ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Formulation and In vitro Evalution of Gellan Gum/Carbopol and Sodium Alginate based Solution to Gel Depot of Ketotifen Fumarate System

Jaya Raja Kumar. K and Selvadurai Muralidharan

Faculty of Pharmacy,
AIMST University, Semeling, Bedong, Malaysia

Abstract
Conventional ophthalmic solutions often eliminate rapidly after administration and cannot provide and maintain an adequate concentration of the drug in the precorneal area. To solve these problems, we developed a pH in situ gelling and mucoadhesive ophthalmic drug delivery system containing ketotifen fumarate based on gellan gum. The pasture of ocular drug delivery is one of the fascinating and challenging endeavors facing the pharmaceutical scientist. The most frequently used dosage forms i.e. ophthalmic solutions and suspensions are compromised in their effectiveness by several limitations, leading poor ocular bioavailability. The viscosity of in situ system was found to be in the range (85 to 92 cps) for the sol, whereas for the gels it was up to (7000 to 17000 cps). The maximum gel strength and mucoadhesion was found to be up to (102 seconds) and (58.33 dynes/cm^2) respectively. In vitro release studies demonstrated diffusion-prolong release of ketotifen fumarate from the combined polymer solutions over a period of 6 h. It appears that ocular bioavailability can be increased more readily by using the in situ gelling and mucoadhesive vehicle.
Keywords: *Ketotifen Fumarate, HPLC, Gellan gum, Carbomer 934, Sodium Alginate*

INTRODUCTION

Mainly conventional ophthalmic dosage forms are simplistic. It is usual that water-soluble drugs are delivered through topical administration in an aqueous solution [1], and water-insoluble drugs are administered topically as an ointment or aqueous suspension. The major deficiencies of these conventional dosage forms include poor ocular drug bioavailability, pulse-drug entry after topical administration, systemic exposure because of nasolacrimal duct drainage, and a lack of effective systems for drug delivery to the posterior segment of ocular tissue. Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, nasolacrimal drainage [2], and the high efficiency of the blood–ocular barrier [3]. It is standard for only 1% or less of a topically applied dose to be absorbed across the cornea and thus reach the anterior segment of the eye [4, 5]. Pulse entry is a common, and yet highly undesirable, pharmacokinetic characteristic associated with eye drops [6]. The initial high drug concentration found in tears, followed by a rapid decline, poses a potential risk of toxicity, and suggests a requirement for frequent dosing. Attempts to overcome the toxicity associated with the high initial concentration without a requirement for frequent dosing form a challenging task, particularly in the case of potent drugs.

Nasolacrimal drainage is the major factor for precorneal drug loss that leads to poor ocular bioavailability. It is also the major route of entry into the circulatory system for drugs that are applied through topical administration [7, 8]. For potent drugs, the systemic exposure through nasolacrimal drainage after topical administration can be sufficiently high to cause systemic toxicity. Over the past two decades, extensive research has been performed in the design of polymeric drug delivery systems. Among them, new series of thermosensitive, ion induced and pH sensitive in situ gel systems are potential carriers of antifungal drugs for oral thrush These systems are made of biodegradable polymers, which can be injected via a syringe into the infected area where the solution translate in to gel depot [9]. Smart polymeric systems represent promising means of delivering the drugs; these polymers undergo sol-gel transition in accordance with the biological stimuli like pH change, temperature modulation and ion exchange.

MATERIALS AND METHODS

Materials

Ketotifen fumarate was obtained as a gift sample from microlabs, India. Gellan gum was a gift sample received from priya multinational, Mumbai, India. Carbopol 934 was purchased from merk, Mumbai, India. Sodium alginate was procured from SD fine chemicals, India.

Methods

Different formulations were prepared with various ratio of (gellan gum: carbopol 934), (gellan gum: sodium alginate). Many experiments were conducted by varying the concentration of those polymers in order to identify the optimum concentration required for polymeric solution. Formulations were prepared with various ratio of polymers was soaked in sufficient quantity of deionised water and kept overnight for swelling and propyl paraben solution was added to the above polymeric mixture. Finally an appropriate amount of ketotifen fumarate was solubilized in above mixture with continuous stirring until uniform solution was obtained. Finally a small amount of triethanolamine was added to adjust pH 6.8. The detailed composition of prepared formulation is depicted in Table1.

