting technique:

The method of solvent casting technique involves preparation of the film base which involves the mixing of suitable film forming excipients along with drug in a suitable solvent or solvent system. Once the solution is prepared, the film casting process is performed where in a film of desired thickness is casted onto a moving inert substrate, where suitable rollers are employed for guiding the solution onto the substrate. The clearance or tolerance between the roller and the substrate determines the required thickness of the film; this process is used in large scale production where in glass or Teflon plates can be used as inert support material to cast a film at the laboratory scale. The formed strip is then subjected to drying process to remove the solvent.

The selection of solvent essentially depends on the active pharmaceutical ingredient to be incorporated into the film.

Hot Extrusion Process:

Hot melt extrusion is commonly used to prepare granules, sustained-release tablet, and transdermal and transmucosal drug delivery system. This technique involves shaping a polymer into a film via the heating process rather than through the traditional solvent cast method. In this process active pharmaceutical ingredient and other ingredients mixed in dry dry state which are subjected to heating process and then extruded out in molten state. These process do not involve use of any solvent system. The molten mass thus formed is used to cast the film. The films further cooled and cut to the desired size. The main disadvantage of this process might degrade thermolabile active pharmaceutical ingredients. The critical step is the casting and the drying process optimization of speed of casting and drying time important from the commercial scale output. Hot-melt extrusion include lower temperature and shown residency time of the drug carrier mix (<2 mint), absence of organic solvents, continues operation possibility, minimum product wastage, good control of operating parameters, and possibility to scale up.

Solid Dispersion Extrusion:

The term “solid dispersion” refers to the dispersion of one or more active ingredients in an inert carrier is 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, obtained below 70^0c without removing the liquid solvent. The selected solvent or dissolved drug may not be miscible with the melt of the polymer.

Rolling method:

In this method the film is prepared by pre- mixing of an active ingredients and excipients fallowed by subsequent addition of the solvent. The pre-mixture or master batch which include the film forming polymer. Polar solvent and any other excipient other than the drug is added to the master batchfeed 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-mixer for a sufficient time to provide a uniform mixer , 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 current or heat on the top surface of the film.

Oral Mucosa[25]**:**

The oral mucosa is the "skin" inside the mouth, and it covers most of the oral cavity apart from the teeth. It has several functions. Its main purpose is to act as a barrier. It protects the deeper tissues such as fat, muscle, nerve and blood supplies from mechanical insults, such as trauma during chewing, and also prevents the entry of bacteria and some toxic substances into the body.

There are three types of oral mucosa within the oral cavity. They are:

1. Masticatory mucosa which includes gingiva and the hard palate (25%).

2. Specialized mucosa which includes the dorsum of the tongue (15%), and

3. Lining mucosa which includes buccal mucosa and the floor of the mouth (60%).

Anatomy and physiology of oral cavity

The mouth also referred to as the oral or buccal cavity is lined with mucus membranes with a total surface area of 200cm2. The oral cavity has distinct areas

1) The floor of mouth (sublingual)

2) The buccal area (Cheeks)

3) The gums (gingival)

4) The palatal region. (Hard palate and soft palate)

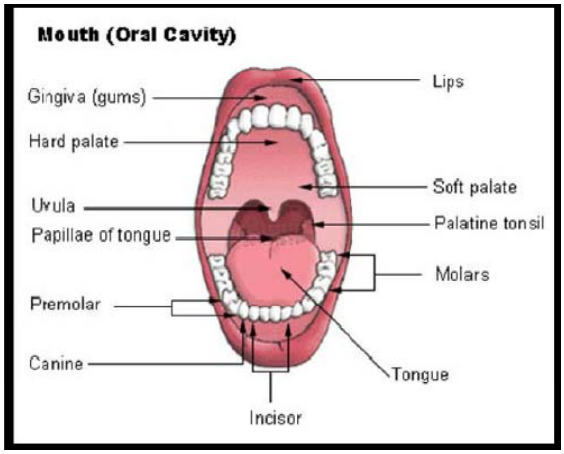

Figure 2: Anatomy and physiology of oral cavity

Cheeks:

The cheeks form the lateral walls of the oral cavity. They covered externally by skin and internally by a mucous membrane, which consist of nonkeratinized stratified squamous epithelium. Buccinator muscles and connective tissue lie between the skin and mucous membrane of the cheeks. The anterior portion of the cheeks end with lips.

Lips/ labia:

The lips are fleshy folds surrounding the opening of the mouth. They contain the orbicularis oris muscle and are covered externally by skin and internally by a mucous membrane. The inner surface of the each lip is attached to its corresponding

gum by a midline fold of mucous membrane called the labial frenulum. This muscles also assist in speech.

Vestibule:

The vestibule of the oral cavity is a space bounded externally by the cheeks and lips and internally by the gums and teeth. The oral cavity proper is a space that extends from the gums and teeth to the fauces, the opening between the oral cavity and the pharynx.

Hard palate:

The hard palate is the anterior portion of the roof of the mouth is formed by the maxillae and palatine bones and is covered by a mucous membrane; it forms a bony portion between the oral and nasal cavities.

Soft palate:

The soft palate which forms the posterior portion of the roof of the mouth, is an arch-shaped muscular partition the roof of the mouth, is an arch-shaped muscular partition between the oropharynx and nasopharynx that is lined with mucous membrane.

Tongue:

The tongue composed of skeletal muscle covered with mucous membrane. Together its associated muscles, it forms the floor of the oral cavity. The tongue is divided into symmetrical lateral halves by a median septum that extends its entire length, and it is attached inferiorly to the hyoid bone, styloid process of the temporal bone, and mandible. Each half of the tongue consists of an identical complement of extrinsic and intrinsic muscles.

- Extrinsic muscle: which originate outside the tongue and insert into connective tissues in the tongue, include the hyoglossus, genioglossus and styloglossus muscles. They also form the floor of the mouth and hold the tongue in position.
- Intrinsic muscles: insert into connective tissue within the tongue. The intrinsic muscle includes the longitudinalis superior, longitudinalis inferior, transverses linguae and verticalis linguae muscles.

The lingual frenulum a fold of mucous membrane in the midline of the undersurface of the tongue, it is attached to the floor of the mouth. The dorsum and lateral surfaces of the tongue are covered with papillae.

Permeability of oral mucosa

The surface of the oral mucosa is permeable for certain drugs which help in its absorption and initiation of its effects. This permeability feature of the oral mucosa is the most important factor that determines the appropriate drug formulations so that the drug gets absorbed and reaches the deeper layers of the oral mucosa. The movement of drug molecules mainly depends on the following features – local variations in mucosal thickness, epithelial keratinization and lipid composition. These features are collectively known as the *barrier region* of the oral mucosa. The permeability of oral mucosa is attributed to intercellular materials derived from membrane coating granules, which are found in the intermediate cell layers of both keratinized and nonkeratinized epithelia. As there is a regional difference in the epithelial thickness of oral mucosa, it has been suggested that the permeability pattern decreases gradually from the sublingual mucosa to the buccal mucosa and palatal mucosa.

MECHANISM OF MUCOADHESIVE DRUG ABSORPTION[26]:

Mucoadhesion is the attachment of the drug along with a suitable carrier to the mucous membrane. It is a complex phenomenon which involves wetting, adsorption

and interpenetration of polymer chains. There are several steps suggested in the process of mucoadhesive bond formation. They are as follows[27]:

1. The first step is the spreading, wetting, and dissolution of mucoadhesive polymer at the interface.
2. The second step is the mechanical or physical entanglement between the polymer and the tissue surface mucus layer, resulting in an interpenetration layer.
3. The next step is the result of chemical interactions, such as covalent and ionic bonds, hydrogen bonding, and Van der Waals' interactions.

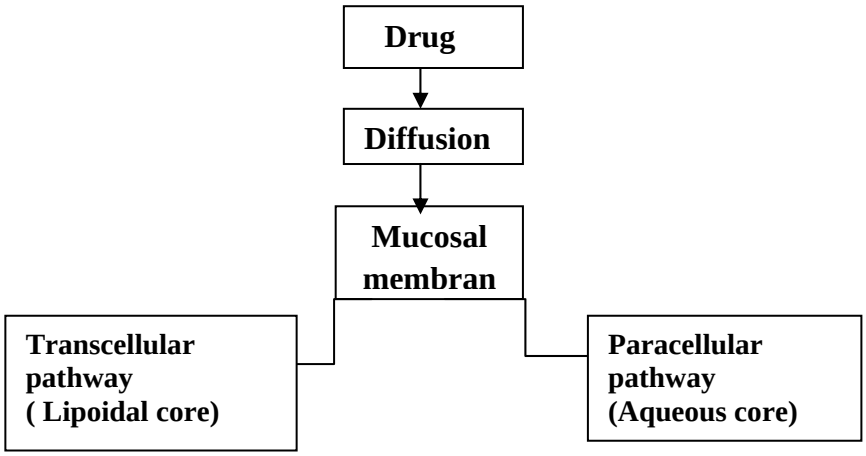

Figure 3: Mechanism of mucoadhesive drug absorption

Advantages of mucoadhesive drug delivery[28].

1. Increases the retention time of the dosage form at the site of absorption.
2. Due to an increased retention time, it enhances absorption and hence the therapeutic efficacy of the drug.
3. Excellent accessibility.
4. Improved patient compliance- ease of drug administration.
5. Faster onset of action is achieved due to mucosal surface.

Mucoadhesive dosage forms can be broadly classified into two, according to the mechanism by which the drug molecule is released from the delivery device. They are as follows[29]:

1. **Monolithic or matrix type:**

   In which the drug is uniformly dispersed or dissolved in the polymer matrix and drug release is affected by diffusion through the polymer network.

2. **Reservoir or membrane controlled type:**

   A drug reservoir is entrapped between an impermeable backing layer and a polymeric membrane that controls the rate of drug release.

   The desirable features of oral adhesive system include:

   (a) High drug loading capacity

   (b) Nonirritant to the tissues

   (c) Good mucoadhesion

   (d) Patient comfort

   (e) Sustained drug delivery

   There are several mucoadhesive systems available for the purpose of oral local drug delivery mainly for the local treatment of variety of oral lesions. These systems include:

   i) Tablets

   ii) Patches

   iii) Films

   iv)Gel or ointment

   v) Sprays

   vi)Oral rinses

MUCOADHESIVE FILMS[30].

Mucoadhesive films are laminates mainly consisting of a polymeric drug-loaded layer which is an impermeable backing layer. This backing layer is used to promote unidirectional drug release. Thin strips of these adhesive films are capable of loading up to 20 mg of drug that can be rapidly delivered to treat certain oral conditions.

Advantages

1. Long term treatment
2. Rapid drug delivery
3. Patient comfort

Disadvantages

1. Deliver drug molecules only to a smaller area

MUCOADHESIVE GEL:

Mucoadhesive gel are the semisolid form of drug dosage mainly introduced for the easy dispersion of drug through the mucosa. These drug systems form an intimate contact with the oral mucosal membrane and facilitate the rapid release of drug molecules at the site of absorption. Major application of mucoadhesive gels is in the treatment of oral conditions such as periodontitis, recurrent aphthous stomatitis, traumatic ulcers, oral mucositis, chronic immunologically mediated oral lesions and to some extent salivary hypofunction.

Advantages

1. Rapid onset of action
2. Ease of use

Disadvantages

1. Inaccurate drug dosing

2. Less retention time of the drug

APHTHOUS ULCER[31]:

Aphthous comes from the greek word “aptha” which means ulcer, the medical literature continues to refer these as oral lesions as aphthous ulcer. Aphthous ulcer are round or oval with grayish yellow, has surrounded by crateriform base surrounded by an erythematous halo of inflamed mucosa. The ulcer usually occurs on the nonkeratinized oral mucosa, including the lips, the buccal mucosa, the floor of the mouth, the soft palate, and the ventral surface of the tongue. Regions of keratinized oral mucosa, such as the hard palate, the gums, and the dorsal surface of the tongue, are uncommon locations.

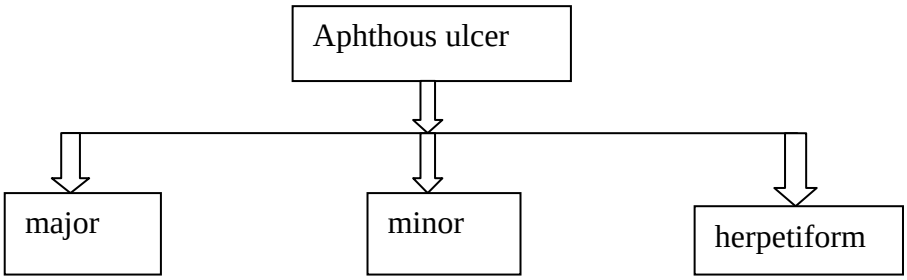

Figure 4: Classification of aphthous ulcer

The aphthous ulcer has been classified into minor, major and herpetiform. Minor aphthous ulcer involves the presence of one to five ulcers at a time, with each ulcer less than 1 cm in diameter. These ulcers are self-limiting and resolve in 7–10 days without scarring. Major aphthous involves 1–10 ulcers at a time, the ulcers exceed 1 cm in diameter, and they persist for up to six weeks. Major aphthae are a cause of significant dysphagia and often result in extensive scarring. In herpetiform aphthae there are 10–100 ulcers at a time, ulcer size is usually 1–3 cm, and the ulcers form clusters that coalesce into widespread areas of ulceration lasting 7–10 days[32].

