

Enhancement of miotic potential of pilocarpine by tamarind gum based *in-situ* gelling ocular dosage form

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Abstract

In-situ gelling solutions are one of the most successful means of delivering the drug at ocular site with maximum bioavailability. Pilocarpine *in-situ* gelling solution based on alginate along with novel bioadhesive tamarind gum and widely used bioadhesive, chitosan were formulated. The formulations were tested for drug content uniformity, bioadhesive strength, gelation and *in vitro* release study. Further *in-vivo* miotic test was carried for all formulations. We found the formulations to be satisfactory in terms of content uniformity, bioadhesion and gelation. The tamarind gum based formulation showed best slow drug release profile compared to the other formulations. It released about 25 % drug in initial hour and about 80 % of the drug was released during the study of 12 h which was slowest then the rest of preparations. *In vivo* miotic study also showed the most significant long lasting decrease in pupil diameter of rabbits with tamarind gum based formulation. Prolonging the drug action and higher pharmacodynamic action clearly indicates enhancement of pilocarpine bioavailability as compared to conventional eye drop solution. Ocular irritation studies indicated that the formulations were well tolerated and non-irritating. This system may provide an excellent potential alternative ophthalmic sustained-release formulation of pilocarpine for clinical use.

Keywords: Alginate glaucoma, *in situ* gelling solution, pilocarpine tamarind gum.

Introduction

Tamarind seeds or kernel is a byproduct of Tamarind pulp industry (Kulkarni et al. 2002). Tamarind gum is obtained from endosperm of seeds of the tamarind tree, which is a seed gum with potential industrial applications (Shankaracharya 1998, Gerarad 1980). Tamarind gum is having applications in paper, food, textile industry etc. Recent year's research has been initiated on the use of tamarind gum in pharmaceutical and cosmetic applications. Tamarind kernel powder disperses and hydrates quickly in cold water but does not reach maximum viscosity unless it is heated for 20-30 min.

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Tamarind gum along with xanthan gum and hydroxyl propyl cellulose (water soluble neutral polymer) used for nasal mucoadhesion studies in powder formulation (Jambhulkar and Shankhapal 1992, Glicksman 1986). Tamarind gum was also evaluated in bioadhesive tablets (Takahashi 2007). Polysaccharide present in tamarind kernel powder is called as tamarind seed polysaccharide. Tamarind seed polysaccharide is having molecular weight 52350 units and monomer of glucose, galactose and xylose in molar ratio of 3:1:2. It is used as potential polysaccharide having high drug holding capacity for sustained release of verapamil hydrochloride. It is also used as suitable polymer for sustained release formulations of low drug loading.

Tamarind seed polysaccharide could be used for controlled release of both water-soluble and water insoluble drugs (Takahashi 2007, Khanna et al. 1997). There are references showing gum's potential in ophthalmic drug delivery system (Khanna et al. 1997, Wise et al. 1991, Zimmerman 1981).

Pilocarpine, a para-sympathomimetic, remains a miotic of choice for open-angle glaucoma mimetic, because it increases the outflow of aqueous humour. The drug penetrates the eye well, with miosis beginning 15–30 min after topical application and lasting for 4–8 h (Zimmerman 1981). Pilocarpine ophthalmic drops are administered as 1 or 2 drops per dose, with 6 drops per day as the maximum recommended dosage. Patients on pilocarpine ophthalmic drops are faced with frequent dosing schedules and difficult drop instillation. While ointment preparations offer a second option, this dosage form causes poor patient compliance because of the blurred vision and discomfort resulting from the messy and greasy properties of the ointment (Lee 1990). Therefore, new and long-acting ophthalmic pilocarpine formulations are needed (Li and Xu 2002).

One of the most conveniently dispensed and produced delivery system is *in-situ* gels. Depending on the method employed to produce the sol to gel phase transition on the ocular surface, the following three types of systems have been used: pH-triggered systems, temperature dependent systems and ion-activated systems. A potential ion activated *in-situ* gelling polymer is sodium alginate. 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 (Smidsrod 1974). In this study, we report an approach for preparing Pilocarpine *in-situ* gelling system based on alginate with tamarind gum. Various physicochemical, bioadhesion, *in vitro* release and *in vivo* miotic studies have been done. No such *in-situ* gelling system based on tamarind gum has been studied before for ocular drug delivery.

