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Development Studies on Gel-forming Erodible Ocular Polymeric Films of Gatifloxacin Sesquehydrate

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Abstract

Gel-forming erodible ocular films of Gatifloxacin sesquehydrate (GS) were prepared with different concentration of sodium alginate and chitosan. The surface of films was treated with 0.2 M calcium chloride solution. Various parameters of formulations including physicochemical properties and bioadhesion were evaluated. Drug release from the prepared films was evaluated using a donor receptor compartment model. The formulation F_5 (2% sodium alginate and 1% chitosan) showed most prolonged drug release of 24 hr indicating the potential of surface cross linking of the film to sustain the drug release. The gelation and residence time studies were carried out for the optimized formulation F_5. The *in-vivo* drug release was also studied and correlated with *in-vitro* release pattern. *In-vivo* drug release and *in-vitro* antimicrobial studies carried out for the formulation F5 indicates the superiority of the ocular films over the marketed eye drop. These results demonstrate that the surface treated alginate-chitosan film could be potential vehicle to enhance ocular GS bioavailability and patient compliance.

Keywords: Gatifloxacin, ocular film, sodium alginate, chitosan, gelation

Introduction

Eye is an easily accessible organ by point of view of topical drug delivery, although it has always presented a challenge to the pharmaceutical scientist when it comes to drug delivery by this route (Laurencin and Langer, 1987; Stjernschntz and Astin, 1993). Most ocular diseases are treated with topical application of eye drops. After instillation of an eye drop, typically less than 5% of the applied drug penetrates the cornea and reaches intraocular tissues, while a major fraction of the instilled dose is absorbed and enters the systemic circulation (Stjernschntz and Astin, 1993; Urtti and Salminen, 1993). There are certain anatomical, physiological, physiochemical and pharmacokinetic factors that leads to inefficient drug delivery and some serious side effects also (Maichuk, 1991; Deshpande et al., 1998). To overcome the poor bioavailability problems many ocular novel drug delivery systems are being developed. Solid ocular drug delivery devices has enjoyed special consideration on account of their solidity, good ocular contact, good drug sustainability, less dosage frequency and excellent therapeutic effects (Pandit et al., 2003; Colo et al., 2001a, 2001b).

Sodium alginate is well known ion activated in-situ gelling agent and has been used in many ocular sol-to-gel transition systems as a means of ocular controlled drug delivery

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systems.(Mishra and Gilhotra, 2008) At the same time, it is also good film forming polymer (Singh and Burgess, 1989; Liu et al., 2006). Chitosan is a deacetylated form of chitin, which is the second-most abundant polymer in nature after cellulose. The potential of chitosan-based systems (chitosan gels, chitosan-coated colloidal systems and chitosan nanoparticles) for improving the retention and bio-distribution of drugs applied topically onto the eye is extensively studied. Besides its low toxicity and good ocular tolerance, chitosan exhibits favorable biological behavior, such as bioadhesion- and permeability-enhancing properties, and also film forming characteristics, which make it a unique material for the design of ocular films (Kas, 1997). Gatifloxacin is a fourth generation fluoroquinolone derivative with a wide spectrum of activity against aerobic and anaerobic bacteria, including most common ocular pathogens as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Takei et al., 1998). It is very effective against external infections of the eye, such as acute and subacute conjunctivitis, bacterial keratitis and keratoconjunctivitis. It affects bacterial DNA gyrase without affecting mammalian DNA activity. Because of its short plasma half-life, it must be instilled as 3–4 drops at least three times a day. Patient compliance and efficacy of drug could be improved by a drug delivery system promoting prolonged release of drug and thus decreasing its application interval.

In the present study, solid drug delivery system based on alginate and chitosan has been explored to enhance gatifloxacin retention and prolong the duration of drug release.

Materials and Methods

Gatifloxacin sesquehydrate (GS) was obtained from Emcure Pharmaceuticals Ltd., Pune. Sodium alginate (250 cps for a 2% solution at 25°C) was a gift sample from Snap Natural and Alginate Products Limited, Ranipet. Water soluble Chitosan (chitosan acetate, 68 cps for a 1% solution at 25°C) was acquired from Indian Sea Foods (Cochin). Calcium chloride was purchased from Sigma Chemicals, Mumbai. All other chemicals used were of reagent grade.

