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Research Article

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Formulation and Evaluation of *Euterpeoleracea* In-Situ Gel for Better Treatment of Conjunctivitis

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ABSTRACT

Introduction: This study is to formulate the Euterpe Oleracea in-situ gel for better treatment of conjunctivitis. The Euterpe Oleracea ophthalmic drug solution can treat the ocular infections effectively.

Aim: Formulation and evaluation of Eutepe Oleracea in-situ gel for better treatment of conjunctivitis

Methodology: Euterpe Oleracea drug containing in-situ gel prepared by using different polymers like carbopol-940, HPMC K15, HPMC K100, HPMC K4M, as a pH triggered gelling system, these polymers are used to controlled release to increase bioavailability.

Result: After the formulation prepared it will undergo evaluation i.e. visual appearance, pH, clarity checking, gel capacity, drug content and rheological studies, in-vitro drug release studies, sterility testing were determined. The prepared in situ gel was clear and transparent, it is having good gelling capacity.

Conclusion: The drug content of these formulations was determined to be 98%. Developed formulation was stable, and non-irritant, effective therapeutically it can be release in the form of sustained release.

Key Words: Euterpe Oleracea, HPMC, Citric acid, Disodium hydrogen phosphate, Tween-80, Benzalkonium chloride

INTRODUCTION

Eye is a sensitive organ in all over the body. It is an isolated organ considered to be a soul window. We can loss the vision based on age, jeans, vitamin deficiency and bacterial, fungal, and viral infections. Drug delivery into eye is most difficult and challenging for pharmaceutical scientists. Eye is a one of sensitive organ in the body it cannot bear if any foreign particle enters into the eye. It will remove by soft cloth but do not rub roughly because it will lead to redness and swelling from the eye. Generally these drug dosage forms having poor bioavailability and therapeutic response cause elimination of pre-cornea and turnover of rapid tear fluid. The anterior and posterior eye can be targeted in the drug delivery system. The eye preparations like eye drops, suspensions and ointments are act as conventional system for anterior part of the eye disease.[1] Mostly 90% of the eye preparations are in the form of eye drops. By using different mechanism the topically applied drugs are washed off resulting low bioavailability. Conventional drugs like solutions, suspensions and eye drops are no longer to sufficient for providing a constant rate drug delivery for prolonged time.[2] Insersion of excess drug into the eye is to maintain the bioavailability it is also dangerous potentially, if the drug is entered systemically absorbed by nasolachrymal duct from the eye.[3] Different types of ophthalmic dosage forms like aqueous gels, solutions, suspensions are being developed to enhance the bioavailability and increases the occupancy time of the drug. These formulations having some disadvantages, blurred vision from ointment and having poor patient compliance that is why, Extensively ophthalmic drug delivery system have not been used.[4]

There are different types of doses like in situ gels, nano particulate system, liposomes, nano suspensions, dendrimers, niosomes, ocular iontophoresis, ocular film, implants, ocuserts etc.[5] For the treatment of various ophthalmic diseases like conjunctivitis, dryness, eye flu, keratitis etc. establishes the drug by topical application. The promising means of delivering drugs have been proved by smart polymeric system.[6]

After administration of these polymers undergoes sol-gel transition. These are like solution before administration, under physiological condition they converted into gels.[7]

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Conjunctiva

The inner eye lid and the eyeball of outer membrane can be infected. It can also cause inflammation or irritation of conjunctiva. It is also known as pink eye. Conjunctiva is a tissue located at inner eyelids and covers the white part of the eye. It can cause different types of bacterial infections and allergic reactions and viral infections.[8,9]

It is vascularized mucosal layer, except cornea it protects the frontal surface of the globe. Cells of goblet promote mucus which are located at conjunctiva is essential for moistening and tear film stability. The globe adding of personal integrity has great ability to fight infection.[10]

Types of Conjunctiva:

1. Palpebral conjunctiva
2. Conjunctiva fornix
3. Bulbar conjunctiva

It is a contagious disease it can cause by bacteria and virus, it spread rapidly from person to person. Mostly for bacterial conjunctivitis treated with antibiotic medications. It is having mainly three type bacterial and viral conjunctivitis, and allergic conjunctivitis.[11] For this treatment, self care is very important. Stop wearing contact lenses which is affected by conjunctivitis. After effected by the conjunctivitis the pupil should be normal and the vision of the patient is also no change. The symptoms started first in one and it gets slowly spread to other eye within 2-5 days.[13]

Symptoms it includes:

Infection of the upper respiratory tract,

Common cold, sneezing,

Itching of the eye,

Swelling, watery eyes

Grayish and yellowish discharge,

Grittiness,

Dryness, Redness of eyelid.

