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Rationally Designed Novel Treatment of Bacterial Conjunctivitis *In-Situ* Gelling Formulation of Ofloxacin with Gelrite® and Monitoring the Ocular Residence Time by Scintigraphic Assessment

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Abstract

This study was carried out to determine the residence time of a drug on the cornea using a gamma camera to image the *in-vivo* distribution of pharmaceutical formulation radiolabelled with a suitable γ-emitting radionuclide. The *In-situ* Gelling formulation of Ofloxacin was prepared using various concentrations of polymer Gelrite® for a sustained release of Ofloxacin under physiological conditions for the treatment of bacterial conjunctivitis. The formulations were optimized based on rheological and *in vitro* release studies. Gamma Scintigraphic assessment is employed to demonstrate the delayed clearance of labeled optimized formulation with respect to the market formulation. The results suggest that the optimized formulation containing 0.5% Gelrite® demonstrated favorable rheological properties for ocular in situ gelations and release of ofloxacin in a sustained manner for 12hrs for the treatment of conjunctivitis, an inflammation of the membrane conjunctiva. The formulation containing Gelrite® 0.5% were therapeutically efficacious, non-irritating, possess excellent ocular tolerance and shows sustained drug release over 12hrs period as measured by gamma Scintigraphy in comparison with a marketed eye drops.

Keywords: Scintigraphic; *In-situ* gelling; Ofloxacin; Monitoring; Ocular Residence Time

Introduction

The unique structure of the eye limits the administration of therapeutically active agents at the site of action. Ocular drug delivery generally involves the delivery of therapeutically active agents into anterior and posterior segments of the eye. The visual bacterial diseases can cause a progression of indications and signs, for example, the arrangement of discharge, conjunctival hyperemia, top edema, and even visual debilitation. The causative microscopic organisms can originate from the outside condition or from foundational contaminations transported by blood [1,2]. Gelrite® is a gellan gum that is a high molecular mass, linear anionic heteropolysaccharide produced aerobically from the bacterium *Auromonas (pseudomonas) elodea*, renamed *Sphingomonas paucimobilis*. The polymer backbone is comprised of a tetrasaccharide repeat unit of Glucose, Glucuronic acid and Rhamnose as shown in molecular structure of Gelrite® in the molar ratio 2:1:1 [3]. Deacetylation of the polysaccharide enables extensive intermolecular association to take place and the formation of strong brittle gels with cations to occur [4]. Human tears contain bicarbonate, chloride, potassium, and calcium ions. Gellan gum forms clear gel in the presence of mono or divalent cations. Because of its ability to form strong clear gels at physiological ion concentration, gellan gum has been widely investigated for use as an *in-situ* gelling agent in ocular formulations. It has a characteristic property of temperature dependent and cation induced gelation. This gelation involves the formulation of an ordered state of gellan chains. X ray diffraction studies have confirmed that a double helix of gellan chains is formed by complexation to cations and hydrogen bonding to water [5].

Conjunctivitis known, also known as "Pink eye" is an inflammation of the membrane (conjunctiva) that covers the inner surface of the eyelid. Hyperactive acute bacterial conjunctivitis most often caused by *N. gonorrhoeae* and *N. meningitides*. Acute bacterial conjunctivitis caused by Staphylococcus species, Streptococcus species, *Pseudomonas, Haemophilus influenza, E.coli* [6]. Approximately 70% of all patients with acute conjunctivitis present to primary care and urgent care

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Table 1: Viscosity studies of 0.5% Gelrite® formulation at 25°C and after addition of STF.

S.No	Shear Rate(1/s)		Shear stress(Pa)		Viscosity			
					Pa.s		Cps	
	At 25ºC	After addition of STF	At 25ºC	After addition of STF	At 25ºC	After addition of STF	At 25ºC	After addition of STF
1	1.79	1.84	23.9	146	15.4	79.3	15400	79300
2	1.81	1.95	27.6	150	15.2	76.9	15200	76900
3	1.91	2.15	29.1	152	15.2	70.6	15200	70600
4	2.22	2.62	30.6	160	13.7	61.8	13700	61800
5	2.57	2.8	31.8	170	12.3	60.7	12300	60700
6	2.83	3.4	33.1	186	11.6	54.7	11600	54700
7	3.19	4.03	35.9	208	11.2	51.6	11200	51600
8	3.47	4.39	38.9	229	11.2	52.1	11200	52100
9	3.79	4.73	41.4	239	10.9	50.5	10900	50500

Table 2: Results of ocular irritation studies.

