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Alginate-chitosan film for ocular drug delivery: Effect of surface cross-linking on film properties and characterization

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The characteristics of alginate-chitosan films intended for ocular drug delivery of gatifloxacin sesquihydrate were compared with the ionically surface cross-linked films of similar compositions. The effect of polymer ratios and cross-linking was studied relatively to various parameters of formulations including physicochemical, mechanical strength, swelling and bioadhesion. The drug release profiles and drug release mechanisms were compared. The folding endurance, tensile strength, bioadhesive strength considerably increased whereas swelling index, elongation at break decreased with surface cross-linking of the films. Surface cross-linked formulation F3 (2% w/v sodium alginate and 1% w/v chitosan) showed most prolonged drug release of 24 h indicating the potential of surface cross linking of the film to sustain drug release. As per the kinetic models both type of films showed a constant drug release, however the drug release mechanism transformed from erosion to diffusion after cross linking. These results demonstrate that the surface treated alginate-chitosan film could be a potential vehicle to enhance ocular GS bioavailability and patient compliance.

1. Introduction

Alginate polymers are anionic polysaccharides composed of blocks of 1,4-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. The blocks may be homopolymeric (MM and GG) or consist of alternating MG sequence (Smidsrød 1974; Smidsrød and Haug 1972). Alginates can interact with polyvalent cations such as Ca^{+2}, Al^{+3} and the cross-linking ability of the alginates is strongly correlated with the composition of the polymer. Sodium alginate is a well known ion activated gelling agent and has been used in many ocular sol-to-gel transition systems as a means of controlled drug delivery systems (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 has extensively been studied (Kas 1997). 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. 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. 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 (Mishra and Gilhotra, 2008).

In recent years much attention has been paid to ocular sustained release drug delivery systems. Polymeric films are considered to be the simplest and cheapest method for production of ocular sustained delivery systems. They have special consideration on account of their solidity, good ocular contact, good drug sustainability, less dosage frequency and excellent therapeutic effects (Maichuk 1991). However researchers have attempted to further enhance the drug bioavailability from solid ocular systems by making minitablets (Weyenberg et al. 2004), one side coated matrices (Sasakia et al. 2003), implementing the rate controlling membranes to drug reservoirs (Sultana et al. 2005) and physically cross-linking the polymeric films (Pandit et al. 2003).

This study was performed in order to evaluate the properties of alginate-chitosan films intended for ocular drug delivery of gatifloxacin sesquihydrate compared to ionically surface cross-linked films of similar compositons. The effect of relative polymer ratio and cross-linking on films' tensile strength, swelling profile, bioadhesion and drug release profile were evaluated.

Table 1: Composition of the drug polymer film and film weight, thickness before and after cross linking

Formulation	Composition(%)			Film weight (mg)			Thickness (mm)		
	Na Alginate	Chitosan	Gatifloxacin	Before cross linking	After cross linking	% Change	Before cross linking	After cross linking	% Change
F1	1.5	1	0.4	9.51 ± 0.21	9.54 ± 0.17	5.8	0.278 ± 0.066	0.270 ± 0.019	2.80
F2	1	2	0.4	10.41 ± 0.20	10.74 ± 0.28	3.17	0.320 ± 0.004	0.318 ± 0.009	0.625
F3	2	1	0.4	12.08 ± 0.70	12.53 ± 0.16	3.72	0.417 ± 0.004	0.405 ± 0.0107	2.87
F4	1.5	1.5	0.4	11.40 ± 0.54	11.58 ± 0.41	1.5	0.391 ± 0.034	0.382 ± 0.107	2.30

2. Investigations, results and discussion

Table 1 shows the composition of the prepared films along with their respective weight and thickness before and after surface cross linking. All 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 0.270 ± 0.019 to 0.417 ± 0.004 mm and weight varying from 9.51 ± 0.21 to 12.53 ± 0.16 mg. The variation in thickness and weight uniformity of the prepared films were within acceptable limits. There was a little weight gain (<6%) and thickness change (up to 2.87%) before and after cross-linking in the films. The films retained their integrity and appearance after cross-linking, but there was a considerable hardening of the film surface. The recorded folding endurance (Table 2) for all the films was greater than 300, which is considered satisfactory and reveals good film properties. After cross-linking tensile strength increased up to approximately 18% indicating an increase in film strength, whereas the elongation at break has decreased up to 32% indicating the loss of flexibility. The extent of cross-linkages between polymer chains determines the level of mechanical strength of the matrix. Clearly, there is a stoichiometric maximal number of cross-links that will depend on the sodium alginate content in this case as the cross linker concentration (0.2M $CaCl_2$) and time of exposure to cross-linking have been the same for all the formulations. Hence the film with higher sodium alginate showed better tensile strength and lower elongation at break on cross-linking. This study indicates that cross-linking increased the mechanical strength and stiffness of the matrix film. The drug content was consistent in all batches and varied from $98.0 \pm 0.10\%$ to $99.5 \pm 0.30\%$.