Corticosteroids are a class of chemicals that includes steroid hormones naturally produced in the adrenal cortex of vertebrates and analogues of these hormones that are synthesized in laboratories. The adrenal cortex secretes glucocorticoids (GC), mineralocorticoids (MC) and androgens. Corticosteroids are involved in a wide range of physiologic processes, including stress response, immune response, and regulation of inflammation, carbohydrate metabolism, protein catabolism, blood electrolyte levels, and behaviour[33].

Glucocorticoids have potent anti-inflammatory actions, including the reduction in the number and function of various immune cells, such as T and B lymphocytes, monocytes, neutrophils, and eosinophils, at sites of inflammation. Glucocorticoids decrease the production of cytokines, chemokines, and eicosanoids and enhances the production of macrophage migration inhibitory factor[34].

Tabel 1: Classification of glucocorticoids based on duration of action

Short acting (8-12hr)	Intermediate acting (12-36 hr)	Long acting(36-72hr)
Cortisol (hydrocortisone)	Triamcinolone	Betamethasone
Cortisone		Dexamethasone
Prednisolone, prednisone		
Methylprednisolone		

Actions of corticosteroids[35].

Glucocorticoids

a. Carbohydrate metabolism: GC increase gluconeogenesis and conserve glucose for use by essential tissues like brain and red blood cells, at the expense of less essential tissues like muscle, during the times of stress or starvation.

b. Protein metabolism: Overall effect is catabolic so that there is negative nitrogen balance with muscle wasting, osteoporosis, growth slowing, skin atrophy, increased capillary fragility, bruising and striae. Healing of wounds is delayed.

c. Fat deposition: It is increased on shoulders, face and abdomen.

d. Maintenance of blood pressure: GC enhances the vascular reactivity to other vasoactive substances such as nor-epinephrine and angiotensin-II.

e. Anti-vitamin D action: They decrease calcium absorption from the gut and increase urinary calcium excretion, thus are useful in treatment of hypercalcemia in sarcoidosis and vitamin D intoxication.

f. Fluid and electrolyte balance: GC exert their effect on tubular function and glomerular filtration rate. They play a permissive role in renal free water excretion.

g. Renal excretion of urate is increased.

h. Anti-inflammatory and immunosuppressive effects: GC decrease recruitment and function of inflammatory cells and vascular permeability at the site of inflammation. They also inhibit prostaglandin and leucotriene synthesis by inhibiting the release of arachidonic acid from the phospholipids. By these mechanisms, GC protect the organism from the damage caused by its own defense reactions and the products of these reactions during stress. Consequently, the use of GC as anti-inflammatory and immunosuppressive agents represent the application of physiological effects to the treatment of diseases.

Hydrocortisone is selected as drug for the treatment of aphthous ulcer as mentioned before it is a short acting drug of glucocorticoid having anti-inflammatory action which is used to decrease the inflammation of the mucous membrane by formulating *in-situ* gels and films of hydrocortisone.

2. OBJECTIVES

Nowadays aphthous ulcer is very common type of disease irrespective of the age. presently available delivery systems (tablet, mouth wash) have minimum effect on the aphthous ulcer and about 90% of the drug are wasted during these delivery systems to avoid wastage of the drug, the alternative ways like *in-situ* gels and films are used. These methods can increase the bioavailability, therapeutic effect and increasing the residency time of the drug by reducing the inflammation of the aphtholmic cells.

The present work is intended to formulate and evaluate the *in situ* gels and films of hydrocortisone(corticosteroid), in view of increasing residence time and bioavailability of drug.

1. Aphthous ulcer are round or oval with grayish yellow of inflamed mucosa. The ulcer usually occurs on the nonkeratinized oral mucosa, including the lips, the buccal mucosa, floor of the mouth, soft palate and the ventral surface of the tongue.
2. Glucocorticoids (Hydrocortisone) have potent anti-inflammatory actions.
3. Selection of drug and excipient using FTIR.
4. To formulate the *in- situ* gels and films of hydrocortisone.
5. To achieve sustained and prolonged release of drug from *in-situ* gels and films.
6. To evaluate the developed gels and films for various characterizations.
7. To carryout the *in-vitro* release studies, kinetic studies.
8. To carryout short term stability studies.

3. REVIEW OF THE LITERATURE

Gulzar M A et al.,[36] has formulated and evaluated the *in situ* gel on the basis of gelation temperature and gelation time system of Rosvastatin and concluded that the formulation posses mucoadhesive properties results of which prolong residence time at the site of application which in turn exhibited better therapeutic effects. In addition in situ gel provides intimate contact between the drug and the absorbing tissue which may results in high drug concentration in local area. The patient compliance may be improved due to the decreases in frequency of drug administration.

Patel.A et al.,[37] has developed Cefpodoxime based on gelation time and temperature *in situ* gelling system and concluded that after oral administration prolonged the release of drug increases the drug bioavailability and diminishes the side effect irritating drugs and delivery the drugs to the infected area.

Gulzar M A et al.,[38] has developed the *in situ* gel on the basis of gelation temperature and gelation time system of Atorvastatin and concluded that the formulation posses mucoadhesive properties results of which prolong residence time at the site of application which in turn exhibited better therapeutic effects. In addition in situ gel provides intimate contact between the drug and the absorbing tissue which may results in high drug concentration in local area. The patient compliance may be improved due to the decreases in frequency of drug administration.

Patidar M.K et al.,[39] has formulated and evaluated the mouth dissolving film on the basis of solvent casting method of Zolpidem and concluded that the formulation containing HPMC as polymer has shown the maximum drug release within 6hrs. Hence this formulation could be used for incessant release of drug , enhance the rate of absorption , efficacy and increases bioavailability.

Aviral K et al.,[40] has formulated and evaluated the oral film by solvent casting technique of Lomefloxacin and concluded that the delivery device provide initially high release and moderate release on the later time in in-vitro study. The developed film was satisfactory in term of drug release and the formulation containing highest concentration of ethyl cellulose has shown the highest percentage of drug release was observed. The patient compliance may be improved due to the decreases in frequency of drug administration.

Maheswari K.M et al.,[41] has developed the mouth dissolving film of Amlodipine Besylate by solvent casting technique from the investigation it can be concluded that formulation containing hydroxyl propyl methylcellulose and methyl cellulose as film formers possessed good physicochemical and dissolution properties. The films showed no change in the homogeneity, transparency, colour and smoothness properties even at the end of six month period. When compared to initial properties and especially no crystallization was observed. The developed film may provide quick onset of action with improved oral bioavailability and enhanced patient compliance and therapeutic efficacy when compared to current marketed formulation like IR and ODTS

Harish NM et al.,[42] has developed a gellan gum based mucoadhesive *in situ* gels for buccal local delivery of fluconazole and evaluated for gelling capacity, viscosity, gel strength, bio adhesive force, microbiological studies and *in vitro* release. They concluded that the formulations were able to release the drug upto 6 hrs and are expected to improve the administration at the site of infection and decrease frequency of administration.

Nirmal H.B[43] has studied new trends in controlled and sustained drug delivery system and concluded that the primary requirement of a successful controlled release

product focuses on increasing patient compliance which the *in situ* gels offer. Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the *in situ* gel dosage forms very reliable.

Chaudhary B et al.,[44] has developed in situ gel containing Acyclovir by gelation time and gelation temperature and concluded that higher the concentration of polymer in the formulation the lower its transition temperature. It is a logical choice for local and systemic delivery of drug, which eventually improves the bioavilability of drug. This approach can be used to treat oral herpes infection locally by improving the patient compliance.

Ambikar R.B et al.,[45] has developed oral dissolving film were prepared by solvent casting technique and concluded that the films having disintegration time within fifteen minits. So they can adhere to the mucosa and shows effect.

Wagh V.D et al.,[46] has evaluated in-situ gel and concluded that provides a sustained release of drug up to eight hours. The drug release was mainly depends on the concentration of plunoric F127 and chitosan.

Throat V.S et al.,[47] has evaluated curcumin loaded mucoadhesive gel and concluded that attempt was done to bridge the indigenes knowledge of medicine with the modern and novel drug delivery system by formulating mucoadhesive thermo reversible int gel system of curcumin using thermo reversible polymers such as pluronic F127/ F68 and mucoadhesive polymer such as carbapol 971P, 974P or xanthum gum. All batches were found to be satisfactory. The formulated drug delivery system was found to be delivering the drugs over an extended period of time for about 4 hrs. Hence it can be concluded that the mucoadhesive gel of curcumin is an ideal candidate for mouth ulcer.

Upreti K et al.,[48] has evaluated paracetamol mouth dissolving film was 2×3cm in size and contained 125mg paracetamol. Thickness of the film was approximately 2mm. The strips disintegrated completely within four minits. In-vitro dissolution studies were carried out in distilled water as well as in stimulated salivary fluid (ph 6.8). The optimized formulation showed 92% drug release with in thirty minits. The prepared strips seems to be attravtive and alternative to conventional marketed formulation.

Rani L.J et al.,[49] has evaluated the oral dispersible film of Lornoxicam and concluded that the formulation f4 containing the polymer SSG with higher concentration has shown better physic- chemical properties with good dissolution profile. Hence it is further studied for the release kinetics and it showed peak plasma concentration of 10.54mg/ml. Hence it was the best formulation.

Harish N.M et al.,[50] has evaluated the in situ gel of clotrimazole and cocluded that gel with mucoadhesive properties was found be promising for prolonging buccal resistance time and thereby better therapatic effects. In addition, they provide intimate contact between the dosage form and the absorbing tissue which may result in high drug concentration in local area. The in situ formulation may improve the patient acceptability, as the formulation is applaid in the form of sols, which upon contact forms the corresponding gels causing less irritation or pain.

3.1 DRUG PROFILE

Hydrocortisone

Chemical Name:

11β,17,21,-trihydroxy pregn-4-ene-3,20-dione.

Structural formula:

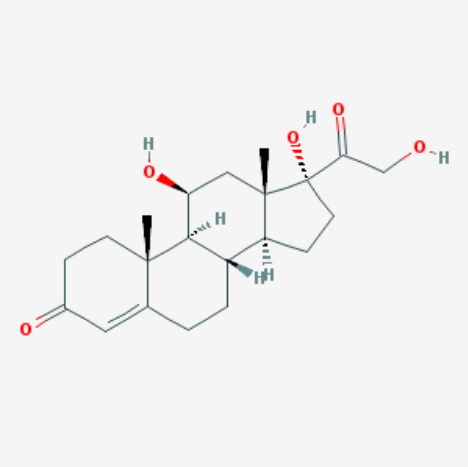

Fig 6 structure of Hydrocortisone

Empirical formula: $C_{21}H_{30}O_5$

Molecular weight: 362.47g/mol

Pharmacokinetics:

Hydrocortisone is well absorbed after oral administration achieving peak blood concentrations after one hour. Plasma protein binding is greater than 90%. Hydrocortisone is primarily bound to plasma globulin. Globulins have a high affinity for hydrocortisone but low binding capacity. Plasma albumin may also bind hydrocortisone. Although albumin has a low affinity for hydrocortisone it does have a high binding capacity. Only unbound form of hydrocortisone is pharmacologically active. Hydrocortisone is metabolised in the liver by hydrogenation to

tetrahydrocortisone and other degraded forms. These are then excreted in the urine as glucuronide conjugates, with a small proportion of unchanged hydrocortisone.

Mechanism of action:

The bactericidal action of sparfloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair, and recombination.

Adverse Reactions

Leucocytosis, hypersensitivity including anaphylaxis, thromboembolism, fatigue, malaise, Congestive heart failure.

Dose and administration:

Hydrocortisone 5mg and 20mg tablets are available in quantities of 100.

Storage/Stability:

Store at or below 30°C. Protect from light and moisture.

3.2 POLYMER PROFILE

3.2.1 METHYLCELLULOSE

Non proprietary names:

- ✓ BP: Methylcellulose
- ✓ PhEur: Methylcellulosum
- ✓ USP: Methylcellulose

Chemical names:

Cellulose Methyl Ether

Empirical formula:

$[C_6H_7O_2(OH)_x(OCH_3)_Y]_n$

Molecular weight: Approx. 20,000 – 38,000g/mol

Description:

Methylcellulose occurs as odourless, tasteless, white to yellowish - white coloured granules or powder.

Structure:

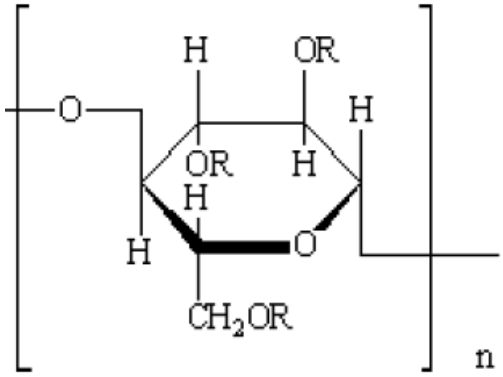

Fig 7 Structure of Methylcellulose

Solubility:

Methylcellulose is Soluble in glacial acetic acid and in mixture of equal volume of ethanol & chloroform, in cold water swells and disappear slowly to form a clear to opalescent, viscous, colloidal suspension. It is insoluble in acetone, chloroform, ethanol, ether, saturated salt solution, taulene and hot water.

