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DESIGN AND EVALUATION OF IN-SITU OPHTHALMIC GEL CONTAINING CARBOPOL AND METHYLCELLULOSE AS VISCOSITY MODIFIER

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ABSTRACT
To increase the bioavailability of the drug in eye it is necessary to increase the residence time of the formulation with minimum solution drainage and untoward disposition of the instilled dose. The reliable method to overcome the above problem is in-situ ophthalmic gel. Combination of carbopol 940, a pH dependent polymer and methyl cellulose A4M, a thermo sensitive polymer were used for present research. The total six batches were prepared and subjected to the evaluation. Rheological studies, gel strength measurement, bioadhesion force measurement, drug content uniformity, in-vitro release study were carried out for all formulations. In-vitro study shows drug release up to 12 hrs. The in-vitro study data treated with various kinetic equations revealed that drug release follows first order diffusion controlled by Fickian diffusion mechanism. The optimized batch is subjected to eye safety evaluation, stability study and comparison with marketed preparation for in-vitro release. The present formulation shows the negligible difference in drug release as compare to the marketed preparation. Hence it is concluded that the present formulation will perform well in the eye upon instillation as in-situ gel.

Keywords: *In-situ ophthalmic gel; pH dependent polymer; thermo-sensitive polymer.*

INTRODUCTION

The eye is a very sensitive organ. The tear flow and the reflex blinking maintain a good environment and remove foreign material from the eye. These protective properties, however, also lead to an effective drainage of drugs when introduce into the eye. This results in low bioavailability and short ocular residence time reducing the desired therapeutic effect of the drug[1]. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the GI tract, leading to side effect[2]. Normal volume of the tear fluid retained in the cul-de-sac of the eye is 7-8il, in non-blinking condition eye can accommodate a maximum fluid volume of 30il, while blinking eye can accommodate 10il. Single drop of ophthalmic solution measure only 50il[3], the above parameter states that more than the half quantity of the instilled dose is drained from the eye. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary.

The residence time of the ophthalmic solution can be prolonged by adding a gel forming polymer to the solution and hence increasing the viscosity of the vehicle. In-situ ophthalmic gels are instilled as low viscosity solution into the conjunctival sac of the eye and, upon contact with the eye, the polymer changes confirmation producing the gel[4]. These systems show the pseudoplastic behavior which resembles the tear fluid and hence less irritating effect as compared to the ophthalmic ointment. The phase transition can be induced by shift in pH, as for carbomers[5] and cellulose acetate phthalate[6], a shift in temperature as for the Xanthan gum[7] and methyl cellulose[8] or by presence of cations as for gellan gum[9] and sodium alginate[4]. This type of gel combines the advantages of a solution, being patient convenient with a favorable residence time of a gel.

In this present paper a combination of pH sensitive polymer carbopol 940 and thermo sensitive polymer methyl cellulose A4M have been studied. Glaucoma is a group of diseases of the optic nerve involving loss of retinal ganglion cells in a characteristic pattern of optic neuropathy. Although raised intraocular pressure is a significant risk factor for developing glaucoma, the intra ocular pressure can be lowered by the drugs in the form of eye drops. Timolol maleate is the most prescribed drug for lowering the intra ocular pressure.

MATERIAL AND METHOD

Timolol maleate was a gift sample from Ajanta pharmaceutical ltd. Mumbai. Carbopol 940 and methyl cellulose A4M was purchased from the Colorcon Asia Pvt. Ltd. Goa, all other chemical and solvent were of analytical grade.

Isotonicity adjustment

Isotonicity calculations were determined for drug and polymers by calculating sodium chloride equivalents (E) method. The sodium chloride equivalents (E) were then converted to boric acid quantities in order to make isotonic buffered in-situ gel systems by using boric acid

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and not the sodium chloride. The sodium chloride equivalent of the polymer was found to be negligible and for timolol maleate it was found to be 0.0394. Hence the quantity of boric acid required to adjust the isotonicity of the formulation was found to be 1.721.

