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Formulation and Evaluation of Cetrimide Based In situ Gelling System for Ocular Drug Delivery

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ABSTRACT

Conventional drug delivery system is a traditional type of drug delivery system which manifests poor bioavailability of drug due to tear turn over, tear flow and blinking of eye which causes removal of drug at the ocular surfaces. To overcome from the drawbacks of conventional drug delivery system In- situ gels come into the fashion of ocular drug delivery system for the treatment of ocular diseases which have enhanced bioavailability and better for patient's compliance. The goal of the present work to express formulation and evaluation of cetrimide in situ gels which have anti-fungal activity used in the treatment of fungal keratitis caused by Fusarium solani. Carbopol 934 is used as viscosity enhancing property. Xanthan gum was used as thickening and stabilizing agent. Sodium alginate was used for gelation ability. The combination of carbopol and xanthan gum showed good gelling capacity and gelation time up to 6hrs period. The developed formulation of cetrimide in situ gels was stable non -irritant which are feasible substitute for conventional drug delivery system such as eye drops by virtue of its stability to increase residence time of drug at the ocular surfaces and provide sustained release of drug with increase bioavailability and better for patient's compliance.

Keywords: *In–situ gelation; Carbopol; Xanthan gum; Sodium alginate; Cetrimide.*

Introduction

The development of conventional drug delivery system became blessings for ocular therapeutics for the treatment of ocular infections but after excessive use of CDDS (Eye drops, ointments, and suspension) it causes blurring vision and treatment of glaucoma with CDDS became difficult. It does not target to the specific area, and it spread to non– specific area as result distribution of drug to Non–target site as a result it causes harmful side effect with Nonproductive absorption, Impermeability of drug to cornea, drainage induced lacrimation, and tear turn over and patient's incompliances [1]. So, to overcome from drawbacks of CDDS In situ gels comes into fashion for the treatment of ocular disease such Fungal keratitis, Conjunctivitis, Dry eyes, Glaucoma, trachoma etc. In–situ gels are solution form before administration into the eye but once administered into the cull – de sac cavity of eye it forms stiff gels by the process of gelation in presence of temperature, pH and Ions by physical and chemical bonding. They have tendency to remain at the ocular surfaces for longer duration to give therapeutics effects with enhanced bioavailability and better for patient's compliances. The basics aim of In–situ gels to achieve maximum concentration of drug at the target site with enhanced pre- corneal absorption [1]. There are various ocular drug delivery In situ gels like

liposomes, Nanosuspension, Nano particulate, Niosomes, Dendrimers, Ocular iontophoresis, collagen shield minidisc, ocular film, occuserts, and implants etc used for delivery of drugs inside the eye [2]. The goal of present study was to develop Cetrimide Antiseptic In–Situ gelling system for ocular delivery system and common ingredients were used during preparations of In situ gels such as Cetrimide, Sodium alginate, Carbopol 934, Xanthan gum, Sodium di hydrogen orthophosphate, Benzalkonium chloride, and purified water. Cetrimide is a category of Antiseptic drugs which are used as Anti-fungal activity for the treatment Fungal keratitis caused by Fusarium solani [3]. Fungal keratitis is one of the major causes of infectious keratitis in ocular areas [4]. cetrimide shows good corneal penetration and may result from the fact that it is a surfactant and may react with the carboxyl group in the fatty acid of the lipid that make up the fungal cell membranes leading to cationic exchange or leakage that is fatal to the pathogen [3]. Sodium alginate is a biodegradable natural polymer used for immediate gelation ability to increase the ocular residence time with enhanced bioavailability. Carbopol 934 is a pH Sensitive polymer which are used for viscosity enhancing property. Xanthan gum used as thickening and stabilizing agent. Sodium di hydrogen orthophosphate used for phosphate buffers and Benzalkonium chloride used for preservative purposes. Polymers play a vital role in the formation of In situ gelling system because by the help of polymers it undergo sol to gel transition and form strong gel after administration in presence of pH, Temperature, Ions etc. As the concentration of polymers increased that result in improved gelling capacity and gelation time [5]. The developed formulation of Cetrimide In Situ gels was stable and Non-irritant which are feasible substitute for conventional drug delivery by virtue of its stability to increase residence time of drug at the ocular surfaces and provide sustained released of drug with enhanced bioavailability and better for patient's compliances.

Material and Methods

Cetrimide and Carbopol 934 was obtained from Loba Chemie Pvt. Ltd, Mumbai. Xanthan gum was obtained from Stride enterprises Pvt, Ltd. Jaipur. Sodium alginate, Sodium dihydrogen orthophosphate and Benzalkonium chloride was obtained from Nice chemical Pvt. Ltd. Kerala.