Typical properties:

- Acidity/alkalinity: pH=5.5-8.0 for a 1% W/V aqueous suspension.
- Angle of repose 40-50^{0}c

Melting point:

280-300^{o}C

Functional categories:

Emulsifying agent, suspending agent; tablet binder; coating agent;tablet and capsule disintigrant; viscosity increasing agent.

Viscosity (dynamic):

A various grades of methylcellulose are commercially available which produce 2% w/v solutions with viscosities of (10-10,000cp). The viscosity of solutions may be increased increasing the concentration of methylcellulose. Increased temperatures reduce the viscosity of solution until gel formation occurs at 50-60^{0}C.The process of thermogelation is reversible, with a viscous solution being reformed on cooling.

Incompatibilities:

Methylcellulose is incompatible with aminacrine hydrochloride, cholesterol, mercuric chloride, phenol, resorcinol, tannic acid etc. Salts of mineral acids and particularly of polybasic acid, phenols and tannins, coagulate solutions of methylcellulose. Complexation of methylcellulose occurs with highly surface active compounds.

Applications in pharmaceutical formulation or technology:

Methylcellulose is widely used in oral, and topical pharmaceutical formulations. In oral products, it is primarily used as a tablet binding agent, disintigrant, coating agent, suspending or thickening agent. Its also used in the preparation of cream, gels and cosmetics etc.

3.2.2 Sodium Citrate

Non proprietary names:

- ✓ BP: sodium citrate
- ✓ USP: sodium citrate
- ✓ PhEur: Natrii citras

Synonyms:

Citric acid trisodium; E331; trisodium citrate.

Chemical name: Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate.

Empirical formula: $C_6H_5Na_3O_7.2H_2O$

Molecular weight: Approx. 294.10

Description:

It is odourless, colourless, monoclinic crystals or a white crystalline powder with a cooling, saline taste.

Structure:

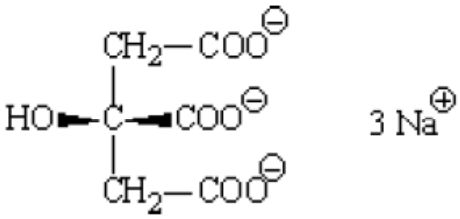

Fig 8 Structure of Sodium citrate

Solubility:

It is soluble in 1 in 1.5 of water, 1 in 0.6 of boiling water, particularly insoluble in ethanol (95%).

Typical properties:

- pH = 7.0 – 9.0 (5% W/V aqueous solution)
- Density : 1.19 g/cm^3

Melting point:

Converts to anhydrous form at 150°C

Functional categories:

Alkalizing agent, buffering agent, sequestering agent.

Incompatibilities:

Aqueous solutions are slightly alkaline and will react with acidic substances. Alkaloidal salts may be precipitated from their aqueous or hydro-alcohol solutions. Calcium and strontium salts will cause precipitation of the corresponding citrates.

Storage conditions: Airtight container in a cool, dry place.

Applications in pharmaceutical formulation or technology:

Sodium citrate is widely used in pharmaceutical formulation. Used in effervescent tablet formulation, blood anticoagulant either alone or in combination with other combination. Therapeutically its used to relive the painful irritation. Its also used as a sequestering agent.

3.2.3 Triethanolamine

Non proprietary names:

- ✓ BP: Triethanolamine
- ✓ USPNF: Trolamine

Synonyms:

TEA, triethylolamine, trihydroxytriethylamine, tris(hydroxyethyl)amine.

Chemical name:

2,2',2''- Nitrilotriethanol

Empirical formula:

$C_6H_{15}NO_3$.

Molecular weight: 149.19 g/mol

Description:

Triethanolamine is a clear, colourless to pale yellow-colored viscous liquid having a slight ammoniacal odor.

Structure:

$N(CH_2CH_2OH)_3$

Solubility:

Triethanolamine is miscible in Acetone, Ethanol, Methanol, water and its soluble in chloroform.

Typical properties:

- pH =10.5 for a 0.1N aqueous solution
- Density (bulk): 1.1242 g/cm^3
- Density (tapped): 1.0985 g/cm^3
- Melting point: approximately 20-21°C

Functional categories:

Alkalizing agent; Emulsifying agent.

Incompatibilities:

Triethanolamine will react with mineral acids to form crystalline salts and esters. With the higher fatty acids triethanolamine forms salts which are soluble in water and have the general characteristics of soap. It will also react with copper to form complex salt.

Applications in pharmaceutical formulation or technology:

Triethanolamine is widely used in topical pharmaceutical formulations primarily in the formation of emulsion. Its also used in the formation of salts for injection, and in topical analgesic preparations. Other general uses are as a buffer, solvent, polymer plasticizer and humectants.

3.2.4 Propylene Glycol

Non proprietary names:

- ✓ BP: Propylene glycol

- ✓ USPNF: Propylene glycol
- ✓ PhEur : Propylenglycolum

Synonyms:

1,2-Dihydroxypropane; 2-hydroxypropanol; methyl ethylene glycol; methyl glycol; propane-1,2-diol.

Chemical name:

1,2- propanediol

(R)-(-)-1,2-propanediol

(S)-(+)-1,2- propanediol

(RS)-(±)-1,2- propanediol

Empirical formula:

$C_3H_8O_2$.

Molecular weight: 76.09

Description:

Propylene glycol is a clear, colourless, viscous, practically odourless liquid with a sweet, slightly acrid taste resembling glycerin.

Structure:

$CH_3CHOHCH_2OH$

Solubility:

Propylene glycol miscible with acetone, chloroform, ethanol(95%),glycerin and water. Soluable 1 in 6 parts of ether; not miscible with light mineral oil or fixed oils, but will dissolve some essential oil.

Typical properties:

- Density : 1.038 g/cm^3
- Melting point: approximately -59°C

Functional categories:

Antimicrobial preservative, disinfectant, humectants, plasticizer, solvent, stabilizer for vitamins, water-miscible cosolvent.

Incompatibilities:

Propylene glycol is incompatible with oxidizing reagents such as potassium permanganate.

Applications in pharmaceutical formulation or technology:

Propylene glycol has become widely used as a solvent, extractant and preservative in a variety of parenteral and non parenteral Pharmaceutical formulations.

4. MATERIALS AND METHODS

PLAN OF WORK

1. Literature survey
2. Collection of drug, polymers & other excipients
3. Standardization of drug
4. Formulation developments

A. Preformulation study

- *Determination of melting point*
- *Solubility*
- *Determination of* λ_{max}
- *Compatibility study*

B. Selection of vehicle

C. Formulation design

D. Evaluation

- *pH*
- *In vitro gelation studies & viscosity*
- *Drug content*
- *Spreadability*
- *Tensile strength*
- *Folding endurance*
- *Disintegration studies*
- *In vitro drug release study*
- *Ex – Vivo study*
- *Stability studies*

4.1 MATERIALS

Table 2 : list of materials

Sl.No	Ingredients	Company Name
1	Hydrocortisone	Yarrow chem. Products, Mumbai
2	Methyl cellulose	S.D Fine Chem. Ltd, Mumbai
3	Sodium citrate	S.D Fine Chem. Ltd, Mumbai
4	Propylene glycol	S.D Fine Chem. Ltd, Mumbai
5	Tri-ethanol amine	S.D Fine Chem. Ltd, Mumbai

4.2 LIST OF INSTRUMENTS

Table 3: List of equipments

Sl no	Equipments	Model/company
1	Electronic Balance	Citizen, India.
2	Magnetic stirrer with hot plate	Almicro, Bangalore.
3	UV-Visible Spectrophotometer	Shimadzu UV"1800, Japan
4	FT-IR Spectrophotometer	Thermo Nicolet 380,india
5	Digital pH meter	Chemi line.
6	Brook field viscometer	RV DV2 T ,India.
7	Stability chamber (106 Model)	LABTOP, SKY Lab Instruments & Engineering Pvt. Ltd
8	Dissolution chamber	LABINDIA, DS 8000, India
9.	SEM	FEI Quanta 200 F.
10.	DSC	DSC 4000.

4.3 PREFORMULATION STUDIES

Preformulation testing is the first step in rational development of dosage forms of a drug substance. Preformulation study is the process of optimizing the delivery of drug through determination of physicochemical properties of the new compound that could affect drug performance and development of an efficacious, stable and safe dosage form. It gives the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the dosage form. Hence, preformulation studies were performed for the obtained sample of drug for identification and compatibility studies.

4.3.1 Determination of λ_{max}

The solution of Hydrocartisone containing the concentration of 10µg/ml was prepared using methanol and UV spectrum was taken using Shimadzu (UV-1700) double beam spectrophotometer. The solution was scanned in the range of 200-400 nm.

4.3.2 Solubility

Solubility is an important consideration in formulations as clarity of the solution is an essential requirement. The solubility of Hydrocartisone was tested in various solvents such as distilled water, methanol, propanol and acetone.

For selection of suitable vehicle for Hydrocartisone excess amount of the drug is added to the following,

a) 10 ml distilled water

b) 10 ml methanol

c) 10 ml phosphate buffer (6.8 pH)

d) 10 ml artificial salivary fluid

After adding maximum amount of the drug till the saturation of the solution is seen, filter it and make dilution as required. Absorbance is measured at 242 nm by using UV-Visible Spectrophotometer .The drug content is calculated by using the standard graph.

4.3.3 Calibration curve of Hydrocartisone:

Accurately weighed 100 mg Hydrocartisone was dissolved in 100 ml of methanol to get the stock-I solution of 1 mg/ml. From this stock solution 10ml is taken and diluted to 100 ml with methanol to get stock-II solution of 100µg/ml. From this stock-II solution aliquots of 0.5, 1.0, 1.5, 2, 2.5 & 3ml were withdrawn and further diluted to 10 ml with methanol to obtain a concentrations range of 5 to 30µg/ml respectively. The absorbance of the solutions was measured at 242 nm by using UV-Vis spectrophotometer. A graph of Concentration vs. Absorbance was plotted.

4.3.4 Compatibility studies

The compatibility studies of the drug with polymers are studied using FT-IR spectroscopy.

- **IR Spectroscopy**

FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The FT-IR spectra of drug with polymers were compared with the standard FT-IR spectrum of the pure drug.

4.4 FORMULATION DESIGN

Table 4: Formulation design of *in situ* gel

Batch Code	Hydrocartisone (%w/v)	Methyl cellulose (%w/v)	Sodium citrate (%w/v)	Triethanolamine	Distilled Water
F1	1	0.25	0.25	Q.S	Q.S
F2	1	0.50	0.25	Q.S	Q.S
F3	1	0.75	0.25	Q.S	Q.S
F4	1	1.00	0.25	Q.S	Q.S
F5	1	1.25	0.25	Q.S	Q.S
F6	1	1.50	0.25	Q.S	Q.S
F7	1	1.75	0.25	Q.S	Q.S
F8	1	2.00	0.25	Q.S	Q.S

4.4.1Preparation of *in situ* gelling system:

For the preparation of methyl cellulose containing *in situ* gel formulations, sodium citrate was added to distilled water with continuous stirring until clear solution was obtained. Methyl cellulose was added to above solution with continuous stirring and allowed to hydrate overnight. Calculated amount of Hydrocartisone (1% w/v) drops triethanolamine was added separately and then added to polymer solution under constant stirring. The formulation design Of Hydrocartisone in situ gel was tabulated. The optimization concentration of methyl cellulose was selected on the basis of gelation temperature and gelation time. Further, the prepared formulations were evaluated for various characterization studies.

4.5 EVALUTION OF *IN SITU* GEL

4.5.1 Gelling Capacity:

All formulations were evaluated for gelling capacity in order to identify the compositions suitable for use as *in situ* gelling systems. The gelling capacity was determined by visual method in which coloured solution of prepared formulations were prepared. Gelling capacity was estimated by placing 2 ml 6.8 pH buffer in a 10 ml test tube and maintained at 37±1°C temperature. One millilitre of coloured

formulation solution was added to the buffer solution. As the formulation comes into contact with 6.8 pH buffer it was immediately converted into a stiff gel-like structure. The gelling capacity of formulation was evaluated on the basis of stiffness of formed gel and time period for which formed gel remains as such. The *in vitro* gelling capacity was graded in three categories on the basis of gelation time and the time taken for the gel formed to dissolve.

4.5.2 Determination of Physical appearance and pH

All the prepared in situ solutions of Hydrocortisone were checked for their clarity and the type of the solutions. The pH of each of the solution of methylcellulose based in situ solutions of Hydrocortisone was measured using a calibrated digital pH meter at room temperature in triplicate.

4.5.3 Viscosity Studies

Brook field digital viscometer (Model RDVT2) was used for the determination of viscosity and rheological properties of Hydrocartisone *in situ* gel using spindle no T- 96. Gel weighing 50 g was taken in a beaker and the spindle was dipped in it. The viscosity of gel was measured at different angular velocities at a temperature of 25°C. A typical run comprised changing of the angular velocity from 10 to 50 rpm. The averages of two readings were used to calculate the viscosity.

4.5.4 Gelation temperature:

A magnetic bead and 10 ml of the sample solution were put into a 30 ml transparent vial placed in a low temperature digital water bath. A thermometer was placed in the sample solution. The solution was heated at the rate of 1oC/m with the continuous stirring. The temperature at which the magnetic bead stopped moving due to gelation was considered as gelation temperature.