Preparation of in-situ ophthalmic gel

The in-situ ophthalmic gel was prepared by taking combination of carbopol 940 and methyl cellulose A4M as gel forming polymers and benzalkonium chloride as preservative. Concentration of Carbopol 940 is varied to evaluate the maximum gel forming efficacy at specified concentration, while keeping the constant concentration of methyl cellulose A4M, because at 1% concentration it form the consistent gel. The methyl cellulose A4M was thoroughly mixed with the half quantity of boric acid to avoid the formation of non dispersed lumps in the distilled water. Then the Carbopol 940 is dispersed in remaining quantity of distilled water and both the polymer solution was equilibrating for 1 hrs by adding the drug and preservative (Table 1).

Table 1: *Formulae for the preparation of in-situ ophthalmic gel*

Sr.no	Ingredient	Batch C-0.1	Batch C-0.2	Batch C-0.3	Batch C-0.4	Batch C-0.5	Batch C-0.6
1	Timolol maleate (g) (Equivalent to 0.5 g of timolol)	0.68	0.68	0.68	0.68	0.68	0.68
2	Carbopol 940 (g)	0.100	0.200	0.300	0.400	0.500	0.600
3	Methylcellulose A4M (g)	1.0	1.0	1.0	1.0	1.0	1.0
4	Benzalkonium Chloride (ml)	0.01	0.01	0.01	0.01	0.01	0.01
5	Boric acid (g)	1.72	1.72	1.72	1.72	1.72	1.72
6	Distilled water up to (ml)	100	100	100	100	100	100

EVALUATION OF IN-SITU OPHTHALMIC GEL

Content Uniformity

Drug content uniformity is an important parameter for any pharmaceutical dosage form. For the determination of content uniformity 1 ml ophthalmic sol was taken from each batch, which contain timolol maleate equivalent to 5 mg. It is diluted up to 100 ml with the distilled water in 100 ml volumetric flask. From this 1 ml was pipette out and then diluted up to 50 ml in 50 ml volumetric flask. Resulting solution was analyzed at 295 nm using distilled water as blank on UV spectrophotometer and content of timolol maleate was calculated.

Rheological properties

The viscosities of the solution and gel formed were determined by Brookfield viscometer (LV model). Different sizes and shapes of the spindles were used for the determination of viscosity dependent upon type of solutions, less viscous or more viscous. For batch C-0.1 and C-0.2 spindle no. 1 were used at 0.3 and 0.6 speed respectively, for C-0.3 spindle no. 2 is used at 1.5 speed, for C-0.4 and C-0.5 spindle no. 3 were used at 3 and 1.5 speed respectively while for C-0.6 spindle no. 4 was used at 3 speed. The viscosity in centipoises was calculated by using following equation.

Viscosity in centipoises = dial reading × Factor

Factor was determined with the help of the factor finding chart supplied by the manufacturer.

The viscosity is measured at different pH after addition of 0.1N sodium hydroxide at room temperature and also at 37 ^{0}C.

Measurement of Gel Strength[10]

The gel strength was determined according to method accepted by Abe Elhady et al[10]. A sample of 50 g of the ophthalmic gel was placed in a 100 ml graduated cylinder which was kept in a thermostatically controlled water bath at 37^{0}C. A weight of 27 g (including the weight of the piston) was then placed onto gel (Fig. 1). The gel strength, which is an indication for the viscosity of the ophthalmic in situ gel at physiological temperature, was determined by time in seconds the weight took to penetrate 5 cm down through the gel.

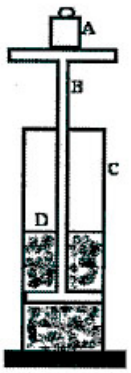

Fig 1: Gel strength measuring device[15]
(A) Weights; (B) Device (Piston); (C) Measuring cylinder; (D) In situ Ophthalmic gel

Determination of Bioadhesive force[10]

The bioadhesive force of all batches was determined on an in-house made assembly. The Bioadhesive force measurement apparatus consisted of a balance. Weight was placed on one arm on a pan. On the other arm of the balance, a glass vial was attached through the copper wire in inverted position to which the conjunctiva was attached with the help of rubber band. Then second glass vial was placed onto the height adjustable pan to which the conjunctiva was attached with the help of a rubber band. The gel was placed on the lower glass vial and contact was made between two vials. It was left for 3 min and weight required to detached the two vials after 3 min was considered as bioadhesion strength of the gel.