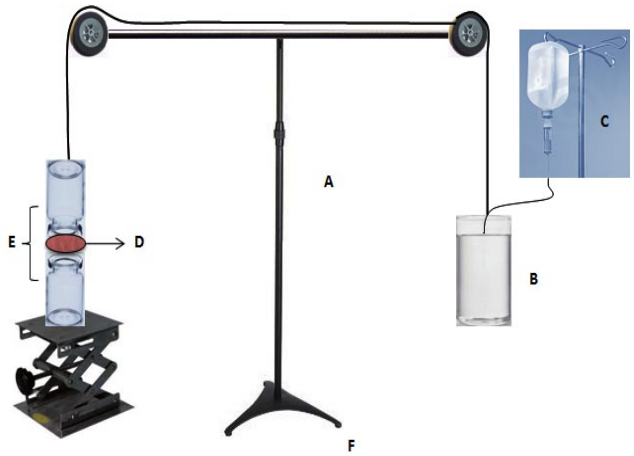
EVALUATION OF IN SITU GEL

Gelling time

The gelling capacity of the prepared formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted [10-11].

Determination of mucoadhesive force

The experimental technique used for determining the mucoadhesive force has been derived from a previously published method [12-13]. The experimental setup is presented in Figure 1. The mucoadhesive force of the formulations was determined as follows; a section of membrane was fixed with mucosal side out onto each glass vial (E) using rubber band. The vial with membrane was connected to the balance in inverted position while first vial was placed on a height adjustable pan (A). Sample was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then, the switch (C) of the infusion apparatus was opened to make the water drop into the glass vial (B) with a constant flow rate of 5 ml/min. The weight of the water in the glass vial (B) kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dynes/cm^2), was determined from the minimal weights that detached the gel. The chicken membrane pieces were changed for each measurement. All measurements were performed in triplicate (n = 3).

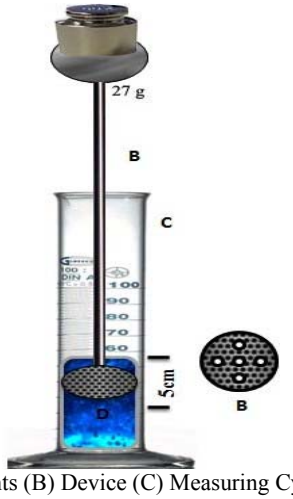


(A) Modified balance (B) Glass reservoir (C) Infusion device (D) Membrane (E) Vial (F) Height adjustment pan.

Figure 1. Assembly of mucoadhesive force measuring device

Measurement of Gel Strength

A sample of 50 gm of gel was placed in a 100 ml graduated cylinder. The apparatus for measuring gel strength (weighing 27 gm) was allowed to penetrate in gel as shown in Figure 2. The gel strength, which means the viscosity of the gels at STF was determined by the time (seconds), the apparatus took to sink 5 cm down through the prepared gel [12].



(A) Weights (B) Device (C) Measuring Cylinder (D) Gel

Figure 2. Assembly of Gel Strength measuring device

Viscosity Studies

The rheological studies were carried out using Brookfield viscometer. The viscosity of *in situ* gel and the solution were determined at different angular velocities and average of two reading was used to calculate the viscosity.

Content Uniformity

The formed gel (1g) was completely crushed with the help of glass road followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution [14]. Final volume was adjusted to 100 ml with fluid. Obtained solution was filtered through 0.45 micron filter membrane and the drug concentration was determined by UV Visible spectrophotometer.

Spreadability

The spreadability of the gel was determined using the following technique: 0.5 g of gel was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed [15]. A weight of 1000 g was allowed to rest on the upper glass plate for 5 minutes. Weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability as shown in Figure 3. The increase in the diameter due to spreading of the gels was noted. The calculation of spreadability (S) is as follows;

$S = ML/T$

Where M = Weight tide to the Upper Slide (g)

L = Length moved on the glass slide (cm)

T = Time taken

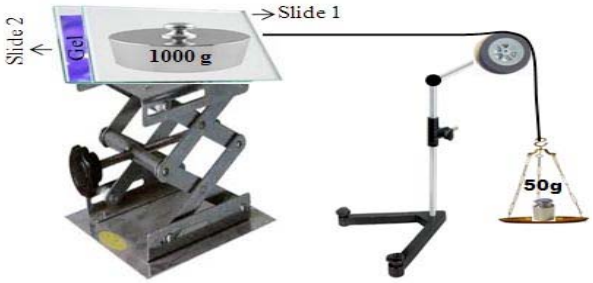


Figure 3. Assembly of spredability measuring device

Ocular Irritation studies

Ocular irritation studies were performed on four male albino rabbits each weighing 2-3 kg. The sterile formulations were instilled twice a day for a period of 21 days and rabbits were observed periodically for redness, swelling in eye.