Materials and Methods

Drugs

Pilocarpine was obtained from Sigma Chemicals (USA), Sodium alginate (250 cps for a 2 % solution at 25 °C) was a gift sample from Snap Natural and Alginate Products Limited, Ranipet, Tamarind gum was a gift sample from Shivam Exim, Ahmedabad. Water soluble Chitosan (chitosan acetate, 68 cps for a 1% solution at 25 °C) was acquired from Indian Sea Foods (Cochin). All other reagents were of analytical grade. Simulated tear fluid (STF) composed of sodium chloride: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride. 2H₂O: 0.008 g, and purified water q.s. 100 g (pH 7.2).

Animals

Rabbits (1.5-3kg) of either sex were used for pharmacological studies. The animals were housed under standard laboratory conditions in polypropylene cages and were provided with food and water *ad libitum*. The animals were acclimatized to the laboratory environment for at least 1 week before the starting of experiments. They were all treated under the appropriate national and university guidelines for the care and use of laboratory animals in research and teaching. The experimental protocol was approved by Institutional Animal Ethical Committee.

Preparation of formulation (Liu et al. 2006)

Formulation A was prepared by dissolving the weighed amount of sodium alginate in distilled water and stirred for 5 h until a solution of uniform consistency is prepared; thereafter the drug, pilocarpine is added in small increments while stirring till it is dissolved. Formulation B and C were prepared by dissolving tamarind gum in warm phosphate buffer pH 7.4 (prepared from potassium dihydrogen ortho phosphate and sodium hydroxide in fresh water for injection at 70 °C under laminar flow), by continuous stirring at 40 °C. After the homogenous solution of gum is prepared, it is added with continuous stirring and in small increments to sodium alginate dispersion. The quantity of drug required to give a final drug concentration of 1% (w/v) was added to the prepared polymeric solution and stirred until dissolved. Formulation D and E was prepared dissolving chitosan in alginate solution prepared (as in formulation A) and then adding the required quantity of drug. (Table 2) Buffering and osmolality adjusting agents were added thereafter. pH of the preparations was checked by pH paper. All the formulations were filled in 10-mL amber colored glass vials, capped with rubber bungs and sealed with aluminum caps. In their final pack, the formulations were terminally sterilized by autoclaving at 121 °C and 15 Pa for 20 minutes. Sterilized formulations were stored in a refrigerator (4–8 °C) until use.

Evaluation of the formulations

Drug content uniformity

The vials ($n = 3$) containing the preparation were shaken for 2–3 minutes manually and 100 μ L of the preparation was transferred aseptically to sterile 25-mL volumetric flasks with a micropipette and the final volume was made up with acetate buffer pH 5.0 (0.2 mol L⁻¹ CH₃COONa + 0.1 mol L⁻¹ CH₃COOH). Pilocarpine concentration was determined at 220 nm (Shimadzu, UV-1601, Japan).

In-vitro gelation study

Gelation studies were carried out in previously described agar gel plates (Gilhotra and Mishra 2008). (2 % w/v agar dissolved in warm simulated tear Fluid STF, pH 7.2). At the centre of the plate a cylindrical reservoir capable of holding 3 ml of gelation solution (STF) was bored. The formulations (100 μ L) were 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.

Bioadhesive strength measurement (Gilhotra and Mishra 2008)

