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In Situ Gelling Ophthalmic Drug Delivery System: Formulation and Evaluation

Neeraj Mittal, Gurpreet Kaur
Department of Pharmaceutical Science and Drug Research, Punjabi University, Patiala 147002, Punjab, India
Correspondence to: G. Kaur (E-mail: kaurgpt@gmail.com)

ABSTRACT: Designing a drug delivery system to target the eye is an interesting and challenging endeavor faced by scientists because of the critical and pharmacokinetically-specific environment that exists in the eye. Topical administration of ophthalmic drugs is used to alleviate the symptoms and signs caused by ocular surface inflammatory disorders, to treat infections, for glaucoma or intraocular inflammation (uveitis). However, the responsiveness toward the conventionally developed drug delivery system is limited because of the presence of several types of barriers in the eye that impede the effective passage of many drugs leading to minimal dose absorption. Ion activated *in situ* gelling ocular systems undergo phase transition in the presence of cations (present in the tear fluid), thus enhancing the residence time of drug in the cornea. In the present study natural polysaccharides (pectin alone or in combination with sodium alginate) or a pectin derivative (thiolated pectin [TP] alone or in combination with sodium alginate) were used to formulate *in situ* gelling eye drops. The formulations were evaluated for their gelling capacity, rheological studies, spreadability, bioadhesion strength, and *in vitro* drug release. Thiolation of pectin was observed to result in an increase in the gelling behavior, viscosity, and bioadhesive strength. The formulations comprising pectin alone (P_7), combination of pectin and sodium alginate (P_5SA_1) or TP_6 demonstrated good *in vitro* release characteristics. The optimized formulations displayed a significant decrease in the intraocular pressure as compared to the marketed formulation upon instillation in rabbit eye. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39788.

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INTRODUCTION

Topical administration is the preferred route for the administration of ocular drugs because of its convenience and affordability. However, the responsiveness toward the conventionally developed drug delivery system is limited. The main reason for this limitation is the presence of several types of barriers in the eye that impede the effective passage of many drugs leading to minimal dose absorption. Further, certain physiological processes also contribute to the poor efficacy of the conventional drug formulations. Blinking and tear drainage through the lachrymal drainage system tend to reduce the residence time of topically applied drugs.[1] This has prompted the scientists to develop a drug delivery system that would circumvent the problems associated with the conventional systems, and provide the advantages of targeted delivery of drugs for extended periods of time and be patient-friendly.

In situ gelling systems comprise of liquid formulations-containing polymers that undergo sol-gel phase transition as a result of change in the physiological environment. These (liquid) vehicles undergo a viscosity increase upon instillation in the eye, thus favoring pre-corneal retention.[2] Such a change in viscosity can be triggered by a change in temperature, pH, or specific ion in the tear fluid. Examples of polymers used are Poloxamer 407 (temperature sensitive), CAP (pH sensitive), and Gelrite (ion induced gelation).[3] Natural polymers with *in situ* gelling and bioadhesive properties (chitosan, pectin) have also been reported to increase the residence time of drug in eye. Further, chemical derivatization with functional groups has also been reported to increase the bioadhesive potential of natural polymers.[4]

Glaucoma is second to cataract as a cause of global blindness and is the leading cause of irreversible visual loss. It has been estimated that by the year 2020, almost 80 million people will be affected with open-angle glaucoma and angle-closure glaucoma.[5] The treatment of glaucoma focuses on preserving vision by slowing down damage to the optic nerve. The therapy for glaucoma aims at preventing further damage by lowering intraocular pressure (IOP) or ocular hypertension and it usually consists of pharmaceutical treatment and laser or surgical procedures.[6] It

has been shown that reducing IOP is effective in preventing disease progression in ocular hypertension, primary open angle glaucoma, and even in normal tension glaucoma.[7] In most glaucoma patients, therapy consists of topical eye drops and oral tablets. However, administration and compliance are often problematic. Eye drops produce low ocular bioavailability, unnecessary systemic exposure and have low patient compliance as well as difficulty of instillation or forgetfulness. Two main strategies have already been used clinically to diminish such effects, namely gel forming (viscous) solutions[8] and controlled drug delivery systems.

The objective of the present study was to develop an ion-activated *in situ* gelling and bioadhesive drug delivery system of brimonidine tartrate using pectin alone or in combination with sodium alginate. These natural polymers undergo gelation in the presence of ions when instilled into the cul-de-sac of the eye and provide sustained release of the drug. Further, to improve the bioadhesive strength of pectin derivatization of pectin was done to form thiolated pectin (TP). Introduction of thiol groups has been reported to increase the bioadhesive strength of polymers.[9] Brimonidine tartrate is among the most promising therapeutic agent for treatment of open-angle glaucoma and ocular hypertension.[10] Its ocular hypotensive efficacy is achieved through increasing uveoscleral outflow and due to its ability to decrease aqueous humour production.[11] Ion activated *in situ* gelling formulations of brimonidine employing pectin alone, pectin and sodium alginate (PSA) or TP alone or TP and sodium alginate (TPSA) were developed and evaluated for their *in vitro* performance (gelling capacity, rheological studies, spreadability, bioadhesion strength, and *in vitro* drug release) and *in vivo* evaluation was carried out by monitoring the reduction in the IOP.