Sterility test

All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed using nutrient agar medium. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to solidified agar medium. The inoculated media were incubated for 24 hrs to see the growth of microbial.

In vitro release studies

In vitro release studies were carried out using bi-chambered donor receiver compartment model (Franz diffusion cell). In vitro release of ketotifen fumarate in situ gels was carried using cellophane membrane. The diffusion medium with phosphate buffer solution pH 7.4 stirred at 200 rpm at 37ºC ± 0.5°C. One end of the diffusion tube was covered by a cellophane membrane. The 2 ml formulation were spread on the cellophane membrane and membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of one hour for the period of 6 hrs from diffusion medium and analyzed by a HPLC method.

HPLC method

HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50 µL loop volume. LC solution version 1.25 was applied for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of methanol -10 mM ammonium acetate buffer (pH 3.0 adjusted with orthophosphoric acid (30:70, v/v), and detection was made at 298.0 nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25 cm × 4.6 mm i.d., 5µ) was used for the separation.

RESULT AND DISCUSSION

The Effect of pH

The pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH [16]. The polymers with a large number of ionizable groups are known as polyelectrolyte. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH sensitive polymers are based on PAA (carbomer) or its derivatives [17]. Formulations which contain ketotifen fumarate were fluid state at pH (6.8) before administration underwent rapid gelation at pH 7.4 after administration to the STF due to neutralization.

Gelation capacity

Gelation capacity is the main prerequisites of *in situ* gels formulation. All the polymers were utilized without compromising the gelation capacity and rheological properties. Rheological properties and gelation capacity of the delivery system may be achieved by the addition of combination of polymers gellan gum: carbopol 934 and gellan gum: sodium alginate. This gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three-dimensional network by complexation with cation and hydrogen bonding with water [17-18]. The optimized formulations were found to have good gelation capacity. The formulations F3 containing gellan gum and CP (0.6: 0.4) exhibited good gelation immediately after addition into the STF and remained up to 4 hours as shown in Figure 4. The formulations F4, F5 and F6 showed moderate gelation capacity and remained for 6 hours

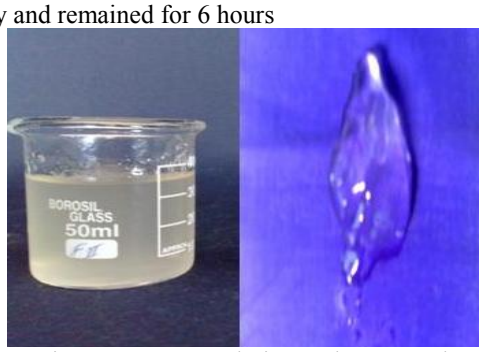


Figure 4. *In Situ* solution and *in situ* gel

Table 1: Composition of *in situ* gel formulation

Gellan gum- GG, Carbopol 934- CP, Sodium Alginate- SG

Formulation Code	***Ingredient (w/v) %***						
	Ketotifine fumarate	***Gellan gum***	***Carbapol 934***	***Sodium Alginate***	***Propyl paraben***	***Triethanol amine***	***Deionized water Up to***
F1	0.0075	0.2	0.3	--	0.001	qs	30 ml
F2	0.0075	0.4	0.4	--	0.001	qs	30 ml
F3	0.0075	0.6	0.5	--	0.001	qs	30 ml
F4	0.0075	0.2	--	0.4	0.001	qs	30 ml
F5	0.0075	0.4	--	0.6	0.001	qs	30 ml
F6	0.0075	0.6	--	0.8	0.001	qs	30 ml

Table 2: Characteristics of optimized formulation of *in situ* gel

Formulation Code	*Bioadhesive force (dynes/cm^2)*	*Gel strength (sec)*	*Spreadability (g.cm/sec)*	*Content uniformity (%w/w)*	*Gelation time (sec)*	*Gelation capacity*
F1	56.51	95	17.7	97.4	52	***
F2	57.41	97	18.0	97.7	50	***
F3	58.33	102	19.3	98.2	49	***
F4	12.12	51	10.4	96.5	85	**
F5	12.43	54	10.5	96.6	87	**
F6	12.42	57	11.7	96.5	89	**

Gel strength

Gel strength of formulation F3 was found to be more as compared to formulation F6. The formulations F1, F2 and F3 exhibited good gel strength which may be due to increase in concentration of gellan gum and carbopol 934 as shown in (Table 2)

Mucoadhesive force

Mucoadhesive force of formulation F3 was found to be more than that of formulations F6. The formulations F1, F2 and F3 showed higher values of mucoadhesive force than F4, F5 and F6 formulations. It may be due to the combination of gellan gum and carbopol polymers as shown in (Table 2).

