Design and characterization of bioadhesive in-situ gelling ocular inserts of gatifloxacin sesquihydrate

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ABSTRACT

Background and purpose of the study: Several polymeric systems have been used to fabricate ocular inserts for better ocular bioavailability and retention to drug of which gelling systems have shown advantages of convenient administration and increased contact time. The purpose of the present study was to develop a bioadhesive in-situe gelling ocular insert of Gatifloxacin using polymeric system of sodium alginate as gelling and chitosan as bioadhesive agent.

Materials and methods: Polymeric ocular inserts of Gatifloxacin sesquehydrate (GS) were composed using sodium alginate and chitosan with glycerin as plasticizer by solvent casting method. The ocular inserts were investigated for physicochemical properties (thickness, weight variation, folding endurance and surface pH), mechanical strength (tensile strength, elongation at break), swelling index, and bioadhesion parameters. *In vitro* release studies were carried using a fabricated donor-receptor compartment model.

Results: Cumulative drug released from the formulation ranged from 95-99% within 8-12h. The formulation D (2% sodium alginate and 1% chitosan) sustained the drug release for the longest period of time (12h). Zero-order release of the drug was from optimized formulation D. A high correlation coefficient (r=0.9845) was recorded between *in vitro* and *in vivo* drug release.

Conclusion: Gatifloxacin sesquehydrate inserts have appreciable film forming properties and were found to posses good antimicrobial efficacy.

Keywords: Gatifloxacin, Ocular insert, Bioadhesion, In situ gelling, Polymers

INTRODUCTION

The surface of the eye is rich in nutrients and consequently, supports a diverse range of microorganisms which constitutes the normal ocular flora(1).However acquisition of a virulent microorganism or uncontrolled growth of an existing organism due to lowered host resistance leads to infections of the external structures of the eye. Bacterial keratitis and conjunctivitis are among the most common ocular infections and in more than 80% of cases, the infections are caused by *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *Pseudomonas aeruginosa* (1). Standard initial treatment consists of frequent instillation of eye drops with a broad-spectrum antibiotic. The drop application schedule requires strict discipline from the patient or care provider since a high and constant antibiotic concentration is intended at the site. However physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs, which results in a short duration of the therapeutic effect. Ocular therapy in the bacterial infections would be significantly improved if the precorneal residence time of drugs could be increased. Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface, but also to slow down drug elimination (2,3). Successful results have been obtained with inserts and collagen shields (4,5). Another approach to optimize bioavailability is the implementation of the mucoadhesive concept. In this method, suitable polymers interact with the mucus layer that coats the external surface of the eye(6). Several polymeric systems are investigated to fabricate ocular inserts for better ocular bioavailability and retention of drugs. Ocular in situ gelling systems offer advantage of convenient administration and increased contact time. These systems undergo sol-to-gel phase transition at ocular surface owing to mainly three mechanisms namely pH triggered system including carbopol (7), temperature dependent systems including pluronics and tetronics(8) and ion activated systems including Gelrite®, gellan and sodium alginate (9).

Alginate is a linear co-polysaccharide isolated from brown seaweeds and certain bacteria. Chemically it is a (1-4)-linked block copolymer of

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â-D-mannuronate (M) and its C-5 epimer R-L-guluronate (G), with residues arranged in homopolymeric sequences of both types and in regions which approximate to the disaccharide repeating structure (MG) (9,10). Commercially alginate is widely used as a gelling agent not only in foods but also in other industries such as pharmaceutical, biomedical, and personal care(11). As it is easy to prepare alginate ionotropic gels under mild conditions, it is possible to entrap drugs and living cells in alginate gels, which allow a wide application of alginate as scaffolds for tissue engineering, drug delivery systems, and cell encapsulation and transplantation(12). When sodium alginate solid matrices are brought in contact with an aqueous medium containing divalent ions e.g. tear fluid, the polymer tend to hydrate, forming a superficial gel, which eventually erodes as polymer dissolves. Drug release from such matrices may be controlled by polymer swelling or erosion or drug diffusion in hydrated gel or by all processes together. All these properties and applications are ultimately dependent on the molecular architecture and gelling mechanism. Its gelling properties have been utilized for making *in situ* gelling systems(13) for ocular delivery. 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 improvement of the retention and bio-distribution of drugs applied topically onto the eye has been extensively studied(14,15).Besides of 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/ inserts(16). The objective of the present study was to develop a bioadhesive, ion activated *in situ* gelling ocular inserts of Gatifloxacin. Polymeric systems of alginate and chitosan were investigated as drug carrier, which undergo hydration and further gelation when instilled into the *cul-de-sac* of eye and provide sustained release of the drug during the treatment of uveitis.