4.5.5 Gelation time:

Gelation time of prepared *in situ* gel formulation was measured by placing 2 ml of the gel in 15 ml borosilicate glass test tube. This test tube was placed in water-bath (37±2^{o}C) and gelation time was noted when there was no flow of the gel when test tube was inverted

4.5.6 Drug content analysis:

Accurately weighed amount of gel equivalent to 2 mg of drug was taken into a 100ml volumetric flask. They were analysed with 25 ml of medium (6.8 pH buffer) for 15 m. The clear solution was diluted to 100 ml of medium. Then 10 ml of this solution was diluted to 100 ml buffer. Aliquots were withdrawn and the absorbance was measured at 242 nm against 6.8pH buffer by using UV-Visible Spectrophotometer-1800 (Shimadzu, Japan) and drug content was calculated from the calibration curve.

4.5.7 Syringeability:

All prepared formulations were transferred into a 5 ml syringe placed with 20 gauge needle to a constant volume (2 ml). The solutions which were easily passed from syringe was termed as pass and difficult to pass were termed as fail

4.5.8 Spreadability:

To determine the Spreadability of the gel, approximately 1 g of gel was placed at the centre of the glass plate (20 cm × 20 cm). This glass plate was covered with another glass plate of the same size. Next, the weight of 1000g was carefully applied on the upper side of the plate; as a result the gel was spread out in between the plates. After one minute the weight was removed and the diameter of the spread area (cm) was measured. This determination was carried out in triplicate.

4.5.9 *In vitro* drug release study

In vitro drug release study of Hydrocortisone from the *in situ* gel formulations was conducted for the period of 8 h using cellophane membrane. The diffusion medium was 6.8 pH buffer. Cellophane membrane, previously soaked overnight in the diffusion medium, was tied to one end of a glass cylinder. Then 1ml of the prepared formulation was placed in cellophane membrane tie in a glass cylinder and make the membrane just touched the receptor medium surface. The diffusion medium was stirred at required 50 rpm using magnetic stirrer. At predetermined time interval one ml of the sample was taken and replaced by an equal volume of the receptor medium. The sample was analysed spectrophotometrically at 242 nm.

4.5.10 Stability study:

Stability study of optimized formulation was carried out at 25± 2 ºC/ 60 ± 5% and 40 ± 2 ºC/75± 5% RH for a period of three months. During stability study *in situ* gel was analysed for pH, viscosity, drug content and *in vitro* drug release

4.6 FORMULATION DESIGN:

Table 5: Formulation design of oral films

Batch Code	Hydrocartisone (%w/v)	Methyl cellulose (%w/v)	Sodium citrate (%w/v)	Propylin glycol	Distilled Water
F1	1	0.25	0.25	0.25	Q.S
F2	1	0.50	0.25	0.50	Q.S
F3	1	0.75	0.25	0.75	Q.S
F4	1	1.00	0.25	1.00	Q.S
F5	1	1.25	0.25	1.25	Q.S
F6	1	1.50	0.25	1.50	Q.S
F7	1	1.75	0.25	1.75	Q.S
F8	1	2.00	0.25	2.00	Q.S

4.7 Evaluation Parameters for Films:

4.7.1 Variation of Mass:

Mass of 0.5cm^2 film from different batches of the formulations was noted on electronic balance. The estimations were carried out in triplicate.

4.7.2 Thickness.

The thickness of film was evaluated using a screw gauge with a range of 0–10mm and revolution 0.001 mm. Anvil of the thickness gauge was turned and the film was inserted after making sure that the pointer was set to zero. The film was held on the anvil and the reading on the dial was noted down. The estimations were carried out in triplicate.

4.7.3 Drug Content.

Zero point five cm^2 film was taken in a 10ml volumetric flask and dissolved in 5ml of methanol and then final volume was made up with methanol. Samples were suitably diluted with artificial saliva and the absorbance was measured at 242 nm. The estimations were carried out in triplicate

4.7.4 *In Vitro* Disintegration Studies.

Disintegration time gives an indication about the disintegration characteristics and dissolution characteristics of the film. In case of films the disintegration and dissolution procedures are hardly distinguishable. If the films disintegrates it concurrently dissolves in a small amount of saliva which makes it difficult to mimic these natural conditions and measures with an adequate method. However, in the present investigation two methods of disintegration were adopted.

Drop Method.

In the first method one drop of distilled water was dropped by a pipette onto the oral films. The films were placed on a glass slide and then the glass slide was

placed planar on a Petridis. The time until the film dissolved and caused a hole within the film was measured. The estimations were carried out in triplicate.

Petridis Method.

In this method 2mL of distilled water was placed in a Petridis and one film was added on the surface of the water and the time required until the oral film dissolved completely was measured. Drug-loaded films were investigated under both methods. The estimations were carried out in triplicate

4.7.5 SEM Analysis.

The morphology and surface topography of the film were examined by scanning electron microscopy. The samples to be examined were mounted on the SEM sample stab using a double-sided adhesive tape. The samples mounted were coated with gold (200˚A) under reduced pressure (0.001 torr) for 5min to improve the conductivity using an Ion sputtering device

4.7.6 Tensile Strength.

Tensile strength is the maximum stress applied to a point at which the film specimen breaks. It is calculated by the load at rupture divided by the cross-sectional area of the film as given below:

$$\text{Tensile strength} = \frac{\text{load at failure} \times 100}{\text{film thickness} \times \text{film width}}$$

It was measured using Shimadzu AG-100kNG (Winsoft tensile and compression testing). The film of size 5 × 5 cm^2 and free of physical imperfections was placed between two clamps held 10mm apart. The film was pulled by a clamp at a rate of 5 mm/min. The whole experiment was carried out in triplicate.

4.7.7 Folding Endurance.

Folding endurance was determined by repeated folding of the film at the same place till the film breaks. This gives an indication of the brittleness of the film. The number of times the film was folded without breaking was computed as the folding endurance value. The estimations were carried out in triplicate.

4.7.8 *In Vitro* Dissolution Studies.

The *in vitro* dissolution studies were conducted using 500ml of artificial saliva as dissolution medium with modified type I dissolution apparatus. A temperature of 37^0C and 50rpm was used. Each film with a dimension of appropriate size equivalent to 5 mg of Hydrocartisone was placed on a watch glass covered with nylon wire mesh. The watch glass was then dropped into a dissolution flask. Five ml samples were withdrawn at 1, 2, 3, 4, 5, 6, 7 and 8hrs time intervals and every time replaced with 5mL of fresh dissolution medium. The samples were analyzed by measuring absorbance at 242 nm. The dissolution experiments were conducted in triplicate.

4.7.9 Ex- Vivo Diffusion Studies.

Ex-vivo release study was conducted using fresh chicken skin, the skin was soaked in the sodium bromide solution for 5-6 hrs and washed with water so as to remove the adhering fat tissue. Than the skin was mounted in the diffusion cell containing phosphate buffer of PH 6.8 .The temperature of the medium thermostatically controlled at 37 ± 10^0C and 5ml of the sample were withdrawn at predetermined intervals and were spectrophotometrically estimated at 242nm against their respective blank formulation.

4.7.10 Drug release kinetics:

Investigation for the drug release from the films was done by studying the release data with zero order, first order kinetics and Higuchi equation. The release mechanism was understood by fitting the data to Kosemeyer Peppas model.

5. RESULTS AND DISCUSSION

5.1 Determination of λ_{max}

The λ_{max} of Hydrocortisone was found to be 242 nm in methanol.

5.2 Drug solubility studies

Drug solubility studies have done by using various solvents. Solubility of Hydrocortisone was found to be in methanol. Results have showed that Hydrocortisone is highly soluble in methanol. It has high solubility in methanol than other solvents and poorly soluble in water compared to acetone.

5.3 Calibration curve of Hydrocortisone

The absorbance was measured in a UV spectrophotometer (Shimadzu UV"1800) at 242 nm in methanol. The absorbance so obtained was tabulated as in Table 7. Calibration curve was plotted as shown in the figure 5.

Table 6: Calibration data of Hydrocortisone

SL .no	Concentration (µg/ml)	Absorbance (nm)
1	0	0
2	0.5	0.132
3	1	0.256
4	1.5	0.412
5	2	0.591
6	2.5	0.772
7	3	0.883

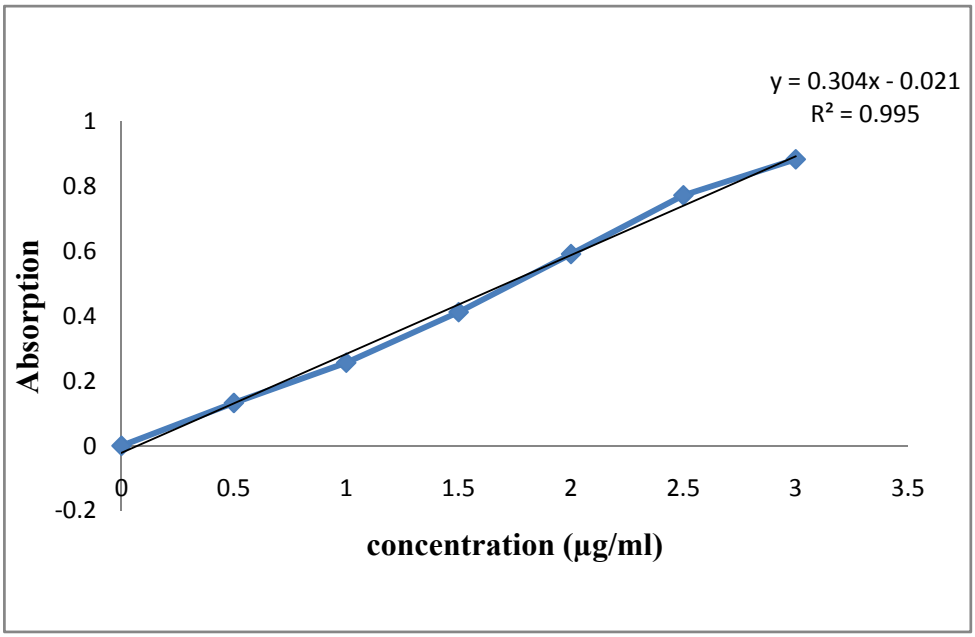

Figure 5: Calibration curve of Hydrocortisone

5.4 FT-IR spectrum

Infra red spectra of pure drug Hydrocortisone and combination of drug with polymers (Methyl cellulose) were obtained ad shown in figures 7 .

All the characteristic peaks of Hydrocortisone were present in spectrum of drug and polymer mixture, indicating compatibility between drug and polymers. The spectrum confirmed that there is no significant change in chemical integrity of the drug.

The functional group peaks of Hydrocortisone were found in all the IR-spectra and was tabulated in Table.

