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Preparation and Characterization of Ion-triggered In-situ Gelling Ocular Formulation Containing Ketorolac in Alginate-Chitosan Mucoadhesive Base

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ABSTRACT: The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions may be overcome by use of in situ gel-forming systems. Alginate has properties that make a sol to gel transition in the presence of multivalent cations. Chitosan has mucoadhesive properties due to its ability to make electrostatic interaction with the negative charges of mucus. Ketorolac is a non-steroidal agent which is administered as a topical ophthalmic solution in ocular inflammatory conditions.
Alginate was used as a gelling agent in combination with chitosan as a mucoadhesive agent. The formulations were evaluated for clarity, gelling capacity, rheological study, mucoadhesive capacity, in-vitro drug release and eye irritation tests.
Results: A concentration of 0.5% chitosan and 0.5% alginate underwent a rapid gelation upon facing with Ca^{2+} in tear, based on its satisfactory viscosity and gelling capacity. The developed formulation showed sustained release of drug for up to 8 hrs.
Alginate-chitosan solution as a base for ion triggered in-situ gel forming eye drop showed appreciable properties via enhanced viscosity and bioadhesion on application in eye.

KEYWORDS:ocular formulation, ion-triggered in-situ gelling, mucoadhesive, ketorolac, chitosan, alginate

I. INTRODUCTION

Ophthalmic drops are the most commonly-used formulations due to the ease of administration and patient compliance. However, the bioavailability of the active substance is less than 5% due to anatomical and physiological constraints such as: static barriers (thickness, hydrophilicity, hydrophobicity and collagen content of biological membranes), dynamic barriers (nasolacrimal and lymphatic drainage, conjunctival blood flow, tear turn over and blinking reflex), as well as metabolic barriers (efflux pumps or enzymes, precorneal drug clearance, low ocular contact time) [1-3].Therefore, short dosing intervals and high drug concentrations are needed to reach effective therapeutic levels which cause both ocular and systemic side effects [4]. Viscous semi-solid preparations, such as, ointments, gels and polymeric inserts have been investigated to extend the ocular residence time of medications for topical application to the eye. However, due to their stinging, burning sensation and vision blurriness, they have not been used extensively [5, 6].

One of the best strategies to increase the residence time of ophthalmic formulations with the corneal tissues is to increase the viscosity of the formulation by using in situ gel-forming ophthalmic drug delivery systems which minimize drug loss through nasolacrimal duct. Pseudoplastic behavior can reduce interference with blinking and frequent dosing regimen, resulting in improved patient compliance [7-9].

Ophthalmic in situ gelling systems are viscous liquid forms, consist of polymers which undergo sol to gel phase transitions based on physiologic stimuli (pH, temperature, ionic strength or ion activation) [10].

Among these stimuli-sensitive or “Smart hydrogels”, hydrogels sensitive to the ionic strength are the most widespread and the most successful ones [7]. These systems can be formulated at the optimal pH for using buffers, can be easily and accurately instilled at room

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temperature and may therefore be less irritating to the ocular tissues than in situ gelling systems depending on a change in pH or temperature [11, 12]. Ion-activated in-situ gelling systems form a crosslink with cations (monovalent and divalent) present in the tear fluid, thus forming a gel on the ocular surface, which give rise to an extended corneal contact time [2, 13].Aqueous solutions of certainpolymers such as alginate, an anionic polysaccharide, forms a viscous gel when it comes in contact with water. Its phase transition from sol-to-gel is due to its interaction with the divalent action i.e., calcium ion (Ca^{2+}) found in the tear fluid [14]. Drug molecules, from small chemical drugs to macromolecular proteins, can be released from alginate gels in a controlled manner. In order to improve mechanical properties and erosion resistance, it is associated with chitosan or its derivatives [1, 15].

Chitosan is a suitable candidate in ophthalmic formulations due to its biocompatibility, biodegradability, non-toxicity, mucoadhesive character, antibacterial and antifungal effects, low immunogenicity and low cytotoxicity. Its solutions have viscoelastic and pseudoplastic properties that do not disturb the tear film. Numerous studies have demonstrated the potential of chitosan as a corneal permeation enhancer for the absorption of hydrophilic drugs. Chitosan is able to adhere to the surface of the mucosa and temporarily open tight junctions between cells [1, 2, 14].

