Original Article

Failure of calcium gluconate internal gelation for prolonging drug release from alginate–chitosan-based ocular insert of atenolol

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Abstract

Background: The aim of the investigation was to develop and evaluate ocular polymeric film of atenolol for the management of glaucoma. **Materials and Methods:** Fixed concentration blends of sodium alginate (NaAlg) and chitosan were combined with the varying concentration of calcium gluconate and the resulting hydrogels were casted as ocular films. Various physicochemical studies and *in vitro* release tests of the prepared films were carried out to study the effect of calcium gluconate addition to alginate–chitosan blend films. **Results:** Cumulative % drug released from the formulations ranged from 95 to 99% within 5- to 12-hour period. The drug release enhanced with incorporation of higher ratios of calcium gluconate. F1 (2% NaAlg and 1% chitosan without calcium gluconate) sustained the drug release for the longest period of time (12 hours). Addition of calcium gluconate to the formulation resulted in faster drug release rather than sustained drug release. **Conclusion:** The results showed that the addition of calcium gluconate leads to a change in the release capacities of the matrices. Despite the presence of calcium ions and thus the possibility of an ionic interaction, the internal gelation of the polymer matrix lead to enhanced drug release and poor sustaining of drug. The sustained release effect of NaAlg–chitosan matrices alone was the best among the formulations studied.

Key words: Atenolol, chitosan, ocular film, prolonged release, sodium alginate

INTRODUCTION

The eye as a portal for drug delivery is generally used for local therapy against systemic therapy in order to avoid the risk of eye damage from high blood concentrations of the drug, which is not intended. The unique anatomy, physiology, and biochemistry of the eye render this organ impervious to foreign substances, thus presenting a constant challenge to the formulator to circumvent the protective barriers of the eye without causing permanent tissue damage. That is why eye drop administration often results in poor bioavailability and therapeutic response due to rapid precorneal elimination of the drug and is also associated with patient compliance problems.[1,2]

One of the new classes of drug delivery systems, polymeric film ocular drug delivery systems, which are gaining worldwide accolade, release drugs at a preprogrammed rate for a longer period by increasing the precorneal residence time. At the same time, there are certain ocular disorders like glaucoma which are better treated by maintaining a constant and longer drug concentration at the ocular cul-de-sac which is appreciably matched with the manner of drug release by ocular films.[3] Glaucoma has characteristic of optic disc cupping and visual field defect. Patients with glaucoma, without appropriate treatment, may ultimately lead to blindness. There are

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more than 67 million glaucoma patients worldwide.[4] For effective glaucoma treatment, patients need multiple-dose eye drops to effectively control Intraocular pressure (IOP) for the further prevention of glaucoma progression. However, some patients develop serious side effects as a result of the systemic absorption of the instilled drug from the nasal and nasolacrimal mucosa through the tear drainage pathway.[5,6] Therefore, it is important to develop a suitable long-term ocular delivery system to control IOP within an appropriate duration or minimizing dosing frequency, reducing systemic absorption, and optimizing therapeutic effect. Polymer ocular films or inserts are potential dosage form with respect to above discussed factor of glaucoma management, as drug-loaded ocular films dissolve slowly to maintain and release drug concentration at cul-de-sac to complement the therapy.[7]

Sodium alginate (NaAlg), a water-soluble salt of alginic acid, is a natural polysaccharide extracted from marine brown algae. It contains two uronic acids, β-D mannuronic acid (M), and α-L-guluronic acid (G), and it is composed of homopolymeric blocks MM or GG, and blocks with an alternating sequence (MG blocks).[8] NaAlg has been used as a matrix for entrapment of drugs[9,10] and macromolecules.[11] Some of the applications of NaAlg relate to a particular property: it can form hydrophilic gels by interaction with bivalent metal ions. Because alginate gel can easily be formed by this ionic interaction in aqueous medium, gel beads are commonly obtained by dropping solutions of NaAlg into solutions of calcium chloride.[12] Gelation occurs by cross-linking of the uronic acids with divalent cations, such as Ca^{+2}. The primary mechanism of this gelation involves extended chain sequences which adapt a regular two-fold conformation and dimerize with specific chelation Ca^{+2}, the so-called 'egg-box' structure.[12] Each Ca^{+2} ion takes part in nine co-ordination link with an oxygen atom, resulting in three-dimensional network of calcium alginate. Therefore, calcium gluconate is used as an excipient to study the possible ionic interaction and cross-linking with alginate. 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 biodistribution of drugs applied topically onto the eye is extensively studied.[13] Besides its low toxicity and good ocular tolerance, chitosan exhibits favorable biological behavior, such as bioadhesion- and permeability-enhancing properties.[13] The authors had demonstrated in their previous work the effect of surface cross-linking on the properties and characterization of alginate–chitosan-based ocular film.[14,15]

In the present study, an attempt has been made to develop atenolol ocular films/inserts and study further the effect of internal cross-linking by using calcium gluconate on alginate–chitosan polymer system. No such ocular inserts of atenolol had been reported before.