MATERIALS AND METHODS

Materials

Water soluble Chitosan (chitosan acetate, 68 cps for a 1% solution at 25°C) was acquired from Indian Sea Foods (Cochin). Sodium alginate (250 cps for a 2% solution at 25°C) was a gift sample from Snap Natural & Alginate Products Limited, Ranipet. Gatifloxacin sesquehydrate (GS) was obtained from Emcure Pharmaceuticals Ltd., Pune. All other reagents and solvents were of analytical grade and used as received.

Preparation of ocular inserts

The Gatifloxacin ocular inserts based on sodium alginate and water soluble chitosan were prepared by solvent casting technique(17). Polymeric solutions were prepared by dissolving sodium alginate and chitosan at distinct compositions (Table 1 Insert codes: A, B, C, D and E) along with 0.4% (m/V) of gatifloxacin sesquehydrate (GS), and glycerin (10% m/m) in distilled water. Chitosan was added in aqueous solution of sodium alginate and GS with constant stirring. The plasticizer was added thereafter and the drug polymer solutions were stirred for 12 h and allowed to stand overnight to remove any entrapped air bubbles. The pH range of the solutions was found to be 5-8. The solutions were then poured into glass rings (4 cm diameter and 12ml volume) placed over mercury in the glass Petri dishes. Solvent was allowed to evaporate by placing the Petri dishes in oven (40 ± 2°C). Dried films were carefully removed from the Petri dish and then cut into oval shaped inserts with the help of a sharp edged die (13.2mm in length and 5.4 mm in width). Each ocular insert contained 2.4 mg of the drug.

Physicochemical Evaluation

Thickness of Insert

Thickness of the inserts (n=3) was measured using dead weight thickness gauge (Prolific). After initial settings, the foot was lifted with the help of the lifting lever fixed on the side of the dial gauge. Insert was placed on the anvil in such a way that the area where the thickness was to be measured lies below the foot. Readings of the dial gauge were recorded after gentle lowering of foot.

Weight Variation Test

Inserts from each batch were randomly selected and weighed individually on electronic balance (AND HR 2000). Mean weight of inserts (n=20) of each formulation was recorded.

Surface pH Determination

Inserts were left to swell for 5 hours on agar plate prepared by dissolving 2% (m/v) agar in warm simulated tear fluid (STF; sodium chloride: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride. $2H_2O$: 0.008 g, and purified water q.s. 100 g(18)) of pH 7.2 under stirring and then pouring the solution into Petri dish till gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of swollen patch.

Folding Endurance value

The folding endurance is expressed as the number of folds (number of times the insert is folded at the same place, either to break the specimen or to develop visible cracks. This test is important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. The specimen was folded in the center, between the fingers and the thumb and then opened. This was termed as one folding. The process was repeated till the insert showed breakage or cracks in center of insert. The total folding operations were named as folding endurance value (19).

Drug Content uniformity

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

Mechanical Strength

Ocular insert with good tensile strength and percent elongation would resist tearing due to stress generated by blinking action of eye. The film was cut into strips (50 x 10mm). Tensile strength and elongation at break was determined by modification of the reported method (20). The apparatus consisted of a base plate with a pulley aligned on it. The film was fixed in insert 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:

Tensile strength (g/mm^2)= break force (g)/ cross-sectional area of the sample (mm^2)

Elongation at break (%) = increase in length at break point (mm) / Original length (mm) ×100

Swelling index

Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared ocular inserts, initial weight of insert was taken, and then placed in agar gel plate (2% *m/v* agar in STF, pH 7.2) and incubated at 37±1°C. For five hours, insert was removed from plate after every one hour, surface water was removed with help of filter paper, and insert was reweighed. Swelling index was calculated (21).