	Number of rabbits							
	1	2	3	4	5	6	Mean (S.D)	Results of the unpaired *t* test
Mucoidal Discharge (Marketed Formulation)	0	0	0	1	0	1	0.334 (0.5164)	The two-tailed P value is 0.5490, considered non-significant. t = 0.6202 with 10 degrees of freedom.
Mucoidal Discharge (Gelrite Formulation)	0	1	0	0	0	0	0.1667 (0.4082)	
Eyelid closure (Marketed Formulation)	1	1	1	2	1	2	1.33 (0.5164)	The two-tailed P value is 0.3632, considered non-significant. t = 1.00 with 5 degrees of freedom.
Eyelid closure (Gelrite Formulation)	1	1	1	2	1	1	1.166 (0.4)	

[7]. The symptoms may be accompanied by hyperemia, increased secretion, itching, redness, sensitivity to light, swelling of lids. The discharge may be purulent or mucopurulent, gritty sensation and matting of Eyelids. Ofloxacin, a fluorinated quinolone, is a pyridone carboxylic acid derivative, which exerts abroad- spectrum antibacterial effect. Ofloxacin inhibits the enzyme *bacterial DNA Gyrase*. Ofloxacin has *in vitro* activity against a broad spectrum of Gram positive and Gram-negative bacteria [8].

The poor bioavailability and therapeutic response exhibited by the conventional ophthalmic solutions due to pre-corneal elimination of the drug may be overcome by the use of in situ gel forming systems, which upon instillation as drops into the eye undergo a sol-gel transition in the cul-de-sac. In situ forming gels (droppable gels) have been developed to prolong pre-corneal residence time of drugs, improve patient compliance, and enhance ocular bioavailability [9]. The aqueous composition that reversibly gel in response to simultaneous variation in at least two parameters, such as temperature, pH, and ionic strength, can be formed by appropriate combination of macromolecular polymers which exhibit reversible gelation properties [10]. At present, certain challenges remain regarding the development of in situ gels for pharmaceutical application.

First, higher concentrations of polymers and non aqueous solvent in the gel may cause eye irritation and safety issues. A number of studies have been done utilizing *in-situ* gelling polymers like chitosan, carboxymethyl chitosan and glycerophosphate [11], Pluronic F-127 in combination with chitosan [12], gelrite [13-16], carbopol-methyl cellulose [17], carbopol-pluronic [18], pluronic F127 [19], alginate-pluronic [20] and carbopol-HPMC [21]. The bioavailability of the drug (s) was enhanced significantly in all these formulations.

The objective of the present work was to develop an ocular in situ delivery system of Ofloxacin, a fluoroquinolone derivative used in bacterial conjunctivitis. In this study the safety of *in-situ* gel carrying drug by ocular irritation studies and precorneal clearance of the formulation was evaluated by non-invasive single photon emission computing tomography technique in rabbits.

Materials and Methods

Ofloxacin was obtained as a gift sample from Cadila, Ahmedabad and Gelrite® from Kelco, Division of Merck, and U.S.A. Other formulation excipients were of pharmaceutical grade and obtained from standard commercial suppliers.

Preparation of formulation

Different concentration of Gelrite® (0.1-0.6%) was dissolved in hot 0.01M Tris Maleate Buffer (pH 6.2) by continuous stirring at 35-40ºC. Ofloxacin (0.27%) was dissolved in buffer and added to the polymer solutions and stirred until dissolved. Finally, preservatives were added and formulations were filled in amber colored glass vials, capped with rubber bungs and sealed with aluminum caps. The formulations, in their final pack were subjected to terminal sterilization by autoclaving at 121ºC and 15 p.s.i.g for 20min. Simulated tear fluid (STF) according to the electrolyte composition of tear fluid was prepared [22].

In Vitro release studies

The drug release kinetics from the prepared formulation we studied using Flow through Apparatus. The dissolution medium used was Simulated Tear Fluid STF pH. Samples were then analyzed for the drug Ofloxacin at 288nm.

Rheological studies

Viscosity measurement was carried out using Cone and Plate Viscometer (Physica Rheolab, Austria) using MK-22 spindle. The Cone and Plate Viscometer consisted of movable spindle. Different shape and size of spindles are used for determination of viscosity dependent upon type of solution less viscous or more viscous.

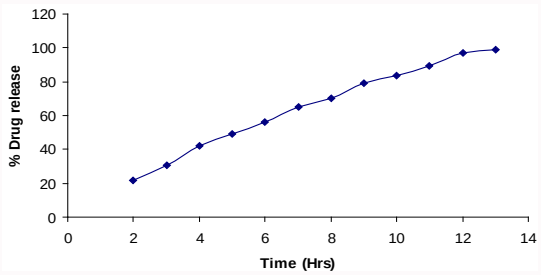

Figure 1: Percentage drug release verses Time of 0.5% Gelrite® formulation.