EXPERIMENTAL

Brimonidine tartrate was received as a gift sample from Farmak, Czech Republic, Olomouc. Pectin (molecular weight 30,000–100,000), sodium alginate (structural unit 198.11, macromolecule 10,000–600,000) and calcium chloride dihydrate ($CaCl_2.2H_2O$) were purchased from LOBA chemie, Mumbai. Thioglycolic acid was purchased from Merck India Limited, Mumbai. Ellman's reagent was purchased from Sigma-Aldrich. All other reagents used were of analytical grade.

Derivatization of Pectin

Thiolation of pectin was done by method already reported by Sharma and Ahuja.[12] Pectin (16 g) was dissolved in 70 mL of hot water, 7.59 g of 80% thioglycolic acid, and 2 mL of 7N HCl were added into this solution. These were allowed to react for 150 min at 80°C. The reaction mixture was poured in 500 mL of methanol. White precipitates of TP so obtained were washed twice with methanol and dried at room temperature.

Characterization of Pectin and Thiolated Pectin

ATR Spectroscopy. The powdered polymers were characterized using ATR spectroscopy. ATR spectroscopy was done using ALPHA-E, ATR/FTIR spectrometer (Bruker IR, Germany) over a frequency range of 4000–500 cm^{-1}.

DSC Studies. The thermal properties of the powdered polymers were evaluated by differential scanning calorimeter (Mettler Toledo 812E, Switzerland). The samples were heated at a heating rate of 10°C/min.

Determination of Thiol Group Content. The degree of thiol group substitution was determined by quantifying the amount of thiol group on TP by Ellman's method.[13]

Preparation of Blank *In Situ* Gelling Formulations

Pectin, TP, and sodium alginate were dissolved in phosphate buffer pH 7.4 using a magnetic stirrer to produce different concentration of polymers (Tables I and II). The formulations were filled in 30 mL glass vials and capped.

Gelling Capacity Studies and Effect of Ions on Gelling

The gelling capacity was determined by dropping 100 μL of *in situ* gelling system in a test tube containing 2 mL of simulated tear fluid (sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g, and purified water sufficient to make 100 mL) pH 7.2, equilibrated at 37 ± 1° C and visually assessing the gel formation. The time taken for gelation of polymers and the time taken for the gel formed to dissolve was observed.[14]

Partial ternary phase diagrams of polymers and Ca^{2+} ions were constructed for polymers in order to investigate the effect of Ca^{2+} ions on the sol to gel transition. The different polymers were dissolved in water, and while cooling down, appropriate amount of $CaCl_2$ solution was added. The solution was left overnight to equilibrate. They were classified as solutions, viscous solutions or gels in terms of their visual appearance and flow the following day.[15]

Rheological Studies. Viscosity measurements of the prepared formulations were carried out using Brookfield viscometer LVDV 1 (spindle no. 00) at different angular velocity ranging from 10 to 100 rpm at a temperature of 37 ± 1°C. The hierarchy of shear rate was reversed and average of two readings was used to calculate viscosity. Evaluations were conducted in triplicate.[16]

Spreadability and Contact Angle. For the determination of spreadability, sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 g weight for 5 min. Weight was added to the pan. The time and the weight in which the upper glass slide moves over to the lower plate was taken as measure of spreadability.[17]

$$S = \frac{ML}{T}$$

Where, M = weight tied to upper slide, L = length moved on the glass slide, T = time taken.

Contact angle measurements were performed on formulations for the determination of relationship between contact angle and spreadability. A volume of 200 μL of each formulation was dropped onto goat conjuctival membrane fixed on glass slide. Images were taken and analyzed using software "Image J".[18] All measurements were performed in triplicate.

Measurement of Bioadhesive Strength. The *in vitro* bioadhesive strength was evaluated using texture analyzer equipment equipped with a 50 kg load cell (TA.XT plus; Stable Micro Systems, UK). Freshly excised conjunctiva membrane of an adult

Table I. Physiochemical Characteristics of Formulations Comprising Pectin or Pectin and Sodium Alginate