Swelling Index (S_w) %=[w_t - w_0/w_o] ×100

(S_w)%= equilibrium percent swelling,

w_t: weight of swollen insert after time t

w_0: original weight of insert at zero time

Ex vivo Bioadhesive Strength

Freshly excised conjunctiva membrane of an adult goat was used as model membrane for the measurement of bioadhesive strength. It was obtained from a local slaughter house, and the underlying skin was removed and placed in aerated saline solution at 4 °C until used. Preparation was placed in an aerated saline at 4°C, which was later washed with distilled water and isotonic phosphate buffer (pH 7.2, 37 °C) before use. Bioadhesive strength of the insert (*n* = 3) was measured on a modified physical balance (22). Membrane was tied to open mouth of a glass vial filled with isotonic phosphate buffer. Vial was fitted in the center of a glass beaker filled with STF (pH 7.2, 37±1°C). Separately, insert 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) which was required to detach the patch from the conjunctival surface was a measure of bioadhesive strength. Force of adhesion was calculated:

Force of adhesion (N) = (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 (23). The commercial semipermeable cellophane membrane, presoaked overnight in the freshly prepared dissolution medium (STF pH7.2), and was tied to one end of a cylinder (open at both the sides) which acted as donor compartment. The ocular insert (n = 3) 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 in the way that touches 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 for 12h and replaced by an equal volume of dissolution medium every time. The samples were analyzed spectrophotometrically at 292 nm. (UV-VIS Systronics Spectrophotometer-106)

In vivo 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 had access to food and water *ad libitum*. Free leg and eye movement was allowed. There were 9 animals in the experimental and 3 animals in the control groups. Both eyes of the animals of the control group received normal saline. The ocular inserts were inserted in both eyes of all animals in the experimental groups. Three ocular inserts were removed at regular interval during 12 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, volume of the formulations (optimized formulation and marketed eye drops) containing equivalent amounts of drug was 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 diameter of zone of inhibition was measured by using an antibiotic zone finder.

RESULTS AND DISCUSSION

Physicochemical data presented in table 2 shows thickness, weight, surface pH, folding endurance and drug content uniformity of the prepared inserts. The prepared inserts were translucent, colorless, smooth in texture, uniform in appearance and show no visible crack or imperfection. Each ocular insert had an area of approximately 77 mm^2. The insert had a thickness varying from 0.199 ± 0.0027 to 0.417 ± 0.0043 mm and weight varying from 7.52±0.18 to 11.40±0.54 mg. It was found that the thickness and weight of the inserts were increased by increase in the total polymer concentration. The inserts were found to possess uniform weight and thickness within the batch. The recorded folding endurance for all batches was greater than 300, which is considered satisfactory and reveals good film properties. Surface pH was within range of 5.5 – 7 which shows that prepared inserts would not cause irritation in the eye. The drug content was consistent in all batches and varied from 97.9 ± 0.10% to 99.7± 0.15%.

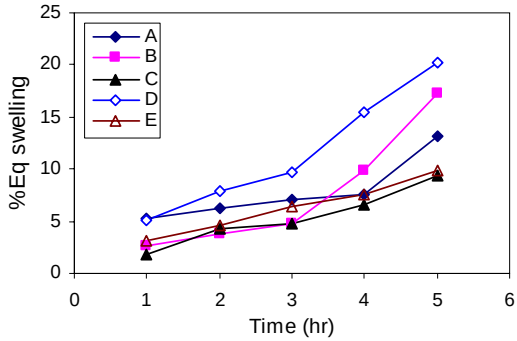

Figure 1. Swelling index of Ocular inserts from batches A to E.

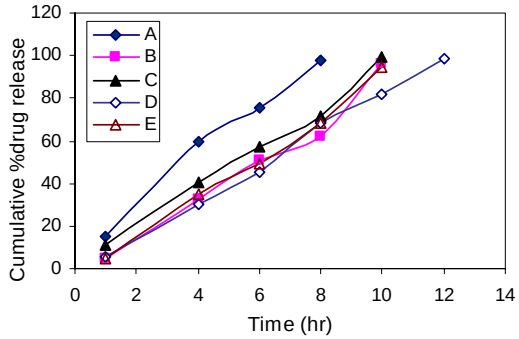

Figure 2. Cumulative % of drug released vs. time for formulations.

The mechanical, swelling and bioadhesive parameters of the prepared ocular inserts are shown in table 3. Formulation D showed maximum tensile strength followed by formulations E, C, B and A (showing least tensile strength). Tensile strength of GS insert increased as the total amount of polymer was increased. However the tensile strength could be related to the sodium alginate content as the inserts with higher sodium alginate content (polymer wt/total polymer content) showed greater tensile strength. Elongation percent was maximum for formulation C followed by E, A, B and D formulation. The equilibrium swelling % varied from 9.75± 0.154% (Formulation C) to 20.69±0.670% (Formulation D). Increase in amount of chitosan in formulation decreased swelling, which may be attributed to its relatively poor water solubility(24).Sodium alginate forms hydrogels and swells considerably in aqueous medium without dissolution(25). This gel forming property and also better aqueous solubility leads to water penetration in polymer matrix and relatively higher swelling. Figure.1

Table 1. Percent composition of gatifloxacin ocular inserts.