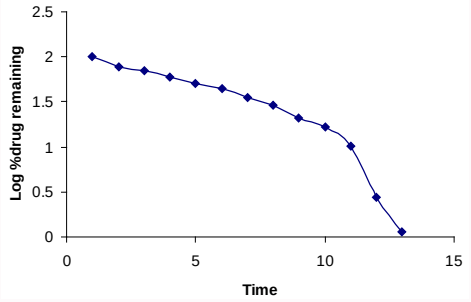

Figure 2: Log % drug remaining verses Time of 0.5% Gelrite® formulation.

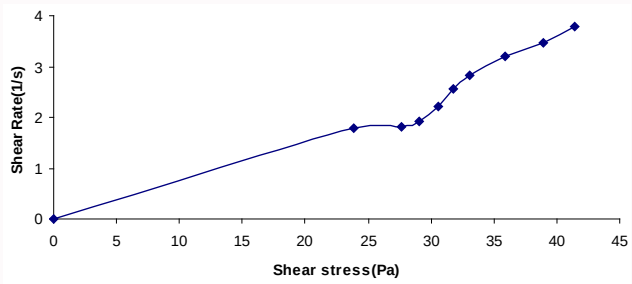

Figure 3: Shear rate verses shear stress of 0.5% Gelrite formulation at 25°C.

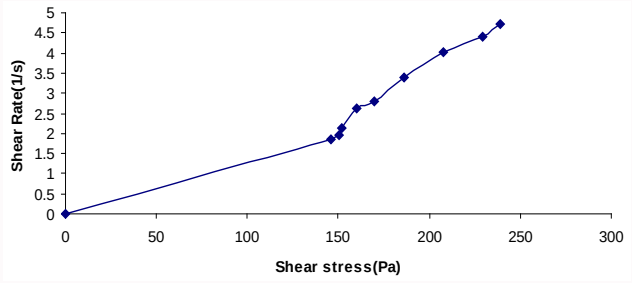

Figure 4: Shear rate verses shear stress of 0.5% Gelrite formulation after addition of STF.

For measurement of viscosity about 1ml of sample was placed on the plate and spindle was touched with the sample, temperature was adjusted at room temperature i.e. 25°C and after addition of STF of pH 7.4 shown in Table 1.

In Vivo studies

Rabbits weighed 2-3 kg were used. Animals were housed 1 per cage and maintained at 20-30% humidity in natural light and dark cycle, with free access to food and water. Permission for the use of animals was obtained from Animal Ethics Committee, of Institute of Nuclear Medicine and Allied Sciences (INMAS) Delhi.

Ocular irritation studies: Discomfort of the rabbit eyes were graded, so that slight irritation was characterized by half closed eyelids and severe irritation by firm closure of the eyelid. The eyelid closure was expressed as the sum of full closure and half closure times of the eyes. Mu coidal discharge was scored from 0-2, where 0 is normal, any clear discharge different from normal is 1, and milky discharge moistening the lids scores 2. Observations are given in Table 2.

Scintigraphic studies: Scintigraphic studies drug was radiolabeled with radionuclide Tc-99m. It was chosen because of its moderate half-life (6hr). Further, it emits gamma rays, that have relatively low energy as compared with α and β rays, and leads to no serious health hazards for the teserrduers.

In Vivo precorneal drainage of Radionuclide was studied using single photon emission computing tomography (SPECT, starcam 3200I X/R General Electric, U.S.A.) auto tuned to detect the 140KeV radiation of Tc99m .The optimized formulation containing 0.5% Gelrite® and marketed eye drops were assessed in three rabbits with a minimum washout period of 3 days. The rabbit was positioned 5 cm in front of the probe and radiolabelled formulation which were stored at 20°C for about half an hour before use, were instilled onto the left corneal surface of the rabbits. The right corneal surface received the market formulation. Radiolabelled formulation was prepared by adding aqueous stannous into the formulation as a reducing agent, pH was set at neutral and after that activity was added. The stability of binding was determined by simple paper chromatography by calculating percentage binding from a Gamma Counter (Electronic Corporation, India).

Recording was started 5sec after instillation and continued for 10 min using 128X128 pixel matrix. Images were analyzed using a Dual Head Gamma Camera (Millinium VG, USA). All the graphs were divided into five regions of interest (ROIs) as shown in Figure 8, and the movement of the gamma emitting material accurately followed within zone.

Results and Discussion

The concentration of Gelrite® was kept at a maximum of 0.5% m/v.

In Vitro release studies

Percentage of drug released versus time and log percentage verses time curves was shown in Figure 1 and 2. The *In Vitro* release studies showed that the gels have the ability to retain the drug and the drugs have higher bioavailability. The coefficient of variation 0.5% Gelrite® formulation was less for first order release constant and higher for zero order release constant which indicated that the release kinetics for this formulation followed first order release rate kinetics.