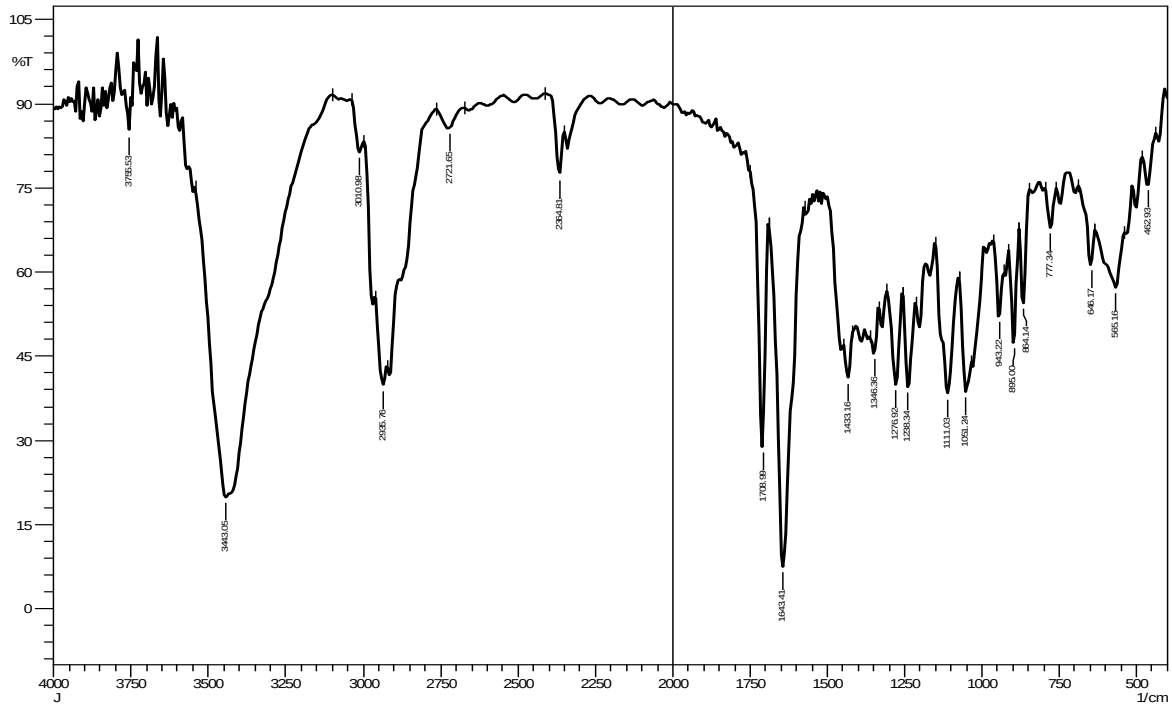

Figure 6: FT-IR spectrum for pure drug

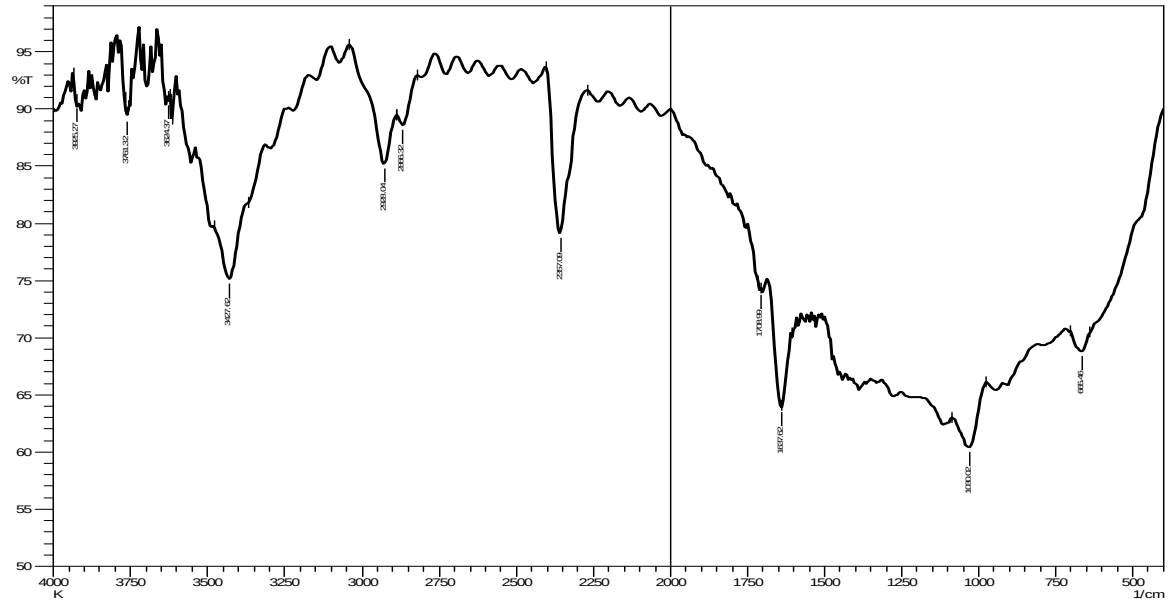

Figure 7: FT-IR spectrum for pure drug with polymer

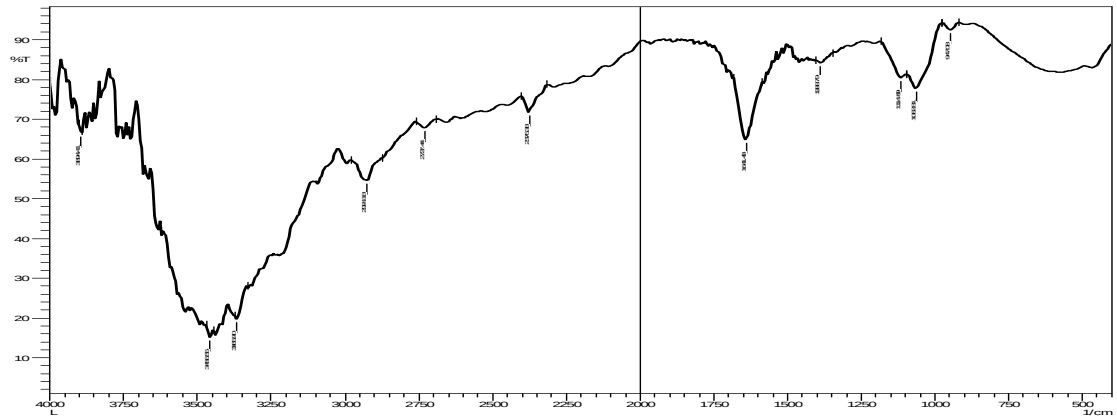

Figure 8: FT-IR spectrum for *in situ* gel formulation

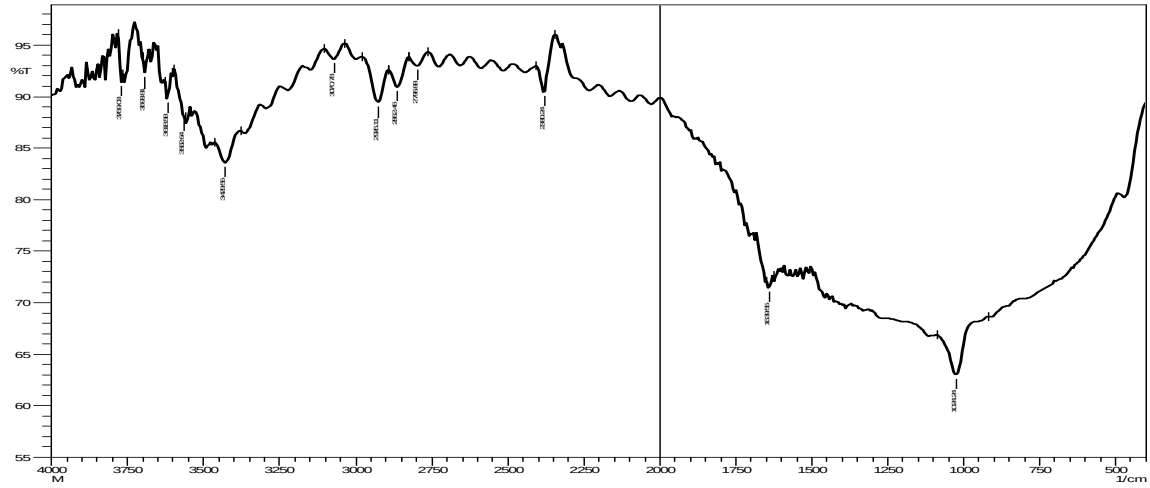

Figure 9: FT-IR spectrum for oral film

Table 7: Interpretations of IR-spectrum

SL. No.	Functional Group	IR Range (cm^{-1})	IR Observed Peaks			
			Pure drug	Drug mixture	*In situ* gel	Film
1	=C-H (s)	3100-3000	3010.98	3102.60	3065.88	3057.90
2	C-H (alkanes)	3000-2850	2906.76	2928.04	2924.18	2924.18
3	CHO	2830-2695	2721.65	2866.32	2727.44	2727.44
4	C=O (s)	1760-1690	1708.99	1700.99	1708.99	1742.71
5	C=C (s)	1680-1600	1643.41	1637.62	1637.62	1641.48
6	C-N (s)	1250-1020	111.03	1030.80	1030.02	1114.89
7	N-H (Wag)	900-675	896.00	665.45	665.46	904.71

5.5 Evaluation of hydrocortisone *in situ* gels

5.5.1 Clarity test

Clarity test for the prepared formulations has done by visual inspection under black and white background. There was no evidence of contamination, the entire formulations passes clarity test.

Table 8: Clarity test for the *in situ* gels

Formulation code	Clarity test
F1	Passes
F2	Passes
F3	Passes
F4	Passes
F5	Passes
F6	Passes
F7	Passes
F8	Passes

5.5.2 Determination of pH

The pH of *in situ* gels was determined using a calibrated pH meter. The readings were taken for average of 3 samples. Methylcellulose exhibited pH values in the range of 5.8 to 6.9 at 25°C which is tabulated in Table 9.

Table 9: Determination of pH for *in situ* gels

Formulation code	pH
F1	5.8±0.008
F2	5.9±0.057
F3	6.2±0.044
F4	6.4±0.072
F5	6.9±0.090
F6	6.5±0.051
F7	6.8±0.018
F8	6.8±0.005

5.5.3 *In vitro* gelling capacity

It was found that the gel intensity was increased when the concentration of polymers was increased. Experimental parts (Table.10) have showed that the formulation F7 and F8 were satisfactory to cause gelation.

Table 10: *In vitro* gelling capacity of the *in situ* gels

Formulation code	Gelling capacity
F1	+
F2	+
F3	+
F4	+
F5	++
F6	++
F7	+++
F8	+++

\+ - Gels after few mints disappear rapidly
++ - Immediately gelling re-disappeared after hour
+++ - Immediately gelling staying for hours

5.5.4 Viscosity and Rheology of the *in situ* sols

The viscosity of *in situ* solutions was determined by Brookfield viscometer (Table.11). Among all formulations, F1 (0.25%) showed least viscosity and F8 (2%) showed more viscosity. This says increase in polymer concentration causes increase in viscosity of the solution.

Table 11: Viscosity of the *in situ* sols

Shear rate (RPM)	Viscosity of the formulation (cps)	
	F1	F8
10	550	1000
20	490	940
30	440	870
40	350	750
50	290	630

The rheological studies of the optimum formulations were studied by plotting a graph of shear rate vs. viscosity (fig 14). This showed that the viscosity of the formulations decreased with increase in shear rate, which indicates the character of pseudoplastic fluids.

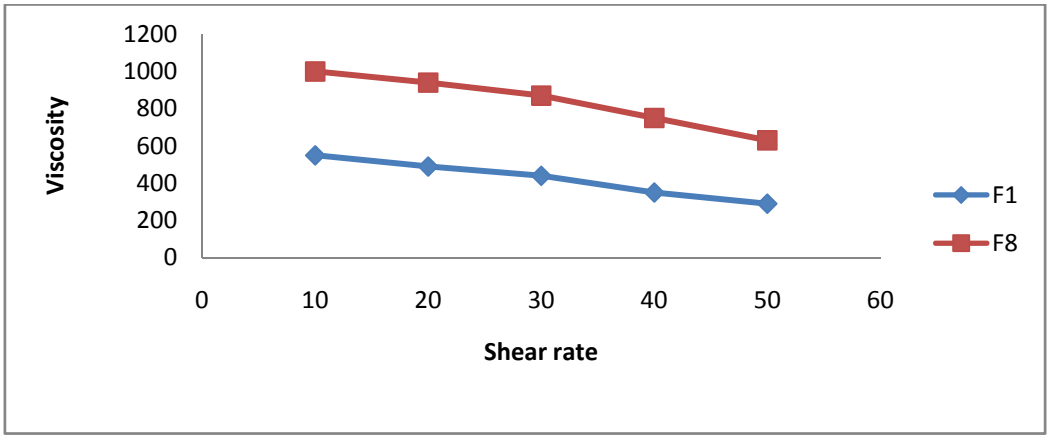

Fig 10: Rheological profile of the *In situ* gelling systems

5.3.5 Syringeability of in situ gel

Formulations F1 to F3 expelled quite easily from the syringe equipped with 20 gauge needle and passes the syringeability test. Formulation F4 and F8 fail the syringeability test may be because they contain higher concentration of methyl cellulose.

Table 12: Syringeability test

Formulation code	Syringeability
F1	Passes
F2	Passes
F3	Passes
F4	Fail
F5	Fail
F6	Fail
F7	Fail
F8	Fail

5.3.6 Spreadability Test.

With increase in the concentration of the polymeric component, viscosity of the solution was increased. At the same time Spreadability of the formulation was reduced. This can be observed from the evaluation tests data compiled in Table 13. F1 formulation showed a higher Spreadability compared to F8 formulation because gel strength and viscosity of F8 formulation were higher. Consequently, its Spreadability was less.

Table 13: Spreadability Test

Formulation code	Spreadability
F1	12 ± 0.03
F2	13.6 ±0.15
F3	14.2 ±0.11
F4	16 ±0.09
F5	18.3±0.05
F6	22.5 ±0.18
F7	26.8 ±0.06
F8	30± 0.011

5.3.7 Drug content:

The drug content estimation was done and the absorbance were measured by UV spectrophotometer (Shimadzu UV"1800), drug content was calculated (Table 14). Drug content of all formulations was found between 76.4±0.051 to 97.6±0.093 w/v.

Table 14: Drug content of *in situ* gel

Formulation Code	Absorbance (nm)	Conc. of drug µg/ml	% of Drug
F1	0.206	0.700	80.3±0.012
F2	0.196	0.66	76.4±0.051
F3	0.208	0.707	81.1±0.076
F4	0.217	0.738	84.7±0.082
F5	0.250	0.850	97.6±0.093
F6	0.218	0.741	85.1±0.091
F7	0.247	0.840	96.4±0.097
F8	0.243	0.826	94.8±0.066

5.6.8 *In vitro* release studies

The *in vitro* diffusion profile of Hydrocartisone from the gels containing different concentration of methylcellulose is shown in fig 15. Formulation F1 (0.25%) showed least drug release (84.86%) and formulation F5 (1.25%) showed maximum drug release (96.819±0.022%). For the first 6 hours of study, initial burst release was higher in *in-situ* gel formulations. From the *in-vitro* release studies we came to know that release rate was maximum for the formulation F5, but when the concentration of gel was further increased the release rate of the drug was decreased. From the experimental results we can say that release pattern was depends up on the concentration of polymer used.