MATERIALS AND METHODS

Materials

Water-soluble chitosan (chitosan acetate, 68 cps for a 1% solution at 25°C) was generously gifted from Indian Sea Foods (Cochin, India). NaAlg (250 cps for a 2% solution at 25°C) was a gift sample from Snap Natural and Alginate Products Limited, Ranipet, India. Atenolol was gifted by Torrent Pharmaceuticals, Baddi (India). Calcium gluconate was purchased from Sigma Chemicals, Mumbai, India.

Methods

Preparation of ocular films

Atenolol ocular films based on NaAlg, water-soluble chitosan, and calcium gluconate were prepared by solvent-casting technique. Polymeric solutions were prepared by dissolving NaAlg and chitosan at fixed compositions (Table 1, film codes: F1, F2, F3, F4, F5) along with 0.4% (m/V) of atenolol and glycerin (10% m/m) in distilled water. Chitosan was added in aqueous solution of NaAlg and atenolol with constant stirring. The plasticizer was added thereafter and the drug polymer solutions were stirred for 5 hours. Calcium gluconate solution was then

Table 1: Composition of ocular films and their respective drug content, weight, thickness, surface pH, and mechanical properties

Formulation	Calcium gluconate % w/w	Drug content (%)	Weight# (mg)	Thickness* (mm)	Surface pH*	Tensile strength* (g/mm2)	Elongation at break (%)
F1	0	99.12 ± 0.13	9.14 ± 0.13	0.40 ± 0.00	7.0 ± 0.5	0.29 ± 0.00	17.5
F2	0.5	97.14 ± 0.05	8.97 ± 0.11	0.37 ± 0.06	6.5 ± 0.5	0.28 ± 0.00	23.2
F3	1	98.40 ± 0.10	9.78 ± 0.20	0.32 ± 0.00	6.5 ± 0.0	0.28 ± 0.00	28.7
F4	1.5	100.24 ± 0.08	10.45 ± 0.40	0.30 ± 0.07	5.5 ± 0.0	0.27 ± 0.00	35.1
F5	2	100.23 ± 0.10	10.56 ± 0.12	0.28 ± 0.05	5.5 ± 0.5	0.26 ± 0.01	46.2

#Value as mean ± SD (n = 20), *Value as mean ± SD (n = 3), Note: All formulations contain 2% sodium alginate and 1% chitosan along with 0.4% of atenolol. The variable concentration of calcium gluconate is only indicated in the table.

added to the above and stirred slowly further for 5 hours. The hydrogel so formed was then poured into glass rings (4-cm diameter and 24-ml 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 films with the help of a sharp-edged die (13.2 mm in length and 5.4 mm in width). Each ocular film contained 4.8 mg of the drug.

Physicochemical evaluation

Thickness, weight variation, and surface pH of film

Thickness of the films (n = 3) were measured using dead weight thickness gauge (Prolific). Films from each batch were randomly selected and weighed individually on electronic balance (AND HR 2000). Mean weight of films (n = 20) of each formulation was recorded. Films were left to swell for 5 hours on agar plate prepared by dissolving 2% (m/v) agar in phosphate buffer saline (pH 7.4) 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. Surface pH was measured by means of pH paper placed on surface of swollen films.

Mechanical strength

Ocular film 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 10 mm). Tensile strength and elongation at break was determined by modifying the method used by Dandagi *et al*.[16] The apparatus consisted of a base plate with a pulley aligned on it. The film was fixed in film holder at one end of base plate and another end was fixed with the 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) X 100

Drug content uniformity

Uniformity of the drug contents was determined by assaying the individual films. Each film was grounded in a glass pestle mortar and 5 ml of phosphate buffer saline (pH 7.4) was added to make a suspension. The suspension so obtained was filtered and the filtrate was assayed spectrophotometrically at 275 nm (UV-VIS Systronics Spectrophotometer-106)

In vitro drug release studies

In vitro drug release study was carried out by using biochemical donor–receptor compartment model.[17,18] The commercial semipermeable membrane cellophane membrane, presoaked overnight in the freshly prepared dissolution medium (phosphate buffer pH 7.4), was tied to one end of a cylinder (open at both the sides) which acted as donor compartment. The ocular film (n = 3) was placed inside the donor compartment in contact with the semipermeable 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 for 12 hours and replaced by an equal volume of dissolution medium every time. The samples were analyzed spectrophotometrically at 275 nm (UV-VIS Systronics Spectrophotometer-106).