Preparation of surface cross linked ocular films

Method used for preparation of films was solvent casting technique (Pandit et al., 2003). Polymeric solutions were prepared by dissolving sodium alginate and chitosan at distinct compositions (alginate:chitosan 0%:1%, 1%:1%, 1.5%:1%, 1%:2%, 2%:1%, 1.5%:1.5%, 1%:0%) along with 0.4% (m/V) of Gatifloxacin sesquehydrate (GS), and glycerin (10% m/m) in doubly distilled water (Film codes: F_1, F_2, F_3, F_4, F_5, F_6, and F_7 respectively). Drug polymer solutions were stirred for 12 h and allowed to stand overnight to remove any entrapped air bubbles. Solutions were then poured into glass Petri dishes. Solvent was allowed to evaporate by placing the Petri dishes in oven (40 ± 2°C). Dried film was carefully removed from the Petri dish and then cut into oval shaped films with the help of a die (13.2 mm in length and 5.4 mm in width). Each film contained 2.4 mg of the drug. The films were dipped into 0.2 M calcium chloride solution and allowed to crosslink with Ca^{+2} for five second. Films were then rinsed with distilled water several times to remove unreacted calcium chloride on surface. Films were dried at 37°C and stored (24±1°C, 60±5% RH).

Determination of the physicochemical properties

Thickness, weight and surface pH of Films

The thickness and weight of the prepared films were measured by using dead weight thickness gauge and by an electronic balance, respectively. Films were left to swell for 2 hours on agar gel plate prepared by dissolving 2% (m/v) agar in warm simulated tear fluid (STF- composition sodium chloride: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride. $2H_2O$: 0.008 g, and purified water q.s. 100 g) of pH 7.2 under stirring and then pouring the solution into Petri dish till gelling at room temperature. Surface pH was measured by means of pH paper placed on surface of swollen films.

Tensile strength determination

Film was cut into strips (50 x 10mm). Tensile strength and elongation at break was determined by modifying the method used by Dandagi et al. (2004). The apparatus consisted of a base plate with a pulley aligned on it. Film was fixed in film holder at one end of base plate and another end was fixed with help of forceps having triangular end to keep the film straight during stretching. A thread was tied to the triangular end and passed over the pulley, to which a small pan was attached to hold weights. A small pointer was attached to the thread that travels over the graph paper affixed on the base plate. The weights were gradually added to the pan till the film was broken. The weight necessary to break the film was noted as break force and the simultaneous distance traveled by the pointer on the graph paper indicated the elongation at break:

$$\text{Tensile strength } (g/mm^2) = \text{break force (g)} / \text{cross-sectional area of the sample } (mm^2)$$

$$\text{Elongation at break } (\%) = \text{increase in length at break point (mm)} / \text{original length (mm) X 100}$$

Drug Content uniformity

Uniformity of the drug content was determined by assaying the individual inserts. Each insert was grounded in a glass pestle mortar and 5 ml of STF (pH 7.2) was added to make a suspension. The suspension so obtained was filtered and the filtrate was assayed spectrophotometrically at 292 nm (UV-VIS Systronics Spectrophotometer-106).

Bioadhesive Strength

Goat conjunctival membrane was used for the measurement of bioadhesive strength. The membrane was placed in an aerated saline at 4°C, which was later washed with distilled water and STF (pH 7.2, 37°C) before use. Bioadhesive strength of the film (n = 3) was measured on a modified physical balance (Sultana et al., 2006). Membrane was tied to open mouth of a glass vial filled with STF. Vial was fitted in the center of a glass beaker filled with STF (pH 7.2, 37±1°C). Separately, film was adhered to the lower side of a rubber stopper, which was attached to lever of physical balance. The mass (put on other limb of balance) required to detach the patch from the conjunctival surface gave the measure of bioadhesive strength. Force of adhesion was calculated:

$$\text{Force of adhesion (N)} = (\text{Bioadhesive Strength X } 9.81) / 1000$$

In-vitro drug release studies

In vitro drug release study was carried out by using biochemical donor- receptor compartment model (Sreenivas et al., 2006). The commercial semi-permeable membrane cellophane membrane, presoaked overnight in the freshly prepared dissolution medium (STF pH 7.2), was tied to one end of a cylinder (open at both the sides), which acted as donor compartment. The ocular insert was placed inside the donor compartment in contact with the semi-permeable membrane. The donor compartment was attached to a stand and suspended in 25 ml of the dissolution medium maintained at 37±1°C so as to touch the receptor medium surface. The dissolution medium was stirred at a low speed using magnetic stirrer. The aliquots of 5 ml were withdrawn at regular intervals and replaced by an equal volume of dissolution medium. The samples were analyzed spectrophotometrically at 292 nm.