Freshly excised goat conjunctival membrane was used as the model membrane for the measurement of bioadhesive strength. Goat conjunctival membrane was obtained from a slaughter house, the underlying skin was removed and it was placed in an aerated saline solution at 4 °C until used. A Pan Balance was modified for this study. The conjunctival membrane was tied to the lower side of the hanging Teflon cylinder which was attached to right pan of the balance. The formulation ($n=3$) was applied to the protrusion on another Teflon block. After balancing the pan balance such that the teflon cylinder with the membrane attached to it, was lowered over the formulation applied to Teflon protrusion. The membrane

was kept in contact of the formulation for 3 min to facilitate the bioadhesion with the ocular tissue. Then the weights on the right hand side were slowly added in the increments of 0.5 g till the formulation just separated from the membrane surface. The weight at which formulation just separated from the tissue was taken as the measure of the bioadhesive strength. Force of adhesion was also calculated using the formula

$$\text{Force of adhesion} = \text{Bioadhesive strength} \times 9.81 / 1000.$$

In vitro release studies

The test solution (n=3) (2 ml) was placed in a circular plastic cup (2.5 cm internal diameter and 1.2 cm depth). This was in turn placed on an inverted USP basket kept inside a 250-ml beaker. Dissolution medium (200 mL of STF of pH 7.2) was added and stirred with a star-headed magnetic bead. Temperature of 37 ± 1 °C was maintained throughout the study. Samples (5 ml) were withdrawn at regular time intervals and replaced with an equal volume of prewarmed medium. The samples were analyzed for drug as stated above.

In-vivo miotic study (n = 3)

Albino rabbits, 1.5–3.0 kg, were used in the *in vivo* experiments. The rabbits were kept in restraining boxes throughout each experiment. All tests were performed in the same room under standard lighting. After 30 min of acclimatization, the difference in pupil diameter between the left and right eyes was measured four times, and the mean value of those measurements was used as a reference (A) for calibration in further experiments. Rabbits were dosed with 100 µL of pilocarpine preparations, always in the right eye, and simulated tear fluid (STF), always in the left eye, as the control. The difference (B) between the left and right eyes was measured at the desired time point. The value obtained from (B) minus (A) is the decrease in pupil diameter at the specific time point. Each preparation was tested in groups of six different rabbits. The rabbits were released from their restraining boxes between the sampling time intervals.

Statistical analysis

Results are given as mean \pm s.d. of at least three measurements. Statistical significance was set at $P < 0.05$. All experiments were run at several time points, each with all pilocarpine formulations handled in a paralleled pattern.

Evaluation of Irritancy- Modified Draize test

An ocular toxicity study was undertaken taking White rabbit as animal of choice as its eyes closely resemble human external eye. 30 rabbits were taken and divided in five groups. They were housed in neat and clean air-conditioned chambers with proper feeding and freedom to relax. The solutions were instilled periodically. Evaluation of irritation is conducted according to a 0 (absence) to 3 (highest) clinical evaluation scale of discharge, conjunctival chemosis and conjunctival redness. The test protocol is carried out in six rabbits after instillation of solution. The untreated eye serves as control. Each animal is observed at 0.5, 1, 2, 3, 6, 9, 12, 24, 32, and 49 h after solution instillation and an index of overall irritation (Iirr) is calculated by summing up the total clinical evaluation scores (A, B, C) over all the observation time points (0.5 to 48 h) (Table 1).

Table 1. Clinical evaluation scores for irritancy study

| | |
|--|---|
| A. Conjunctival redness | |
| Normal vessels | 0 |
| Injected vessels | 1 |
| Diffuse crimson red vessels | 2 |
| Diffuse beefy red | 3 |
| B. Conjunctival chemosis | |
| No swelling | 0 |
| Swelling above normal | 1 |
| Swelling with partial eversion of lids | 2 |
| Swelling with half closed lids | 3 |
| C. Discharge | |
| No discharge | 0 |
| Slight discharge without moistness out of eye | 1 |
| Discharge with moistness just adjacent to lids | 2 |
| Discharge with moistness on an area around the eye | 3 |

Results and Discussion

Preparation of formulation and physicochemical evaluation

The present work aimed at enhancing the ocular bioavailability of antiglaucoma agent, Pilocarpine by formulating an in-situ gelling solution based on sodium alginate. Further authors aimed to study the potential of a novel mucoadhesive tamarind gum and well established mucoadhesive chitosan to enhance the ocular bioavailability of the drug. The prepared formulations were characterized for the clarity, drug content, gelation and mucoadhesive strength. The clarity of the formulations, determined by the visual examination against white and black backgrounds under illuminated conditions, was found to be good. The uniformity of drug content was also found to be good in range of 98-100%. pH of the formulations was optimum and in non irritation range (Table 2). The force of bioadhesion for formulations ranged from 0.023N to 0.058N indicating an appreciable bioadhesive potential of formulations. However the tamarind gum based formulations were better bioadhesive than the chitosan based formulation.

