International Standard Serial Number: 0976-1403

Deccan J. Pharmaceutics and Cosmetology 1(2): April-June 2010

DECCAN PHARMA JOURNAL SERIES

ARMS Online Publications

www.deccanpharmajournals.com

In Situ Ocular Gel of Pilocarpine Nitrate: Formulation, Rheological Studies, *In-Vitro* and *In-Vivo* Evaluation

P. C. Satbhai*, Rita.R. Lala

Prin. K.M.Kundnani College of Pharmacy, Cuffe Parade, Mumbai-400005, Maharashtra, India

Abstract:

To achieve therapeutic goals in ophthalmic therapy, increased contact time is important for increasing the amount of drug penetrating through the cornea. To increase the contact, the timely gelation and retention of in situ gelling ophthalmic formulations would be effective in improving the efficacy of drugs. In this study, Pilocarpine Nitrate gels were formulated and evaluated for various properties including rheology, bioadhesion, gelation time, *in-vitro* and *in-vivo* release. *In-vivo* performance of these gels was assessed using rabbit as the animal model. Different kinds of polymers including those which are thermosensitive, viscosity modifying and bioadhesive were used to prepare the formulations. Our formulation was composed of thermosensitive polymers such as poloxamer 407 (P407), poloxamer 188 (P188), viscosity imparting agents such as sodium alginate and bioadhesive polymers such as polyox WSR301 NF in appropriate concentrations. The formulations exhibited Newtonian behavior at 25 °C but were non-Newtonian at 37 °C. This is most suitable for such ophthalmic delivery systems. The liquid formulations showed differences in gelation time and viscoelastic properties depending on the polymer concentrations used. Upon dilution with simulated tear fluid, the formulations exhibited the rheology and viscoelastic properties typical for a gel. These results indicate that the rheological evaluation at physiologic conditions needs to be carefully studied and evaluated to develop more effective in situ-gelling ophthalmic formulations. *In-vivo* studies showed that this drug delivery system can be used successfully for the treatment of Glaucoma.

Keywords: Cornea, in situ gel, bioadhesion, gelation temperature, rheology, Intra Oular Pressure (IOP)

Introduction:

The development of improved ocular drug delivery systems is very challenging. Significant obstacles that need to be overcome are the rapid turnover rate of the tear film, which quickly flushes the drug out of the pre-corneal area, and the low permeability of the corneal tissue.[1]
Increased contact time is important for increasing the amount of drug entering in to the cornea. In therapies intended for precorneal diseases, e.g. infections and inflammations, the contact time might be decisive for the successful outcome of the treatment. Ophthalmic drug delivery systems such as inserts can provide almost unlimited contact time and can hold almost constant dosage levels during this time. Foreign body sensation, expulsion and difficulties in handling and insertion are problem areas for use of ocuserts.[2] More common methods used to prolong ocular residence times for drugs are viscosity enhancement with polymers or by using suspensions and ointments which retain drugs in the eye much better than solutions.[3] However, large deviations may result due to excessive lacrimation resulting in washing away of the drug.[4]
In situ gels are ophthalmic delivery systems that respond to environmental changes, such that the liquid formulation upon instillation undergoes phase transition in the ocular cul-de-sac to form a viscoelastic gel. The long retention time of the viscous gels is attributable to their high yield stress values, which allow them to withstand in vivo shearing action of the eyelid and eyeball movements.[5]
In this study we present formulations of in situ gels made from pluronics, sodium alginate and polyox. Pluronics (poloxamer 407 and poloxamer 188), are nonionic triblock copolymers composed of a central hydrophobic chain of polyoxypropylene (poly (propylene oxide)) flanked by two hydrophilic chains of polyoxyethylene (poly (ethylene oxide)). Sodium alginate, which is the sodium salt of alginic acid, increases viscosity and also is a bioadhesive. Polyox WSR 301 NF is a water soluble resin known for its bioadhesive properties.
The rheological properties of a gel are characterized by its viscoelastic properties that are measured at low shear. How well the gel stays in the eye is most likely dependent on not only the bioadhesive properties of the gel, but also the bulk rheological properties of the gel, i.e. how well the gel is held together in situ. In this paper we have sought a relationship between the rheology and the ocular contact times of the polymeric gels prepared.

Materials and Methods

The following materials were used as received:
Pilocarpine Nitrate IP/USP (Gift sample from Centaur Pharmaceuticals Ltd, Mumbai, India), poloxamer 407/P407 (Lutrol 127,Signet Chemical Corporation Pvt. Ltd, Mumbai, India), poloxamer 188/P188 (Lutrol 68 Signet Chemical Corporation Pvt. Ltd, Mumbai, India), polyvinyl alcohol, PVA (West Coast laboratories, Mumbai); sodium alginate (Loba Chemie Pvt. Ltd, Mumbai, India); Polyox WSR 301 (Colorcon Ltd, Goa), acetonitrile for chromatography (Merck Specialties, Mumbai, India), Methanol for HPLC (Merck Specialties, Mumbai, India), disodium orthophosphate (HPLC grade, Qualigens Fine Chemicals, Mumbai, India).

