WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 5, 1077-1105. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF *IN SITU* OCULAR GEL FOR DRY EYE SYNDROME

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Article Received on
08 March 2017,

Revised on 28 March 2017,
Accepted on 19 April 2017

DOI: 10.20959/wjpr20175-8404

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ABSTRACT

Ophthalmic drug delivery must be able to sustained the drug release and to remain in the vicinity in front of the eye for prolong period of time. The topical application of drugs is the method of choice under most circumstance because of its convenience and safety for ophthalmic chemotherapy. To develop the in situ ocular gel of a polymixin B sulphate which release the drug in sustained manner by using high esterified pure pectin, guar gum and hydroxyl propyl methylcellulose. Ocular in situ gel was prepared by dispersion method. In the present research work it can be shown that polymixin B sulphate, an antibacterial agent used in the treatment of dry eye syndrome was successfully formulated as an ion-activated in situ forming ophthalmic solution using pectin, guar gum in combination with HPMC as a viscosity enhancer. The in situ gel was subjected to content uniformity and was found to be between 97% to 98%. The developed formulation is a viable alternative of conventional eye drop due its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain release drug.

KEYWORDS: Opthalmic drug, Antibacterial agent, Bioavailability, Polymixin B.

INTRODUCTION

Eye is most interesting organ due to its drug deposition characteristics. Generally, topical application of drug is the method of choice under most circumstances because of it's or convenience and safety for ophthalmic chemotherapy.[1] A significant challenge to the formulator is to circumvent (by pass) the protective barrier of the eye without causing

permanent tissue damage.[2] Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Conventional ophthalmic formulations like solution, suspension and ointment have many advantages which result into poor bioavailability of drug in the ocular cavity. The specific aim of designing a therapeutic system is to achieve an optimal concentration of a drug at the active site for the appropriate duration.[2] Ocular disposition and elimination of a therapeutic agent is dependent upon its physicochemical properties as well as the relevant ocular anatomy and physiology. A successful design of a drug delivery system, therefore, requires an integrated knowledge of the drug molecules and the constraints offered by the ocular route of administration.[3] The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drug can be divided into two categories. The first one is based on the use of sustained drug delivery system, which provides the controlled and continuous delivery of ophthalmic drugs. The second involves maximizing corneal drug adsorption and minimizing precorneal drug loss.[3] Ideal opthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity in front of the eye for prolong period of time. Consequently it is imperative to optimize ophthalmic drug delivery. One of the ways to do so is by addition of polymer of various grades, development of *in situ* gel or colloidal suspension or using erodible or non-erodible insert to prolong the precorneal drug retention.[4]

THE ANATOMY OF THE EYE[5,6,7,8,9]

The human eye, elegant in its detail and design, represents a gateway to the process we call vision. The eyeball is spherical in shape and about 1 inch across. It houses many structures that work together to facilitate sight. The human eye is comprised of layers and internal structures, each of which performs distinct functions. The detailed description of each eye part is given below.

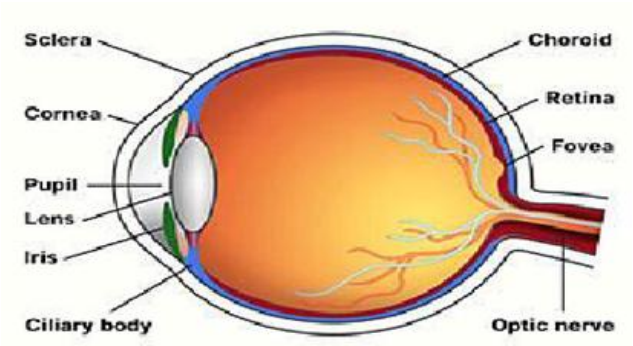


Figure 1: Structure of eye ball

A. Sclera

The sclera (white portion of the eye) is the tough white sheath that forms the outer-layer of the ball. It is a firm fibrous membrane that maintains the shape of the eye as an approximately globe shape. It is much thicker towards the back/posterior aspect of the eye than towards the front/anterior of the eye.

B. Conjunctiva

The conjunctiva is a thin transparent mucous epithelial barrier, lines the inside of the eyelids, and covers the anterior one-third of the eyeball. The respective portion of conjunctiva is referred to as the palpebral and bulbar conjunctiva. The conjunctiva is composed of two layers: an outer epithelium and its underlying stroma (substantia propria). The exposed surface of the eye includes conjunctiva and cornea and is covered with the tear film. The conjunctiva contributes to the formation of the tear film by way of secreting substantial electrolytes, fluid and mucins.

C. Cornea

The cornea is a strong clear bulge located at the front of the eye. Surface of the adult cornea has a radius of approximately 8mm. It has an important optical function as it refracts light entering the eye which then passes through the pupil and onto the lens (which then focuses the light onto the retina). The cornea, a non-vascular structure (does not contain any blood vessels) gets the necessary nutrients from the capillaries that terminate in loops at its circumference. It is supplied by many nerves derived from the ciliary nerves. These enter the laminated tissue of the cornea. It is therefore extremely sensitive.

D. Aqueous humor

The aqueous humor is a jelly-like substance located in the outer/front chamber of the eye. It is a watery fluid that fills the "anterior chamber of the eye" which is located immediately behind the cornea and in front of the lens. The aqueous humor is very slightly alkaline salt solution that includes tiny quantities of sodium and chloride ions. It is continuously produced, mainly by the ciliary processes, flows from the posterior chamber through the pupil into the anterior chamber, and exits via the trabecular route at the angle and the uveoscleral route. Schlemm's canal (canal of Schlemm or the scleral venous sinus), is a circular channel that collects aqueous humour from the anterior chamber and delivers it into the bloodstream via the anterior ciliary veins. It is located at the junction of the cornea and the sclera. In human, the rate of aqueous humor turnover is approximately 1% -1.5% of the anterior chamber

volume per minute. The rate of aqueous formation is approximately 2.5 µl/min. Aqueous humor consists of pressure dependent and pressure independent pathways. The pressure dependent outflow refers to the trabecular meshwork-schlemm's canal-venous system, while pressure independent outflow refers to any non-trabecular outflow and is called as uveoscleral outflow.[10]

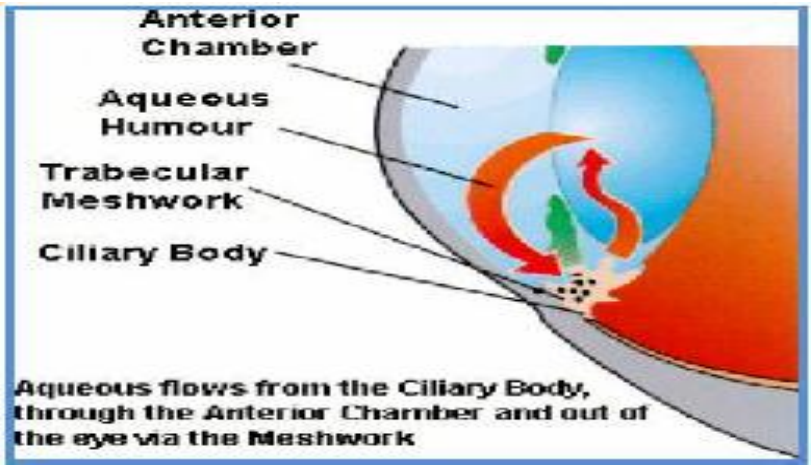


Figure 2: Aqueous flow from ciliary body, through the anterior chamber and out of eye via the mesh work.