In-vitro gelation study

Gelation studies were carried out in previously described agar gel plates (2 % m/v agar dissolved in warm STF, pH 7.2). At the centre of the plate a cylindrical reservoir capable of holding 3mL of gelation solution (simulated tear fluid, STF) was bored. The formulation was carefully placed into the cavity of

the cylindrical reservoir and 2 mL of gelation solution was added slowly. The plates were covered with transparent cover and the gelation was assessed by visual examination.

In-vivo gelation and residence time study

Approval for the use of animals in the study was obtained from the local Animal Ethics Committee. New Zealand rabbits of either sex weighing 3 to 4.5 kg were used to measure the *in vivo* release of the drug in the eye. The rabbits were housed singly in restraining boxes during the experiment and allowed food and water *ad libitum*. Free leg and eye movement was allowed. The ocular films were inserted in the right eyes of 3 animals and the left eyes received normal saline. The extent of gelation and residence time was assessed by visual examination for 24 h.

***In vivo* drug release studies**

Approval for the use of animals in the study was obtained from the local Animal Ethics Committee. Adult New Zealand rabbits of either sex weighing 3 to 4.5 kg were used to measure the *in vivo* release of the drug in the eye. The rabbits were housed singly in restraining boxes during the experiment and allowed food and water *ad libitum*. Free leg and eye movement was allowed.

There were 9 animals in the experimental group and 3 animals in the control group. Both eyes of the control group animals received normal saline. The ocular films were inserted in both eyes of all animals in the experimental group. Three ocular films were removed at regular interval during 24 h study from eyes of animals of the experimental group. The amount of drug remaining in each ocular insert was determined and cumulative percent drug released *in vivo* was calculated.

In-vitro antimicrobial efficacy

The microbiological studies were carried out to ascertain the biological activity of the optimized formulation and marketed eye drops against microorganisms. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used as the test microorganisms. A layer of nutrient agar (20 mL) seeded with the test microorganism (0.2 mL) was allowed to solidify in the petri plate. Cups were made on the solidified agar layer with the help of sterile borer of 4 mm diameter. Then the optimized formulation and marketed eye drops were poured into the cups. After keeping petri plates at room temperature for 4 h, the plates were incubated at 37 °C for 24 h. The zone of inhibition was obtained. The diameter of zone of inhibition was measured by using an antibiotic zone finder.

Results and Discussion

Physicochemical data presented in Table 1 shows thickness, weight, surface pH, tensile strength and bioadhesive strength of films. The prepared films were smooth in appearance, uniform in thickness, weight and show no visible crack or imperfection. Each ocular film had an area of approximately 77 mm^2. The film had a thickness varying from 0.114±0.0058 to 0.405±0.0070 mm and weight varying from 6.52 ± 0.430 to 12.13±0.160 mg. The variation in thickness and weight uniformity of the prepared films were within acceptable limits. Surface pH was within range of 5.5 – 7. This shows that prepared films would not alter the pH of tear fluid in the eye. Formulation F_5 showed almost two fold tensile strength than formulation F_1 (Table 1). Tensile strength of the films increased as the amount of sodium alginate increased. The formulation F_1 showed maximum % elongation at break, whereas the least was shown by formulation F_7. The films with higher sodium alginate content showed relative poor elongation at break than the one with chitosan. Mechanical strength pattern shown by film indicates the correlation of surface cross linking of sodium alginate with the tensile strength and elongation parameters. The surface cross-linking of sodium alginate with calcium ion leads to surface hardening of the film, which seems to have decreased the flexibility of the film in terms of % elongation, whereas tensile strength was better because of formation of cross links within the

polymer matrix. The drug content was consistent in all batches and varied from 98.0± 0.10% to 99.5 ± 0.30%.