Batch code	Conc. of P/SA (%w/w)	Gelling capacity	Spreadability (gcm s^{-1})	Contact angle (°)
P_1	1/0	–	[a]	[a]
P_2	2/0	–	[a]	[a]
P_3	3/0	–	[a]	[a]
P_4	4/0	+	[a]	[a]
P_5	5/0	++	[a]	[a]
P_6	6/0	+++	307.10 ± 2.14	20.15 ± 1.21
P_7	7/0	+++	288.71 ± 3.05	24.52 ± 2.16
$P_1SA_{0.5}$	1/0.5	–	[a]	[a]
P_1SA_1	1/1.0	+	[a]	[a]
$P_2SA_{0.5}$	2/0.5	–	[a]	[a]
P_2SA_1	2/1.0	++	[a]	[a]
$P_3SA_{0.5}$	3/0.5	–	[a]	[a]
P_3SA_1	3/1.0	++	[a]	[a]
$P_4SA_{0.5}$	4/0.5	+	[a]	[a]
P_4SA_1	4/1.0	++	[a]	[a]
$P_5SA_{0.5}$	5/0.5	++	[a]	[a]
P_5SA_1	5/1.0	+++	264.32 ± 2.11	29.64 ± 3.04
$P_6SA_{0.5}$	6/0.5	+++	243.92 ± 1.42	31.42 ± 2.11
P_6SA_1	6/1.0	+++	210.14 ± 2.16	36.21 ± 1.20
$P_7SA_{0.5}$	7/0.5	+++	188.25 ± 3.28	44.49 ± 0.86
$P_7SA_{0.5}$	7/1.0	+++	171.19 ± 2.64	51.42 ± 1.34

P, pectin; SA, sodium alginate; –, no gelation; +, immediate gelation, but gel dissolves rapidly, ++, immediate gelation, gel remains for a few hours however, less stiff gel is formed; +++, immediate gelation, remains for extended periods and forms stiff gels;
[a] batches not evaluated.

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.[19] The conjunctival membrane was mounted securely in place on a cylindrical probe (using double sided adhesive tape) which was fixed to the mobile arm of the texture analyzer. A sample of the prepared polymeric formulation was placed to the lower arm. The cylindrical probe with the membrane attached to its base was lowered at a speed of 0.5 mm/s at a force of 1 N for a contact time of 2 min. It was then withdrawn at a rate of 0.5 mm/s to a distance of 10 mm. The mucoadhesive performance of the samples was determined by measuring the resistance to the withdrawal of the probe (maximum detachment force) reflecting the mucoadhesion characterization of the polymeric formulations with conjuctival membrane. At least three repetitions were obtained for each measurement.

Preparation of *In Situ* Gelling System of Brimonidine Tartrate

In situ gelling formulations of brimonidine tartarate (final drug concentration 1.5% w/v) were prepared employing pectin or TP alone, combination of PSA or TPSA were dissolved in phosphate buffer pH 7.4 using a magnetic stirrer. The solutions were made isotonic by the addition of sodium chloride. The pH of the solution was adjusted to 7.4. The formulations were filled in 30 mL glass vials, capped and terminally sterilized by autoclaving at 121°C and 15 Pa for 20 min. Sterilized formulations were stored in a refrigerator (4–8°C) until use.[16,20]

Appearance and Determination of pH. The appearance of the formulations, that is, clarity, color of solution was observed visually and pH was noted. The clarity of formulated solutions was determined by visual inspection alternatively against black and white backgrounds. pH was measured using pH meter.[21]

Estimation of Drug Content. The vials ($n = 3$) containing the investigational formulations were shaken for 2–3 min manually, 1 mL of the preparation was transferred to volumetric flasks the final volume was made up with phosphate buffer pH 7.4.[16] One micro liter of this solution was transferred to 10 mL volumetric flask with a pipette and the volume was made up with phosphate buffer pH 7.4 and stirred for 2–3 min. The concentration of brimonidine tartrate was determined spectrophotometrically at 250 nm.

Sterility Test. Two micro liter of the formulation from test container was removed with a sterile syringe. The test formulation was aseptically transferred to fluid thioglycolate medium (20 mL) and soya bean-casein digest medium (20 mL), separately. The inoculated media were incubated for not less than 14 days at 30–35°C in the case of fluid thioglycolate medium and 20–25°C in the case of soya bean-casein digest medium.[22]

Isotonicity Study. Isotonicity has to be maintained to check tissue damage or irritation of eye. Formulations were mixed with

Table II. Physiochemical Characteristics of Formulations Comprising Thiolated Pectin or Thiolated Pectin and Sodium Alginate

Batch code	Conc. of P/SA (%w/v)	Gelling capacity	Spreadability (gcm s^{-1})	Contact angle (°)
TP_1	1/0	–	[a]	[a]
TP_2	2/0	–	[a]	[a]
TP_3	3/0	–	[a]	[a]
TP_4	4/0	++	[a]	[a]
TP_5	5/0	+++	304.19 ± 2.05	21.08 ± 1.06
TP_6	6/0	+++	295.15 ± 1.15	22.15 ± 2.85
TP_7	7/0	+++	275.03 ± 1.25	26.85 ± 1.26
$TP_1SA_{0.5}$	1/0.5	–	[a]	[a]
TP_1SA_1	1/1.0	+	[a]	[a]
$TP_2SA_{0.5}$	2/0.5	–	[a]	[a]
TP_2SA_1	2/1.0	++	[a]	[a]
$TP_3SA_{0.5}$	3/0.5	+	[a]	[a]
TP_3SA_1	3/1.0	++	[a]	[a]
$TP_4SA_{0.5}$	4/0.5	++	[a]	[a]
TP_4SA_1	4/1.0	++	[a]	[a]
$TP_5SA_{0.5}$	5/0.5	+++	238.52 ± 3.12	33.46 ± 0.95
TP_5SA_1	5/1.0	+++	201.32 ± 2.34	39.72 ± 3.06
$TP_6SA_{0.5}$	6/0.5	+++	198.09 ± 2.18	41.43 ± 2.45
TP_6SA_1	6/1.0	+++	185.31 ± 3.01	46.16 ± 1.61
$TP_7SA_{0.5}$	7/0.5	+++	168.42 ± 2.61	49.19 ± 0.58
$TP_7SA_{0.5}$	7/1.0	+++	149.65 ± 1.02	53.72 ± 3.96