Preparation of in-situ Gel:

The buffer salts like disodium hydrogen phosphate 1.127 gm of 0.1 gm of NaOH were dissolved in a 75 ml of purified water. Different grades of Hydroxypropyl methyl cellulose (HPMC K15, HPMC K100 M, HPMC K4) were added and allowed to hydrate. Carbopol 940 was sprinkled over this solution allowed to hydrate overnight. Then stir the solution with overhead stirrer. 0.1 gm of drug is dissolved in a small quantity of water and benzalkonium chloride added to polymer solution. Then add 100 ml of distilled water to make up the volume. The solution was filtered through 0-2mm of filter paper as showing table no.1.

Evaluation of in-situ gel

➤ Testing of appearance and clarity :

Clarity is one of the most important characteristic features of eye preparations. Optical appearance and clarity test can be determined under white and black environment to check whether the presence of clear solution or not.[14]

➤ pH:

pH is one of the most important characteristic parameter involved In ophthalmic formulation. pH of prepared in-situ gel system is to ensure formulation stability and at the same time causes no irritation to eye, patient upon administration of the formulation should have pH ranging between 5 and 7.4. After addition of all the ingredients in each formulation and measured by using pH meter.[15,16]

➤ Rheological studies:

It is to determine residence time of drug, in eye viscosity of instilled preparation is a huge factor. The designed formulation accepted as gel by adding of simulated tear fluid then the viscosity determination and viscosity determination were carried by using Brooke field viscometer DV-E model in spindle no S-32, the spindle was rotated around its own axis about 10-100 rpm. When increases the polymer concentration viscosity of formulation is also increases.

➤ Sterility testing:

Sterility testing is used to find the presence of any microbial contamination. It was also performed for aerobic and anaerobic bacteria and fungi. The 2 ml of the medium thioglycolate and soya bean casein medium. Both are incubated for 14 days, thioglycolate is incubated at 30-35 °C and soya bean casein medium incubated at 20-25 °C to detect growth of aerobic and anaerobic bacteria.[17,18]

➤ FTIR Studies:

Infrared spectroscopy was used determination of organic materials. Reciprocation studies of formulated in-situ gelling system were evaluated. There is nothing interrelation came within drug, polymer. As long as identification stability of drug in prepared formulation IR Spectra occupied and match with pure drug. Results of these research showed that there is no definite changes found in the bands of drugs in respect of pure drug.[19]

➤ *In-vitro* gelation:

Gelling capacity of in-situ gel evaluated to identify the formulation is suitable for gelling system. The formulated gel solution was mixed with simulated tear fluid to know the gelling capacity.

The simulated tear fluid was made with sodium chloride (0.670g), sodium bi carbonate (0.2g), calcium chloride dihydrate and bi-distilled water quantity sufficient up to 100ml. adjust the physiological pH (7.4±0.2) by adding of required amount of 0.1N HCL.[20]

➤ Drug content analysis:

Estimation of drug by spectrophotometric method:

Estimation of drug by using of UV-Spectrophotometric method was developed in a simulated tear fluid. In Euterpe oleracea for anthocyanins in simulated tear fluid at the pH of 7.2 the maximum wavelength show that 385 nm.[21, 22]

➤ *In-vitro* drug release:

In-vitro drug release for in-situ gel performed by using Franz diffusion cell. It implement via simulated tear fluid containing pH of 7.4. Franz diffusion cell contains two compartments donor compartment and receptor compartment. The receptor compartment contains simulated tear fluid is present in the receptor compartment and the formulation kept in a donor compartment. Between the donor and receptor compartment dialysis membrane was placed. The solution containing Franz diffusion cell was placed on the magnetic stirrer. Maintain temperature medium about 37°C. Withdrawn sample according to the time intervals up to 8 hours. Again replace the same volume of the fresh medium. The withdrawn sample was diluted with 10ml volumetric flask by simulated tear fluid. These withdrawn solutions are undergone UV-visible spectroscopy according to the respective wavelength of drug. The drug content was calculated by using standard equation. Calculate the % cumulative drug release.

RESULTS AND DISCUSSION

Evaluation test for in-situ gelling system:

Drug and Polymer compatibility studies of FTIR Spectra:

The FTIR spectrum shows stretching vibration of O-H bonds associated with a band at 3362.07cm. The C-H stretching vibration was observed at 2924.21cm. Stretching vibration of Ester carbonyl group was observed at 1753.67cm. The stretching vibration of C-C aromatic ring band at 1608.70cm. Similarly, the axial deformation of C=C bond in aromatic ring band at 1512.25cm was observed.[12] Spectrum shows the deformation of C-H bonds at 1440.88cm and another C-O bonds stretching of phenol band at 1026.17cm. At 1456-1419cm, 1377-1340cm, 1155-889cm other bands were observed as showing fig 1.