Rheological studies

The Steady flow viscosity results of 0.5% system was chosen from the *In Vitro* Studies. This formulation has optimum viscosity. Reading was observed and graph was plotted between shear rate and shear stress (Figure 3 and 4) and viscosity and shear rate (Figure 5 and 6) of the formulation 0.5% Gelrite® at 25°C and after addition of STF. The formulation exhibited pseudo plastic rheology, as evident by shear thinning and increase in shear stress with increased angular velocity. The viscosity was directly dependent on the polymeric content. No change in viscosity was observed after autoclaving. The desirable conversion of sol to gel was obtained on addition of STF of pH 7.4, which was supported by viscosity studies.

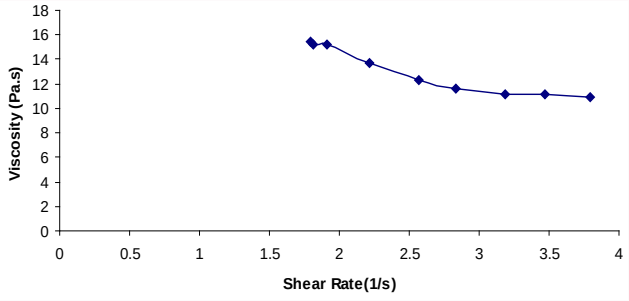

Figure 5: Viscosity verses shear rate of 0.5% Gelrite formulation at 25°C.

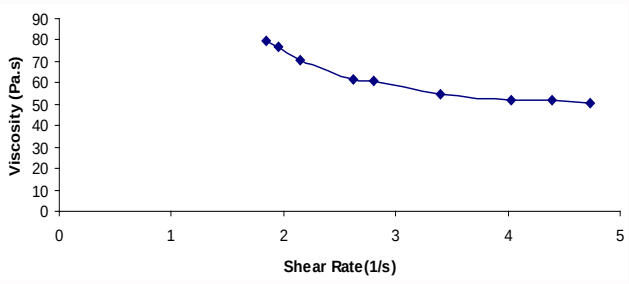

Figure 6: Viscosity verses shear rate of 0.5% Gelrite® formulation after addition of STF.

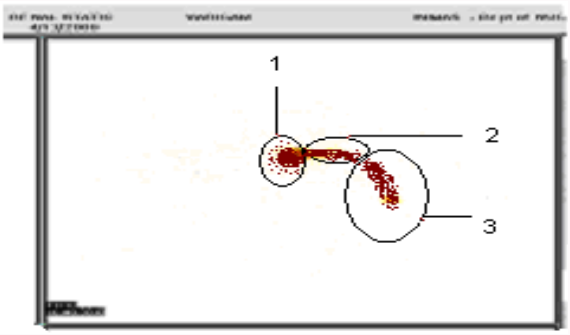

Figure 7: Precorneal retention of 0.5% Gelrite® formulation for 10mins.

Ocular irritation studies

The results of ocular irritation studies indicated that formulation was non- irritant. Excellent ocular tolerance was noted. Abnormal clinical signs to Cornea, iris, or conjunctiva were not seen. Results of ocular irritation studies are given in Table 2.

In Vivo results

Gamma Scintigraphy is a well-established technique for *in vivo* evaluation of the ophthalmic drug delivery systems. Gelrite® 0.5% showed at all times a significantly higher retention of the radioactive tracer in the corneal region of interest in comparison to marketed eye drops as shown in Figure 7. A marketed eye drop was rapidly cleared due to tear turn over, giving decrease bioavailability. Presence of polymer in the optimized formulation prolonged the residence of the radioactive tracer in the precorneal region (Figure 8).

Conclusion

In ocular in situ sol to gel system of Ofloxacin was successfully formulated using Gelrite® as ion-activated polymer. The formulation were therapeutically efficacious, the gel formed in situ afforded

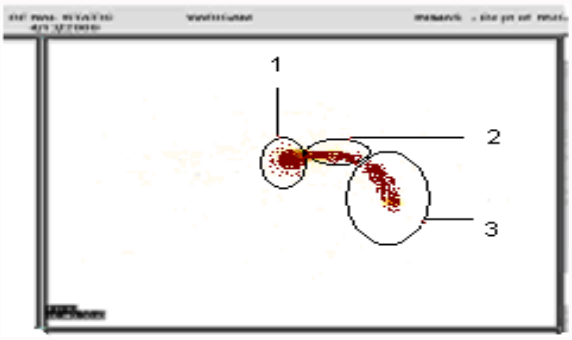

Figure 8: Scintigraphic image divided into three region of interest (ROIs); 1. Corneal surface, 2. Inner canthus, 3. Nasolacrimal duct.

sustained drug release over 12hrs period. Gelrite® 0.5% formulation was non irritating, possess excellent ocular tolerance as determined by ocular irritation studies and prolonged the corneal contact time as measured by gamma scintigraphy.

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