E. Pupil

Pupil generally appears to be the dark "centre" of the eye, but can be more accurately described as the circular aperture in the centre of the iris through which light passes into the eye. The size of the pupil (and therefore the amount of light that is admitted into the eye) is regulated by the pupillary reflex (also known as the "light reflex").

F. Iris

The iris is a thin circular contractile curtain located in front of the lens but behind the cornea. The iris is a diaphragm of variable size whose function is to adjust the size of the pupil to regulate the amount of light admitted into the eye. It is the coloured part of the eye (shades may vary individually like blue, green, brown, hazel, or grey).

G. Ciliary Muscle

The ciliary muscle is a ring of striated smooth muscles in the eye's middle layer that controls accommodation for viewing objects at varying distances and regulates the flow of aqueous humour into schlemm's canal. The muscle has parasympathetic and sympathetic innervation. Contraction and relaxation of the ciliary muscle alters the curvature of the lens. This process

may be described simply as the balance existing at any time between two states: Ciliary Muscle relaxed (This enables the eye to focus on distant objects) and Ciliary Muscle contracted (This enables the eye to focus on near objects).

H. Lens

The lens is a transparent structure enclosed in a thin transparent capsule. It is located behind the pupil of the eye and encircled by the ciliary muscles. It helps to refract light travelling through the eye (which first refracted by the cornea). The lens focuses light into an image on the retina. It is able to do this because the shape of the lens is changed according to the distance from the eye of the object(s) the person is looking at. This adjustment of shape of the lens is called accommodation and is achieved by the contraction and relaxation of the ciliary muscles.

I. Vitreous Humour

The vitreous humour (also known as the vitreous body) is located in the large area that occupies approximately 80% of each eye in the human body. The vitreous humour is a perfectly transparent thin-jelly-like substance that fills the chamber behind the lens of the eye. It is an albuminous fluid enclosed in a delicate transparent membrane called the hyaloid membrane.

J. Retina

The retina is located at the back of the human eye. The retina may be described as the "screen" on which an image is formed by light that has passed into the eye via the cornea, aqueous humor, pupil, lens, and finally the vitreous humour before reaching the retina. The function of the retina is not just to be the screen onto which an image may be formed but also to collect the information contained in that image and transmit it to the brain in a suitable form for use by the body. The retinal "screen" is therefore a light-sensitive structure lining the interior of the eye. It contains photosensitive cells (called rods and cones) and their associated nerve fibers that convert the light they detect into nerve impulses that are then sent onto the brain along the optic nerve.

K. Macula

The center of the retina is called the macula. The macula contains a high concentration of photoreceptor cells which convert light into nerve signals. Because of the high concentration

of photoreceptors, we are able to see fine details such as newsprint with the macula. At the very center of the macula is the fovea, the site of our sharpest vision.

L. Choroid

The choroid layer is located behind the retina and absorbs unused radiation and nourishes the outer portions of the retina. It is a thin, highly vascular (i.e. it contains blood vessels) membrane that is dark brown in colour and contains a pigment that absorbs excess light and so prevents blurred vision (due to too much light on the retina). The choroid has one of the highest blood flows in the body. The choroid is loosely attached to the inner surface of the sclera by the lamina fusa.

M. Optic nerve

The optic nerve (a bundle of over 1 million nerve fibers) is responsible for transmitting nerve signals from the eye to the brain. These nerve signals contain information on an image for processing by the brain. The front surface of the optic nerve, which is visible on the retina, is called the optic disk.

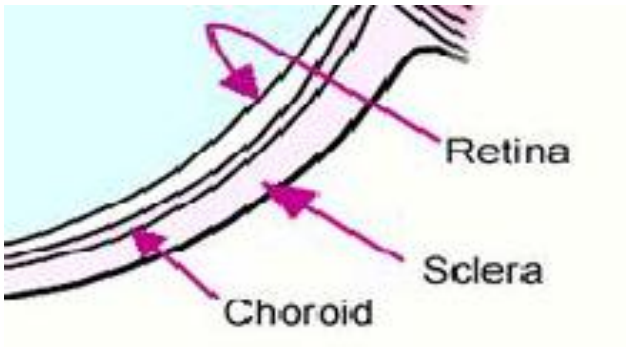


Figure 3: Showing optic nerve of eye

ROUTS OF OCULAR DRUG DELIVERY[11]

There are several possible routes of drug delivery into the ocular tissues. The selection of the route of administration depends primarily on the target tissue.

Topical route

Typically topical ocular drug administration is accomplished by eye drops, but they have only a short contact time on the eye surface. The contact, and thereby duration of drug action, can be prolonged by formulation design (e.g. m gels, gelifying formulations, ointments, and inserts).

Subconjunctival administration

Traditionally sub-conjunctival injections have been used to deliver drugs at increased levels to the uvea. Currently this mode of drug delivery has gained new momentum for various reasons. The progress in materials sciences and pharmaceutical formulation have provided new exciting possibilities to develop controlled release formulations to deliver drugs to the posterior segment and to guide the healing process after surgery.

Intravitreal administration

Direct drug administration into the vitreous offers distinct advantage of more straightforward access to the vitreous and retina. It should be noted; however that delivery from the vitreous to the choroid is more complicated due to the hindrance by the RPE (Retinal Pigment Epithelium) barrier. Small molecules are able to diffuse rapidly in the vitreous but the mobility of large molecules, particularly positively charged, is restricted.

BARRIERS FOR OCULAR DELIVERY[11]

Drug loss from the ocular surface

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of eye. Even though the lacrimal turnover rate is only about 1µl/min the excess volume of the instilled fluid is flown to the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity.

Lacrimal fluid-eye barriers

Corneal epithelium limits drug absorption from the lacrimal fluid into the eye. The corneal epithelial cells form tight junctions that limit the paracellular drug permeation. Therefore, lipophilic drugs have typically at least an order of magnitude higher permeability in the cornea than the hydrophilic drugs. In general, the conjunctiva is leakier epithelium than the cornea and its surface area is also nearly 20 times greater than that of the cornea. Blood-ocular barriers The eye is protected from the xenobiotics in the blood stream by blood-ocular barriers. These barriers have two parts: blood-aqueous barrier and blood-retina barrier. The anterior blood-eye barrier is composed of the endothelial cells in the uveam (The middle layer of the eye beneath the sclera. It consists of the iris, ciliary body, and choroid). This barrier prevents the access of plasma albumin into the aqueous humor, and also limits the access of hydrophilic drugs from plasma into the aqueous humor. The posterior barrier

between blood stream and eye is comprised of retinal pigment epithelium (RPE) and the tight walls of retinal capillaries. Unlike retinal capillaries the vasculature of the choroid has extensive blood flow and leaky walls. Drugs easily gain access to the choroidal extravascular space, but thereafter distribution into the retina is limited by the RPE and retinal endothelia.

Dry eye syndrome

Dry eye syndrome (DES) is a disorder of the preocular tear film that results in damage to the ocular surface and is associated with symptoms of ocular discomfort. DES is also called keratoconjunctivitis sicca (KCS), keratitis sicca, sicca syndrome, xerophthalmia, dry eye disease (DED), ocular surface disease (OSD), or dysfunctional tear syndrome (DTS), or simply dry eyes.[12] Keratoconjunctivitis sicca is a Latin word and its literal translation is "dryness of the cornea and conjunctiva." It may be helpful to know that "sicca" is part of the English word "desiccate." dry eye syndrome in which the eyes do not produce enough tears is also known as "sjogrens syndrome". Dry eye disease is characterized by istability of the tear film that can be due to insufficient amount of tear production or due to poor quality of tear lm, which results in increased evaporation of the tears. Dry eye therefore can mainly be divided into two groups, namely,

(1) Aqueous production deficient dry eye disease;

(2) Evaporative dry eye disease.

