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Methyl Cellulose Based Sustained Release Thermosensitive *in situ* Fast Gelling Ocular Delivery of Ketorolac Tromethamine

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The purpose of this study is to develop methyl cellulose (MC) based *in situ* gelling formulations of ketorolac tromethamine (KT) for enhancing its ocular bioavailability. The gelation temperature of 1 % w/v MC solution was 60 ºC. Sodium chloride was added to reduce the gelation temperature of MC solution below physiological temperature, *i.e*., 37 ºC. The effect of NaCl concentration on the rheological property and drug release profile of prepared formulations were examined. It was observed that 5-7 % w/v NaCl lowered the gelation temperature below 37 ºC and the solution was free flowing liquid at 25 ºC for proper instillation to the eye as drop (s) and gave sustain drug release profile. In an attempt to increase drug release time by increasing viscosity of gel at 37 ºC without compromising the *in situ* gelation capability, hydroxy propyl methyl cellulose (HPMC K15 M) was added, replacing MC that gave finally 1 % w/v polymer (MC and HPMC mixture) solution containing 0.5 % w/v KT. The viscosity and drug release profile of prepared formulations containing HPMC were evaluated. The highest viscosity at 37 ºC and slowest drug release was obtained from MC:HPMC::2:1 solution containing 7 % w/v NaCl.

Key Words: Methyl cellulose, Hydroxy propyl methyl cellulose, Ketorolac tromethamine, *in situ* Gelling, Sodium chloride.

INTRODUCTION

Poor bioavailability of conventional ophthalmic liquid formulation is caused by the rapid elimination of drug through lacrimal secretion and normal tear turnover[1-3]. Ocular bioavailability can be significantly improved by increasing precorneal residence time of drug[4,5] or by instillation of concentrated drug solution[6]. To increase the ocular residence time of drug, many formulations were developed such as viscous solutions, ointments, gels, suspensions and inserts[7,8]. The concentrated drug solutions causes both ocular and systemic side effects[9]. This problems can be overcome by using *in situ* gel forming ocular drug delivery systems prepared from polymers that

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exhibit reversible phase transition (sol-gel-sol) and pseudoplastic behaviour to minimize interference with blinking[6,10]. Such system can be formulated as liquid dosage form suitable to be administered by instillation into the eye which, upon exposure to physiological conditions, changes to the gel phase, thus increasing the precorneal residence time of the delivery system and enhancing ocular bioavailability of drug[11-13]. Poloxamer, a block copolymer is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature[14]. The aqueous solution of methyl cellulose undergoes sol-gel-sol phase transition depending on the temperature and addition of salt can reduce the gelling temperature of methyl cellulose solution[15]. Generally three concepts employed for the development of *in situ* gelling systems are pH dependent[16], temperature sensitive[17] and ion-activation[18]. The mixture of 0.3 % carbopol and 14 % pluronic solutions showed a significant enhancement in gel strength in the physiological condition of eyes, this gel mixture is also found to be free flowing at pH 4.0 and 25 ºC[5]. The aqueous solution of gellan and Na-alginates readily undergoes gels in presence of Ca^{2+} ions[19,20].

The objective of the present study was to develop an temperature dependent *in situ* gelling system for ketorolac tromethamine, a NSAID used to treat seasonal allergies such as itching, swelling and inflammation of the eyes and to improve patient compliance. Sodium chloride containing methyl cellulose and MC-HPMC solutions were investigated as vehicles for the formulation of ketorolac tromethamine eye drops (0.5 % w/v) which will show fast gelation when instilled into the cul-de-sac of the eye and provided sustained release of the drug.

EXPERIMENTAL

Metolose SM 4000 (methyl cellulose, 29.6 % methoxyl content) was obtained from Shinetsu Chemical Co. Ltd., Japan. Hydroxy propyl methyl cellulose (HPMC K15 M) from Colorcon Asia Pvt. Ltd., Verna, Goa, India and Ketorolac tromethamine from Sun Pharma, Baroda, Gujrat, India were gift samples. Sodium chloride, calcium chloride and sodium bicarbonate were purchased from E. Merck India Pvt. Ltd., Mumbai, India. All chemicals were of analytical grade.

Preparation of sample solutions: Methyl cellulose (MC) solution (1 % w/v) was prepared by dispersing the MC in about half of the desired volume of hot water at 70 ºC to slurry and then remaining portion of cold water was added to the slurry in order to reduce the temperature below 20 ºC with continuous stirring until homogenous dispersion[21]. The dispersion was kept in a refrigerator for 48 h to get clear, transparent solution. Sodium chloride was dissolved in the MC solution and evaluated for gelling temperature in order to identify the compositions suitable for *in situ* gelling systems. Ketorolac tromethamine was then added to the *in situ*-gelling systems and effect of KT was evaluated on gelling temperature. Hydroxy propyl methyl cellulose solution (1 % w/v) was prepared by same procedure as MC solution. The MC-HPMC solutions were prepared by homogeneous mixing of required amount of MC and HPMC solutions which finally gave MC:HPMC as 4:1, 3:1,

2:1. The MC-HPMC *in situ* gelling solutions were prepared by adding required amount of NaCl and KT. All the solutions were prepared with deionized double distilled water.