Insert code	Polymers		Drug	Plasticizer
	Sodium alginate (%)	Chitosan (%)	Gatifloxacin (%)	Glycerin (% m/m of total polymer weight)
A	1.0	1.0.	0.4	10
B	1.5	1.0	0.4	10
C	1.0	2.0	0.4	10
D	2.0	1.0	0.4	10
E	1.5	1.5	0.4	10

Table 2. Physicochemical parameters of the ocular inserts.

Insert code	Weight# (mg)	Thickness* (mm)	Folding* Endurance	Surface pH*	%Drug content*
A	7.52 ± 0.18	0.199 ± 0.0027	372 ± 8	7.0±0.5	99.7 ± 0.15
B	9.15 ± 0.21	0.278 ± 0.0667	381 ± 9	5.5±0.5	99.4 ± 0.45
C	10.41 ± 0.20	0.320 ± 0.0040	390 ± 11	5.5±0.0	98.0 ± 0.30
D	12.08 ± 0.70	0.417 ± 0.0043	367 ± 7	6.0±0.0	98.5 ± 0.36
E	11.40 ± 0.54	0.391 ± 0.0345	400 ± 9	6.0±0.5	97.9 ± 0.10

*Value as Mean ± SD (n=3); # Value as Mean ± SD (n=20)

Table 3. Mechanical, Swelling and Bioadhesive parameters of ocular inserts

Insert code	Tensile strength* (g/mm^2)	Elongation at break (%)	Equilibrium Swelling* (%)	Bioadhesive strength* (g)	Force of adhesion (N)
A	0.224 ± 0.0067	29.2	13.78 ± 0.220	8.9 ± 0.60	0.087
B	0.234 ± 0.0031	28.1	17.89 ± 0.210	8.5 ± 0.50	0.083
C	0.238 ± 0.0020	39.9	9.75 ± 0.154	10.5± 0.25	0.102
D	0.423 ± 0.0070	24.4	20.69 ± 0.670	8.7± 0.40	0.085
E	0.300 ± 0.0022	35.5	10.55 ± 0.235	10.2 ± 0.30	0.099

*Value as Mean ± SD (n=3)

Table 4. Kinetic model for the formulations A, B, C, D and E.

		Formulations				
		A	B	C	D	E
ZERO ORDER	R	0.9753	0.9654	0.9764	0.9853	0.9792
	K	-0.1310	-0.9816	-3.1596	0.1643	-2.6643
	t-Test	10.817	9.070	10.657	8.213	11.800
FIRST ORDER	R	-0.9235	-0.9501	-0.9471	-0.9261	-0.9751
	K	-0.0002	-0.0002	-0.0002	-0.0002	-0.0001
	t-Test	7.174	8.334	10.219	8.506	15.238
MATRIX	R	0.9438	0.9343	0.9297	0.9386	0.9381
	K	-37.780	-23.4018	-31.875	-19.289	-26.170
	t-test	6.990	6.148	6.182	6.665	6.634
PEPPAS	R	0.9836	0.9797	0.9750	0.9402	0.9670
	K	0.6699	0.2587	0.4053	-0.2322	0.2240
	t-test	13.371	11.978	10.755	6.761	9.292
HIXON CROWELL	R	-0.9201	-0.9331	-0.9470	-0.9361	-0.9751
	K	-0.001	-0.0001	-0.0001	-0.0001	0.000
	t-Test	6.172	7.331	11.217	10.506	15.232
t-Table at p<0.05 (Two tails) DF =n– 2		2.306	2.356	2.176	2.179	2.179

shows the swelling profiles of the ocular inserts for 5 hr. Formulation C showed maximum bioadhesive strength and hence maximum force of adhesion. It is evident from the results (Table 3) that inserts with higher chitosan content show better bioadhesive strength and force of adhesion.

Table 5. Zone of inhibition produced by the optimized formulation D.

Micro organisms	Area of the zone of inhibition (mm^2) after 24 h of incubation	
	Formulation D	Marketed eye drops
S. aureus	585 ± 3.0	455 ± 1.5
E. coli	665 ± 1.5	500 ± 1.2

Number of observations, n=3.