Preparation:

An ideal in situ gel should be a free flowing liquid at room temperature to allow reproducible administration into the eye as a drop and then undergo in situ phase transition to form a strong gel capable of withstanding shear forces in the cul-de-sac and sustain drug release at physiological conditions.[6] A gel with gelation temperature between 30–36°C is considered optimum for ophthalmic use.[7] Based on this P407 and P188 were selected due to their thermo-sensitive gelling properties. In order to determine the optimum concentrations of P407 and P188, various mixtures of poloxamer gels were prepared to obtain the desired gelation temperature.
The preparation of the ocular solutions involved following steps:

I. Preparation of the thermoresponsive polymeric solution using Poloxamer 407 and Poloxamer 188 in various ratios,
II. Addition of viscosity imparting agents such as sodium alginate,
III. Addition of the bioadhesive polymer such as polyox NF,
IV. Incorporation of 1% drug in the polymeric solutions.

Prepared formulations were sterilized by UV radiation and refrigerated overnight for further dissolution of the pluronics.

The compositions of 1% formulations coded A, B, C, D, E, F, G, H, and I are as shown in Table 1.

Table1: Compositions of 1% batches A, B, C, D, E, F, G, H, and I

Batch	P407 (%)	P188 (%)	Sodium alginate (%)	Polyox WSR 301(%)
A	15	7	0.25	0.5
B	15	7	1.25	0.5
C	15	7	2.5	0.5
D	15	11	0.25	0.5
E	15	11	1.25	0.5
F	15	11	2.5	0.5
G	15	15	0.25	0.5
H	15	15	1.25	0.5
I	15	15	2.5	0.5

Evaluation:

The gelation temperature was determined by heating the test solution at a rate of 2^0C/min with stirring at 150rpm in a water bath.[8] The results are presented in Table 2.

To determine the bioadhesive force, in situ gel was applied as a thin film between two excised portions of goat cornea attached to tissue holders of plexiglass. The upper holder was hung by means of an aluminium wire, while a light weight polypropylene bottle was hung from the lower tissue holder. Water (at a rate of 1 drop/s) was added to this bottle till the corneal tissue surfaces detached to give the bioadhesive force in gms.[9] (Table 2).

Table2: Table showing gelation temperature & bioadhesion

Batch	Gelation Temperature (^{o}C)	Bioadhesion (g/cm^2)	
		Temperature* 25^{o}C	Temperature* 37^{o}C
A	56	7.2±3.5	30±3.1
B	50	7.8±3.1	32.7±2.6
C	52	8.3±2.9	33.7±2
D	44	8.4±3	34.1±2.3
E	43	8.9±2.3	34.9±2.1
F	42	9.2±2.3	36±3.1
G	39	9.8±2.5	37.3±3.4
H	38	12.4±3.0	44±2.8
I	35	13.9±4.1	46±2.7

***data represented as mean: n= 3**

Rheologies of the prepared in situ gels were studied by using the Brookfield Cone and Plate Viscometer with CAPCALC software at 25.0°C (room temperature), and at 35.0°C (the temperature in the conjunctival sac of the eye). For the measurement of viscosity, one drop of the formulation was placed on the plate of the viscometer and the resistance to the rotation of the cone was measured directly. Statistical software (Design Expert 7.1) was used for validating the results as shown in Table 3.

Table 3: Table showing viscosities of batches F, G, H & I

BATCH	RPM													
	150		300		450		600		450		300		150	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
F	560	2000	523	4000	552	6000	512	8000	540	6000	520	4000	540	2000
G	120	500	150	1000	190	1500	210	2000	190	1500	160	1000	120	500
H	3230	1333	3891	2667	5168	5333	5380	6667	4890	5333	3720	2667	3230	1333
I	3270	1333	3750	2667	4330	5333	4480	6667	4128	5333	3131	2667	2950	1333

A: Shear Stress (N/m^2)
B: Shear Rate (1/s)

Results and discussions

Gelation temperature was found to decrease as the polymer concentration increased (Table 2). Schick et al. [10] have explained the temperature dependent gelation on the basis of configuration change. Since the corneal temperature is about 34^0C, formulation 'I' with a gelation temperature of 35^0C was considered most suitable.

Bioadhesive strength increased with increase in polymer concentration. Maximum detachment force was observed with formulation 'I' as shown in Figure 1 as it contained high concentrations of polyox WSR 301 and sodium alginate Table 2.