RESULTS

Each ocular film had an area of approximately 75 mm^2. Table 1 shows various physicochemical and mechanical properties of the films. Thickness of films ranged between 0.28 and 0.40 mm, surface pH ranged from 5.5 to 7.0, and weight of films was found to be between 10.56 and 8.97 mg. The average tensile strength of the films was 0.28 g/mm^2. Percent elongation at break ranged from 46.2 to 17.5%. Figure 1 shows the *in vitro* drug release from the ocular films over a period of 12 hours. Film F1 sustained the drug release for 12 hours, followed by the formulation F2 which sustained the drug release for 8 hours, followed by

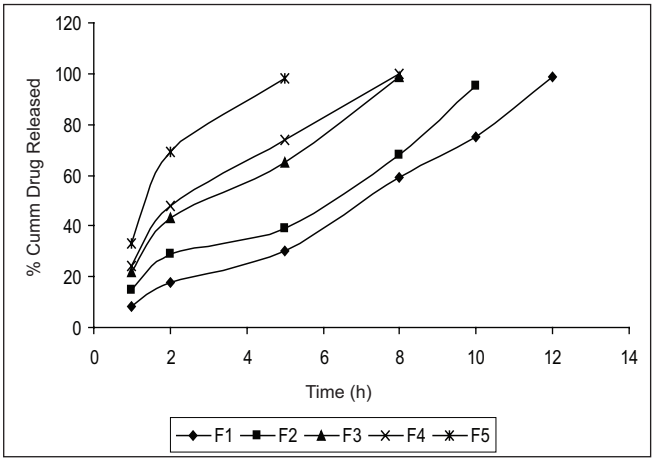

Figure 1: Cumulative % drug released *in vitro* with time

the formulation F3 and F4 for 6 hours, and F5 sustaining for 4 hours approximately.

DISCUSSIONS

The prepared batches were found to be uniform and flexible, proving the efficiency of glycerin as a plasticizer. Physicochemical evaluation studies revealed that all the batches were uniform with respect to thickness, weight of individual insert, and drug content, proving the suitability of the solvent-casting method for preparing the inserts. The minimum standard deviation values suggest that the method adopted for casting the films on the mercury surface was quite satisfactory. The gel films had undergone shrinkage on drying. The tensile strength was optimum and comparable for all inserts, although tensile strength slightly decreased from formulation F1 to F5. The elongation at break however increased from formulation F1 to F5 owing to internal gelation of the polymer matrix.

Ocular film F1 was prepared with 2% NaAlg and contained 1% of chitosan but no calcium gluconate. Formulations F2 to F5 were prepared with same NaAlg and chitosan concentration, with increasing concentration of calcium gluconate. The formulation F1 showed maximum potential to prolong the drug release for 12 hours, followed by the formulation F2 which sustained the drug release for 8 hours, followed by the formulation F3 and F4 for 6 hours, and F5 sustaining for 4 hours approximately [Figure 1]. Despite their different compositions, all the matrices had shown sustained drug release and a fairly constant rate of drug release. Calcium gluconate was chosen as an additional constituent because calcium ions are important to the alginate gelling mechanism. As the concentration of calcium gluconate is increased from the formulation F2 to F5, the drug release rate increased leading to sustaining the drug for 8 hours in F2, 6 hours for F3 and F4, and 4 hours for F5. All these results showed that the presence of calcium gluconate in combination with NaAlg generally led to a lower capacity of the systems in controlling the release of the drug for a prolonged time. The observation that uncross-linked systems had slower release rates than systems cross-linked with calcium was at first sight somewhat contradictory to our previous study.[14,15] However, it was observed that behavior of polymer matrix at the time of dissolution is determined by the hydrated layer of the matrix and its subsequent physical properties at that phase. NaAlg was effective as a carrier with chitosan alone, but internal calcium ions, which ionically cross-link alginate at a molecular level, gave rise to changes in its characteristics, particularly in its hydration. After placing the matrix in contact with the dissolution medium, calcium cross-linking in the gelled layer of the polymer matrices probably determined inhomogeneities in the structure of the hydrated gel. The presence of the calcium salt could also have a channeling effect that determined more rapid media penetration into the inner layers of the matrix. This resulted in an open structure of the matrix that failed to affect diffusional pathways. All this caused irregular disintegration (instead of slow and progressive erosion) and in formulation F5 a catastrophic failure of the system; the final result of this process was a drug release rate which occurred quicker. Second, alginate gel undergoes shrinking during formation. As the stoichiometric proportion of Ca^{+2} ions was increased, it leads to more cross-linking of polymer and faster and higher shrinkage; this shrinking and squeezing effect of gel draw drug out of the gel structure and cause it to lie superficially. When the dried gel film undergoes dissolution, this is the superficial drug on the film surface that is released in the starting phase of dissolution, causing the evident brust effect in the formulation with calcium gluconate.

CONCLUSION

This research shows that NaAlg–chitosan system can be used to prolong the atenolol release from the matrices. The results showed that the sustained release effect of NaAlg–chitosan matrices alone was the best among the formulations studied. The addition of calcium gluconate leads to a change in the release capacities of the matrices. Despite the presence of calcium ions and thus the possibility of an ionic interaction, the internal gelation of the polymer matrix lead to enhanced drug release and poor sustaining of drug.

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