Rheological studies of in-situ gel for before gelation and after gelation:

Rheological studies of in-situ gels viscosity of the formulation should be measured with shear rate from 0 to 100 rpm. Viscosity of in-situ gel for all the formulations, before gelation and after gelation will be decreases according to shear rate. Rheological studies of in-situ gels after gelation will be higher than(i.e 190-2500) rheological studies of in-situ gels before gelation(i.e 30-480).[23]

➤ Evaluation of visual appearance, clarity, pH, and drug content:

Prepared in-situ gel was subjected to different examinations and it gives clear gel and transparent as showing table2.

➤ pH

The pH of formulation was found to be satisfactory and was in the range of 7.1-7.4 the formulation were liquid at room temperature as showing table2.

➤ Drug content determination

The drug content of the ophthalmic formulation of Euterpe Oleracea in situ gel was found to be 98.02-92.74%.

Sterility testing:

All prepared in-situ gel formulations were sterile to avoid new contamination of the eye. From this sterility studies, in-situ gels did not show any turbidity in media after 7 days. It was not evidenced any microbial growth at 20-25°C in case of soya bean casein digest medium. There is no microbial growth after 7 days of incubation of all the formulations. Fluid as showing table 2.

➤ Rheological studies:

The viscosity and gel capacity both are taken into account for development of in-situ gelling system. The visco-elasticity fluid with viscosity namely low underneath high shear rate condition and high under lower shear rate condition called as pseudo plastic fluid as showing table 3.

➤ *In-vitro* drug release:

The in situ gelling formulations of Euterpe Oleracea, F1-F12, were subjected to in-vitro release studies, which were carried out using simulated tear fluid (STF) of pH 7.4 as dissolution medium. In vitro release data indicated that formulation F8 showed a better sustained effect than the other formulations did. The prolonged release in the later stage could be attributed to the slow diffusion of the drug through the polymer matrix. The initial burst release of the drug could be explained by the fact that the in situ gelling system was formulated in water and hence the polymer was completely hydrated. When they came in contact with STF, gelation oc-

curred. However, the gels are having ability to release the drug in a sustained period of time up to 8 hours. These gels having more dissolution rate due to shearing action of eyelid and eyeball movement. Dissolution in the eye proceed more slowly in than that seen in the in-vitro experiment. In the course of experiment no disruption in the gels were observed. After succesive withdrawal of gradual dissolution of the polymers comprising the gels was that the filtration of aliquote release medium became increasingly difficult. Hence, this formulation follows the diffusion mechanism.

CONCLUSION

The ophthalmic in-situ gel of *Euterpeoleracea* was formulated successfully it is an antimicrobial agent it can reduces the bacterial infection in the eye. By using of different grades of gelling agents they are HPMC K15, HPMC K100, and HPMC K4M. Prepared in- situ gel undergone visual appearance and it found to be clear and transparent. The pH of formulation was ranging from 7.0-7.4. The sterility studies were checked there is no microbial growth after 7 days of incubation. The drug content from the in-vitro evaluation studies was obtained within limits. When the gel contacted simulated tear fluid F1, F5, F9 formulations showed instantaneous gelation except other formulations. F4, F8, F12 are the formulations which are prolong the action of drug for up to 8 hours of period. The F8 formulation which shows most sustained drug release.

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Ramineni Shireesh Kiran **-** Procere and Manuscript preparation

Ganti Subrahmanya Sharma - Manuscript editing
Dr. Tadikonda Rama Rao - Manuscript editing.

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Table 1: Composition of in situ gelling Formulation:

Ingredients (gm)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Euterpe oleracea(gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol 940(gm)	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
HPMC K15(gm)	1.5	1.5	1.5	1.5	-	-	-	-	-	-	-	-
HPMC K100(gm)	-	-	-	-	0.6	0.6	0.6	0.6	-	-	-	-
HPMC K4M(gm)	-	-	-	-	-	-	-	-	0.5	0.5	0.5	0.5
Citric acid(gm)	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407	0.407
Disodium hydrogen phosphate (gm)	1.127	1.127	1.127	1.127	1.127	1.127	1.127	1.127	1.127	1.127	1.127	1.127
Sodium hydroxide(gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tween-80(ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Benzalkonium chloride	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Distilled water (ml)	100	100	100	100	100	100	100	100	100	100	100	100

Table 2: Preliminary examination of gelling capacity, pH, and drug content.