Table 1. Physicochemical and mechanical parameters of the ocular films

Film code	Weight* (mg)	Thickness * (mm)	Surface pH	Tensile strength* (g/mm^2)	Elongation at break (%)	Bioadhesive strength* (g)	Force of adhesion (N)
F_1	6.52 ± 0.430	0.114 ± 0.0058	6.5	0.234 ± 0.0020	46.6	9.6 ± 0.30	0.094
F_2	7.84 ± 0.117	0.197 ± 0.0025	7.0	0.320 ± 0.0024	23.3	9.3 ± 0.57	0.091
F_3	9.54 ± 0.178	0.270 ± 0.0196	5.5	0.264 ± 0.0010	20.0	9.1 ± 0.76	0.089
F_4	10.4 ± 0.281	0.318 ± 0.0098	6.0	0.258 ± 0.0005	36.6	12.3 ± 0.30	0.120
F_5	12.53 ± 0.160	0.405 ± 0.0070	6.0	0.497 ± 0.0009	16.6	9.2 ± 0.30	0.090
F_6	11.58 ± 0.411	0.382 ± 0.0107	6.5	0.245 ± 0.0017	26.6	11.1 ± 0.30	0.108
F_7	6.93 ± 0.160	0.152 ± 0.0050	6.5	0.396 ± 0.0004	13.3	4.2 ± 0.40	0.412

*Value as Mean ± SD (n=3)

Formulation F_4 showed maximum bioadhesive strength and hence maximum force of adhesion. It is evident from the results (Table 1) that films with higher chitosan content show better bioadhesive strength and force of adhesion, however, formulations with sodium alginate also exhibited appreciable bioadhesive performance. The results show the superiority of chitosan as promising bioadhesive material. It was suggested that at neutral and alkaline pH, chitosan has numerous amine and hydroxyl groups as well as number of amino groups that may increase the interaction with the negatively charged group in biological membrane (Henriksen et al., 1996). Cross linked sodium alginate has a unique gelling characteristic which is responsible for its adhesive properties in addition to its high mechanical strength, tack and high elasticity.

The cumulative % of GS released from polymeric films F_1, F_2, F_3, F_4, F_5, F_6, and F_7, as a function of time is shown in Fig. 1, formulation F_1 and F_7 could sustain the drug release for 8 h, formulations F_2, F_3, F_4 and F_6 could sustain the drug release for 12 h. Only formulation F_5 showed a sustained release of the drug for 24 h. Hence, F_5 could be considered to be studied as an optimized "once a day" formulation of GS. The surface cross linking of the sodium alginate in polymer film with Ca^{+2} could be explained through the "egg box model"(Grant et al,1973) . Two G blocks of adjacent polymer chain cross link with Ca^{+2} through interaction with the carboxylic groups in the sugars, which leads to formation of a gel network. When this polymer system undergoes dissolution the superficial gel network layer will first rehydrate and serve as a rate controlling layer for the drug embedded in the film. The drug diffusion from the system will depend on the pore size of Ca-alginate gel which further will depend on the extent of cross-linking related to the sodium alginate content present in the film. As the dissolution fluid penetrates the polymer matrix it further causes the gelation of core polymer layer owing to the Ca^{+2} ions present in STF. The gelled state and the presence of additive like chitosan would be expected to cause gel to dissolve much slower and to release the drug slower. The bioadhesive nature of chitosan present in the formulation also helps to improve the retention of the drug in the pre-corneal area, thereby facilitating the reservoir effect. The results of gelation and residence time study for the formulation F_5 (Table 2) complements the drug release study. The film underwent a constant and slow gelation both in vitro and in vivo leading to formation of an in situ gel of the solid polymer matrix. The residence time study indicated that the film remains

intact through out the study. The results are in unison with the bioadhesive strength (9.2 ± 0.30g) of the formulation which seems to be optimum to retain the formulation *in vivo*. This could be attributed to the bioadhesive nature of chitosan as well as the viscous and consistent in situ gel forming nature of sodium alginate.

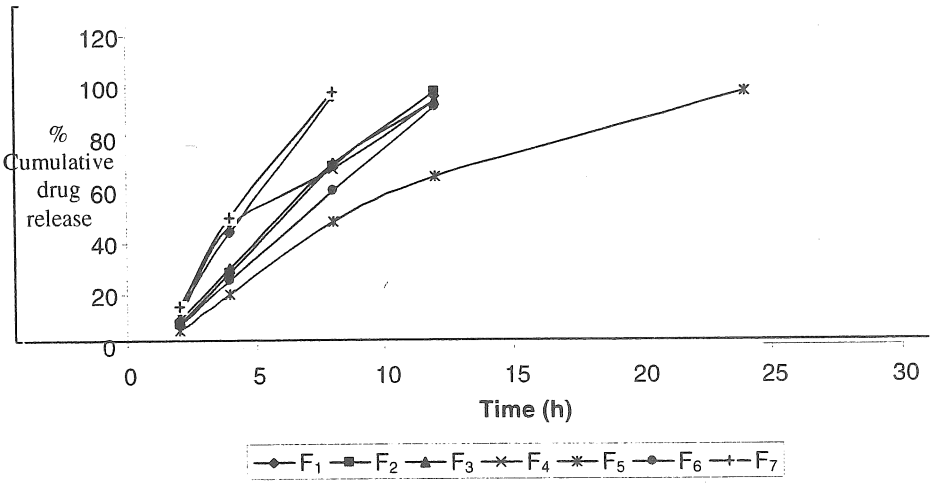

Figure 1. Percent Cumulative drug release Vs time.