Table 2. Composition and pH of *in-situ* gelling systems of Pilocarpine and evaluation parameters

| Composition | A | B | C | D | E |
|------------------------|-------------|-------------|-------------|-------------|-------------|
| Pilocarpine (%w/v) | 1 | 1 | 1 | 1 | 1 |
| Sodium alginate(%w/v) | 1 | 1 | 1 | 1 | 1 |
| Chitosan (%w/v) | --- | --- | --- | 0.75 | 1 |
| Tamarind gum (%w/v) | --- | 0.5 | 1 | --- | --- |
| % Drug content* | 98.0 ± 0.2 | 99.1 ± 0.1 | 100 ± 0.1 | 100 ± 0.3 | 99.7 ± 0.3 |
| pH | 6.8 | 6.7 | 6.8 | 6.8 | 6.8 |
| Force of adhesion (N)* | 0.023±0.003 | 0.055±0.001 | 0.058±0.002 | 0.039±0.002 | 0.043±0.006 |

Note All the formulations contain Benzylkonium chloride—0.01% W/V, Citric acid—0.2% W/V, Boric acid—0.3% W/V, Sodium chloride —0.9 % W/V, Disodium EDTA 0.0625% W/V, Sodium Metabisulfite---0.02% W/V.

* (n = 3, mean± standard deviation)

The *in vitro* gelation studies were observed as in Table 3. All preparations underwent optimum gelation; however the formulations B and C made more viscous gel. Alginate which was the

basic polymer matrix in all formulation is responsible for the well established in-situ gelling phenomenon based on egg box model (Grant 1973). However the tamarind gum, because of its viscolising properties and gummy consistency added up to the gel consistency. Water soluble chitosan in formulations D and E could not add to the consistency of gel and was comparable to formulation A.

Table 3. Gelation studies of formulations

| Code | Physical characteristics | |
|------|--|---|
| | Solutions before gelation | Gels formed after addition of STF at 37 °C, pH 7.4 |
| A | Buff colored and transparent solution | Viscous solution of gel like consistency was formed |
| B | Buff colored translucent slightly viscous solution | thick gel was formed immediately on addition of few drops |
| C | Buff colored translucent viscous solution | Consistent and pronounced gel was formed |
| D | Clear transparent pourable liquid | Less viscous gel was formed |
| E | Slightly clear transparent and viscous solution | Viscous gel was formed |

In vitro release study

Formulation F in *in-vitro* release study was marketed Pilocarpine eye drop. The *in-vitro* study Pilocarpine loaded in eye drops (Formulation F) was released very quickly, and more than 90% of the loaded pilocarpine was released and reached a plateau within 5 h (Figure 1). Formulation A to E, all showed an initial burst effect by releasing 25-40 % of the drug in first 5 h. The formulations B and C proved a better reservoir in initial drug release. These formulations released only 30 and 25 % drug respectively (Lower than other preparations). Formulation A showed a significant drug release up to 40 %. This observation indicates that although alginate makes a potential matrix system on undergoing gelation in divalent ions; however addition of other polymers enhances the gel consistency and capacity to sustain drug release. This was also a finding in Liu et al. (2006) study. The initial fast release of drug from the prepared systems could be explained by the fact that these systems were formulated in an aqueous vehicle. The matrix formed on gelation was already hydrated and hence hydration and water permeation could no longer limit the drug release. A similar release pattern was reported for pilocarpine, wherein the initial fast release (burst effect) decreased with an increase in polymer concentration from alginate systems (Cohen et al. 1997).