Insufficient tears cause damage to the interpalpebral ocular surface and are associated with symptoms of discomfort. the International Dry Eye Workshop (2007) defined dry eye as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular sjogren's syndrome". Healthy eyes are covered with fluid all the time, known as tear film, which is designed to remain stable between each blink. The stable tear film prevents the eye from becoming dry and keeps eye clear and comfortable vision. If a tear gland produces a lower quantity of tear, the tear film can become destabilized. The tear film can break down completely creating dry spots on the eye surface. surface. It is accompanied by increased osmolarity of the tear film and in ammation of the ocular surface DES is associated with decreased ability to perform certain activities such as reading, driving, and computer related work, which requires visual attention. Patients experience dry eyes symptoms constantly and severely, affecting their quality of life.[13,14]

SIGN AND SYMPTOMS OF DRY EYE SYNDROME

- A stinging sensation in eye.
- A burning sensation of eye.
- Feeling dryness in eye.
- Feeling grittiness and soreness in eye.
- Stingy mucous in or around the eye.
- Eye sensitivity to smoke.
- Redness of eye.
- Eye fatigue.
- Blurred vision.

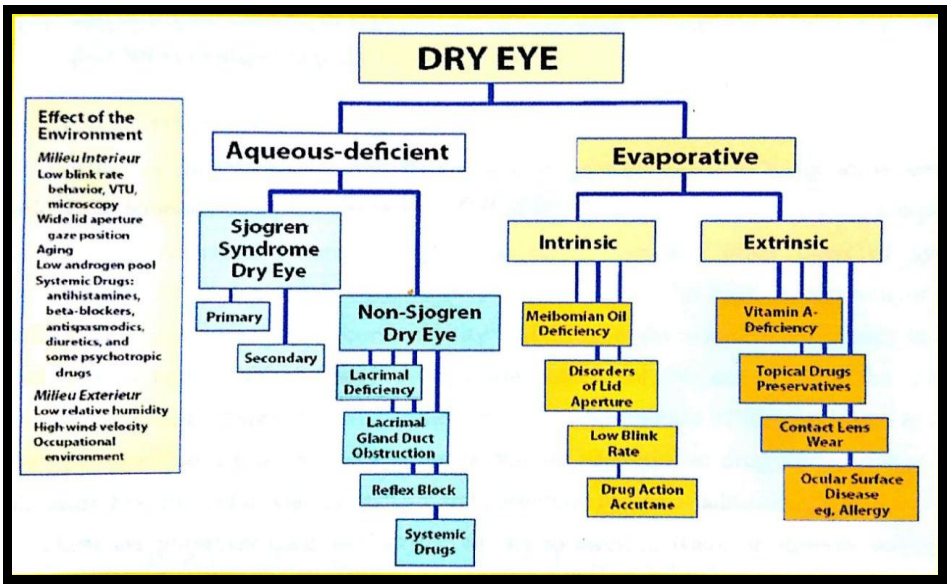


Figure 4: Causes of dry eye syndrome

***IN SITU* HYDROGELS**

Hydrogels are polymeric networks that absorb large quantities of water while remaining insoluble in aqueous solutions due to chemical or physical crosslinking of individual polymer chains. They resemble natural living tissue more than any other class of synthetic biomaterials due to their high water content; furthermore, the high water content of the materials contributes to their biocompatibility.[15] hydrogels show minimal tendency to adsorb proteins from body fluids because of their low interfacial tension. Further, the ability of molecules of different sizes to diffuse into (drug loading) and out of (drug release) hydrogels allow the possible use of dry or swollen polymeric networks as drug delivery

systems for oral, nasal, buccal, rectal, vaginal, ocular and parenteral routes of administration.[16]

These are polymers endow with an ability to swell in water or aqueous solvents and induce a liquid-gel transition.[17] Currently, two groups of hydrogels are distinguished, namely performed and *in situ* forming gels. Preformed hydrogels can be defined as simple viscous solutions which do not undergo any modifications after administration. The use of preformed hydrogels still has drawbacks that can limit their interest for ophthalmic drug delivery or astear substitutes. They do not allow accurate and reproducible administration of quantities of drug and, after administration; they often produce blurred vision, crusting of eyelids, and lachrymation. Thus *in situ* hydrogels can be instilled as eye drops and undergo an immediate gelation when in contact with the eye. *In situ* forming hydrogels were liquid upon instillation and undergo phase transition in the ocular cul-de-sac to form viscoelastic gel and this provides a response to environmental changes. Three methods have been employed to cause phase transition on the surface: change in temperature, pH, and electrolyte composition[13]. Increase in solution viscosity by using polymers improves retention of product on the corneal surface. More recently, the approach to improve precorneal retention is based on the use of mucoadhesive polymers. The principle for use of bioadhesive vehicles relies on their ability to interact with the mucin coating layer present at the eye surface. The polymers chosen to prepare ophthalmic hydrogels should meet some specific rheological characteristics. It is generally well accepted that the instillation of a formulation should influence tear behavior as little as possible. Because tears gave a pseudoplastic behavior, pseudoplastic vahicles would be more suitable as compare to Newtonian formulations, which have constant viscosity independent of the shear rate, whereas pseudoplastic solution exhibit decreased viscosity with increasing shear rate, therefore offering lowered viscosity during blinking and stability of the tear film during fixation.[18,19]

Drug release from hydrogels[20]

As discussed in the previous sections, hydrogels have a unique combination of characteristics that make them usefull in drug delivery applications. Due to their hydrophilicity, hydrogels can imbibe large amount of water. Therefore, the molecule release mechanisms from hydrogels as very different from hydrophobic polymers. Both simple and sophisticated models have been previously developed to predict the release of an active agent from a hydrogel device as a function of time. These models are based on the rate limiting step for

controlled release and therefore categorized as diffusion, swelling and chemically controlled mechanism.

SMART HYDROGELS[18]

"Smart**"** hydrogels, or stimuli-sensitive hydrogels, are very different from inert hydrogels in that they can "sense" changes in environmental properties such as pH and temperature and respond by increasing or decreasing their degree of swelling. The volume changing behavior of "smart" hydrogels is particularly useful in drug delivery applications as drug release can be triggered upon environmental changes. These "intelligent" or "smart" polymers play important role in the drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released. The stimuli that induce various responses of the hydrogel systems include physical (temperature) or chemical (pH, ions) ones.

The aim of present study is to develop *In situ* ocular gel of polymixin B sulphate which releases the drug in sustained manner by using high esterified pure pectin and guar gum and hydroxypropyl methyl cellulose. The objective of the study is:

1. To characterize natural/semisynthetic polymers as *In situ* gelling agent.
2. To formulate *In situ* ocular gel by using natural/semisynthetic polymers.

MATERIALS AND METHODS

POLYMIXIN B SULPHATE.[21,22]

Polymixin B is a low molecular weight cationic polypeptide antibiotics.[16] It is powerful bactericidal agent and is used clinically. Polymixin B is obtained from bacillus polymyxa. They are active against gram negative bacteria only. They are rapidly acting bactericidal agents, have a detergent like action on the cell membrane. They have high affinity for phospholipid: the peptide molecules orient between the phospholipid and protein films in gram negative bacterial cell membrane distortion. As a result ions, amino acids, etc, leak out. They may also inactivate the bacterial endotoxin.