The results show the superiority of chitosan as promising bioadhesive material at neutral or slightly alkaline pH (26), which is found to be advantageous for adsorption on the ocular surface. It was suggested that at neutral and alkaline pH, chitosan has numerous amine and hydroxyl groups as well as a number of amino groups that may increase the interaction with the negatively charged group in biological membrane (27).

The cumulative percent of GS released from in situ gelling polymeric inserts A, B, C, D and E, as a function of time is shown in Figure. 2, which reveal that 98% of drug was released from formulation A in 8 h, 96% of drug was released from formulation B in 10 h, 99% of drug was released from formulation C in 10 h, 98% of drug was released from formulation D in 12 h, and 95% of drug was released from formulation E in 10 h. These results suggested that GS was released in a sustained manner from formulation D, when the content of polymers was 2% Sodium alginate and 1 % of chitosan. The formulation D showed the potential of sustaining the drug release for the longest period of time and hence formulation D was selected as optimized formulation. In order to understand the drug release mechanism, the release data was tested assuming common kinetic model (28) (Table 4). The best- fit kinetic model for the optimized formulation D was the zero order kinetic model (R = 0.9853, k = 0.1643). There was not sufficient linearity for Peppas, Hixon Crowell and first order kinetic models. The drug release form such system is controlled by the dissolution fluid, which permeate through the superficial polymer layer and create sufficient internal pressure to drive the drug out. During dissolution the sodium alginate present in the film absorbs a significant amount of water to hydrate, swell and form a stable hydrogel upon exposure to the divalent cations Ca^{+2} present in STF. The Gel formation in the presence of Ca^{+2} has been explained through the "egg box model"(29). Two G blocks of adjacent polymer chain can be cross linked with Ca^{+2} through interaction with the carboxylic groups in the sugars, which leads to formation of a gel network. The drug GS which is embedded in the chitosan alginate film is now immobilized in the polymer matrix because of the cross linked gelation. The in-situ gel forming inserts acts as a drug reservoir which release drug from the matrix depending on the pore size of the Ca-alginate gel. 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.

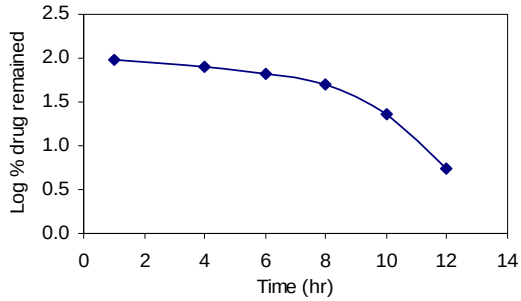

Figure 3. Log % of drug remaining vs. time for optimized formulation at different time intervals.

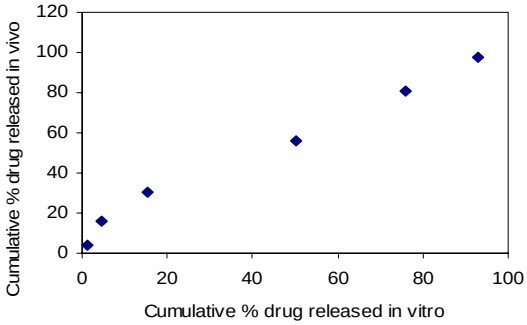

Figure 4. Scatter diagram showing in vitro and in vivo correlations of ocular insert D (r=0.9845).

In vivo release of the drug from the optimized ocular inserts D was studied in rabbit's eyes by measurement of the content of the drug remaining in the ocular inserts at particular time intervals. For 12 hr study, total observed release was 95.4% as shown in Fig. 3. Correlation coefficient (r) values for the cumulative percentage of drug released in vivo were found to be very high, and a positive correlation was found (r= 0.9845). Scatter diagrams Fig 4 also showed high correlation between *in vitro* and *in vivo* release studies for the formulation. The optimized gel forming ocular

insert D showed antimicrobial activity when tested microbiologically by cup plate technique. Clear zones of inhibition were obtained in case of formulation C and eye drops in the market. The diameter of zone of inhibition produced by formulation C against both test organisms were greater than those produced by marketed eye drops of the market (Table 5). The 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 to the target site relatively slowly.

CONCLUSION

Sodium alginate–chitosan ocular inserts of Gatifloxacin sesquehydrate showed appreciable film forming properties. The inserts were found to be uniform, tough, elastic and bioadhesive. On the basis of in vitro, in vivo, and microbiological studies, it could be concluded that Gatifloxacin sesquehydrate could be successfully administered through gel forming controlled release ocular inserts for treatment of bacterial keratitis and conjunctivitis.

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