The hydrated form of chitosan also showed good mucoadhesive properties. The structure of chitosan is important in determining its mucoadhesive properties due to the formation of ionic interaction between the positively charged amino groups of chitosan and negatively charged sialic acid residues of mucin supported by other forces such as hydrogen bonding and hydrophobic association [16, 17].

Ketorolac is a non-steroidal agent with potent analgesic and moderate anti-inflammatory activity and is FDA-approved for seasonal allergic conjunctivitis, post cataract inflammation and ocular discomfort after refractive surgery. A 0.5% solution of Ketorolac has been administered topically in ocular inflammatory conditions in 3 or 4 times daily [18, 19].

The aim of the present study was to find and evaluate the best combination of alginate and chitosan concentration for enhanced ocular drug delivery.

II. MATERIAL AND METHOD

Ketorolac tromethamine was kindly obtained from SinaDarou laboratories, chitosan (low molecular weight) and sodium alginate (medium viscosity) were purchased from sigma labs. All of the other materials were of analytical grade.

III. PREPARATION OF FORMULATION

Different combinations of chitosan and sodium alginate were examined (Table 1), and optimized composition was selected based on gelation and clarity. A dose of 0.5% of ketorolac was used in the formulations as the commercial ophthalmic preparations. Polymer solution was prepared by dispersing sodium alginate in deionized water and heating up to 40 °C for 30 minutes while stirring. The weighed quantity of chitosan and acetate buffer was added to the beaker. After cooling, prepared drug solution in water was added to the polymer solution and the volume was adjusted to 100 ml.

INGREDIENTS	F1	F2	F3	F4	F5
Alginate (g/100ml)	0.7	0.6	0.5	0.4	0.3
Chitosan (g/100ml)	0.3	0.4	0.5	0.6	0.7
Ketorolac (g/100ml)	0.5	0.5	0.5	0.5	0.5
Distilled water (q. s.)	100	100	100	100	100

Table 1- Different formulations of gels.

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IV. EVALUATION OF FORMULATIONS

- **Appearance**

Physical appearance and clarity were determined by visual inspection under a good light before and after gelling, alternatively, against a black and white background. It was observed for formation of turbidity or any unwanted particles dispersed in the solution [20, 21].

- **pH and Gelation studies**

pH is one of the most important parameterinvolved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability [22].

For each formulated batch, pH was measured using pH meter which was previously calibrated using standard buffers. Determinations were carried out in triplicate and an average of these determinations was taken as the pH of the gel [21,23].

Gelling capacity was evaluated in order to identify the suitable formulations for use as in situ gelling systems. Gelling was determined by placing a drop of the formulation in a vial containing freshly prepared simulated tear fluid and visually observed [23].

Simulated tear fluid was prepared according to previous studies as follows:

Sodium chloride 0.670g, Sodium bicarbonate 0.200g, Calcium chloride dihydrate: 0.008g, deionized water q.s. to 100ml.[20, 22]

- **In vitro drug release**

In vitro release tests are valuable tools for monitoring the release of active substances from semisolid formulations following product development and quality control. In the relevant pharmacopoeias there are no compendial apparatus, procedures or requirements for in vitro release testing [20].

The in vitro release of ketorolac from the formulations was studied through cellophane membrane as stated elsewhere [9]. Freshly prepared artificial tear fluid (pH 7.4) was used as the dissolution medium. Cellophane membrane previously soaked overnight in the dissolution medium. 5 ml of the gel formulation was accurately placed in and then was tied at both ends. The prepared bag was suspended in 1000 ml of dissolution medium and maintained at 35°C so that the membrane just touched the receptor medium surface. Aliquots, each of 2 ml volume, were withdrawn at 30 minutes intervals and replaced by equal volumes of the receptor medium. The aliquots were analyzed by UV-spectrophotometer at 323 nm[24].