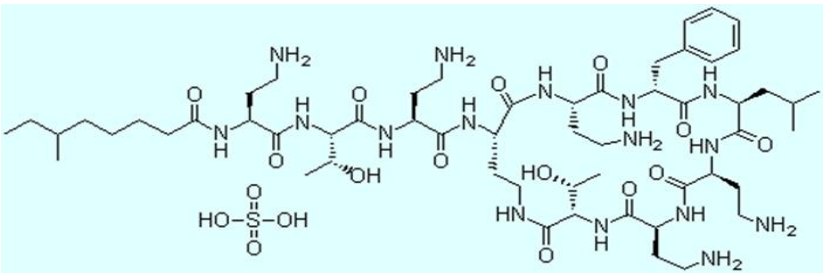


Figure 5: The chemical structure of polymixin B sulphate

PECTIN[23]

Pectin is a polysaccharide comprising mainly esterified D-galacturonic acid residue in an a-(1-4) chain. The acid groups along the chain are largely esterified with methoxy groups in the natural product. The hydroxyl groups may also be acetylated. The pectin is soluble in water; insoluble in ethanol (95%) and other organic solvents. Pectin gelation characteristics can be divided into two types; high methoxy and low methoxy gelation, and sometimes the low methoxy pectin may contain amine groups.

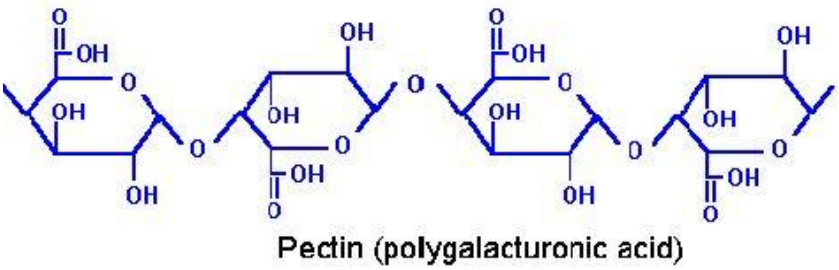


Figure 6: Chemical structure of pectin.

GAUR GUM.[23]

Gaur gum as a gum obtained from the ground endosperms of cyamopsis tetragonolobus (L.) Taub. (Fam. Leguminose). It consists chiefly of a high molecular weight hydrocolloidal polysaccharide, composed of gelactan and mannam units combined through glycoside linkage, which may be describe chemically as a galactomannam.

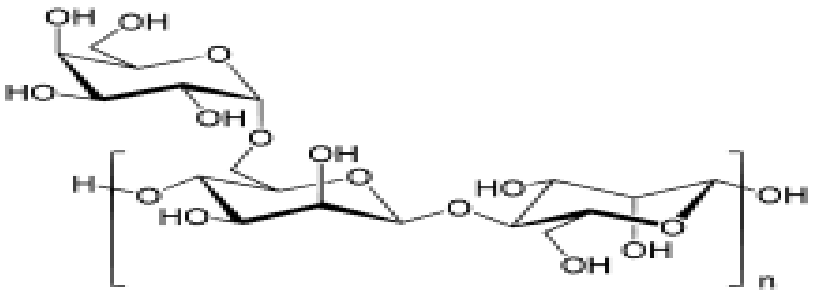


Figure 7: Chemical structure of guar gum.

HYDROXY PROPYLMETHYL CELLULOSE (HPMC)[23]

HPMC is a partly O- meyhylated and O-(2-hydroxypropylated) cellulose. It is available in several grades that vary in viscosity and extend of substitution. Grades may be distinguished by appending a number indicative of the apperent viscosity, in mPa s, of a 2% w/w aqueous solution at 208C. HPMC is an odorless and tasteless, white or creamy-white fibrous or granular powder. Soluble in cold water, forming a viscous colloidal solution. Practically

insoluble in hot water, chloroform, ethanol (95%), and ether, but soluble in mixture of methanol and dichloromethane.

CHARACTERIZATION OF POLYMER OF PECTIN, GAUR GUM AND HPMC.

1. Standardization of polymer as per I.P.1996

Pectin, gaur gum and HPMC were evaluated as per I.P.1996 following parameters were evaluated.

Determination of Ash value[24]

Samples of pectin, gaur gum and HPMC weighed (1g) in a metal crucible and incinerated at a temperature not exceeding 450^0C to constant weights, cooled and weighed. The present ash with reference to the air dried was calculated.

Determination of Acid insoluble matter.[25]

Samples of pectin, gaur gum and HPMC equivalent to 1.5gm were weighed accurately and dispersed in 150ml of water in beaker. 1.5ml of conc. Sulphuric acid was added to mixture and warmed on a hot water bath for 6 hrs. The water evaporated was replaced at regular intervals. At end of heating period the mixture was filtered through weighed ashless filter paper was dried at 150^0C for 3 hrs. Cooled in a desicator, weighed and weight of filter paper was substracted to obtain the actual weight of residue.

Loss on drying.[26]

1gm of samples was accurately and taken in dried, glass stoppered weighing bottle. The loaded glass bottle was placed in drying chamber (oven). The stopper was removed and bottle was left in the chamber. The samples were dried to constant weight in the oven at 105^0C. After drying, the chamber was opened, the bottle was closed promptly and allowed to cool to room temperature in a desicator. The bottle and the content were weighed and loss on drying was calculated.

Chemical test[27]

1. Heated 1gm of sample with 9ml of water on a water-bath until a solution was formed, replacing the water lost by evaporation and cooled; there was formation of stiff gel.
2. To a 1% w/v solution of pectin, gaur gum, HPMC. Added an equal volume of ethanol (95%); a translucent, gelatinous precipitate was produced.

3. To 5 ml of a 1% w/v solution of pectin, gaur gum, HPMC added 1 ml of a 2% w/v solution of potassium hydroxide and kept aside at room temperature for 15 minutes; a transparent gel or semi-gel formed. Acidified the gel with dilute hydrochloric acid and shake well; a voluminous, colourless, gelatinous precipitate formed, which upon boiling white and flocculent.

Pathogenic Test (For presence of salmonellae Typhi)[28]

1gm of samples was mixed with 50 ml of cysteine broth. Allowed to stand for 1 hr. and then incubated at 37^0C for 24 hrs. 1 ml of enriched media was added to (a) Bismuth sulphite agar (5ml) and (b) xylose lysine esoxycholate agar (5ml) taken in different petri dishes, samples were incubated at 37^0 C for 24 hrs. The colonies formed, if any, were observed.

2. Determination of physicochemical properties of pectin

Following parameters were evaluated.

Study of physical parameters

This parameter is useful in identification of specific material. Following properties were evaluated: Colour, Odour, and Texture.

Determination of pH

By using digital pH meter, pH of pectin, gaur gum and HPMC (0.5%w/v) was determined. Average of three determinations was reported.

Determination of viscosity

Viscosity of pectin, gaur gum and HPMC (1%w/v) was determined by using Brookfield's viscometer with the spindle no. 63 and at the rate of 100 rpm at constant temperature i.e. at room temperature. Polymer was allowed to hydrate for 4 hrs. and then readings were taken. Averages of three determinations were reported.