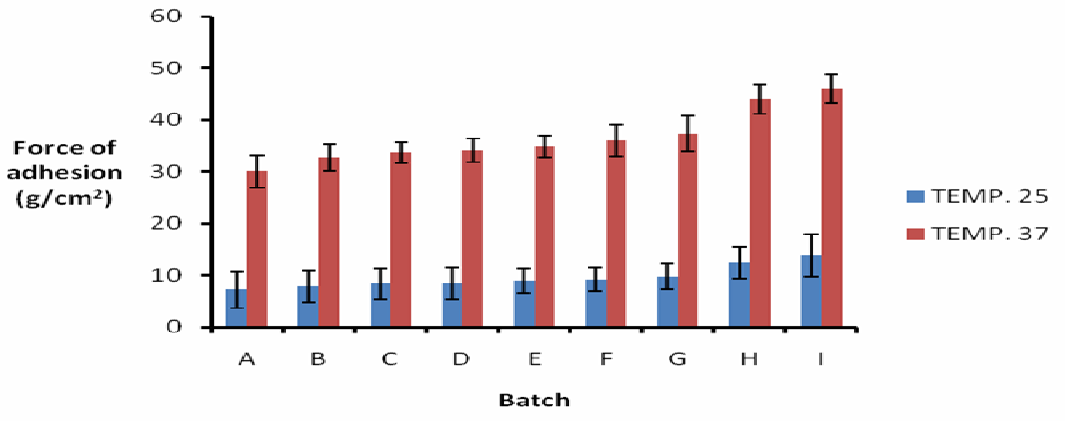

Figure: 1

This formulation is therefore expected to provide extended release of the drug being mucoadhesive.There was not much difference between the viscosities of various batches at 25.0°C and at 35.0°C. Optimized formulation 'I' showed the maximum viscosity of 2.5 Pas. Table 3 shows the rheological studies on batches F, G, H and I as an example. The formulations showed near Newtonian flow before instillation into the eye. However, once in contact with the cations present in the tear fluid and on the ocular surface, formulations containing anionic polysaccharides exhibit pseudo plastic (shear-thinning) behavior. This is a favorable characteristic as the secondary bonds and physically entangled polymer networks of pseudo plastic systems can be easily destroyed by the shear stress applied during blinking, while they may regain their structures during inter blinking periods, therefore retaining the formulation in front of the eye.

Formulations which give increased contact time with the cornea are gaining importance in ophthalmology. Various combinations of natural polymers such as sodium alginate, synthetic polymers such as polyox WSR 301 and thermosensitive polymers can be used to attain desired drug release pattern extending to several hours. From the data it can be concluded that the ophthalmic in situ gel formulated provided constant drug levels in the tear film cornea compartment for a long period of time.

Conclusion:

Poloxamer, sodium alginate and polyox can be used to develop in situ gel for ophthalmic systems to treat glaucoma. This system can release drug upto 6-8hr with increased patient compliance.

Acknowledgement:

We are grateful to All India Council of Technical Education (AICTE) for providing the grant for conduct of this research.

References:

1. Lee VHL, Robinson JR. Topical Ocular drug delivery: Recent developments and future challenges. J Ocul Pharmacol 1986; 2: 67-72.
2. Katarina Edsman, Johan Carlfors and Kaisa Harju. Rheological evaluation and ocular contact time of some carbomer gels for ophthalmic use. Int J Pharm 1996; 137: 223-241.
3. J.Y. Chang et al. Rheological evaluation of thermosensitive and Mucoadhesive vaginal gels in physiological conditions. Int J Pharm 2002; 241: 155–163.
4. Sieg, J.W., Robinson, J.R. Vehicle effects on ocular drug bioavailability. J Pharm Sci 1975; 64: 931–936.
5. C.H. Lee et al. Thyrotrophic property in pharmaceutical formulations. Journal of Controlled Release 2009; 136: 88-98.
6. Eun-Young Kim, Zhong-Gao Gao, Jeong-Sook Park, Hong Li, Kun Han. rhEGF/HP-CD complex in poloxamer gel for ophthalmic delivery. Int J Pharm 2002; 223: 159-167.
7. Gang Wei, Hui Xu, Ping Tian Ding, San Ming Li and Jun Min Zheng. Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies. Journal of Controlled Release 2002; 83: 65-74.
8. Varsha Agarwal and B. Mishra. Design, Development, and Biopharmaceutical Properties of Buccoadhesive Compacts of Pentozocine. DDIP 1999; 25: 701-709.

9. Ashutosh Jani, Ramesh K Goyal, Gaurang B Shah and Anita A Mehta. Effect of Calcium Channel Blockers on Intraocular Pressure in Rabbits. Institute of Pharmaceutical Education & Research, Gandhinagar. IJPT 2005;4: 95-99.
10. Schick, M.J. Configuration of the polyoxyethylene chain in bulk, nonionic surfactant. Surfactant Sci. 1966; 1: 753–793.