TP, thiolated pectin; SA, sodium alginate; –, no gelation; +, immediate gelation, but gel dissolves rapidly; ++, immediate gelation, gel remains for a few hours however, less stiff gel is formed; +++, immediate gelation, remains for extended periods and forms stiff gels;
[a] batches not evaluated.

few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation-containing brimonidine tartrate.[23]

***In Vitro* Diffusion Study.** Keshary-Chien Franz diffusion cell was used for evaluation of drug release. The formulation containing brimonidine tartrate was placed in donor compartment and freshly prepared simulated tear fluid in receptor compartment. The diffusion study was carried out using dialysis membrane (0.22 μm pore size).[24] The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37 ± 0.5°C. One micro liter of sample was withdrawn at predetermined time interval of 1 h for 8 h and same volume of fresh medium was replaced. The withdrawn samples were diluted to 10 mL in a volumetric flask with simulated tear fluid and analyzed by UV spectrophotometer at 250 nm. The drug content was calculated using the equation generated from standard calibration.

Ocular Irritation Studies. The optimized formulations were evaluated for *in vivo* performance in animal model (Rabbits). All animal experiments were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi, India (approval number 107/99/CPCSEA-2011-19). Six rabbits were used for this study. The animals were placed in cages and the eyes were marked as test and control. The control group received no sample and the test eye received the formulation (50 μL), and the eyes were observed for the ocular irritancy periodically for redness, swelling, watering of the eye (includes the macroscopic observation of cornea, iris, and conjunctiva).

***In-vivo* Studies.** *In vivo* studies were carried out using Reichert PT100 Tonometer. IOP lowering activity of selected formulation of brimonidine tartrate was studied in normotensive albino rabbits weighing 1.5–2.0 kg. This experimental protocol was approved by Institutional Animal Ethical Committee. The animals were housed under well controlled conditions of temperature (20–25°C), humidity and given access to food and water.[25] Rabbits were divided into five groups each comprising three animals. The first group was administered formulations P_7, second group TP_6, and the third group was administered P_5SA_1. The fourth group of animals received Brimodin™ (marketed formulation). The formulations were instilled on the corneal surface of one eye and contra lateral eye was kept as control. The fifth group received investigational formulation without drug (blank). The IOP was measured with tonometer as a function of time. Ocular pressure (IOP) changes were recorded before drug administration and every hour for a period of 8 h till the pressure difference between the control eye and treated eye is zero. The ocular hypotensive activity was expressed as the average difference of IOP between "0" time to "t" time to

minimize the diurnal, seasonal, individual variation commonly observed in rabbits.

Change in IOP (ΔIOP) =IOP "0" time – IOP "t" time

Statistical Analysis

ANOVA was used to determine statistical significance. Differences were considered to be significant for values of $P < 0.05$.

RESULTS AND DISCUSSION

Derivatization of Pectin

The yield of TP was found to be 58.62% and the thiol group content of TP was found to be 0.62 ± 0.005 mmol of thiol groups/g of polymer.

The ATR spectra of TP showed a peak at 2367 cm^{-1} indicating the presence of mercaptan group. Further, the presence of –SH stretch at 2367 cm^{-1} and increased intensity of C=O stretch of ester confirms the formation of TP (Figure 1). Similar peaks have already been reported by Sharma and Ahuja, 2011.[12] The DSC thermograms of pectin and TP are shown in Figure 2. The samples were analyzed by DSC studies at a heating rate of 10°C/min. The DSC thermograms of pectin (A) (Figure 2) revealed one endotherm at 83.09°C, second at 198.22°C, and exotherm at 239.34°C. The endothermic peak at 83.09°C can be ascribed to glass transition temperature (T_g), at 198.22°C can be assigned to melting temperature and exothermic peak at 239.34°C can be attributed to thermal decomposition of pure pectin.[26] The DSC thermogram of TP (B) (Figure 2) exhibited first endotherm at 229.24°C followed by second endothermic transition at 345.11°C. It was observed that the peak temperature corresponding to first endotherm shifted from 83.09 to 229.24°C and second endotherm shifted from 198.22 to