Percentage water uptake.[29]

The water uptake capacity of powder bed (ml/g) was determined for a period of 3hrs.

$$\mathbf{WU}=\frac{W1-W0}{W0}\times\mathbf{100}$$

Degree of swelling

1 g of powder of pectin, gaur gum and HPMC was added to measuring cylinder and swelling of powder with respect to time was calculated using following equation:

$$S=\frac{ht-ho}{ho}\times 100$$

3. Micromeretics studies[30]

Angle of repose

Flowability of pectin, gaur gum and HPMC was determined by calculating angle of repose by fixed height method. A funnel with 10mm inner diameter of stem was fixed at a height of 2 cm over the platform. About 20 gm of samples was slowly passed along the wall of funnel till the tip of the pile formed touches the stem of the funnel. A rough circle was drawn around the pile base and the radius of powder cone was measured. Angle of repose was calculated using following formula:

$$\Theta = \tan^{-1} h/r$$

Bulk density

Approximately 20gm of pectin, gaur gum and hydroxypropyl methyl cellulose was introduced in to the graduated cylinder. The volume occupied by the sample was recorded. Bulk density was calculated using following formula:

Bulk density = mass of powder /bulk volume

Tapped density

Sample of pectin, gaur gum and HPMC (20gm) was poured through a glass funnel into 100 ml of graduated cylinder. The cylinder was tapped gently from the height of 2 inches until a constant weight was obtained. The volume occupied by the sample after tabooing was recorded and tapped density was calculated using following formula:

Tapped density = mass of powder/tapped volume compressibility

Carr's compressibility index

$$\text{Carr's index}=\frac{\text{Tapped density}-\text{Bulk density}}{\text{Tapped density}}\times 100$$

Effect of pH on solubility

All the pH solutions like 1, 2, 3, 4, 5, 6, 6.8, 7.4,8, etc were made as per I.P. 1996. 1 g of pectin, gaur gum and HPMC were shaken for 2 hrs. in water bath shaker at $37^0 \pm 0.5^0$ C. After shaking, 2 ml was pipette out and transferred in to the petri dish. The petri dishes were dried

at 105^0 C in oven till they attained constant weight. Finally the weight of petri dish was subtracted from the weight of empty petri dish and also from blank.

Solubility

The solubility of drug was determined in water and tear fluid.

Determination of melting point

The melting of drug sample was determined using the digital melting point apparatus (four tech digital melting point apparatus). The melting point was noted.

Determination of λmax

The UVspectrum of polymixin B solution in water was scanned at 400 nm to 200 nm using standard stock solution of 100µg/ml.

Infrared absorption spectra

The IR spectrum of polymixin B was recorded by using KBr pellet method. The drug was triturated in porcelain mortar pestle with dry potassium bromide in ratio 1:100. The pellets were prepared in KBr press at a pressure of 8 tones. The pellet was scanned over the range of 45000-500 cm^{-1} in FTIR and the spectrum was obtained.

DSC Thermogram

DSC thermogram of drug was separately recorded on a Mettler thermal analyzer. The sample was placed in aluminum pan with a reference pan heated at a rate of 20^0C/min. over a range of 0-300^0C. Inert atmosphere was maintained with purge of nitrogen gas.

COMPATIBILITY STUDIES BETWEEN DRUG AND EXCIPIENT

FT-IR study

The excipient compatibility of drug and the polymer was studied using their physical mixture. The mixtures were prepared by triturating the drug complex with pectin and gaur gum. Mixtures were stored for a week at 40^0C temperature. FT-IR spectrum of pure drug and the mixtures were recorded.

Differential scanning calorimetry

DSC thermogram of pure polymixin B, pure polymer (pectin,gaur gum and hydroxypropyl methyl cellulose), and drug and combination of all i.e. mixture was separately recorded on a Mettler thermal analyzer. The sample was placed in aluminium pan with a reference pan

heated at a rate of 20^{0}C/MIN over a range of 0-300^{0}C. Inert atmosphere was maintained with purge of nitrogen gas.

FORMULATION OF OCCULAR IN SITU GEL[31]

Ocular *In situ* gel was prepared by dispertion method by using pectin, gaur gum and hydroxypropylmethyl cellulose.

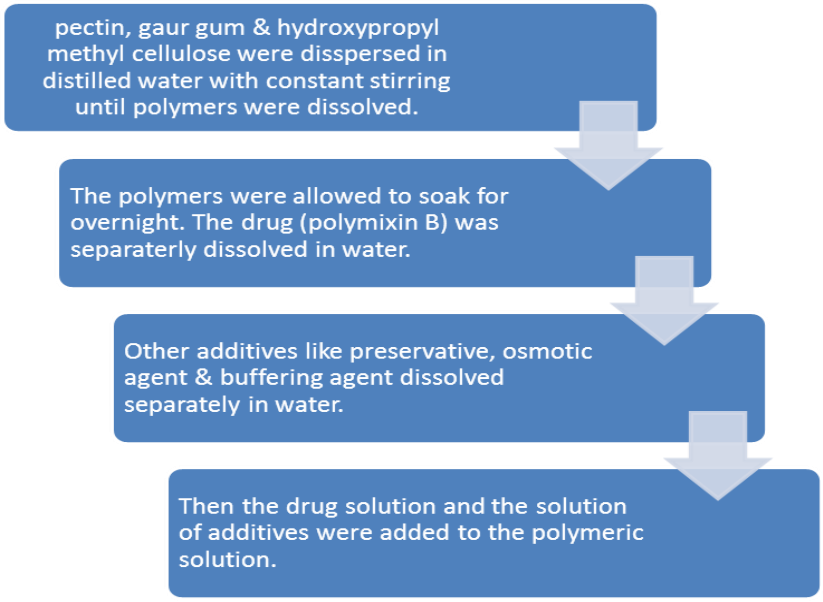


Table 1: Formulation of *in situ* ocular gel

Formulation code	F1	F2	F3	F4	F5	F6	F7
Drug (mg)	50	50	50	50	50	50	50
Pectin(%w/w)	1.5	1.5	1.5	1	1	1.5	2
Gaur gum (%w/w)	1	1	0.5	1	0.5	1	0.5
HPMC(%w/w)	3	2	2	2	2	1.5	-
Benzalconium cloride(%v/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sodium chloride (%w/w)	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Sodium acetate(%v/v)	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled water(ml)	5	5	5	5	5	5	5

Evaluation of ocular in situ gel[31]

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background.

Determination of pH

pH is one of the most important parameters involved in ophthalmic formulations. The two areas of critical importance are the effect of pH on solubility and stability. The pH of an ophthalmic formulation should be such as to ensure formulation stability and at the same time to cause no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have a pH ranging between 5 and 7.4. The developed formulations were evaluated for pH by using a digital pH meter.

Determination of drug content

The drug content was determined by diluting 1 mL of the formulation to 50 mL with freshly prepared simulated tear fluid having pH 7.4. An aliquot of 5mL was withdrawn and further diluted to 50 mL with simulated tear fluid. Polymixin B concentration was then determined at 272 nm using a UV-Visible spectrophotometer.

In Vitro Gelation Studies

The gelling capacity of the prepared system containing different concentrations of polymixin B was evaluated. It was performed by placing a drop of system in vials containing 1 ml of simulated tear fluid, freshly prepared and equilibrated at 37^0C, and visually assessing the gel formation and noting the time for gelation as well as time taken for the gel formed to dissolve. The Composition of Simulated tear fluid (STF) was sodium chloride (0.670 g), sodium bicarbonate (0.200 g), calcium chloride dihydrate and double distilled water q.s.100.0 g. Physiological pH (7.4 0.2) was adjusted by adding the required amount of 0.1 N HCl.