***In vitro* gelation studies:** The gelation studies were conducted with a cell, equipped with a thermojacket to maintain a constant temperature. The cell is a 3 mL cylindrical reservoir containing 2 mL of gelation solution. A 250 µL transparent plastic cup present in the bottom of the cell to hold the gel sample in place, after its formation. 100 µL of the formulated solution was injected into the cavity of the cup, with a thermal cycle of 20-70 ºC. The gelation temperature was recorded by visual inspection repeatedly[22]. The temperature was verified with test tube tilting method by observation of non flowing state of the solution[18].

Rheological studies: The rheological studies were carried out on a viscometer (TV-10 viscometer, TOKI Sangyo Co. Ltd., Japan). The viscosity of the formulated solutions was measured at shear rate of 20 rpm at 37 ºC. The temperature was maintained by a thermally controlled water bath. The samples were equilibrated for 10 min to reach the running temperature prior to each measurement.

Swelling studies: The studies were conducted with a cell, equipped with a thermojacket to maintain a constant temperature. The cell containing artificial tear fluid [composition: 0.67 g NaCl, 0.20 g $NaHCO_3$, 0.008 g $CaCl_2 \cdot 2H_2O$ and distilled water qs to 100 g][5] was used as swelling medium equilibrating at 37 ºC. The 1 mL of formulated solution was packed in a dialysis bag and put into the swelling medium. At specific time interval the bag was removed from the medium and weight of the wet gel was recorded. The swelling of the polymer gel as a function of time was determined by using the following relationship:[23]

$$\% \text{ Swelling (t)} = \{\text{Gel weight (t)} - \text{Gel weight (0)}\} \times 100/\text{Gel weight (0)}$$

***In vitro* drug release studies:** A Franz diffusion cell was used for *in vitro* release study of ketorolac tromethamine from prepared formulations. The dissolution medium used was artificial tear fluid. The dialysis membrane, previously soaked overnight in the dissolution medium, was tied to one end of the specifically designed glass cylinder, *i.e*., doner of the Franz diffusion cell. The cylinder was suspended in 40 mL of dissolution medium maintained at 37 ºC so that the membrane just touches the medium surface and the stirring rate was maintained 50 rpm. 1 mL of formulation was accurately pipetted and placed over the dialysis membrane. Aliquots, each of 1 mL starting from first minute, were withdrawn at hourly interval and replaced by an equal amount of artificial tear fluid. The ketorolac tromethamine content of the aliquots was analyzed by UV spectrophotometry at 323 nm.

RESULTS AND DISCUSSION

***In vitro* gelation studies:** The gelation temperature of 1 % w/v MC solution is about 60 ºC. Fig. 1 shows the effect of NaCl on gelling temperature of 1 % w/v MC solution. When salt concentration is varied from 1-10 % w/v, the gelling temperature is decreased below body temperature, since the salt causes conformational changes

in polymer chain because of water-structure formation properties of the salts. The addition of a salt affects the structure of water, which is mainly due to the interactions between ions and water molecules. Sodium chloride is a salt-out salt, exhibits stronger interaction(s) with water than those hydrogen bonds between water molecules. Thus, some of the original hydrogen-bonding network (including cage like structures) formed by water molecules is destroyed by the salt. In other words, ions tend to compete with MC chains for the water molecules and they succeed in attracting more water molecules surrounding them due to their stronger hydration abilities. This competition causes the decrease of MC solubility in water. As a result, at the same temperature, there are more hydrophobic aggregates of MC in a salted MC solution than in a non salt MC solution.

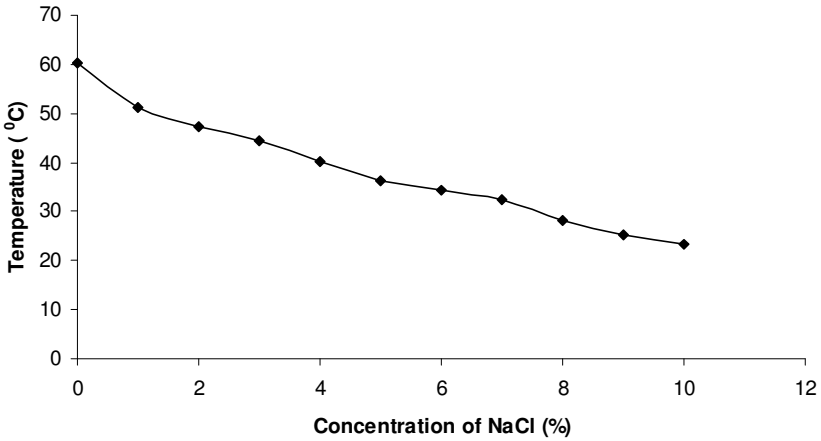

Fig.1. Variation of gel temperature of 1 % w/v MC solution with NaCl (% w/v) concentration (average of three runs)