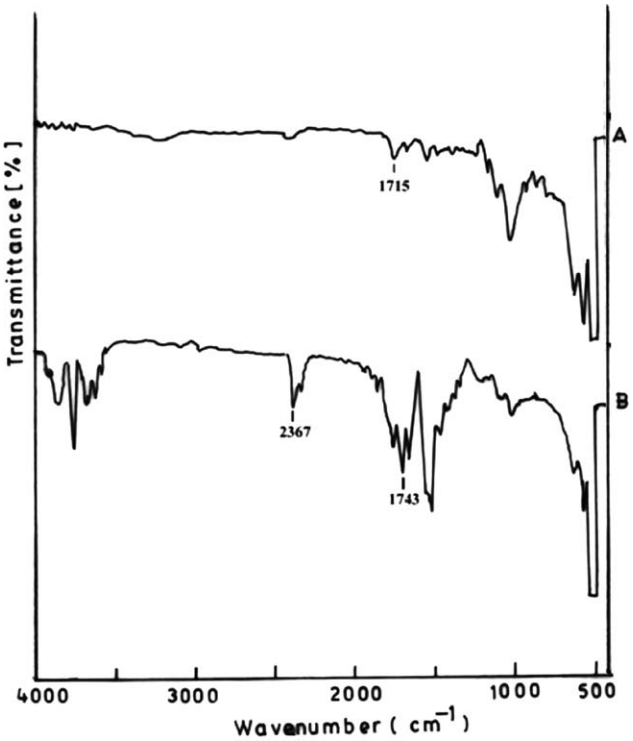

Figure 1. ATR analysis of pectin and thiolated pectin.

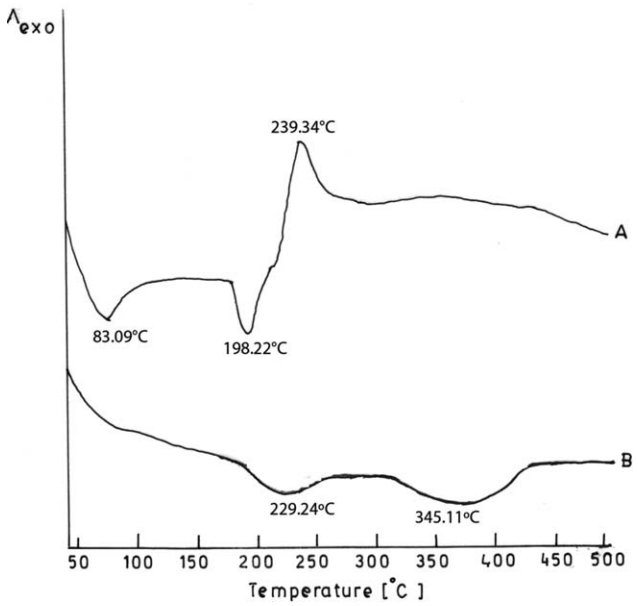

Figure 2. DSC analysis of pectin and thiolated pectin.

345.11°C. Thus, the shift in the endothermic peaks and disappearance of exothermic peak in the thermal curve of TP indicates the thiolation of pectin. Similar results demonstrating the changes in the peak transition temperature in thiolated tamarind seed polysaccharide have already been reported.[27]

Gelling Capacity Studies. Preliminary studies were carried out to determine the concentration of pectin, TP, and sodium alginate necessary for formulation of *in situ* gelling drug delivery. Tables I and II depicts the gelling capacity of various investigational formulations.

The formulations comprising pectin alone (P_1–P_3), or TP alone (TP_1–TP_3), PSA ($P_1SA_{0.5}$, $P_2SA_{0.5}$, $P_3SA_{0.5}$), or TPSA ($TP_1SA_{0.5}$, $TP_2SA_{0.5}$) were not showing any gelation. Although gelation was observed in formulation P_4, TP_4, PSA (P_1SA_1, P_2SA_1, P_3SA_1, $P_4SA_{0.5}$, P_4SA_1, $P_5SA_{0.5}$) or TPSA (TP_1SA_1, TP_2SA_1, $TP_3SA_{0.5}$, TP_3SA_1, $TP_4SA_{0.5}$, TP_4SA_1) but it was observed that the gel dissolved immediately. Stable gelation was observed in formulations P_6, P_7, TP_5, TP_6, TP_7, PSA (P_5SA_1, $P_6SA_{0.5}$, P_6SA_1, $P_7SA_{0.5}$, P_7SA_1), or TPSA ($TP_5SA_{0.5}$, TP_5SA_1, $TP_6SA_{0.5}$, TP_6SA_1, $TP_7SA_{0.5}$, TP_7SA_1). The gels were stiff and the gelation was maintained for extended period of time.