Swelling behavior was assessed by measuring equilibrium degree of swelling by the weight method (Table 3). The swelling extent of the formulation decreases up to 30% after the cross-linking. This indicates that the film dissolution has considerably decreased because of surface treatment. Figure 1 shows the swelling profiles of the ocular films for 5 h. There was an enhancement of both bioadhesive strength and force of adhesion in the films with cross-linking. Formulation F2 showed maximum bioadhesive strength and hence maximum force of adhesion. It is evident from the results (Table 3) that films with higher chitosan content show better bioadhesive strength and force of adhesion. However, the surface cross-linking of alginate also enhanced the bioadhesive performance. It is

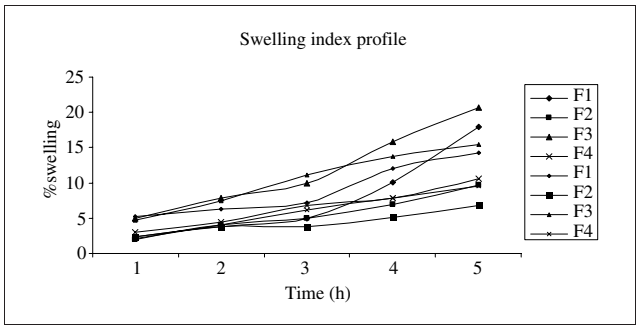

Fig. 1: Swelling index for the formulations before and after surface cross-linking
____ line indicate non cross-linked film
------ line indicate cross-linked film

Table 2: Folding endurance, tensile strength and elongation at break of films before and after cross-linking

Formulation	Folding endurance			Tensile strength (g/mm^2)			Elongation at break		
	Before cross Linking	After cross Linking	% Change	Before cross Linking	After cross Linking	% Change	Before cross Linking	After cross Linking	% Change
F1	381 ± 9	390 ± 10	2.3	0.234 ± 0.003	0.264 ± 0.001	12.8	28.1	20.0	28.5
F2	390 ± 11	400 ± 14	2.5	0.238 ± 0.002	0.258 ± 0.000	8.4	39.9	36.6	8.5
F3	367 ± 7	385 ± 12	4.9	0.423 ± 0.007	0.447 ± 0.000	17.5	24.4	16.6	32
F4	400 ± 9	410 ± 9	2.5	0.300 ± 0.002	0.295 ± 0.001	1.6	35.5	26.6	24

Table 3: Equilibrium swelling, bioadhesive strength and force of adhesion of films before and after cross-linking

Formulation	Eq. swelling (%)			Bioadhesive strength (g)			Force of adhesion (N)		
	Before cross linking	After cross linking	% Change	Before cross Linking	After cross Linking	% Change	Before cross linking	After cross linking	% Change
F1	17.89 ± 0.21	14.34 ± 0.10	20	8.5 ± 0.50	9.1 ± 0.76	7	0.083	0.089	7.2
F2	9.75 ± 0.15	6.78 ± 0.37	30	10.5 ± 0.25	12.3 ± 0.30	17	0.102	0.120	17.6
F3	20.69 ± 0.67	15.54 ± 0.53	25	8.7 ± 0.40	9.2 ± 0.30	6	0.085	0.090	5.8
F4	10.55 ± 0.23	9.65 ± 0.15	10	10.2 ± 0.30	11.1 ± 0.30	9	0.099	0.108	9.0

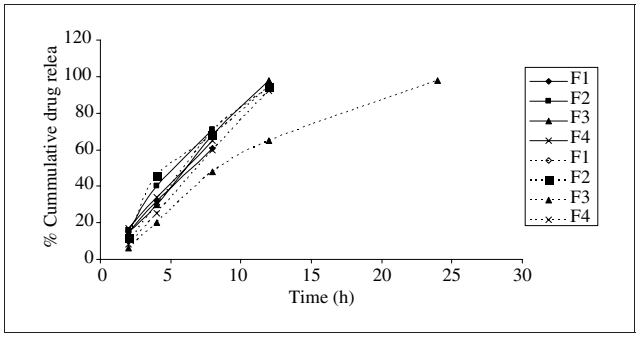

Fig. 2: Cumulative % drug released vs. time for the formulations before and after surface cross-linking
____ line indicates non cross-linked film
------ line indicates cross-linked film

quite evident 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 membranes (Henriksen et al. 1996). Cross linked sodium alginate has unique gelling characteristics which are responsible for its adhesive properties in addition to its high mechanical strength and tack.