Test for sterility

Test for sterility on sterilized in-situ gel was performed according to I.P[11]. Alternative fluid thioglycollate

medium and soyabean casein digest medium was used as culture medium. Half the quantity of each container was added to the fluid thioglycollate medium and remaining half was added to the soyabean casein digest medium and after appropriate dilution it was kept for incubation for 14 days. Sterile media without inoculation was also incubated for same period.

Preservative efficacy study

All the formulations were tested for the effectiveness of the preservative as per IP 1996 method. The test was carried out for the detection of viable forms of bacteria and fungi. The preservative properties of the preparation are adequate if, in the conditions of the test, there is a significant fall or no increase, as appropriate in the number of microorganisms in the inoculated preparation after the times and at the temperature prescribed.

In-Vitro Release Study[12]

The diffusion cell which was used by Bottari et al[12] for the analysis of semi solid formulations was modified and used. The home made assembly consisted of a stainless steel body and a plate which was kept over the body and tightened with screws. The body consisted of a well 5 cm in a diameter and can hold up to 10 ml of gel. For release studies, 10 ml of sol-to-gel (The solution form of the eye drop was converted into gel for in-vitro release study) was kept in the well of diffusion cell. Then muslin cloth was kept over it and the plate was placed over and it was secured with screws. The diffusion cell was placed at the bottom of one liter capacity beaker already filled with 500 ml of simulated tear fluid (STF) of pH 7.4 at 37±0.5 ^{0}C kept in USP paddle dissolution test apparatus. The paddle was lowered into the beaker and rotated at 50 rpm. Sampling was done at various time intervals. 3 ml of sample was withdrawn and replaced with equal quantity of fresh buffer. The samples were filtered through Whatman filter paper no. 42 and analyzed spectrophotometerically at 295 nm using STF of pH 7.4 as blank. Drug concentration was determined from standard curve and cumulative % of drug released was calculated.

Eye safety evaluation[13]

Eye is a very sensitive organ. While developing the novel drug delivery system for the eye it is mandatory to evaluate the eye safety studies to check the any damage to the delicate parts of the eye i.e. conjunctiva, cornea and iris. The modified Draize Technique (scoring method) was used for the eye safety evaluation. The optimized batch was subjected to Draiz test.

Comparison with the marketed preparation

The optimized formulation was compared with the marketed preparation i.e. Timolet GFS for in-vitro drug release study. The release rate was also determined for the marketed preparation.

Stability study

Stability studies were carried out on optimized C-0.4 batch according to ICH guidelines[14]. Sufficient numbers of in-situ gel systems were kept in the stability chamber, packed in its final container (eye drop bottles). The temperature of the stability chamber was adjusted at 40±0.5 ^{0}C and relative humidity at 75%. The study was performed for a period of 3 month as per ICH guidelines.

RESULT AND DISCUSSION

In the present study the total six batches were successfully prepared by adjusting the isotonicity using the modified boric acid buffer as vehicle. The prepared formulations were subjected to different evaluation parameters such as drug content uniformity, rheological study, gel strength measurement, bioadhesion force measurement, sterility test, preservative efficacy study, in-vitro release study and eye safety evaluation.

The content uniformity of all batches have been checked by UV spectrophotometer and it was found that batches C-0.2 to C-0.6 shows content uniformity between 95 to 105%, while the batch C-0.1 showed 90% drug content. The batch C-0.4 showed 99% drug content. Results are given in Table 2.