Thiolation of pectin was observed to enhance the gelling capacity of pectin. The *in situ* gelling process in thiolated polymers occurs as a result of oxidation of thiol moieties on thiomers. Therefore, introduction of thiol groups improves the *in situ* gelling properties due to the formation of disulfide bonds[28] and immobilization of thiol groups.[29]

The ternary phase diagrams for the polysaccharides are depicted in Figure 3. The ternary phase diagrams and gelling capacity studies demonstrated that the mixture of TPSA form better gels at low concentration followed by PSA as compared to the same concentration of TP or pectin alone. The degree of esterification of the pectin and the mannuronic acid (M)-guluronic acid (G) ratio (M:G) of the alginate are the factors which affect the

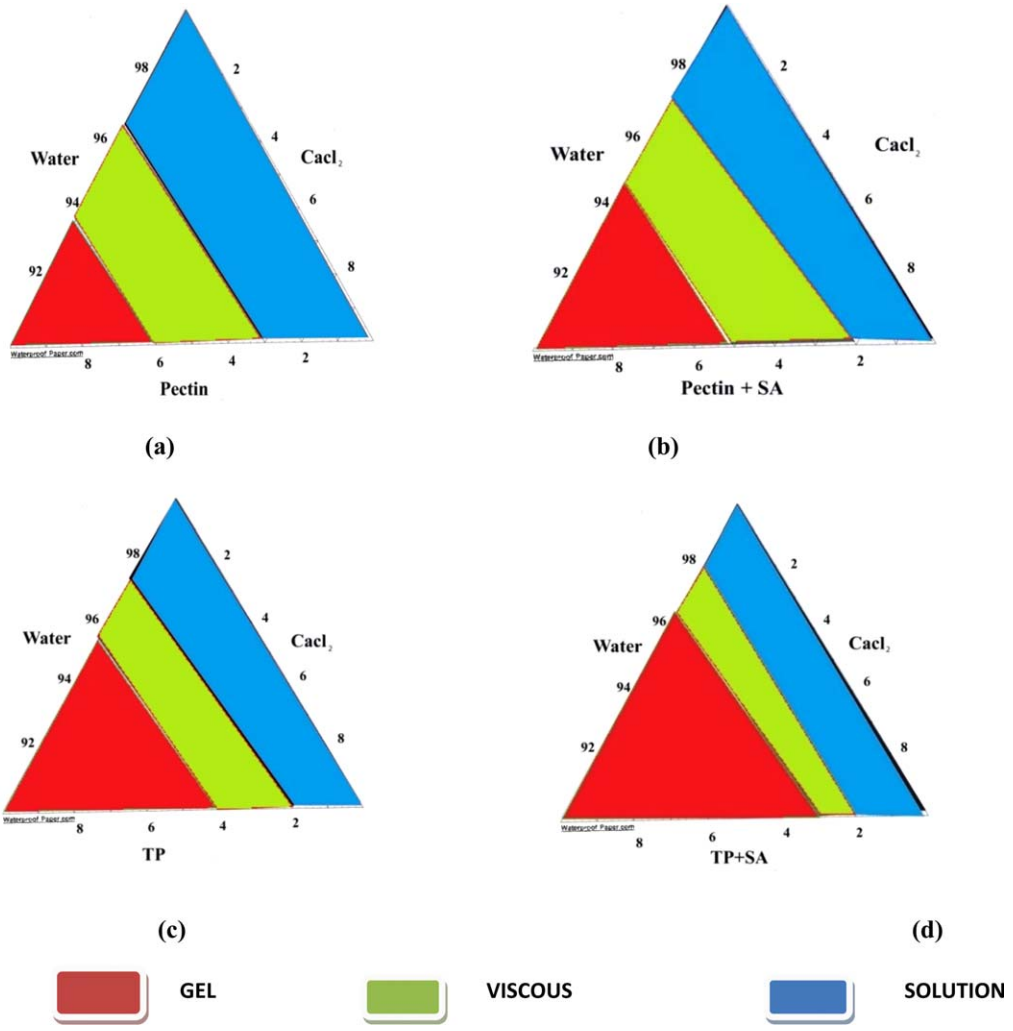

Figure 3. Partial ternary phase diagrams showing the effect of Ca^{2+} on the sol-to-gel transition of (a) pectin, (b) pectin and sodium alginate (PSA), (c) thiolated pectin (TP), and (d) thiolated pectin and sodium alginate (TPSA). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

stability of the gels. The synergistic interaction between pectin and alginate can be attributed to heterogeneous associations of the G blocks of alginate with the methyl esters of pectin. This result in the formation of a more cohesive gel network as compared to those formed from alginate and pectin alone.[30] This co-operative binding of Ca^{2+} in polyguluronate (regions of alginates) and polygalacturonate (regions of pectin) gels, is through "egg-box" complexes with the polysaccharide chains.[31] The oxidation of thiol groups at physiological pH value causes thiolated polymers to form extremely viscous gels by the process of formation of disulfide bonds between the polymer chains.[32]

On the basis of gelling capacity, formulations showing immediate gelation that persisted for extended periods and formed stiff gels, that is, P_6, P_7, TP_5, TP_6, TP_7, P_5SA_1, $P_6SA_{0.5}$, P_6SA_1, $P_7SA_{0.5}$, P_7SA_1, $TP_5SA_{0.5}$, TP_5SA_1, $TP_6SA_{0.5}$, TP_6SA_1, $TP_7SA_{0.5}$, and TP_7SA_1 were selected for further studies.