Further in the present work the additives like tamarind gum and chitosan enhanced polymers' reservoir capacity for drug. Further formulation C sustained the drug release for the longest period of time whereas marketed eye drop did it for the least, the order being C > B > D > E > A > F. This indicates the overall better performance of *in situ* system as compared to conventional eye drop F. Secondly better potential of tamarind based formulation than the chitosan and alone alginate based system. This is attributed to highly branched carbohydrate polymer, tamarind gum. It disperses and hydrates quickly in cold water to give a viscous non-newtonian pseudoplastic fluid, so does it do in STF. The tamarind gum and alginate in conjugation, lead to a highly consistent gel (Owing to alginate) and highly viscous gel (Owing to tamarind). This matrix give rise to a polymer bed of gum and alginate, in which drug is

entrapped on gelation and is further immobilized because of cross linking and high viscosity of matrix.

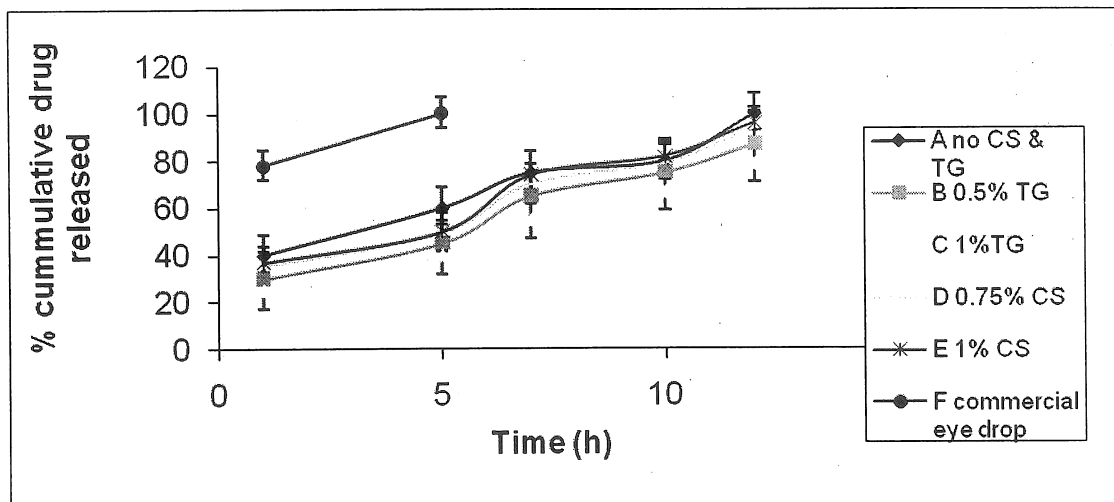


Figure 1. *In-vitro* release of pilocarpine eye drop and in situ gelling formulations (n=3) as a function of time in STF at pH 7.2 and 37 °C for 12 h

Note All the formulations contain 1% pilocarpine, 1% sodium alginate, Benzylkonium chloride—0.01% W/V, Citric acid—0.2% W/V, Boric acid—0.3% W/V, Sodium chloride —0.9 % W/V, Disodium EDTA 0.0625% W/V, Sodium Metabisulfite—0.02% W/V. concentration of bioadhesives are shown in figure. CS – Chitosan and TG- Tamarind gum.

To understand the mechanism of drug release the release profile of formulations (A-E) was analyzed using following equations:

$$Mt/M_{\infty} = Kt^n$$

$$\text{Log}(Mt/M_{\infty}) = \text{log}K + n \text{log} t$$

Where Mt/M is the amount (%) of Pilocarpine released at time t (min), n is the diffusional exponent, and K is the apparent release rate ($\% \text{ min}^{-1}$). Our data show that the release index (n) of the formulations studied ranged from 0.39 to 0.45, this low value of n is related to a high initial burst effect of drug. The data suggest an overall combination of diffusion and dissolution controlled release kinetics followed by the dosage form.

In vivo miotic study

The *in vivo* miotic study complemented the release patterns as the decrease in pupil diameter was greatest for the tamarind based formulation (Figure 2). The decrease in pupil diameter was in the order of $C > B > D > E > A > F$ in perfect unison with *in vitro* data. The miosis efficiency of formulation C (and B) lead to prolonged pharmacological effect upto 12 h. Pilocarpine in the tamarind based formulation had the largest AUC (0→12) of the other formulations, a difference that was statistically significant. This indicates that formulation C is the most efficient delivery vehicle for pilocarpine.