- **Rheological Evaluation**

Viscosity and rheological behavior of the instilled formulation is an important factor in determining residence time of drug in the eye. Viscosity of the formulations before and after addition of simulated tear fluid was evaluated using Brookfield R/S plus Rheometer in Room temperature 250C for solutions and 350C for gels. The prepared solutions were allowed to gel in the simulated tear fluid. The instrument continuously sheared the material at various angular velocities from 10 to 300 rpm [6, 7, 23].

- **Determination of mucoadhesive force**

The experimental technique used for determining the mucoadhesive force has been derived from a previously published method. In this method excised calf corneal membrane was immediately fixed with the mucosal side outwards onto glass pieces. Sample was added onto the mucosa of one glass while the height of the other glass was so adjusted that the mucosal surfaces of both glasses come in intimate contact. One minute time of contact was given. Mucoadhesive force, the detachment stress (dynes/cm2), was determined from the maximal weights that detached the gel. The corneal membrane pieces were changed for each measurement. All measurements were performed in triplicate [24, 25].

- **Rabbit eye irritation test**

In order to reduce the risk of exposure to harmful substances, all products manufactured for instillation into the eye, as well as their ingredients, must be tested and evaluated for the potential of ophthalmic irritation.

Based on appearance test, the selected formulations (F1, F2 and F3) was used for in vivo studies, according to Draize rabbit eye irritation test which is the oldest eye irritation test developed by Draize et al. in 1944. It continues to be widely used and approved by the Organization for Economic Cooperation and Development as well as by the FDA [20].

The amount of substance applied to the eye was normally 100µl placed into the cornea and cul-de-sac with observation of the various criteria made at the interval of 1hr, 24hrs, 48hrs, 72hrs, and 1 week, while the other eye serves as control. The formulation was instilled twice a day for a period of 7 days, and a cross-over study was carried out. Rabbits were observed periodically for redness,

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swelling, watering of the eye [5, 20].

- **Short term stability study**

Selected formulation was stored at 4±1˚C for a period of three and six months. The formulation was evaluated at periodic intervals for drug content, visual appearance, clarity and pH, gelling capacity, viscosity and in vitro drug release [26].

V. RESULT AND DISCUSSION

The preparation of in situ ocular gelling system was carried out by using alginate/chitosan polymers in different concentrations (Table 1). The formulations were subjected to general appearance, pH, gel strength, drug content rheological studies, in-vitro release behavior and mucoadhesion studies. Based on physical examinations, among the different formulations, F3 (a combination of 0.5% chitosan with 0.5% of sodium alginate) was selected as the favorite gel base.

The color of all formulations was light yellow and Clarity of three formulations (F1, F2 & F3) were found to be satisfactory (Table 2), so that they were chosen for further tests. The pH was near 7 and did not cause any irritation upon administration. The selected formulations (F1, F2 & F3) had an optimum viscosity which would allow their instillation into the eye as a liquid and would then undergo rapid sol to gel transition due to ion exchange. Moreover, all the formulations formed gels instantaneously on contact with simulated tear fluid.

	F1	F2	F3	F4	F5
Appearance	Clear	Clear	Clear	Not clear	Not clear
Gel strength	+	+	+	----	----
Mucoadhesion (dyne/cm^2)	268.8	420	915.5	----	----

Table 2- Evaluation of in situ gelling systems of Ketorolac.

The in vitro release studies were carried out for F1, F2, F3 using simulated tear fluid as the dissolution medium. The percent of ketorolac released in comparison to marketed formulation, as a function of time, is shown in Figure 1. The in vitro drug release conditions may be very different from thoselikely to be encountered in the eye. However, the results clearly show that the gels have the ability to retain ketorolac for 8 hours and that the drug release will not occur instantaneously. In the cul-de-sac, the gels will probably undergo faster dissociation due to the shearing action of the eyelid and eyeball movements.