Table 15 *In vitro* release studies of *in situ* formulations

TIME (Hour)	% CUMULATIVE DRUG RELEASE							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	4.078±0.012	8.588±0.013	12.238±0.023	10.520±0.014	16.962±0.021	13.956±0.016	0.191±0.046	15.67±0.023
2	8.156±0.025	23.059±0.017	24.598±0.012	21.575±0.027	27.006±0.008	23.541±0.033	19.706±0.023	25.49±0.015
3	21.794±0.014	35.225±0.022	38.675±0.032	32.848±0.034	39.128±0.014	35.014±0.021	39.075±0.035	39.97±0.033
4	33.135±0.022	44.362±0.018	44.610±0.016	43.050±0.006	55.565±0.019	44.145±0.025	43.788±0.017	45.91±0.009
5	51.094±0.021	56.690±0.007	58.624±0.019	55.389±0.015	64.959±0.004	62.270±0.037	64.489±0.004	64.00±0.017
6	65.170±0.014	64.970±0.021	64.989±0.025	67.748±0.023	76.645±0.024	68.246±0.005	73.650±0.033	69.12±0.036
7	77.268±0.016	77.504±0.016	79.651±0.005	79.464±0.027	85.992±0.037	79.039±0.003	88.103±0.052	80.550±0.034
8	84.861±0.015	85.357±0.005	86.66±0.010	88.596±0.012	96.819±0.022	94.525±0.021	91.233±0.018	90.472±0.022

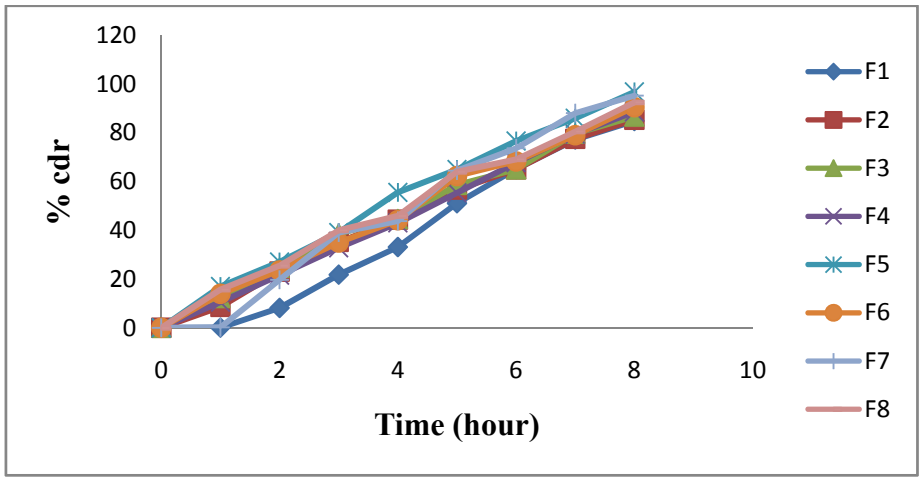

Fig 11: Comparative drug release profile of the *in situ* gel formulation

5.6.9 Release kinetics:

The regression values of zero order kinetics of *in-situ* gel for all the formulations ranges from 0.916 to 0.993 respectively where as first order kinetics 0.155 to 0.553 respectively. When subjected to Higuchi's model, R^2 value ranges from 0.730 to 0.898 respectively. Korsemeyer-peppas model showed R^2 value of 0.501 to 0.611 respectively for all the 'n' value ranges from 1.592 to 1.6011 indicating that the drug release was by Super case II release mechanism.

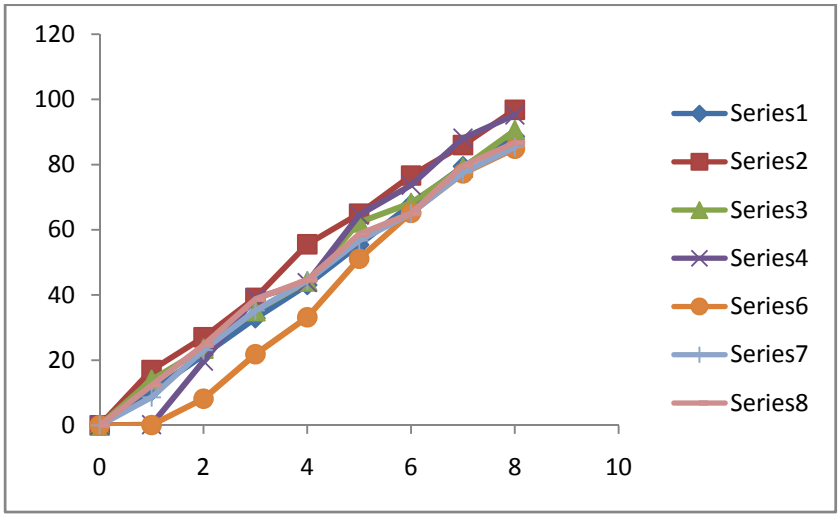

Fig 12: Comparative Zero Order release profile of formulations

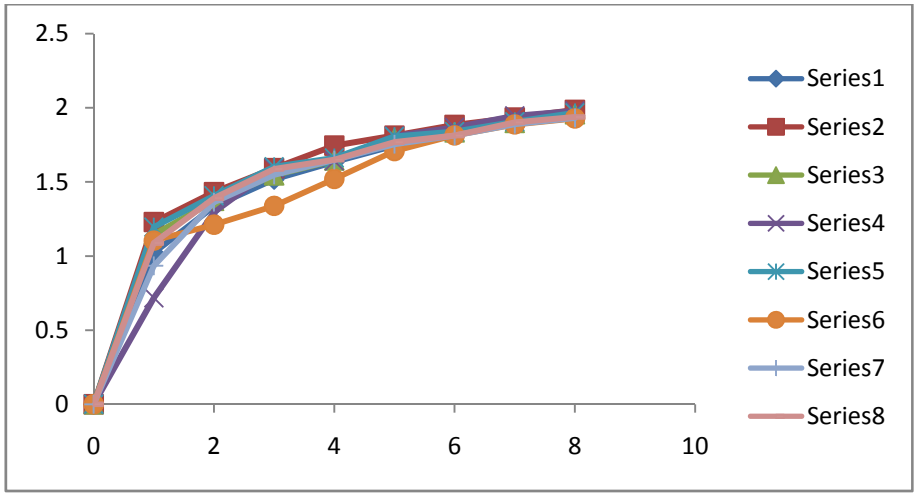

Fig 13 Comparative First Order release profile of formulations

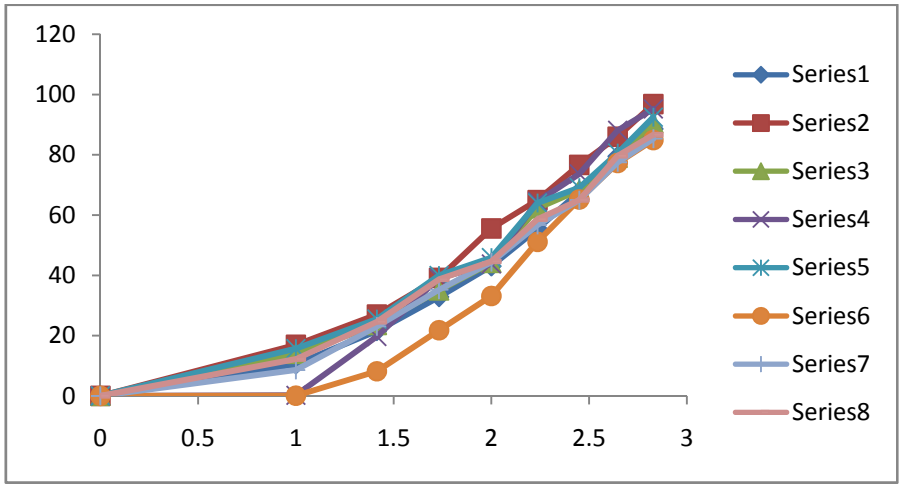

Fig 14 Comparative Higuchi release profile of formulations

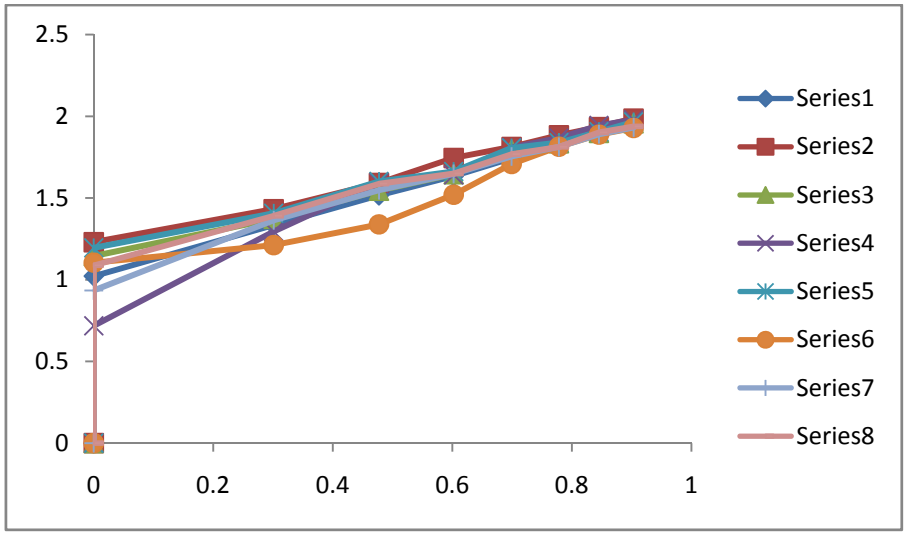

Fig 15: Comparative Peppas release profile of formulations

Table 16: Release exponent values and rate constant values for different formulations

Formulation Code	KINETIC MODELS				
	Zero order	First order	Higuchi	Korsmeyer et al.	
	R^2	R^2	R^2	N	R^2
F**1**	0.956	0.436	0.730	1.538	0.452
F**2**	0.995	0.377	0.879	1.611	0.492
F**3**	0.993	0.254	0.898	1.530	0.350
F**4**	0.995	0.236	0.867	1.583	0.387
F**5**	0.990	0.171	0.898	1.514	0.250
F6	0.993	0.155	0.898	1.522	0.275
F7	0.982	0.552	0.815	1.806	0.688
F8	0.986	0.186	0.902	1.498	0.264

5.6.10 Stability studies:

The stability studies were carried out for prepared *in situ* gelling systems. All the formulations were analysed for visual appearance, clarity, pH, gelling capacity, drug content and *in vitro* release studies. 90days of stability studies revealed that there was no change in visual appearance and clarity. All the formulations have shown slight changes in pH which was in acceptable limits (±0.3). Study of drug content and *in vitro* drug release revealed that there were no definite changes observed to justify for drug degradation.

Table 17: Stability studies of formulations stored at 40 ±1°C/ ambient humidity

No. of days	Drug content%							
	F1	**F 2**	**F 3**	**F 4**	**F 5**	**F6**	**F7**	**F8**
15	80.3	76.4	81.10	84.70	97.61	85.15	96.40	94.80
30	80.29	76.39	81.09	84.69	97.59	85.12	96.38	94.79
45	80.27	76.37	81.08	84.67	97.58	85.11	96.37	94.77
60	80.26	76.35	81.06	84.66	97.56	85.10	96.36	94.75
75	80.25	76.34	81.04	84.65	97.55	85.08	96.35	94.74
90	80.24	76.32	81.05	84.63	97.52	85.06	96.32	94.73

6.0 Evaluation of oral films

6.1. Surface PH of the films

The surfaces PH of all films were found to be in the range of 6.37±0.08 to 6.79 ± 0.01. It assured that there will not be any kind of irritation to the mucosal lining of the oral cavity.

Table 18 Surface PH of the films:

Formulation Code	Surface PH
F1	6.48±0.013
F2	6.37±0.027
F3	6.50±0.018
F4	6.52±0.016
F5	6.79±0.024
F6	6.55±0.022
F7	6.62±0.015
F8	6.49±0.019

6.1.1 Variation of Mass

Films of 0.5 cm^2 were cut from different batches and weighed. The results are given in Table 19. Same mass of film was obtained with three batches of films indicating reproducibility of preparation method and formulation.

Table 19: Variation of Mass

Formulation Code	Variation of Mass
F1	0.0095±0.0003
F2	0.0094±0.0005
F3	0.0098±0.0002
F4	0.0098±0.0004
F5	0.0100±0.0007
F6	0.0094±0.0006
F7	0.0100±0.0003
F8	0.0097±0.0005

6.1.2 Thickness

The thickness was measured with a screw gauge at different places of the MDFs in order to evaluate the reproducibility of the preparation method. Around 90% of wet film thickness was lost during drying. The results are given in Table 20. For the prepared film a good uniformity of thickness was observed.

Table 20: Thickness:

Formulation Code	Thickness (μm)
F1	450±0.06
F2	467±2.04
F3	458±1.08
F4	470±0.09
F5	490±0.03
F6	475±0.07
F7	476±1.66
F8	480±2.04

6.1.3 Drug Content

Films of 0.5 cm^2 were cut from different places of the whole films and Hydrocortisone content was estimated. The results are given in Table 21. These results indicated a good uniformity of Hydrocortisone within films, and overall good solubilisation of Hydrocortisone in the formulations was observed.

Table 21: Drug Content

Formulation Code	Conc. Of polymer % w/v	Absorbance (nm)	Conc. of drug µg/ml	% of Drug
F1	0.25	0.240	0.816	93.7
F2	0.50	0.239	0.812	93.2
F3	0.75	0.242	0.823	94.5

F4	1.00	0.245	0.833	95.6
F5	1.25	0.252	0.857	98.4
F6	1.50	0.246	0.836	96.0
F7	1.75	0.250	0.850	97.6
F8	2.00	0.248	0.843	96.8

6.1.4 Disintegration Time

The results of disintegration time are given in Table 22.

With the petridish method F1, F2 and F3 formulations disintegrated/dissolved faster than the other formulations.