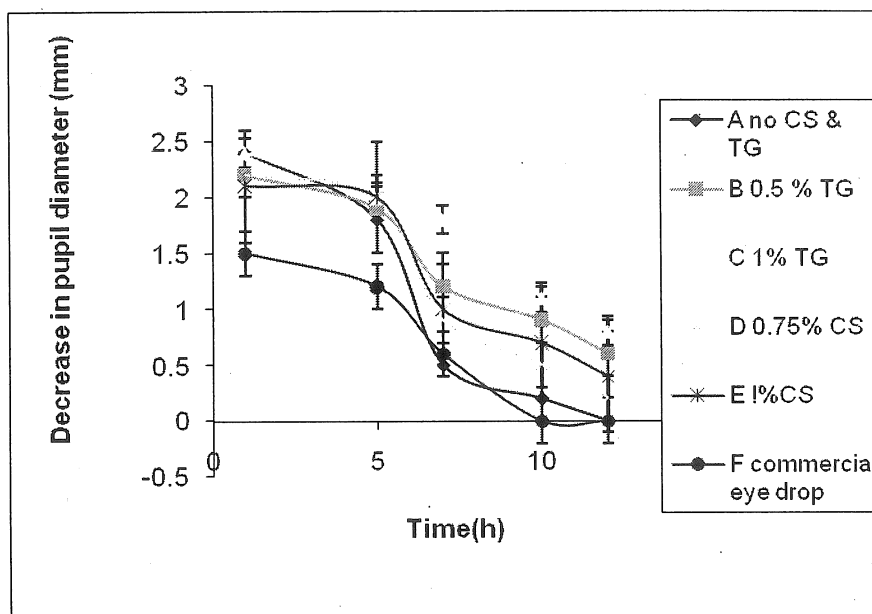


Figure 2. In vivo decrease in pupil diameter versus time profiles for Pilocarpine eye drop and in situ gelling formulations (n=3) as a function of time at 25 °C for 24 h.

Note All the formulations contain 1% pilocarpine, 1% sodium alginate, Benzylkonium chloride—0.01% W/V, Citric acid—0.2% W/V, Boric acid—0.3% W/V, Sodium chloride —0.9 % W/V, Disodium EDTA 0.0625% W/V, Sodium Metabisulfite—0.02% W/V. concentration of bioadhesives are shown in figure. CS – Chitosan and TG- Tamarind gum.

At first sight these observations are justified on the fact that we have already discussed while understanding its potential for *in vitro* drug release, i.e. the tamarind gum is good viscosity enhancer and hence it prevents the spillage of the ocular solution out of *cul de sac* thereby preventing loss by drainage and reduction of wash out of topically administered drug (Glicksman 1986, Khanna et al. 1997, Takahashi et al. 2007). Further more, it is reported to be a mucomimetic, mucoadhesive and bioadhesive, which further justifies its sustaining of miotic effect for longer period (Gheraldi et al. 2000). Polymer is based on polysaccharide consisting of a cellulose like backbone that carries xylose and galactoxylose substituents, chemical residues similar to those of mucin MUC-1 and episialin (Hilkens et al. 1992). There are reports indicating that being similar to mucins, it helps to bind to the cell surface and intensify the contact between drugs and the adsorbing biological membrane (Burgalassi et al. 2000). As previously reported for ocular delivery of ofloxacin and gentamicin (Gheraldi et al. 2000), another study demonstrated that it enhances transcorneal disposition and intraaqueous penetration of rifloxacin in healthy rabbits when administered topically in a drop regimen (Wise et al. 1991).

At the same time, Chitosan based systems also sustained the drug action although less than the gum based formulations. Chitosan must have enhanced the drug action because of chitosan has numerous amine and hydroxyl groups as well as a number of amino groups that may increase the interaction with the negative mucin (Gilhotra and Mishra 2008). A study of the rheological interaction between chitosan and mucin suggested a positive rheological synergism in the presence of excess mucin, which caused a strengthening of the mucoadhesive interface.