Rheological Studies. Figure 4 depicts the viscosity of different investigational formulations. The formulations prepared with TPSA were showing maximum viscosity followed by PSA > TP > P (pectin). Thiolation of polysaccharides results in extensive cross linking process by the formation of inter- and intramolecular disulfide bonds within the polymer thus leading to increase in viscosity. Viscosity values in the range of 15–50 cps have been shown to significantly improve the contact time in the eye.[33] However, an increase in the viscosity above 50 cps has shown to result in eye irritation.[34] Therefore, the formulations possessing viscosity <50 cps were selected for drug loading.

Spreadability and Contact Angle. An increase in the spreadability of polymeric formulations decreases the contact angle. A significant difference (P <0.05) was observed in spreadability between the various investigational formulations. Formulations prepared with pectin (P) showed maximum spreadability with minimum contact angle followed by TP > PSA > TPSA shown in Tables I and II. Further, the contact angle was found to be a function of polymer concentration. An increase in polymer concentration leads to an increase in viscosity of formulation thus leading to decrease in spreadability or increase in contact angle. In general, the formulations which showed lower contact angle

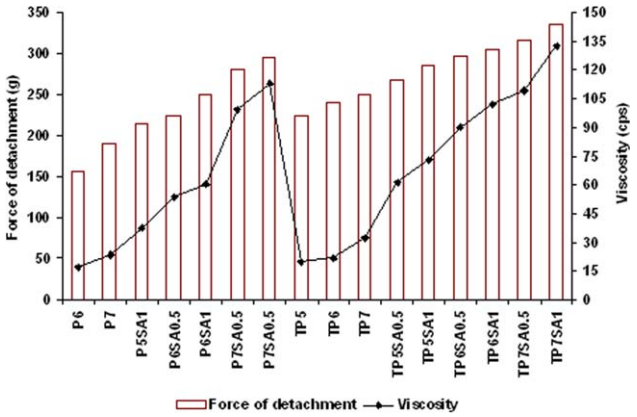

Figure 4. Force required for detachment of formulated *in situ* gels from conjunctival goat membrane and viscosity of investigational formulations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

values, exhibit better spreading on the ocular surface and vice-versa. A high contact angle value of polymeric formulations has been indicated to possess poor spreadability on the ocular surface.[15]

Measurement of Bioadhesive Strength. Bioadhesive strength studies were carried out with selected formulations using texture analyzer. PSA have been reported as mucoadhesive polymers. The mucoadhesive property of PSA can be attributed to the formation of hydrogen bonds with mucin-type glycoproteins through carboxyl–hydroxyl interactions.[12]

TP was found to possess greater bioadhesive strength as compared to pectin (Figure 4). The superior mucoadhesive property of TP can be attributed to the formation of disulfide bonds between the —SH groups of TP and cysteine-rich subdomains of mucus glycoproteins.[12] The immobilization of thiol groups on mucoadhesive polymers, results in an improvement in mucoadhesive properties from 2- to 140-fold.[9] The investigational formulations TPSA required maximum force for detachment as compared to PSA. The G-blocks of the alginate have been reported to interact with methylated regions of pectin chains resulting in increase in bioadhesion strength.[30] Although, PSA alone are reported to possess bioadhesive property however, their combination was displaying increased bioadhesive strength. A combination of polymers has been reported to possess better bioadhesive strength as compared to individual polymers due to synergistic action[35] and formation of more cohesive gel networks than those formed from polymer alone.[30] Further, bioadhesive strength was observed to increase with a corresponding increase in the polymer concentration because the more concentrated polymer results in a longer penetrating chain length and better adhesion.[36]

On the basis of gelling capacity, viscosity, spreadability and bioadhesion strength formulations P_7, TP_6, and P_5SA_1 were selected for drug loading and subjected for further evaluation parameters. Although the force required for detachment in TPSA formulations was good however, due to high viscosity these formulations was rejected for further studies.

Evaluation of Drug Loaded Formulations

All the formulations were found to be light yellow in color. The pH of the formulations was found to be within ± 0.8 units of the neutral pH. Therefore, these formulations should not cause any irritation to eyes. The drug content was found to be in the range of 97.10–99.11%. No microbial growth was observed in sterilized ophthalmic *in situ* gels. Based on these observations, it was concluded that moist heat sterilization of formulations could be done to achieve the sterility. The shape and size of blood cells was found to be same or nearly same as that of blood cells with standard marketed formulation.

In Vitro Diffusion Study

In vitro release studies were conducted using Keshary-Chien franz diffusion cell. Simulated tear fluid 22.5 mL (pH 7.4) was used as dissolution medium. The formulation P_7, TP_6 and P_5SA_1, respectively, released 90.15%, 85.16%, and 76.04% of brimonidine tartrate in 8 h as shown in Figure 5. An inverse relationship was observed between viscosity of the formulation and drug release. An increase in the viscosity of formulation (P_5SA_1) resulted in delayed release of brimonidine tartrate. The influence of viscosity on the diffusion of the drug can be described on the basis of the Stokes–Einstein equation which demonstrates that an increased viscosity of the formulation results in slower diffusion of the drug across the gel matrix and into the receptor medium. A one way ANOVA revealed that there was no significant difference ($P < 0.05$) in the release profile from the investigational formulations P_7 and TP_6. However, the release from P_5SA_1 was significantly different from the other two formulations.