Rheological studies

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. The rheological studies of the formulations were carried out with a Brookfield viscometer and angular velocity was increased gradually from 10 to 100 rpm. Then, the hierarchy of angular velocity was reversed (100 to 10 rpm). The average of two readings was used to calculate the viscosity.

Sterility Testing

All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameters. Direct inoculation method was used. Sterility testing was intended for detecting the presence of viable form of microorganisms and performed for aerobic/anaerobic bacteria and fungi by using fluid thioglycolate medium and soybean-casein digest medium,

respectively as per the Indian Pharmacopoeia 1996. Both the media were observed every day for the presence or absence of turbidity and compared with a positive and negative control.

Antimicrobial efficacy studies

This was determined by the agar diffusion test employing "cup plate technique". Sterile solutions of polymixin B (standard solution) and the developed formulations were diluted at different concentration (test solution) these solutions were poured into cups bored into sterile nutrient agar previously seeded with test organisms. The agar plates were incubated at 37^0C for 24 hrs. The zone of inhibition measured around each cup was compared with that of control.

Stability studies

Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the formulations were subjected to stability studies at ambient humidity conditions at 2^0C TO 8^0C, and 40 ± 1^0C for a period of one month. The samples were withdrawn after 7, 15 and 30 days and were evaluated for following parameters.

1. Physical appearance
2. Drug content
3. Viscosity
4. In vitro drug release

RESULT AND DISCUSSION

Table 2: Standardization of polymer as per I.P. specification

S.no	Parameters	Pectin	Gaur gum	HPMC
1	% ash value	≤4%w/w	≤2%w/w	_
2	Acid insoluble matters	≤0.4%w/w	≤3%w/w	_
3	loss on drying	≤10%w/w	≤13%w/w	NMT 10.0%
4	Presence of enteric pathogens	Negative	Negative	Negative
5	Sulphated ash value	_	_	NMT 3.0%

Table 3: Chemical parameters of Pectin, Gaur gum, and HPMC (mean ± S.D, N=3)

S.NO	Physical evaluation	Pectin	Gaur gum	HPMC
1	pH (0.5% aq.solution after 3 hrs.)	3.8±0.21	6.0±0.4±5	7±1.5
2	Viscosity (1%aq. solution) with spindle no.	97±2 cps	310±12 cps	34±3 cps

	64 at 100 rpm			
3	% water uptake.	0.33±0.12	0.53±0.21	0.23±0.11
4	Swelling factor.	54.5±0.92	69.8±0.87	41.3±0.34

Table: 4 Micromeretic parameters of pectin, gaur gum, and HPMC

S. NO.	Parameters	Pectin	Gaur gum	HPMC
1	Angle of repose (º)	35.21±1.5	32.19±1.05	26.35±1.23
2	Bulk density (g/ml)	0.53±0.01	0.623±0.005	0.76±0.25
3	Tapped density (g/ml)	0.645±0.015	0.753±0.015	0.82±0.12
4	% compressibility	17.82±1.46	17.26±0.94	7.31±0.45

Fourier Transform Infra Red Spectroscopy (FT-IR)

The study was conducted with an intension to check the compatibility of polymer with drug.

Pectin-Drug

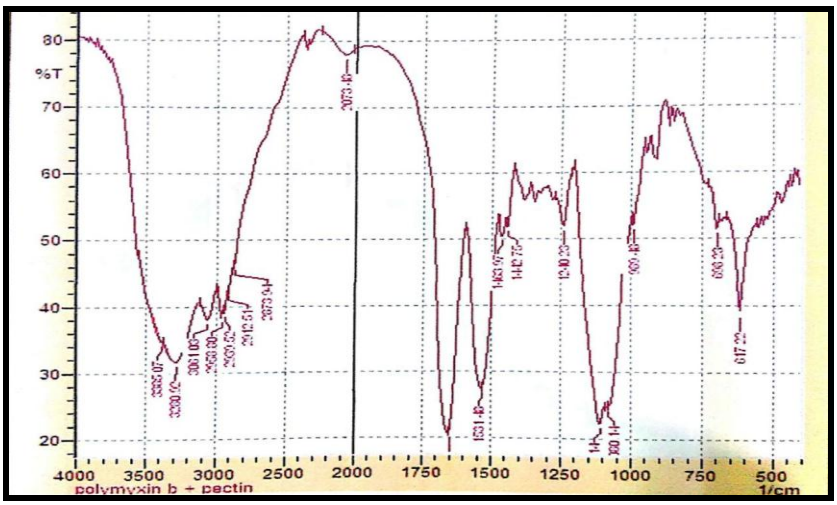


Figure 8: FT-IR spectrum of Pectin-Drug

Gaur gum-Drug

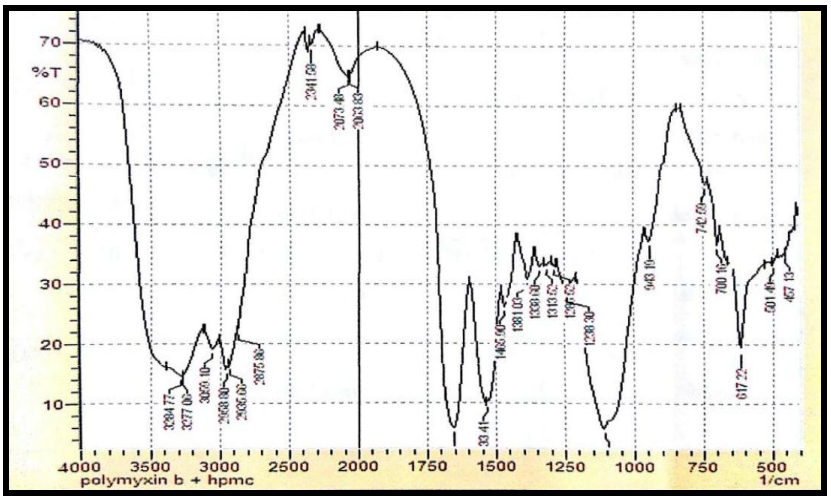


Figure 9: FT-IR spectrum of Gaur gum-Drug

HPMC-Drug

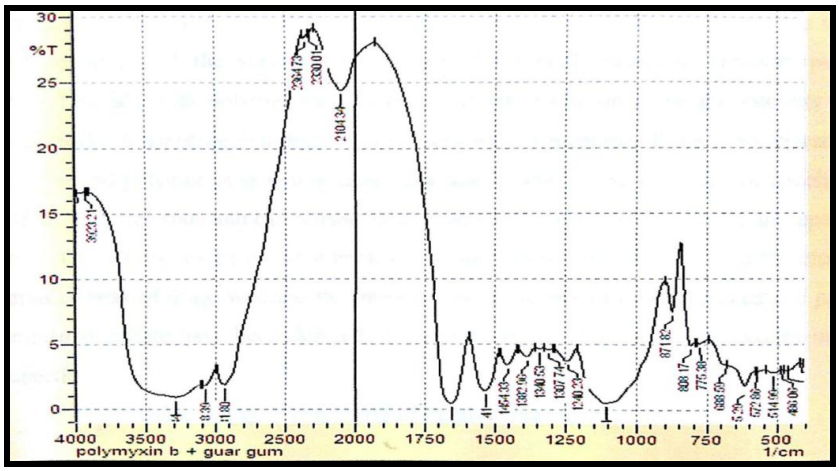


Figure 10: FT-IR spectrum of HPMC-Drug

Table 5: comparative FTIR study of drug, drug-Pectin, drug-Gaur gum and drug-HPMC

Peaks (cm^{-1})	Polymixin B (drug)	Drug-pectin	Drug-Gaur gum	Drug-HPMC	Peak assignment
3500-2500	2958.80	2958.80	2958.80	2958.80	O-H stretching
2500-2000	2873.94	2073.48	2104.28	2073.48	O-H stretching
1550-1250	1384.89	1442.30	1382.96	1381.72	C=O stretching
1000-750	972.12	989.48	871.82	943.12	υC-O/δO-H stretching
750-500	617.22	688.22	688.40	617.22	Ar-H Stretching

Differential scanning calorimetry (DSC)

Thermal analysis has been done to know the interaction of polymer and drug.