Preparation of Ocular In Situ Gels

Compatibility Studies

Compatibility studies are important factor during preformulation studies between drug and polymer. The compatibility studies were conducted by Fourier transform infrared spectroscopical methods [6-7].

Preparation of Formulation No 1 (F1)

Accurately weighing sodium alginate 1 .5 g and carbopol 1g and triturate in motor pestle for becoming finely divided particles further phosphate buffer (1.125 g) solution was adding to the polymer solution with constant stirring for 1 hr until homogenous solution was obtained [6-7]. Cetrimide (0.3 g) was dissolved in small quantity of water and benzalkonium chloride (0.02 ml) was added to the drug solution further the drug solution mixed with polymer solution with constant stirring until homogenous solution was obtained [6-7].

Preparation of Formulation No 2 (F2)

Accurately weighing sodium alginate 1.5 g and xanthan gum 1.0 g and triturate in motor pestle for becoming finely divided particles further phosphate buffer (1.125 g) solution was adding to the polymer solution with constant stirring for 1 hr until homogenous solution was obtained [6-7]. Cetrimide (0.3 g) was dissolved in small quantity of water and Benzalkonium chloride (0.02 ml) was added to drug solution further the drug solution mixed with polymer solution with constant stirring until homogenous solution was obtained [6-7].

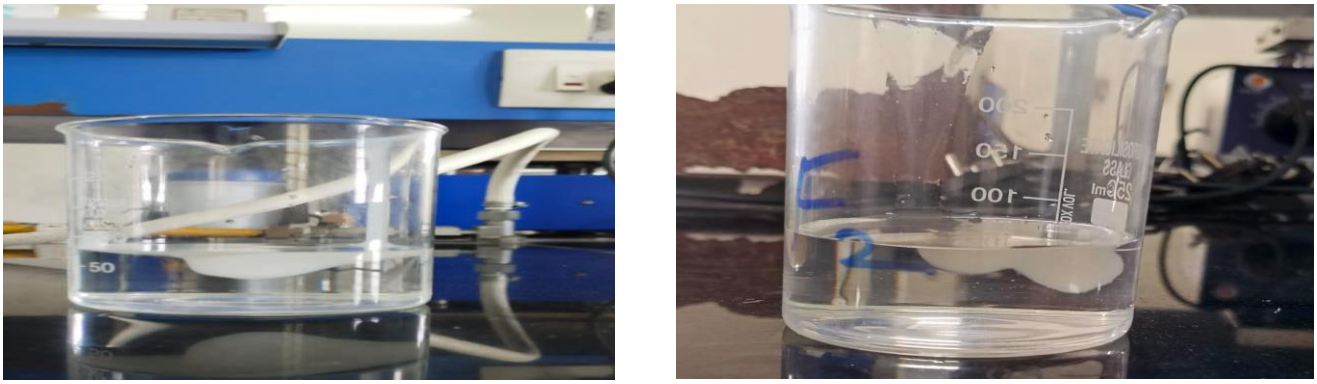

Figure 1: Formulations F1 and F2 forming gel in the simulation tear fluid.

Preparation of Formulation No 3 (F3)

Accurately weighing carbopol (1.5 g) and xanthan gum (1 g) and triturate in motor pestle for becoming finely divided particles Further phosphate buffer (1.125 g) solution was adding to the polymer solution with constant stirring for 1hrs until homogenous solution was obtained [6-7]. Cetrimide (0.3 g) was dissolved in small quantity of water and benzalkonium chloride (0.02 ml) was added to drug solution further the drug solution mixed with polymer solution with constant stirring until homogenous solution was obtained [6-7].

Preparation of Formulation No 4 (F4)

Accurately weighing sodium alginate 0.5 g and carbopol 0.2 g and triturate in motor pestle for becomes finely divided particles further phosphate buffers (1.125 g) solution was adding to the polymer solution with constant stirring for 1hrs until homogenous solution was obtained [6-7]. Cetrimide (0.3 g) was dissolved in small quantity of water and benzalkonium chloride (0.02 ml) was added to the drug solution further the drug solution mixed with polymer solution with constant stirring until homogenous solution was obtained [6-7].

Evaluation

Appearance and pH: All prepared formulas are evaluated for clarity by Visual observations against a black and white background and the pH was measured using digital pH meter [6-7].

Rheological studies: The rheological studies of solution determined by using a Brookfield synchoelectric viscometer. The solution is gushed inside a brook field synchoelectric viscometer and slowly increase angular velocity from 10 to 100 rpm. The standard of two readings is used to calculate viscosity. The solution is poured inside the ointment jar and pH was increased to 7.4 by putting on initiated lachrymal fluid [6-7].

Drug content: Studies on drug content were conducted using UV / visible spectrophotometric techniques. Cetrimide formulation in an amount equal to 1.0 mg was dissolved in 25 ml distilled water. 2 ml samples were taken from this solution and appropriate dilution were prepared before they were analyzed spectrophotometrically at 281 nm. The collected information was used to determine the drug content [6-7].