The *in vitro* release data were kinetically analyzed according to zero-order, first-order, and diffusion-controlled release mechanism (Higuchi model). The correlation coefficient (r^2) value was used as criteria to choose the best model to describe drug release from *in situ* gelling formulations. In most of the formulations the r^2 values were higher in Higuchi model. The relative high correlation coefficient values obtained from the analysis of

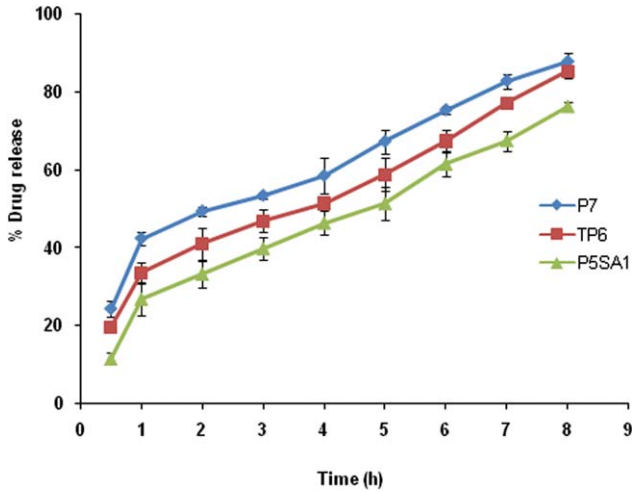

Figure 5. *In vitro* release profile of brimonidine tartrate from P_7, TP_6, and P_5SA_1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

percentage of drug released versus square root of time indicated the fitting of the data to the Higuchi kinetic model, that is, diffusion-controlled release mechanism.[37]

Ocular Irritation Studies. The possibility of eye irritation due to the *in situ* gel administration was evaluated in rabbits. Rabbits were graded for ocular lesions and no symptoms of ocular irritation such as redness, tearing, inflammation, or swelling were observed after *in situ* gelling formulations administration.

In Vivo Studies. The mean percentage decrease in IOP profiles after the instillation of brimonidine solution or application of its *in situ* gelling formulations into the rabbit's eye for 8 h following administration is shown in Figure 6. Three formulations P_7, TP_6, and P_5SA_1were selected for *in vivo* studies after their *in vitro* release. Both the test products (designed *in situ* gelling formulations) and marketed (mkt) eye drop solution (Brimodin^{TM}) was administered only once to obtain relative comparison between single-dose administrations.

There was no reduction in IOP with the blank formulation. It was observed that the marketed formulation produced an immediate decrease in IOP and this decrease was maintained for 2 h. However, after 2 h the IOP was again found to increase. The investigational formulation TP_6 was found to decrease IOP within 1 h and this decrease was maintained for 5 h. There was a significant difference ($P < 0.05$) in IOP between the marketed formulation and the investigational formulation. The decrease in IOP was maintained for 5 h indicating a sustained release of brimonidine. Although, pectin alone formulation (P_7) produced a reduction in IOP value, the maximum reduction in intraocular pressure (ΔIOP) was produced by TP6 and was found to be 4.1 mmHg as compared to 3.3 mmHg in P_7. The TP_6 formulations was found to reduce the IOP till 5 h however the pectin formulation produced a maximum effect till 3 h The investigational formulation P_5SA_1 produced only a small reduction in IOP. The considerable less reduction in IOP value could be due to the high viscosity and hence low spreadability of formulations P_5SA_1 and P_7.

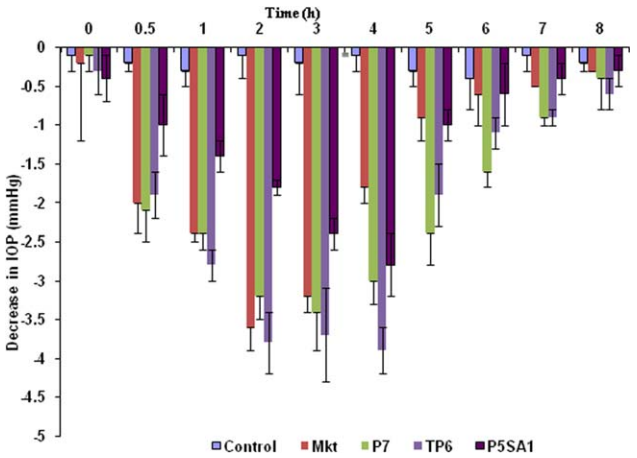

Figure 6. Decrease in intraocular pressure (IOP) after administration of marketed formulation and *in situ* gelling formulations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSION

Brimonidine tartrate was successfully formulated as an *in situ* gelling system using pectin, TPSA. The formulation was found to be suitable for sustained topical drug delivery to eyes. The *in situ* gelling formulations were found to decrease the IOP in rabbits for a longer period of time when compared to conventional eye drop formulation. Thus, this new formulation is a viable alternative to conventional eye drops by virtue of its longer precorneal residence time and ability to sustain drug release.

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