Ocular irritancy study

Ocular irritation studies indicated that the formulations were well tolerated (total score with formulations ranged between 0.33-0.66 which was less than 10 % of the maximum possible total score of 9). Thus, the developed ocular drug delivery systems were apparently free of any ocular irritation potential and could be safely administered to humans.

References

- Burgalassi, S., Raimondi, L., Pirisino, R., Banchelli, G., Boldrini, E. and Saettone, M.F. (2000) Effect of xyloglucan (tamarind seed polysaccharide) on conjunctiva cell adhesion to laminin and on corneal epithelium wound healing. *Eur. J. Ophthalmol.* 10: 71.
- Cohen, S., Lobel, E., Trevigoda, A. and Peled, Y. (1997) A novel in situ forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. *J. Control. Rel.* 44: 201.
- Gerard, T. Handbook of Water soluble gums and resins. New York: McGraw Hill; 1980, Chapter 23.
- Ghelardi, E., Tavanti, A., Celandroni, F. and Senesi, S. (2000). Effect of novel mucoadhesive polysaccharide obtained from tamarind seeds on intraocular penetration of gentamicin and ofloxacin in rabbits. *J. Antimicrob. Chemother.* 46: 831.
- Gilhotra, R.M. and Mishra, D.N. (2008). Alginate-chitosan film for ocular drug delivery: Effect of surface cross-linking on film properties and characterization. *Pharmazie* 63: 576.
- Glicksman, M. (1986). Tamarind seed gum in food hydrocolloids. *Florida CRC Press*, 3: 191.
- Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C. and Thom, D. (1973). Biological interactions between polysaccharides and divalent cations. *FEBS Lett.* 32: 195.
- Hilkens, J., Ligtenberg, M.J., Vos, H.L. and Litvinov, S.V. (1992). Cell membrane-associated mucins and their adhesion modulating property. *Trends Biochem. Sci.* 17: 359.
- Jambhulkar, V. and Shankhapal, K.V. (1992). Effect of minerals on lipid production by *Rhizopus nigricans* and *Penicillium nigricans* on tamarind kernel powder. *J. Food Sci. Technol.* 29: 333.
- Khanna, M., Nandi, R.C. and Sarin, J.P. (1997). Standardization of Tamarind seed powder for pharmaceutical use. *Ind. Drugs* 24: 268.
- Kulkarni, G.T., Gowthamarajan, K., Brahamajirao and Suresh, B. (2002). Evaluation of binding properties of selected natural mucilages. *J. Sci. Ind. Res.* 61: 529.
- Lee, V.H. (1990) New directions in the optimization of ocular drug delivery, *J. Ocul. Pharmacol.* 6: 157.
- Li, J. and Xu, Z. (2002). Physical characterization of a chitosan-based hydrogel delivery system. *J. Pharm. Sci.* 91: 1669.
- Liu, Z., Li, J., Nie, S., Liu, H., Ding, P. and Pan, W. (2006). A study of alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin. *Int. J. Pharm.* 315: 12.
- Saettone, M.F., Burgalassi, S., Boldrini, E., Bianchini, P. and Luciani, G. Ophthalmic solutions viscosified with tamarind seed polysaccharide, International patent application PCT/IT97/00026.
- Shankaracharya, N.B. (1998). Tamarind-chemistry, technology and uses a critical appraisal. *J. Food Sci. Technol.* 35: 193.

Smidsrød, O. (1974). Molecular basis for some physical properties of alginates in the gel state. *J. Chem. Soc. Faraday Trans. 57*: 263.

Takahashi, Y., Takeda, C., Seto, I., Kawano, G. and Marinda, Y. (2007). Formulation and evaluation of Lactoferrin bioadhesive tablets. *Int. J. Pharm.* 343: 220.

Wise, R., Johnson, J., O'Sullivan, N., Andrews, J.M. and Imbimbo, B.P. (1991). Pharmacokinetics and tissue penetration of rifloxacin, a long acting quinolone antimicrobial agent. *J. Antimicrob. Chemother.* 28: 905.

Zimmerman, T.J. (1981). Pilocarpine. *Ophthalmology* 88: 85.

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