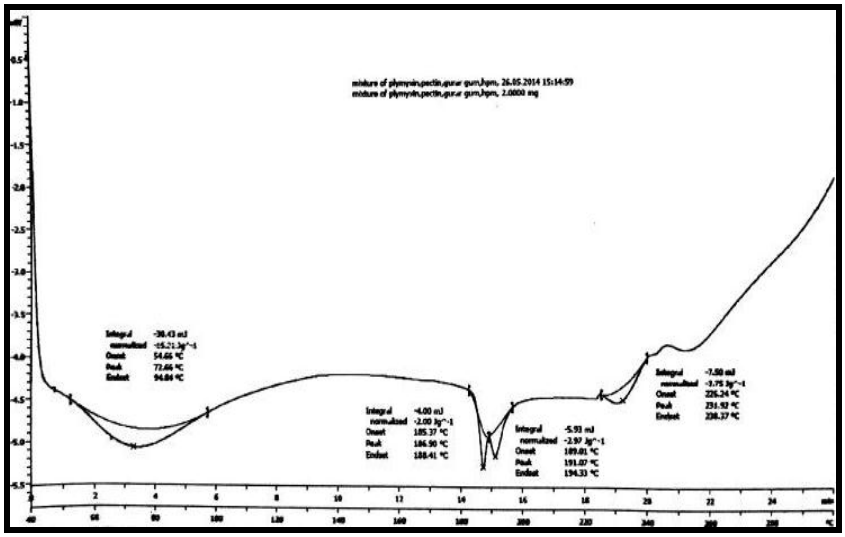


Figure 9: DSC thermogram of polymixin B sulphate-pectin-gaur gum-HPMC

The peak of polymixin B sulphate in drug complexed with excipients was found to be at 186^0c. the shortening of peak was due to pectin dissolution state. Thus it is proved that there were no major difference in thermogram, hence the excipients were compatible with drug.

Preparation of standared calibration curve of polymixin B sulphate

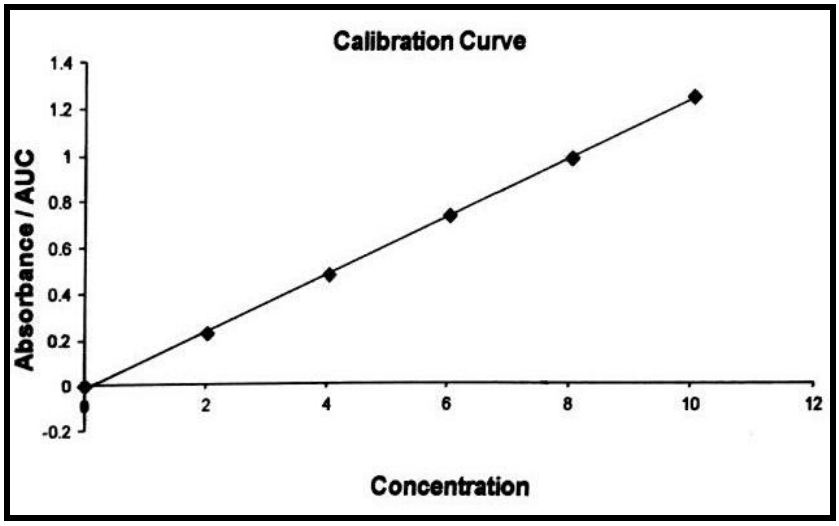


Figure 10: Standard calibration curv of polymixin B sulphate

The standered calibration curve yields a straight line, which shows that drug obeys beer lamberts law in the concentration range 2-10µg/ml at the pH of 7.4(tear fluid) The result are as follows:

Slope = 7.97

Intercept = 0.095

Correlation coefficient (R) = 0.9998

A straight line equation (y = mx + c) was generated for the calculation.

Evaluation of ocular *in situ* gel

Table 5: Evaluation parameters of in situ gel of different formulation F1-F7

Evaluation parameter	F1	F2	F3	F4	F5	F6	F7
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Ph	6.9±0.21	7.2±0.23	6.8±0.38	7.0±0.22	6.9± 0.11	6.8±0.32	7.0±0.20
Gelation capacity	+	+++	++	+++	+++	++	++
Residence time (hrs)	6	10	9	10	8	9	7
Drug content (%)	98.18±0.10	98.37±0.29	98.10±0.32	97.13±0.11	98.02±0.13	98.10±0.22	98.30±0.12

Rheological studies

Figure 11 and 12 shows that the viscosity values obtained for formulation F1-F7 before gelation and after gelation respectively using Brookfield DV-111+ rheomoter at different angular velocity formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity. The result obtained from the rheological study of prepared in situ gelling system F1-F7revealed that the viscocity decreases as the angular velocity increases. The administration of ophthalmic preparation should influence as little as possible the pseudoplastic character of the precorneal tear film.