Table 22: Disintegration Time

Formulation Code	Disintegration time (sec)	
	Drop method	**Petridish method**
F1	30±0.56	360±1.32
F2	32±0.58	389±0.59
F3	38±1.02	400±0.54
F4	40±0.045	430±1.57
F5	44±1.27	480±2.56
F6	47±0.55	520±1.32
F7	50±0.58	550±1.70
F8	55±1.32	568±2.54

6.1.5 Tensile Strength

MDFs should possess moderate tensile strength, high % elongation (% E), low EM, and high percent of drug release. The results revealed that all the films showed moderate tensile strength values. Among all formulations, F5 formulation showed highest % E and tensile strength when compared with other formulations. The nature

and concentration of polymer affects tensile strength and % elongation. Formulation F5 having optimum concentration of methylcellulose (1.25%) showed highest % of tensile strength and % elongation. The results were given in Table 23.

Table 23: Results of Tensile strength

Formulation code	**Tensile strength** (kg/ mm^2)	**% Elongation**
F1	0.454±0.015	5.67±0.013
F2	0.476±0.093	5.92±0.043
F3	0.479±0.081	5.99±0.042
F4	0.614±0.034	6.67±0.071
F5	0.672±0.044	7.67±0.005
F6	0.534±0.072	6.1±0.008
F7	0.510±0.008	6.01±0.072
F8	0.489±0.023	5.52±0.003

6.1.6 Scanning electron microscopy of oral film

One of the oral film formulations was subjected to SEM studies to assess changes in its surface morphology (Fig.). Initially prepared film revealed smooth and compact surfaces but, after dissolution studies, the film appeared porous and showed significant changes in texture and clearly visible pores. This might be due to the uptake of water resulting from the presence of methylcellulose in the formulations. Based on these results, it can be concluded that the methylcellulose in patches absorbs water and significantly affects their surface morphology and leads to the formation of pores in accordance with the in vitro dissolution data.

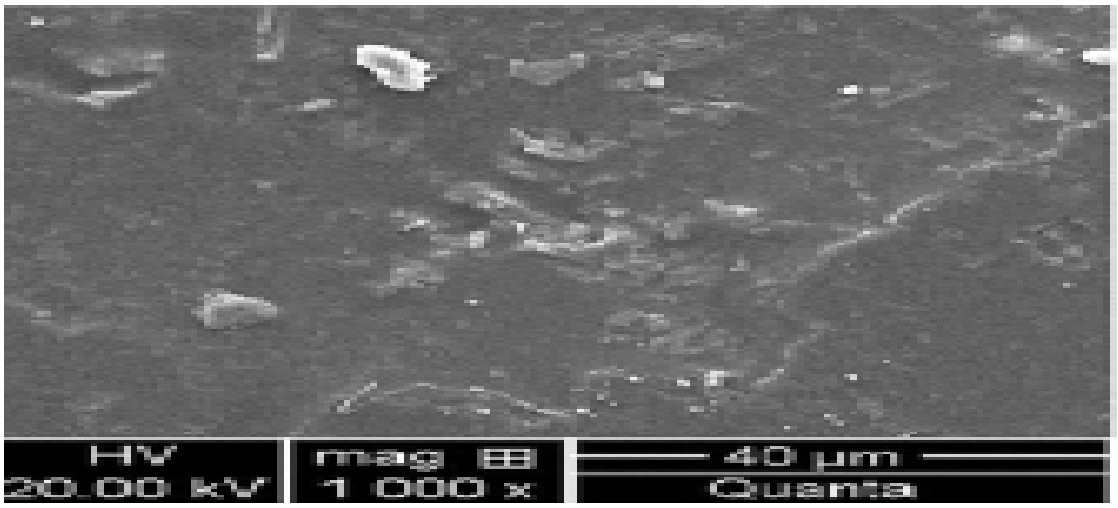

Figure 16: SEM image of oral film

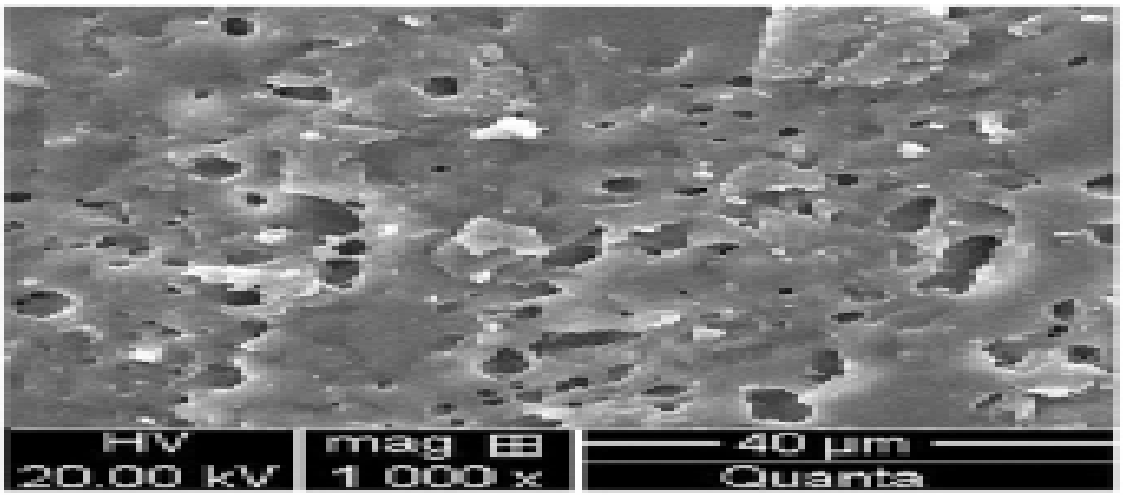

Figure17 : SEM image of oral film after dissolution

6.1.7 DSC study.

The DSC thermogram of hydrocortisone exhibited an endothermic peak of 224.46^{0}C corresponding to its melting point. The DSC thermogram of hydrocortisone with other excipients does not show profound shift in peak (224.46^{0}C) which indicates compatibility. The DSC of individual drug and final formulation is shown in the figure.

6.1.8 Folding Endurance.

All the prepared MDFs have an acceptable folding endurance. In folding endurance test no films developed any visible cracks or breaks, thus showing good folding endurance. F8 has higher folding endurance when compared with other MDFs. The results were shown in Table 24.

Table 24: Folding Endurance

Formulation Code	Folding endurance
F1	80
F2	95
F3	113
F4	122
F5	130
F6	134
F7	140
F8	146

6.1.8 *In Vitro* Dissolution Studies

The *in vitro* dissolution profiles of Hydrocartisone buccal film are shown in Table 25. In total, 8 different formulations of Hydrocartisone were prepared using Methyl cellulose as film forming polymers with sodium citrate. The cumulative percent released Hydrocartisone at the end of 8 hrs. The release rate from different films shows that, release of drug was increased with increase in the concentration of release retardant polymer at certain level i.e. 1.25%, further increase in concentration of the polymer concentration decreases the release behaviour of formulation significantly. The release of drug from these films exhibits two phases. There is a initial burst effect is followed by the completion of a stable gel layer which in turn, controls the release of drug from the delivery system.

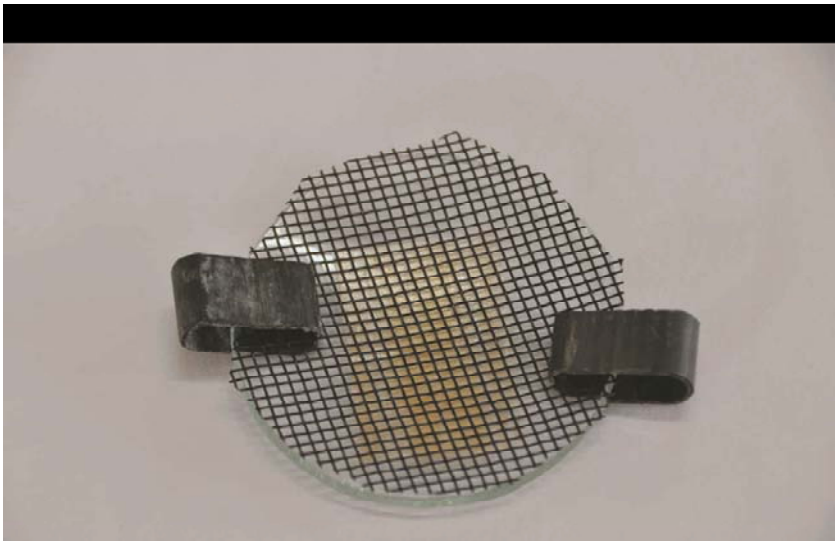

Table 25: *In vitro* dissolution studies

TIME (min)	% CUMULATIVE DRUG RELEASE							
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1	7.557±0.013	8.244±0.022	10.306±0.016	11.336±0.034	0.122±0.012	7.901±0.008	10.306±0.015	8.931±0.024
2	27.214±0.026	31.343±0.015	31.363±0.021	32.748±0.015	12.721±0.027	28.248±0.018	25.867±0.017	31.006±0.006
3	34.622±0.034	39.129±0.032	43.595±0.017	43.608±0.022	36.258±0.033	41.159±0.017	37.357±0.026	42.217±0.010
4	49.809±0.014	53.632±0.043	55.394±0.019	58.142±0.037	54.241±0.006	53.996±0.020	49.493±0.043	57.098±0.029
5	60.608±0.018	65.455±0.017	66.503±0.027	64.125±0.025	66.380±0.015	69.238±0.054	61.979±0.018	70.299±0.043
6	66.210±0.023	71.411±0.015	74.513±0.032	73.802±0.017	78.159±0.028	76.945±0.034	72.064±0.022	79.360±0.029
7	77.258±0.014	78.683±0.033	79.744±0.028	82.829±0.025	88.214±0.018	85.952±0.021	82.812±0.038	87.350±0.036
8	85.612±0.016	86.312±0.025	87.697±0.007	89.102±0.014	97.554±0.035	94.285±0.036	92.316±0.019	90.329±0.017

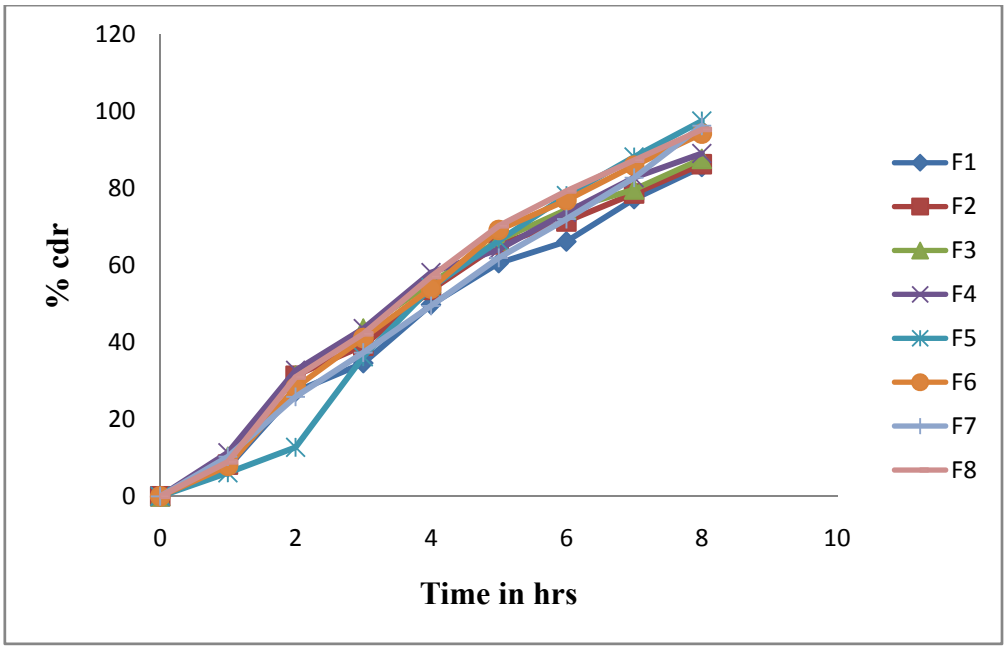

Figure 18: Comparative drug release profile of the formulations

6.1.9 Release kinetics:

The regression values of zero order kinetics of oral films for all the formulations ranges from 0.966 to 0.986 respectively where as first order kinetics 0.367 to 0.596 respectively. When subjected to Higuchi's model, R^2 value ranges from 0.818 to 0.933 respectively. Korsemeyer-peppas model showed R^2 value of 0.333 to 0.511 respectively for all the 'n' value ranges from 1.543 to 1.835 indicating that the drug release was by Super case II release mechanism.