Spreadability

Increased ratio of gellan gum and carbopol in the formulation F1, F2 and F3 showed good spreadability (gellan gum: carbopol 934), in comparison with F4, F5 and F6 (gellan gum: sodium alginate) combination polymers as shown in (Table 2).

Gelation Time

Gelation time of prepared formulations were showed gel-like depot at STF (pH 7.4). The gelation time of F6 was observed at 89 sec. The formulations F1, F2 and F3 showed gelation time at (52, 50 and 49 sec.) as shown in (Table 2).

Rheological properties

Aqueous solutions of *in situ* system containing polymers in various ratio and combinations such as gellan gum: CP and gellan gum: SG was prepared and evaluated viscosity in order to identify the composition suitable for as *in situ* gel systems. Many experiments were conducted by varying the concentration of these polymers in order to identify the optimum concentration required for the gel forming solution. The *in situ* system containing gellan gum in the range of 0.2 to 0.6 % of CP and 0.4 to 0.8% of sodium alginate were utilized in the F code formulation. Rheological properties of the delivery system may be achieved by the addition of viscosity enhancing polymers such as gellan gum and pH induced polymer CP. It also helped the gels for its adhesion property to prolong the precorneal drug retention. All the formulations were behaving as shear thinning systems as shown in Figure 5 and 6.

Drug release

The formulation F1 containing the lower polymer ratio of carbopol showed the release profile only up to 4 h with 80% release, whereas formulation having higher polymer ratio i.e., F3 showed only 90 % release at the end of 4 h. since we were inclined the formulate in situ gel which show 90% release profile within 4 h, F1, F2 and F3 formulations were not found to be ideal formulations for in situ gels for prolonged delivery. Formulations of carbopol disintegrated rapidly and released the drug within 4 h in comparison with F4, F5 and F6 the gels containing sodium alginate having the maximum concentration F6 could show release up to 6 h with 81% release as shown in Figure 7.

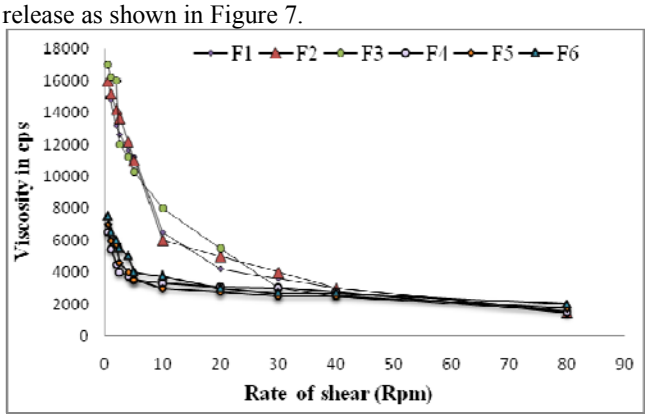


Figure 5. Showing the viscosity of in situ gel

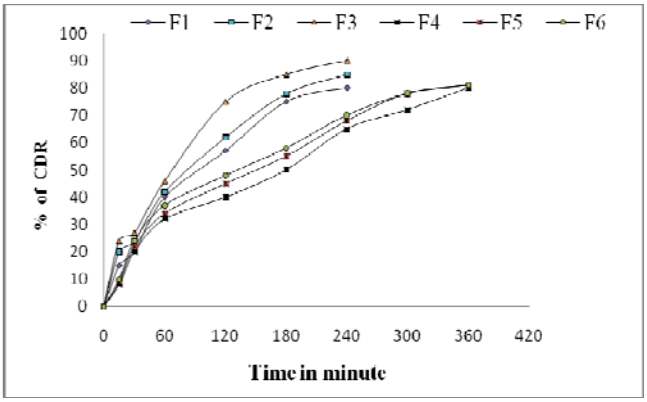


Figure 6. Showing the drug release

Ocular irritation studies

The results of the ocular irritation studies showed that formulations of combination of gellan gum: sodium alginate and carbopol were nonirritant. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible as shown in Figure 8. There was no appearance of turbidity and hence no evidence of microbial growth when the formulations were incubated for not less than 14 days at 30 ºC to 35 °C in case of nutrient agar medium. The preparations being examined therefore passed the test for sterility.