Table 2. *In vitro* and *in vivo* gelation and residence time study of formulation F_5

Time	*In-vitro* gelation	*In-vivo* gelation	Residence time
30 min	Superficial hydration	complete film hydration	film intact
1h	complete film hydration	superficial gelation	intact
5h	superficial gelation	partial gelation	intact and start eroding
7h	partial gelation	partial gelation	intact and undergoing erosion
12h	complete film gelation	complete film gelation	eroding and still intact
24h	----	traces of the gel left	complete erosion

In vivo release of the drug from the optimized ocular film F_5 was studied in rabbit's eyes by measuring the content of the drug remaining in the ocular inserts at particular time intervals. For 24 h study, total release observed was 97.5% as shown in Fig. 2. Correlation coefficient (r) values for the cumulative percentage of drug released *in-vivo* and *in-vitro* were found to be very high, and a positive correlation was found (r = 0.9967).

The optimized gel forming ocular film F_5 showed antimicrobial activity when tested microbiologically by cup plate technique. Clear zones of inhibition were obtained in case of the tested formulation and marketed eye drops. The diameter of zone of inhibition produced by formulation against both test organisms were greater than that produced by marketed eye drops (Table 3). Greater antimicrobial effect of the GS *in situ* gelling formulation is probably due to a

fairly constant release of drug from the cross-linked hydrogel drug reservoir which permits drug to be released slowly.

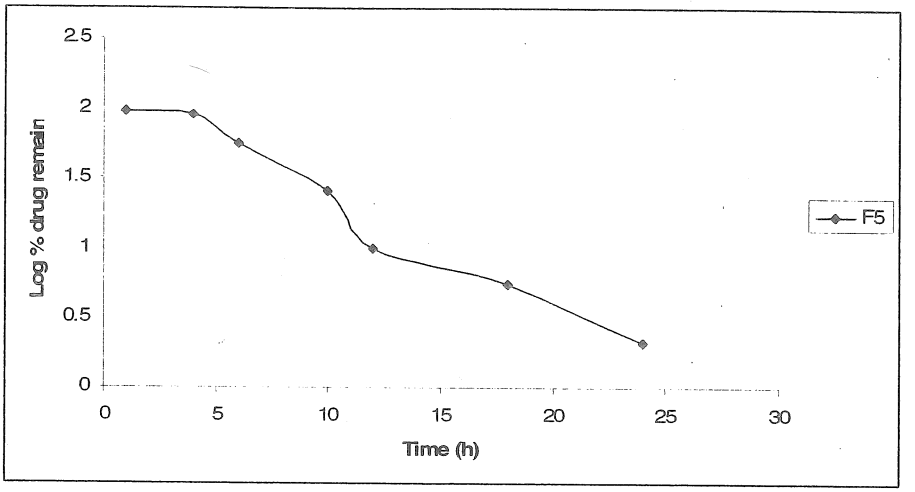

Figure 2. Log % drug remaining Vs Time (h) for optimized formulation F_5 at different time intervals

Table 3. Zone of inhibition produced by the optimized formulation F_5

Micro organisms	Area of the zone of inhibition* (mm^2) after 24 h of incubation	
	Formulation C	Marketed eye drops
S. aureus	615 ± 3.0	450 ± 1.5
E. coli	685 ± 1.5	510 ± 1.2

*Value as Mean ± SD (n=3)

Conclusion

Surface cross-linked sodium alginate–chitosan composite films showed appreciable film forming properties. The films were found to be tough, elastic and bioadhesive, showing potential for use as drug delivery vehicle. *In vitro* drug release , *in vivo* residence time and antimicrobial efficacy study indicates the gel forming surface cross-linked ocular films could be a potential "once a day" dosage form for the treatment of bacterial keratitis and conjunctivitis.

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