Table 2: *Gel strength, Bioadhesion force and Content uniformity*

Batch	Content Uniformity (%)	Gel strength (sec)	Bioadhesion force (dyne/cm^2)
C-0.1	90.10	16±2	0.0054
C-0.2	105	25±2	0.0058
C-0.3	96.0	38±3	0.0092
C-0.4	99.4	49±2	0.0097
C-0.5	95.0	60±3	0.010
C-0.6	97.4	70±3	0.013

The viscosity was determined at different pH by adding 0.1N NaOH at room temperature as well as at 37 ^{0}C. Viscosity values in centipoises at pH 4.7 and room temperature, at pH 7.4 and room temperature and at pH 7.4 and 37 ^{0}C for the batch C-0.1 to C-0.6 are given in Fig. 2. The viscosity result shows the viscolizer behavior of the methyl cellulose A4M upon addition to Carbopol 940. The results showed that batch C-0.1 and C-0.2 form the less viscous gel with solution like

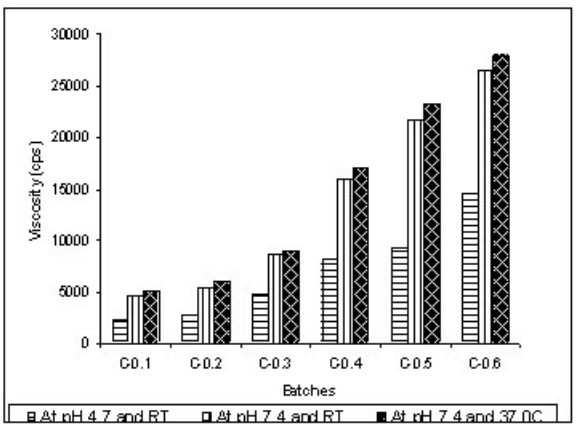

Fig 2: *Viscosity of the formulations at different pH and temperature*

consistency and batch C-0.5 and C-0.6 form the rigid gel upon addition of the few drops of the 0.1N NaOH. While batch C-0.3 and C-0.4 shows the optimum viscosity.

Gel strength of the formulations was determined using in-house made gel strength apparatus. The gel strength values between 25-50 seconds were considered sufficient[15]. Gel strength less than 25 seconds may not preserve its integrity and may erode rapidly while gels with strength greater than 50 seconds is too stiff and may cause discomfort. Results show that formulations C-0.1, C-0.2 and C-0.3 were found to have suitable gel strength and were easily administered as drops[15]. Results are shown in Table 2.

The bioadhesion strength in terms of detachment stress showed that carbopol 940 and methyl cellulose posses the adhesive properties that increases with the concentration of carbopol 940. More bioadhesive strength of carbopol may be due to availability of carboxylic groups[10]. These groups may form hydrogen bonding with glycoprotein of mucin. Results are shown in Table 2.

The formulation C-0.1 to C-0.6 were found to passes the sterility test when carried out for 14 days for the growth of bacteria and fungi, as no colony formation was seen on the Alternative fluid thioglycollate medium and soyabean casein digest medium. The preservative efficacy study was carried out for 28 days and substantial decrease in the number of bacteria and fungi was calculated at the interval of 7, 14, 21 and 28 days. The preservative system used was found to be effective against bacteria as well as fungi.

In-vitro release studies were performed by using modified paddle over disc, diffusion cell method. Results are shown in Table 3. It was observed that release rate decreases in the formulations in order of C-0.1 > C-0.2 > C-0.3 > C-0.4 > C-0.5 > C-0.6 i.e. In C-0.1 release rate was faster and dissolution completed in 9 hours, up to 95% while in C-0.6, release rate was lowest and up to 88% of drug released in 12 hours. In formulation C-0.4 uniform and maximum release occurred up to 12 hours as compared to other formulations. So it was considered as optimized one. In the study a relationship was found in terms of concentration of carbopol and release rate i.e. as concentration of carbopol increased, release rate decreases. This might be due to the consistent viscosity effect of carbopol while increasing its concentration. As release was observed following first order kinetics it was predicted that mechanism of drug release of drug from gels was due to diffusion and erosion. The above results of the content uniformity, gel strength, bioadhesion force and in-vitro release study reveals that the optimized batch is C-0.4 among all batches, and hence it is used for the further study.