Formulation	Gelling capacity	Clarity	pH	Drug content
F1	+++	Clear	7.10	98.02
F2	+++	Clear	7.12	98.02
F3	+++	Clear	7.15	97.92
F4	+++	Clear	7.19	97.90
F5	++	Clear	7.23	97.60
F6	++	Clear	7.26	96.93
F7	+++	Clear	7.29	95.98
F8	+++	Clear	7.31	94.96
F9	+++	Clear	7.33	94.57
F10	+++	Clear	7.35	93.96
F11	+++	Clear	7.37	92.96
F12	+++	Clear	7.39	92.74

++ Gelation immediately dissolve rapidly.
+++ Gelation immediately but extended some period of time.

Table 3: Sterility testing

Formulation code	Days of incubation						
	1	2	3	4	5	6	7
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-	-	-	-	-
F5	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-
F8	-	-	-	-	-	-	-
F9	-	-	-	-	-	-	-
F10	-	-	-	-	-	-	-
F11	-	-	-	-	-	-	-
F12	-	-	-	-	-	-	-

"-" Sign indicates no growth of microbes

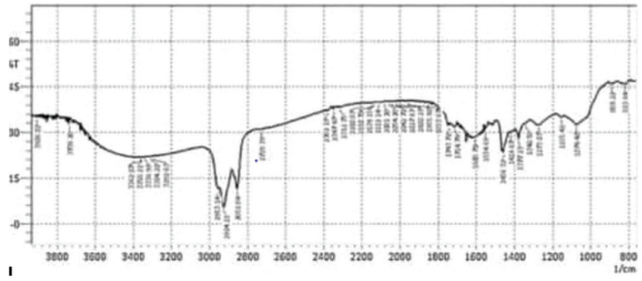

Figure 1: FTIR Spectra of drug.

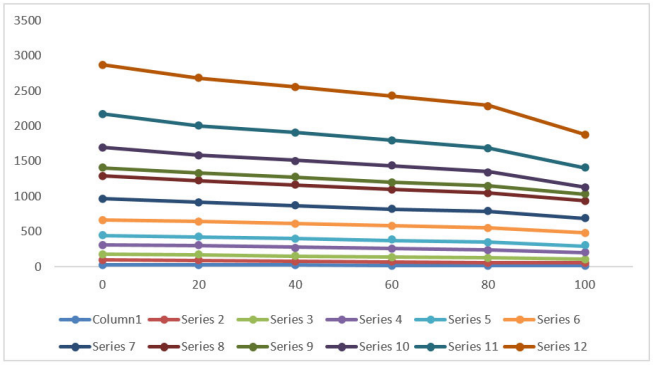

Figure 2: Rheological studies of in-situ gel before gelation.

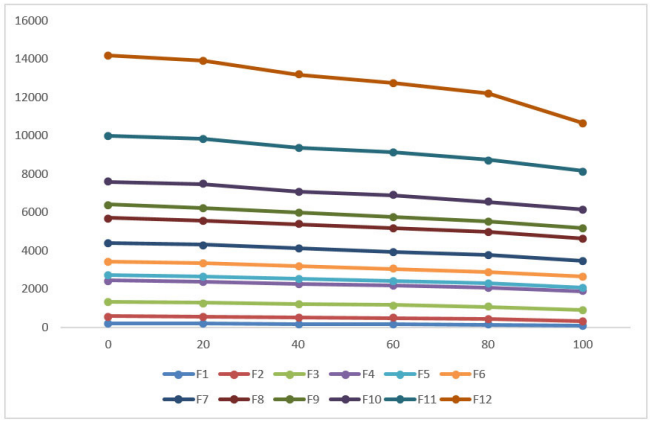

Figure 3: Rheological studies of in-situ gels after gelation.

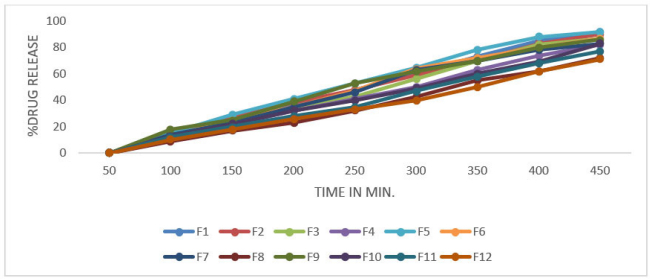

Figure 4: % Drug release of in-situ gel.