Gelling capacity: The gelling capacity is determined by placing one drop of the formula in a test tube containing 2ml of freshly prepared simulated tear fluid and equilibrated at 37 ℃ and visually assessing the formation of a gel noting the time for gelation and the time taken for formed gel to be dissolved [6-7].

In vitro release studies: The in vitro studies are done by using USPXXIII dissolution apparatus pH 7.4 initiated artificial tear fluid as a medium for a period of 6 hr at 100 rpm and 37°C. The sample is withdrawn at the interval of 30 minutes. The dissolution medium used as artificial tear fluid freshly prepared of pH 7.4 buffer cellophane membrane previously soaked overnight in the dissolution medium is tied to one end of a specifically designed glass cylinder an assembly. The cylinder is attached to the metallic drive shaft and suspended in 100 ml of dissolution medium maintained by 37℃ ± 1℃ so that the membrane just touched the receptor medium surface. The shaft is rotated at 50 rpm aliquots each of 1ml volume is withdrawn at hourly intervals and replaced by an equal volume of the receptor medium [6-7].

Anti-microbial efficacy studies: The anti-microbial studies are determined by using cup plate method. The potent solution of Cetrimide (standard solution) is diluted at different concentrations (test solution) is poured into cup bored into sterile agar medium where (test organism) pseudomonas aeruginosa and staphylococcus aureus is also present. The solution is allowing for diffusion for 2 hr and agar plate is incubated at 37 °c for 24 hrs. The zone of inhibitions is measured around each cup. The whole procedure was carried out in laminar flow except incubations [6-7].

Accelerated stability studies: The accelerated stability studies are done at 40°C, 50°C and 60°C as well as room temperature and freezing temperature. The sample is stored at different temperatures such as 40°C and room temperature at RH 75%. The sample is withdrawn at weekly intervals and calculated drug content by using UV visible spectrophotometer at 272 nm fluorescent light [6-7].

Result and Discussion

The goal of the present study to developed cetrimide in situ gels for treatment of fungal keratitis caused by Fusarium solani by increasing the residence time of drug at the ocular surfaces with enhance bioavailability. The developed formulation evaluated for Appearance, pH, Rheological properties, Drug content, Gelling capacity, In vitro drug release, antimicrobial studies and accelerated studies. The obtained result is discussed below [6-7].

Appearance and clarity: All the formulation were visual examined and found to be clear and transparent [6-7] (Table 1).

pH: pH of the formulation is an important factor in ophthalmic preparation because eye is the most

sensitive part of our body and when the formulation of pH are in the range of the pH of the eye it decreases displeasure and irritation of the eye. The normal pH range of the eye was between 6.5 and 7.6. The pH of the all formulation were measured by using digital pH meter and found to be 5.95 to 7.0 and this pH range acceptable in ophthalmic preparation [6-7] (Table 1).

Table 1: Data of visual appearance, clarity and pH.

Test	F1	F2	F3	F4
Visual appearance	Transparent	Transparent	Transparent	Transparent
Clarity	Clear	Clear	Clear	Clear
pH	5.95	6.01	7	6.75

Rheological Studies: The Brookfield synchoelectric viscometer was used to determine the rheological studies of the solution. The solution greatly increases angular velocity from 10 to 100 rpm inside a Brookfield synchoelectric viscometer. Calculating viscosity requires the use of standard of two readings. The solution was poured into an ointment jar, where it is activated with lachrymal fluid to raise the PH to 7.4 [6-7] (Table 2).

Table 2: Evaluation of viscosity of different formulations.

Formulation Code	Viscosity (cp) at 20 rpm	
	Before gelation	After gelation
F1	118	907
F2	210	1203
F3	670	1483
F4	690	1510

Drug content: Studies on drug content were conducted using UV / visible spectrophotometric techniques. Cetrimide formulation in an amount equal to 1.0 mg was dissolved in 2ml samples were taken from this solution and appropriate dilution were prepared before they were analyzed spectrophotometrically at 281.0nm. The collected information was used to determine drug content [6-7] (Table 3).

Gelling capacity: Gelling capacity of all prepared formulation measured by placing 1 ml of formulation solution into simulated tear fluid (sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride 0.008 g and purified water q.s). As the formulation solution comes in contact with gelation solution it was immediately converted into stiff gels. The gelling capacity was evaluated on the basis of stiffness of formed gels and time period for which formed gel remain as such [6-7] (Table 4).