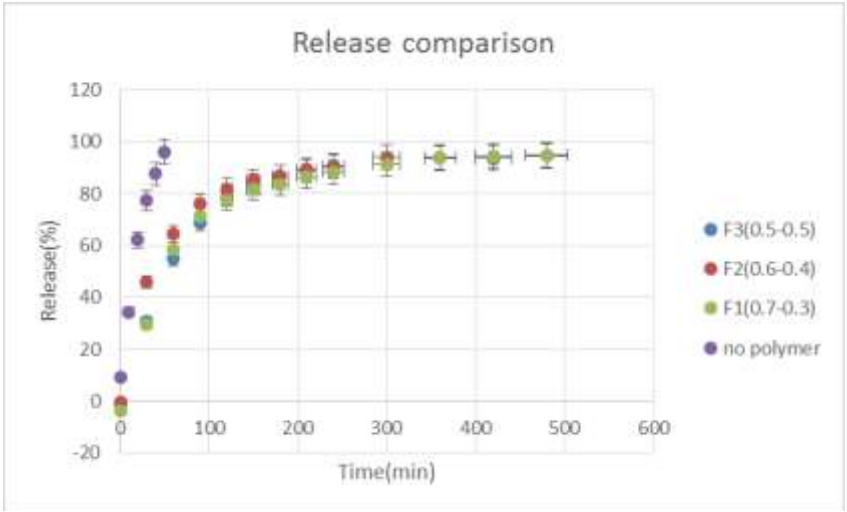

Fig. 1. In vitro drug release profile (n=3, ±SD).

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In rheological studies, formulation F1, F2 and F3, all presented a pseudoplastic as well as thixotropic behavior, which were suitable for ocular uses.
Mucoadhesive force of formulation F3 was found to be more than that of formulations F1 and F2. It may be due to the higher percent of chitosan as shown in Table 2.

Ocular irritancy study results indicated that the formulation F1, F2 and F3 had appropriate viscosity for instillation. Moreover, all the formulations formed gel quickly and no ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible. Watering of eyes, redness or inflammation was not observed.
Stability studies for formulation F3 were performed for up to 6 months and were found to be clear with no change in pH, drug content, viscosity, in vitro release and gelling capacity (Table 3 and Figure 2).

	Appearance	Gelling capacity
0 month	Clear	+
3 month	Clear	+
6 month	Clear	+

Table 3- Stability test results.

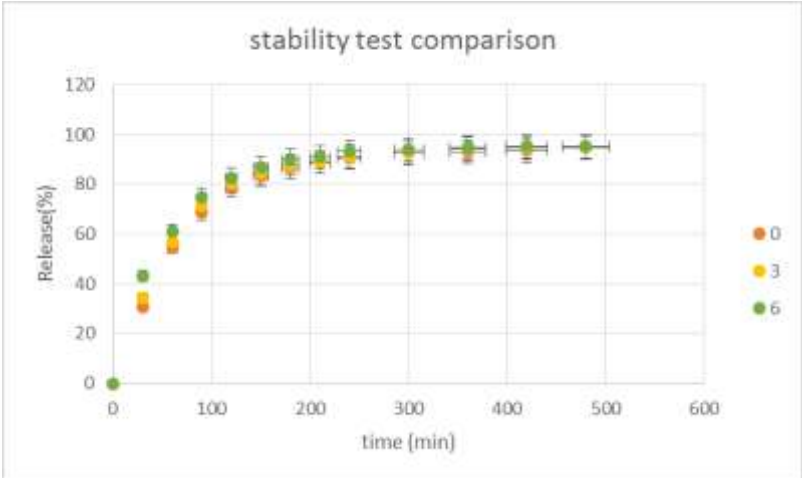

Fig. 2. In vitro drug release after six months (n=3, ±SD).

VI. CONCLUSION

The objective of the present research was to develop a sustained release-mucoadhesive ocular drug delivery system with improved patient compliance and longer precorneal resistance time to enhance bioavailability as an alternative to conventional eye drops.

The developed formulation (F3) contained ketorolac (0.5%) as an anti-inflammatory agent and sodium alginate (0.5%) as a gelling agent in combination with chitosan (0.5%) as a mucoadhesive and viscosity enhancing agent.
Based on in vitro characterization, it is concluded that the developed in situ gelling formulation is a nonirritant, mucoadhesive and sustained release system for durable topical drug delivery to eyes. This new formulation of ketorolac is a viable option for effective and controlled management of conjunctivitis and other eye related disorders.

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