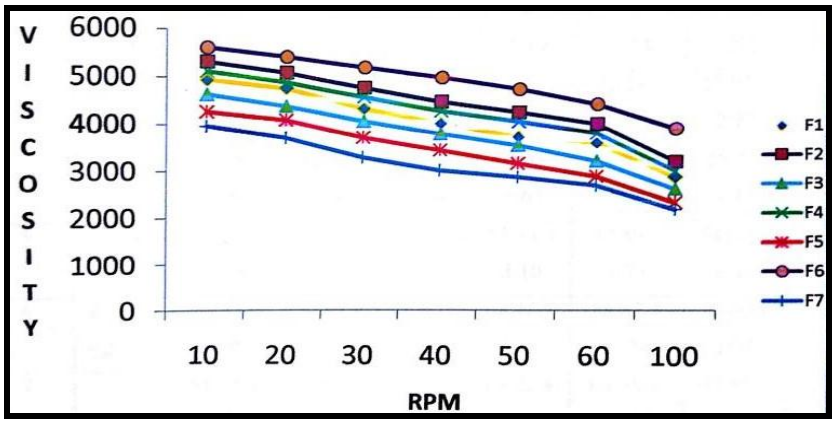


Figure 11: Viscosity of gel

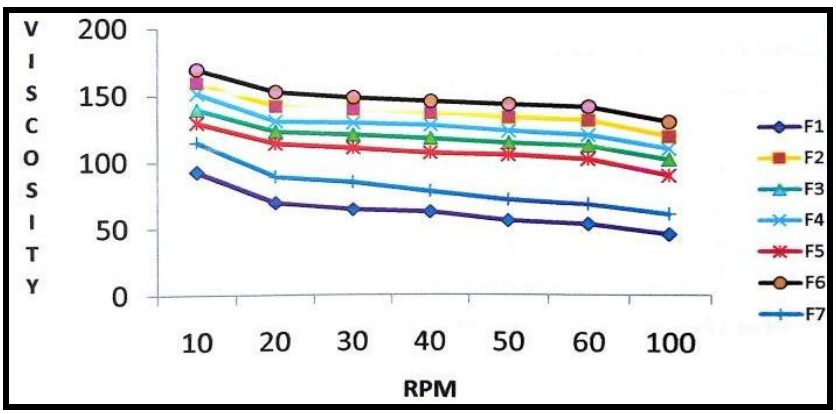


Figure 12: Viscosity of solution

In vitro drug release studies

Table 6: In vitro drug release studies of *in situ* gel of formulation F1-F7

Time (hrs)	% cumulative dug release						
	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
1	6.99±1.69	6.51±3.76	5.23±2.93	5.07±1.28	5.39±1.34	5.39±3.98	4.92±3.90
2	22.11±2.04	13.76±2.99	12.3±32.87	12.63±2.13	14.46±2.19	13.10±3.34	10.65±2.91
3	38.31±1.23	22.49±1.36	20.77±1.99	21.63±3.19	25.78±2.42	21.69±2.42	17.95±2.77

4	58.08±3.76	31.28±2.44	31.32±1.60	29.88±2.64	37.95±3.65	31.56±2.13	26.2±3.43
5	78.84±2.45	40.57±2.76	42.28±1.23	39.30±2.32	53.11±3.10	42.89±1.23	54.17±2.46
6	97.24±2.02	50.24±2.88	54.93±1.78	51.00±1.26	68.16±1.69	55.67±1.79	76.66±1.33
7		61.05±1.76	68.65±3.42	61.92±3.65	84.22±2.66	68.94±2.65	97.41±1.21
8		72.96±1.03	84.36±3.98	73.62±2.10	98.71±1.09	83.53±3.98	
9		85.86±2.55	97.55±2.41	85.85±1.07		97.77±1.12	
10		98.65±1.11		97.50±1.66			

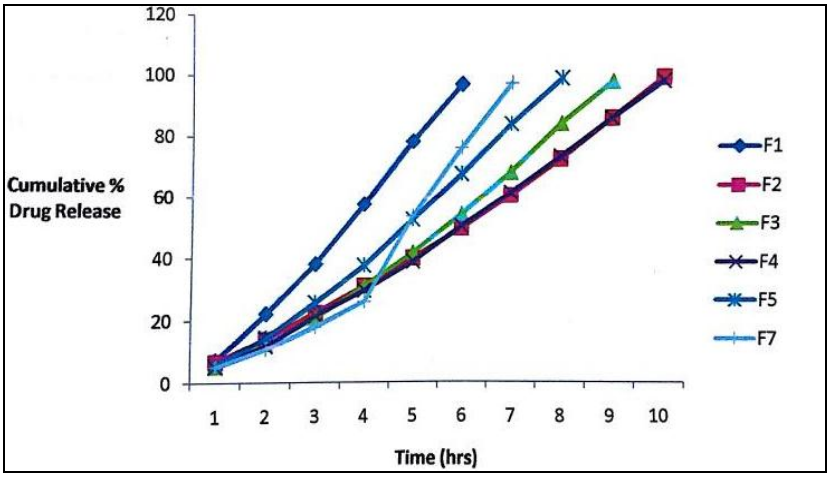


Figure 13: Cumulative percentage drug release of *in situ* gel from formulation F1-F7.

Drug release kinetics of *in situ* gel F1-F7

Table 7: Drug release kinetics

Formulation	Release kinetics model					Diffusion Coefficient (n)
	Zero order	First order	Matrix	Peppas	Hixon-crowell	
F1	0.7574	0.7699	0.9348	0.8946	0.7659	0.5553
F2	0.4961	0.5097	0.8649	0.7927	0.5055	0.3021
F3	0.8026	0.8156	0.9485	0.9082	0.8115	0.4810
F4	0.7768	0.7843	0.9202	0.8639	0.7820	0.3725
F5	0.7020	0.7832	0.9343	0.8805	0.7796	0.5004
F6	0.8002	0.8127	0.9480	0.9168	0.8088	0.4595
F7	0.7824	0.8045	0.9663	0.9479	0.7975	0.3623

The release kinetics for each batch was analyzed by using various release kinetics models. Formulation F2 was considered as optimized batch having peppas released kinetics order with 0.3021 n value. The 0.3021 value indicates the non-fickian release of drug.

Sterility test

There was no appearance of turbidity and hence no evidence of microbial growth when the formulations were incubated for not less than 14 daysat 30^0C to 35^0C in case of fluid thyoglycolate medium and at 20^0C to 25^0C in the case of soyabean- casein digest medium.

Figure 14: (a) Sterility testing of Polymixin B sulfate in situ gel in soyabean casein digest media.

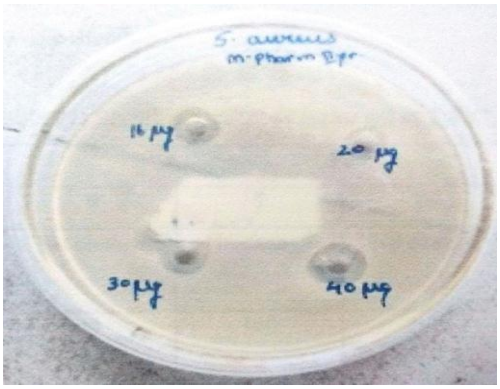
Figure 15: (a) Sterility testing of Polymixin B sulfate in situ gel in fluid thioglycolate media.

Antimicrobial studies

Table: 8 Antimicrobial activities of Polymixin B

Organism	Concentration (µg/ml)	Zone of inhibition (mm)		Percent efficacy(%)
		Standard	Test	
Staphylococcus Aureus	16	3	2.8	93.33
	20	3.8	3.7	97.36
	32	5	5	100
Pseudomonas Aeruginos	16	2	1.8	90
	20	3	2.9	96.66
	32	5	4.8	96

The result of the antimicrobial efficacy tests are shown. The study indicates that polymixin B sulphate retained its antimicrobial efficacy when formulated as an in situ gelling system.



(a)

(b) **(c)**

Figure: 16 antimicrobial activity of standered solution on (a) S. aureus, (b) P. aureginosa, (c) antimicrobial activity of test solution.

CONCLUSION

Most eye disease are treated with topical application of eye drops. The poor bioavailability and therapeutic response exhibited by these conventional eye drops due to rapid precorneal elimination of the drug may be overcome by the use of in situ gelling systems that are instilled as drops into the eye and undergo a sol-to-gel transition in the cul-de-sac. The FTIR studies indicate that polymixin B sulphate showed complete entrapment within the polymer carrier bonding and there were no physical as well as chemical interaction. The prepared gel was evaluated for various parameters like physical appearance, pH, gellation capacity, residence time, viscosity, antimicrobial activity, drug excipient interaction study, in-vitro drug release study and stability. From the result of the research work it can be concluded that polymixin B sulphate, an antibacterial agent used in the treatment of dry eye syndrome was successfully formulated as an ion-activated in situ gel forming ophthalmic solution using pectin, gaur gum in combination with HPMC as a viscosity enhancer which sustained the drug release over a period of 8 hours. The formulation also promises to reduce the frequency of drug administration, thus improve patient compliance.

ACKKNOWLEDGEMENT

We thanks to Shefa Health Care Pvt Ltd, Mumbai for providing drug as a gift sample for manufacturing of *in situ* gel.

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