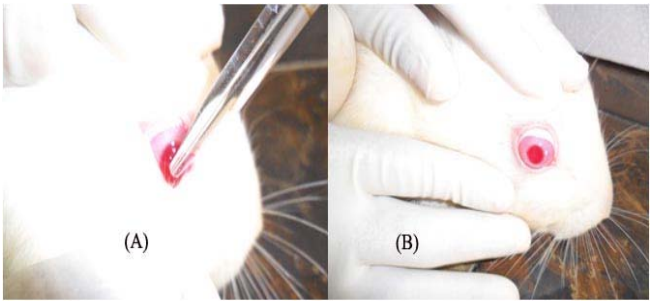


Figure 7.
(A) Ocular instillation of formulated *in situ* gel-
(B) Visual observation of eye after administration of *in situ* gel

CONCLUSION

In this study, a pH sensitive in situ gelling and mucoadhesive ophthalmic drug delivery system containing ketotifen fumarate based on gellan gum: sodium alginate and gellan gum: carbopol 934 was developed. We have demonstrated that incorporating either 0.3 % or 0.5 % CP 934 into *in situ* solution under physiological condition did not affect the rheological properties and would enhance the mucoadhesive force significantly. The combined solutions which were free flowing liquid at acidic pH would shift to firm gels after administration, which could ensure suitable gel strength and prevent rapid precorneal elimination, and attach to the ocular mucosal surface for a relative long time, which could improve the retention of the drug and promise a high bioavailability. Therefore, the combined systems can be used as the ocular *in situ* gelling and mucoadhesive vehicles to enhance bioavailability.

REFERENCES

[1]. Lang, J.C. (1995) *Adv. Drug Deliv. Rev.* 16, 39–43
[2]. Desai, S.D. and Blanchard, J. (1995) in *Encyclopedia of Pharmaceutical Technology* (Swarbrick, J. and Boylan, J.C., eds), pp. 43–75, Marcel Dekker, New York, NY, USA
[3]. Peyman, G.A. and Ganiban, G.J. (1995) *Adv. Drug Deliv. Rev.* 16, 107–123
[4]. Lee,V.H.L. and Robinson, J.R. (1986) *J. Ocul. Pharmacol. Ther.* 2, 67–108
[5]. Mezei, M. and Meisner, D. (1993) in *Biopharmaceutics of Ocular Drug Delivery* (Edman, P., ed.), pp. 91–104, CRC Press, Boca Raton, FL, USA
[6]. Shell, J.W. (1985) *Drug Dev. Res*. 6, 245–261
[7]. Urtti, A. and Salminen, L. (1993) *Surv. Ophthalmol*. 37, 435–456
[8]. Chang, S-C. and Lee,V.H.L. (1987) *J. Ocul. Pharmacol. Ther.* 3, 159–169
[9]. Hatefi A, Amsden B. Biodegradable injectable in situ forming drug delivery systems. Journal of Controlled Release 2002; 80:9-29
[10]. Mitan R, Gokulgandhi Jolly R, Parikh, Megha B and Dharmesh MM. A pH triggered in- situ gel forming ophthalmic drug delivery system for tropicamide. Drug Deliv Technol 2007; 5: 44–49.
[11]. Pandit D, Bharathi A, Srinatha DN, Ridhurkar S.Singh, Long acting ophthalmic formulation: Evaluation of alginate gel systems, Ind.J. Pharm.Sci. 2007; 25: 37-. 40.
[12]. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK, Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int J Pharm.1998; 165:33-44.
[13]. Koffi FAA, Agnely G, Ponchel JL, Grossiord,Modulation of the rheological and mucoadhesive properties of thermosensitive pluronic-based hydrogels intended for the rectal administration of quinine. European Journal of Pharmaceutical Sciences.2006; 27:328–335.
[14]. Jagdish Balasubramaniam, In vitro and in vivo evaluation of the Gelrite gellan gum-based ocular delivery system for indomethacin, Acta Pharm. 2003; 53: 251–261.
[15]. Gupta GD, Gound RS, Release rate of nimesulide from different gellants. Indian J Pharm Sci. 61: 229-234 (1999).
[16]. Qiu Y, Park K. Environment-sensitive hydrogels for drug delivery. Adv Drug Deliv Rev 2001; 53:321-39.
[17]. Soppimath KS, Aminabhavi TM, Dave AM, Kumbar SG, Rudzinski WE. Stimulus-responsive "smart" hydrogels as novel drug delivery systems. Drug Dev Ind Pharm 2002; 28: 957-74.