The cumulative % of GS released from polymeric films as a function of time is shown in Fig. 2, non cross-linked formulations sustain the drug release for 8–12 h, however the surface cross-linked formulations F1, F2, F4 sustained the drug release for 12 h which was higher than their non cross-linked forms. Formulation F3 showed a sustained release of the drug for 24 h. Hence, F3 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 by 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 will further 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.

Table 4 shows the rate constants and correlation coefficients of the fits of release data to different kinetic models for matrices prepared before and after cross-linking. The correlation coefficient for the best statistical line revealed that the Higuchi model was better applicable to the release data. The reduction in release rate by surface cross-linking was due to the increased thickness of diffusional path on the surface of film over which the drug must diffuse and also due to production of harder films, which retarded the diffusion of water into the matrices and consequently prolonged the time required for disintegration of matrices. The value of n for non cross-linked film indicates that mostly erosion controls drug release from these matrices while diffusion was the main mechanism for the cross-linked films. This transition in drug release mechanism from erosion to diffusion owes to polymer cross-linking resulted in delayed drug release.

3. Experimental

3.1. Materials

Gatifloxacin sesquihydrate (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.

3.2. Methods

3.2.1. Preparation of surface cross linked ocular films

Ocular films of GS were prepared using film casting method (Sultana et al. 2005; Pandit et al. 2003). Polymeric solutions were prepared by dissolving sodium alginate and chitosan at distinct compositions (Table 1) along with 0.4% (w/v) of GS, and glycerin (10% w/w) in doubly distilled water. 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 an oven (40 ± 2 °C). The 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). The films were dipped into 0.2 M calcium chloride solution and allowed to cross-link with Ca^{2+} for 5 s. 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).

3.2.2. Determination of thickness, weight and tensile strength

The thickness and weight of the prepared films were measured with a dead weight thickness gauge and an electronic balance, respectively. Films were left to swell for 2 h on an agar gel plate prepared by dissolving 2% (w/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 the petri dish until gelling at room temperature. Surface pH was measured by means of pH paper placed on the surface of swollen films (Mishra and Gilhotra 2008).

Film strips (50 × 10 mm) were evaluated for tensile strength and elongation at break by modifying the method used by Dandagi et al. (2004). The apparatus consisted of a base plate with a pulley aligned on it. The film was fixed in a film holder at one end of the base plate and the other end

Table 4: Values of various parameters obtained from fit of release data to different kinetic models for films prepared before and after cross-linking

Formulation	Non-cross linked films				Cross linked films			
	Higuchi model		Zero order model		Higuchi model		Zero order model	
	r	n	R	k	r	n	R	k
F1	0.951	0.87	0.965	−0.9816	0.903	0.42	0.833	1.1130
F2	0.970	0.99	0.964	−3.1596	0.882	0.44	0.884	0.8412
F3	0.990	0.89	0.985	0.1643	0.966	0.47	0.904	0.8422
F4	0.944	0.87	0.979	−2.6643	0.942	0.44	0.784	1.0120

was fixed with forceps having a 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 until 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:

$$\text{Tensile strength } (g/mm^2) = \text{break force (g)}/\text{cross-sectional area of the sample } (mm^2) \quad (1)$$

$$\text{Elongation at break } (\%) = \text{increase in length at break point (mm)}/\text{original length (mm) X } 100 \quad (2)$$

3.3. 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).

3.4. Swelling index

To determine the swelling index of prepared films, initial weight of film was taken, and then it was placed in an agar gel plate (2% w/v agar in STF, pH 7.2) and incubated at 37 ± 1 °C. For 5 h, film was removed from plate after every hour, surface water was removed with the help of filter paper, and the film was reweighed. The swelling index was calculated as follows (Wan et al. 1995).

$$\text{Swelling Index } (S_w)\ \% = [w_t - w_0/w_o] \times 100 \quad (3)$$

(S_w) % = equilibrium percent swelling, w_t = weight of swollen film after time t, w_0 = original weight of film at zero time.

3.5. 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. The 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 was regarded as bioadhesive strength. Force of adhesion was calculated:

$$\text{Force of adhesion (N)} = (\text{Bioadhesive strength} \times 9.81)/1000 \quad (4)$$

3.6. In vitro drug release studies

In vitro drug release study was carried out by using the biochemical donor-receptor compartment model (Sreenivas et al. 2006). A commercial semi-permeable 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.

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