Eye irritancy potential of a substance is evaluated on the basis of its ability to cause injury to the cornea, iris and conjunctivae, when the substance is applied to the eyes. The cornea was scored on the basis of density

Table.3: *Cumulative % of drug release*

Time (hrs)	Batch C-0.1	Batch C-0.2	Batch C-0.3	Batch C-0.4	Batch C-0.5	Batch C-0.6
0	0	0	0	0	0	0
0.5	20.798 ±1.258	28.796 ±1.358	20.798 ±1.265	20.788 ±0.265	20.423 ±1.351	20.673 ±1.215
1	35.401 ±1.325	40.226 ±1.654	28.088 ±1.123	28.838 ±1.158	25.088 ±1.859	25.463 ±1.159
2	42.781 ±1.659	57.803 ±1.498	35.517 ±1.012	37.518 ±0.659	31.012 ±1.482	31.263 ±1.459
3	54.489 ±1.654	65.042 ±1.145	41.837 ±1.987	44.342 ±1.569	37.572 ±1.695	36.574 ±1.594
4	60.346 ±1.258	69.546 ±1.035	51.818 ±1.369	50.679 ±0.025	40.896 ±1.983	41.271 ±1.698
5	68.339 ±1.469	74.183 ±1.801	58.020 ±1.859	55.654 ±1.456	46.976 ±1.259	47.852 ±1.498
6	75.599 ±1.265	81.204 ±1.098	63.010 ±1.125	61.888 ±1.985	50.819 ±1.698	52.071 ±1.158
7	81.373 ±1.548	86.114 ±1.698	68.509 ±1.498	69.758 ±0.369	55.669 ±1.364	54.781 ±1.251
8	88.283 ±1.796	89.909 ±1.031	73.643 ±1.659	75.896 ±1.644	61.653 ±1.875	61.031 ±0.012
9	94.456 ±1.789	97.594 ±1.369	78.788 ±1.125	81.421 ±0.598	65.149 ±1.365	66.401 ±0.699
10			87.819 ±1.099	86.031 ±1.982	72.776 ±1.695	75.905 ±0.843
11			92.990 ±1.979	94.376 ±0.498	76.670 ±1.549	78.930 ±0.125
12			96.922 ±1.912	97.936 ±0.624	87.694 ±1.145	89.083 ±0.695

± *Standard Deviation*

of opacity and total area involved. Iris on the basis of degree or intensity of inflammation exhibited and the conjunctiva was scored on the extent of chemosis, redness and discharge. The total score given to cornea, iris and conjunctiva was 5, 5 and 2 respectively. The results showed that the score obtained from the test is less than the total graded score, indicating no irritation or severe damage to the delicate parts of the eye i.e. cornea, iris and conjunctiva.

The marketed product (Timolet GFS) was evaluated for in-vitro drug release and showed 98.505% cumulative drug release while batch C-0.4 showed 97.936% cumulative drug release in 12 hrs. It was revealed that Batch C-0.4 found good in cumulative % drug release when compare with Timolet GFS.

The stability study was carried out as per ICH guidelines, and shelf life of the product is determined upon exposure to the accelerated stability study. The physical parameters were checked after three months of study and it shows negligible changes in the consistency, viscosity and drug content. On performing stability study, the degradation rate constants for formulation C-0.4 were found to be 7.235×10^{-4} and 2.339×10^{-4} day^{-1} respectively at 40 ^{0}C and at room temperature. Since these values are negligible, it could be concluded that very less degradation has occurred.

CONCLUSION

It is concluded that the formulation prepared in our laboratory is good to produce the sustained drug release for 12 hours from the formulation. Rheological and bioadhesion study data reveals the synergistic effect of both the polymers, the in-vitro release study data treated with various kinetic equations and it reveals

that drug release follows first order diffusion controlled by Fickian diffusion mechanism. The optimized batch was found to be C-0.4 which was confirmed by the bioadhesion force, content uniformity and in-vitro release study, C-0.4 also shows the comparative release study with marketed preparation as well as found stable at accelerated stability study for the period of three months.

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