In-vitro drug release: The USP XXIII dissolution apparatus pH 7.4 Started artificial tear fluid was used as a medium for the in vitro investigations for a duration of 6 hours at rpm and 37°C at an interval of 30 min the sample was removed. The pH 7.4 Buffered cellophane membrane that had been soaked overnight in the dissolution medium was connected to one end of a specifically made glass cylinder assembly with the dissolution medium utilized as tear fluid. The cylinder was suspended in 100 ml of dissolving medium kept at 37°C±1°C with the membrane just touching the receptor media surface. The cylinder was connected to the metallic drive shaft. The shaft rotates at 50 rpm and hourly aliquots of each 1ml volume were drawn [6-7] (Table 5).

Anti-microbial studies: The cup plate method was used to determine the antimicrobial studies pseudomonas aeruginosa and staphylococcus aureus two additional test organisms are contained in the sterile agar medium along with the strong cetrimide solution (standard solution) which was diluted at various quantities. The agar plate was incubated at 37°C for 24 hours while the solution was allowed to diffuse for two hours around each cup, the zone of inhibitions was measured except for incubations, all process were carried out in laminar flow [6-7] (Table 6).

Accelerated stability studies: The accelerated stability investigations are conducted at 40, 50 and 60 degrees Celsius in addition to ambient and subfreezing temperatures. The sample was kept at various temperature including 40˚C with 75% relative humidity. The sample was taken out at weekly intervals and the drug content was determined using a UV visible spectrophotometer and fluorescent light with a 272 nm wavelength [6-7] (Table 7).

Table 3: Drug content of all prepared formulation.

Formulation code	Drug Content
F1	99.53±1.1
F2	99.46±1.2
F3	99.39±0.9
F4	99.45±1.3

Tabe 4: Gelation profile of all formulations.

F1: Gelation after few secs and dispersed rapidly.
F2: Gelation after few min and dispersed rapidly.
F3: Gelation immediate remains for an extended period of time.
F4: Gelation after few secs and dispersed rapidly.

Table 5: In vitro Drug Release Profile of Formulations (F1 to F4).

Time (h)	F1	F2	F3	F4
1	3.20±1.150	9.61±2.423	14.5±2.995	18.9±1.885
2	4.81±2.000	13.9±2.905	18.2±3.756	27.1±4.108
3	7.11±2.189	18.4±3.196	25.1±1.015	32.5±3.392
4	10.8±2.469	23.2±3.007	30.6±2.027	37.2±3.407
5	14.3±2.852	28.2±4.467	35.6±2.955	42.6±4.107
6	18.9±3.569	32.1±3.049	41.6±3.307	48.8±4.657
7	23.2±4.400	34.4±3.881	46.5±3.968	54.2±3.510
8	28.2±4.914	36.8±4.883	51.9±4.086	60.0±2.885

Table 6: Comparison of anti-microbial activity of different formulations (S. aureus).

Concentration (µg/ml)	Standard Zone of inhibition (cm)	Tests Zone of inhibition (cm)			
		F1	F2	F3	F4
20	2.5	2.5	2.7	2.6	2.8
40	3.0	2.9	3.1	2.8	2.9
60	3.2	3.2	3.3	3.2	3.1
80	3.4	3.5	3.5	3.4	3.3
100	3.8	3.9	3.9	3.5	3.4

Table 7: Stability studies of Cetrimide in-situ gels (F1, F2, F3, F4) at 40°C.

S. No.	No. of weeks	% Drug remaining			
		F1	F2	F3	F4
1.	0	99.53	99.46	99.39	99.45
2.	3	99.10	98.97	98.99	99.13
3.	5	98.83	98.54	98.67	98.99
4.	7	98.32	98.21	98.35	98.43

Conclusion

The development of novel drug delivery system in – situ gel was to attain maximum concentration of drug at the target site for longer duration of action and increase bioavailability and better for patient compliancy. Cetrimide used as Anti-fungal activity for the treatment of fungal keratitis caused by Fusarium solani. It shows good corneal penetration and may result from the fact that it is a surfactant and may react with the carboxyl group in the fatty acid of the lipid that make up fungal cell membrane leading to cationic exchange or leakage that is fatal to the pathogen. Sodium alginate is a biodegradable natural polymer used for immediate gelation ability to increase the ocular residence time and increase the bioavailability. Carbopol 934 is used as viscosity enhancing property. Xanthan gum is used as a thickening and stabilizing agent. Sodium dihydrogen orthophosphate is used as phosphate buffers. Benzalkonium chloride is used as a preservative purpose. The developed formulation of cetrimide In situ gels are stable and non- irritant which are feasible substitute for conventional drug delivery systems such as eye drops by virtue of its stability to increase residence time of drug at the ocular surfaces and provide sustained release of drug with increase bioavailability and better for patients compliance. The results suggest strong potential of in situ gelling system as a successful ocular drug delivery system. However, further clinical data will be required to support this research work.

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Conflict of Interest

None declared.

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