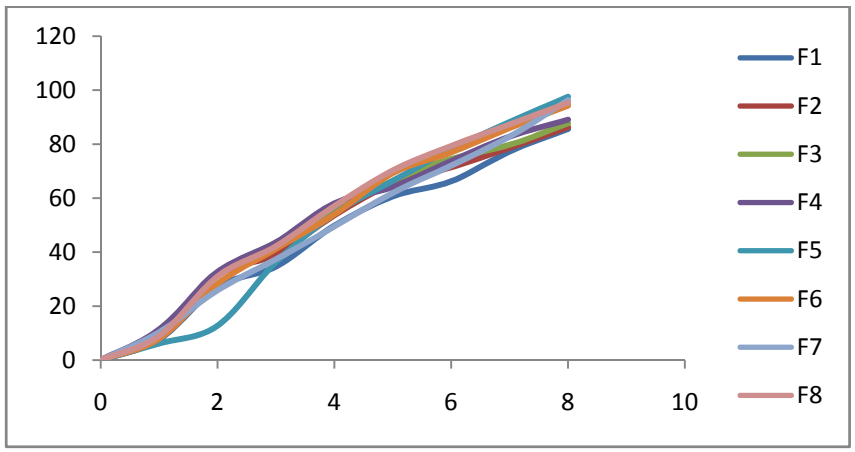

Figure 19: Comparative Zero Order release profile of formulations

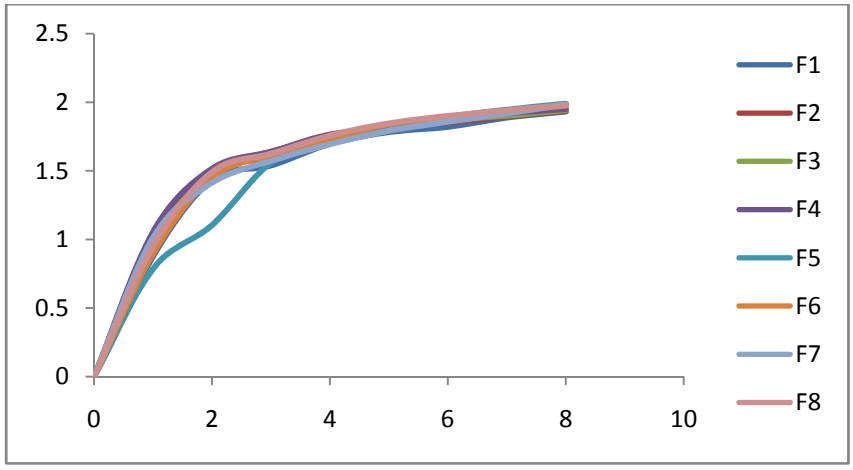

Figure 20: Comparative First Order release profile of formulations

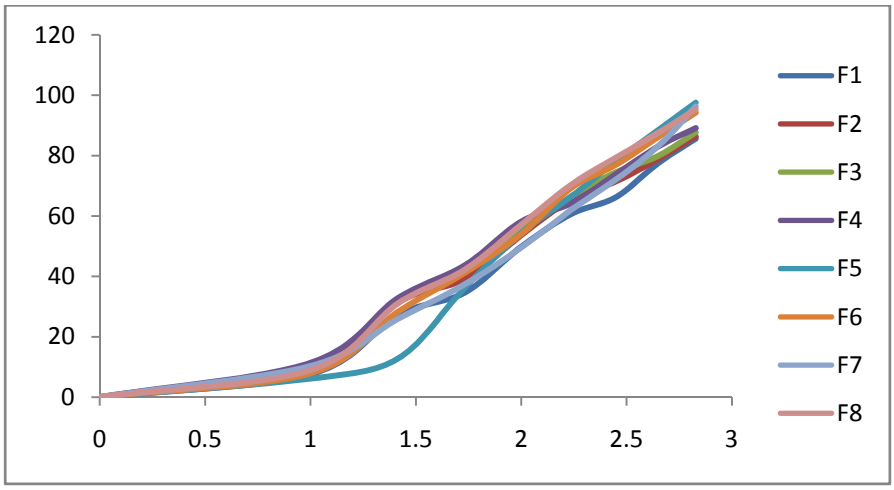

Figure 21: Comparative Higuchi release profile of formulations

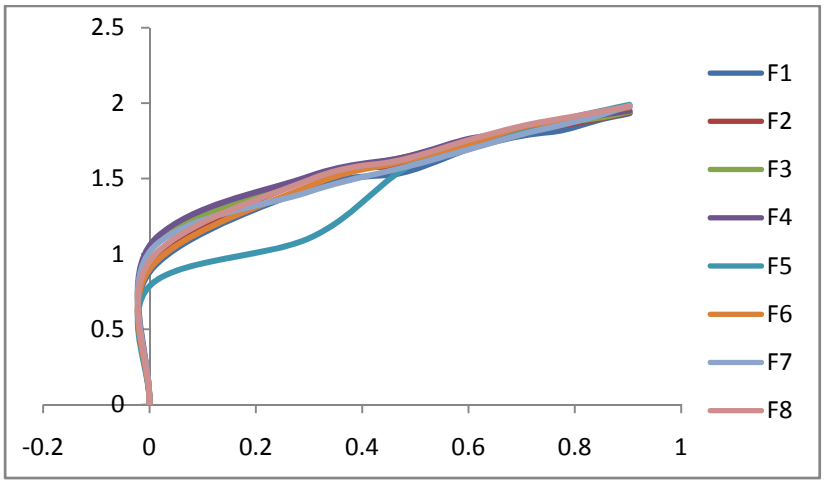

Figure 22: Comparative Peppas release profile of formulations

Table 26 Release exponent values and rate constant values for different formulations

Formulation Code	KINETIC MODELS				
	Zero order	**First order**	**Higuchi**	**Korsmeyer et al.**	
	R^2	**R^2**	**R^2**	**R^2**	**N**
F1	0.986	0.505	0.894	0.506	1.543
F2	0.966	0.367	0.854	0.451	1.622
F3	0.983	0.545	0.921	0.515	1.584
F4	0.976	0.323	0.933	0.333	1.564
F5	0.977	0.596	0.818	0.713	1.835
F6	0.984	0.334	0.878	0.511	1.688
F7	0.998	0.317	0.904	0.440	1.613
F8	0.979	0.319	0.856	0.459	1.662

6.1.10 *Ex-vivo* studies:

The best formulation was selected from the in situ gel and film were subjected to ex-vivo release study through chicken skin using diffusion cell. The ex-vivo release would give a better estimate of drug permeation characteristics through animal skin. The amount of drug permeated through skin after 8 hrs from the formulation was shown in the figure .

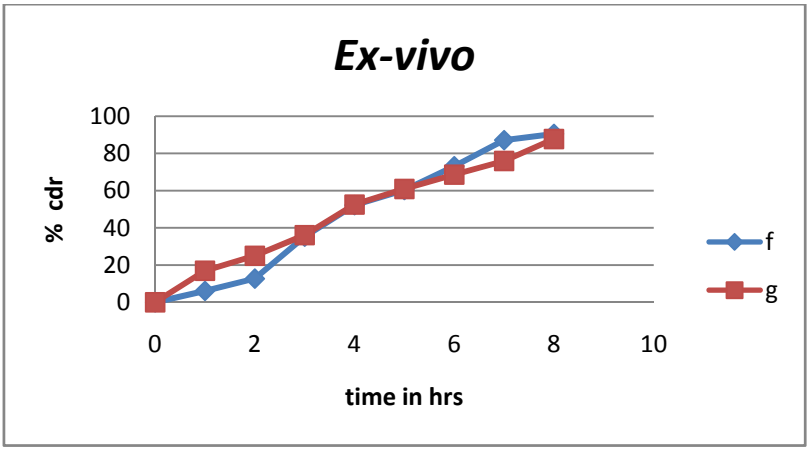

Figure 23: *Ex-vivo* studies

6.1.11 Stability studies:

The stability studies were carried out for prepared oral films. All the formulations were analysed for surface pH, folding endurance, drug content and *in vitro* release studies. 90 days of stability studies revealed that there was no change in visual appearance. All the formulations have shown slight changes in pH which was in acceptable limits (±0.3). Study of drug content and *in vitro* drug release revealed that there were no definite changes in drug content to justify for drug degradation, hence stable formulation.

Table 27: Stability studies of oral films stored at 40 ±1°C/ ambient humidity

No. of days	Drug content%							
	F1	**F 2**	**F 3**	**F 4**	**F 5**	**F6**	**F7**	**F8**
15	93.75	93.2	94.52	95.61	98.48	96.09	97.6	96.8
30	93.73	93.19	94.50	95.59	98.47	96.07	97.59	96.78
45	93.71	93.17	94.49	95.58	98.46	96.05	97.58	96.76
60	93.70	93.16	94.47	95.56	98.44	96.04	97.66	96.74
75	93.68	93.15	94.46	95.54	98.42	96.01	97.64	96.73
90	93.66	93.12	94.45	95.52	98.40	96	97.62	96.71

Comparison between release behaviour of *in-situ* gel and oral film

In-vitro release behaviour of both *in-situ* gel and oral film were preformed in same medium. The data obtained from the experiment reviled that the film based formulation released all most all percentage of drug within 8 minutes, but it took almost 8 hours for same amount of drug release from the *in-situ* gel formulation. Longer time period for the drug release from the gel might be the diffusion controlled mechanism. When we see the release pattern it was higher and faster for oral film i.e. within 8 minutes, but the formulation was designed for aphthous ulcer, so we consider *in-situ* gel formulation was suitable formulation for the treatment of aphthous ulcer, as it sustain the drug release up to 8 hours. The frequency of the drug application also reduced significantly and also improves patient compliance.

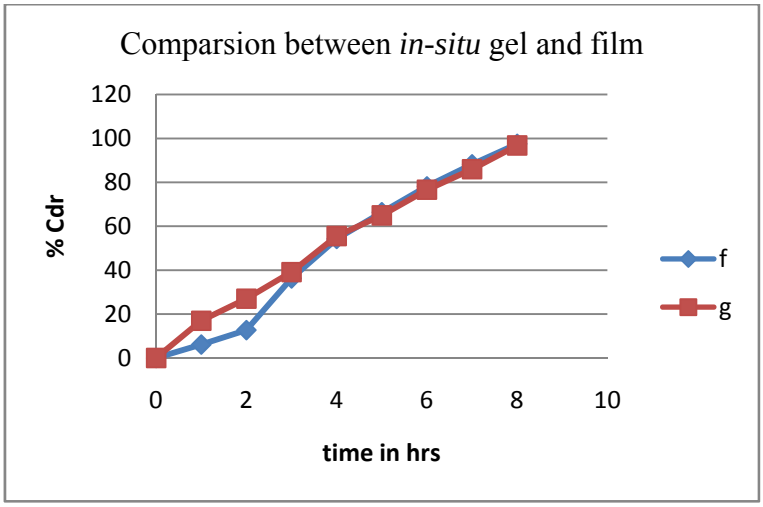

Figure 24: Comparsion between *in-situ* gel and film

6. CONCLUSION

The present work is an attempt to develop a *in situ* gels and films of Hydrocortisone from temperature induced gelling system. The study has demonstrated various aspects and from the results obtained, it was concluded that

- ✓ *In situ* gel formulation of Hydrocortisone with mucoadhesive properties is useful to prolonging residence time in mouth.
- ✓ The developed formulation can release the drug at controlled rate for prolonged duration.
- ✓ Local drug delivery may be an advantageous in treatment, since it would probably eliminate side effects, which occur with systemic dosing.
- ✓ Effective and prolonged release of drug could be achieved without much systemic load with comparatively less frequency of administration.
- ✓ This type of drug delivery system can serve as a novel approach for treating mouth infections with better patient compliance.
- ✓ The optimized formulations F5 (Methylcellulose 1.25%), F6 (Methylcellulose 1.50%), F7 (Methylcellulose 1.75%) were liquid before instillation into mouth and underwent rapid gelation upon instillation into mouth.
- ✓ The formulations were found to be clear, having good *in situ* gelling capacity and a drug content 84-96%.
- ✓ Optimised formulations were sterile and showed sustained drug release over 8 hrs.

Hence from the above results we can conclude that it is possible to formulate *in situ* gels and films of Hydrocortisone using Methylcellulose for treating aphthous ulcer. In that the (F5) film formulation has shown the best release studies when compared to other formulation, and when compared between the *in-situ* gels and films formulation the films is having sustained and prolonged release of the drug compared to the *in-situ* gel.

7. SUMMARY

Oral diseases are commonly encountered in day to day life, which are cured or prevented through the conventionally used dosage forms like tablets, ointments, gels etc., Delivery to the surface of the mucous layer of the mouth remains troublesome due to anatomical and protective structure of the mucous layer.

Poor availability of drug which is less than 1% for conventional oral lesions, because of plenty of secretion of saliva from the salivary glands approximately 2.5 liters per day washed out drug which is applied on the surface of the mucous layer. Most of the topically applied drugs in the form of ointment or gel enter the lower digestive tract like pharynx and esophagus. Due to the frequent movement of the tongue the drug which is applied on the surface of the infected area will decrease the residence time.

Oral efficiency is closely related to oral drug bioavailability which may be enhanced by increasing mucousa drug penetration and prolonging pre-oral drug residence time. Oral *in-situ* drug delivery systems consists of polymer that exhibit sol-to-gel phase transitions due to temperature induced gelling system and where as the film consist of the polymers that exhibited is prepared by the solvent casting method results in ease of application, reduction in frequency of administration, improved patient compliance and comfort.

Present work focused on preparation and evaluation of *in situ* gels and films containing Hydrocortisone as a local drug delivery for the treatment of Aphthous ulcer. Hydrocortisone is a synthetic broad spectrum corticosteroid group of anti-inflammatory agent, for the treatment of aphthous ulcer. So Hydrocortisone has been selected as a model drug for the project.

In this study preparation of *in situ* gels and films was carried out by temperature induced gelling system and solvent casting mechanism by using various polymers like methylcellulose and sodium citrate. The optimum formulations were subjected to various evaluation tests like drug content of sols, clarity test, pH, viscosity, gelling capacity, *in vitro* drug release studies, folding endurance, tensile strength. All the results were found to be satisfactory.

Hence, it can say that *in situ* gels and films containing Hydrocortisone may be used for the treatment of aphthous ulcer. In that the F5 film formulation containing Hydrocortisone has shown to be the best for the sustained release of drug for the treatment of aphthous ulcer.

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