Thus, upon heating it will be easier for a salted MC solution to meet the requirement for the critical number of hydrophobic aggregates to form a gel, so that the sol-gel transition occurs at a lower temperature, or it is better to say that the salt accelerates the formation of MC gel. The increased salt content results in fewer free water molecules available around MC chains and a stronger hydrophobic environment for MC. It has been observed that 5-7 % w/v NaCl is capable to ensure gelling temperature below body temperature and the solutions are free flowing liquid to allow reproducible instillation into the eye as drops at 25 ºC. HPMC does not significantly alter the gelation temperature of MC solution but in case of F6 there is slightly lowering effect on gelation temperature. All the developed formulations are evaluated for clarity by visual observation and satisfactory clarity is found. Table-1 shows that increased concentration (5-7 % w/v) of NaCl decreases the pH of the formulations from pH 6.62 to pH 6.14. The addition of HPMC increases the pH of formulation and increase concentration of HPMC also increases the pH from 6.28 to pH 6.47. This type of variation in pH of the formulations does not create

any problems to the eye due to the buffering power of tear to neutralize quickly the unbuffered solutions over a wide range (pH 3.5 to pH 10.5) provided the normal volume instilled is 1 to 2 drops (0.05-0.1 mL)[24].

TABLE-1
CHANGE IN VISCOSITY AND pH WITH THE DIFFERENT COMPOSITION OF *in situ* GELLING SYSTEMS

Formulation	Composition (1 % w/v polymer solution containing 0.5 % w/v ketorolac tromethamine)		Viscosity (cP) at 20 rpm and 37 ºC	pH
	MC:HPMC	NaCl (%)		
F1	1:0	5	16700	6.62
F2	1:0	6	18400	6.28
F3	1:0	7	20800	6.14
F4	4:1	7	22100	6.28
F5	3:1	7	24400	6.44
F6	2:1	7	29700	6.47

Rheological studies: The rheological behaviour of formulations is investigated as a function of temperature. All measurements are performed in triplicate with good reproducibility. At 25 ºC, the formulations are in a liquid state and exhibited low viscosity. An increase in temperature at 37 ºC, the solutions convert into gels with high viscosity. This is because of the polymers are fully hydrated and polymer-polymer interactions exist through simple entanglement at low temperature. But when temperature increases, hydration of polymer by water is gradually weakened and polymer-polymer association becomes more pronounced, thereby resulting in the formation of gel structure. From the Table-1, it is clear that the viscosity enhancing capability of HPMC is much more than MC alone.

Swelling studies: All the formulations are observed to be stable gel throughout the period of swelling (7 h). On comparing the rate of swelling for F1, F2 and F3, it is observed that F3 swelled less than others (Table-2). As 7 % w/v NaCl in 1 % w/v MC solution gives higher viscosity at 37 ºC leading to decrease in water penetration. So rate of swelling of F3 is less than F1 and F2. For F4, F5 and F6, F6 has less swelling rate because of increasing HPMC concentration shows high viscosity leading to decrease swelling rate. If water penetration is faster, swelling rate will be increased and for highly viscous prehydrated gel matrix, water penetration is less that leading to slower rate of swelling. From Table-2, it is clear that swelling rate of F4 is similar to F5 because of their almost similar viscosity. The result shows that HPMC containing *in situ* gelling system is less swelled than MC alone. This is due to the highly viscous gel formation property of HPMC.

***In vitro* drug release studies and release kinetics:** The cumulative percentage release of ketorolac tromethamine as a function of time profiles from different *in situ* gel formulations of MC and MC solution and *in situ* gel formulations of MC-HPMC has been shown in Fig. 2. For MC solution, almost all the drug released within 1.5 h. It has been observed that addition of salt in MC solution will increase

the drug release time, *i.e*., sustain drug release. When the formulations (F1 to F6) come in contact with the artificial tear fluid at 37 ºC and gelation occurs, a prehydrated gel matrix is formed in which water penetration and hydration is the rate limiting step of drug release. If water penetration is faster, hydration and drug release will be faster, *i.e*., sustained drug release will not be achieved. In case of F1, F2 and F3, with the increase in the NaCl concentrations from 5 to 7 % w/v, drug release time increases upto 5 h. The reason behind the increase in drug release time with the increase viscosity as increase in concentration of NaCl is the increase in viscosity and decrease in percentage swelling as a result of slower solvent penetration with the increase in NaCl concentration. This is further supported by the observation that when HPMC concentration is increased from MC:HPMC::4:1 to 2:1, the drug release time increases upto 7 h due to more increasing viscosity.

TABLE-2
in vitro DRUG RELEASE AND SWELLING KINETICS OF *in situ* GELLING SYSTEMS CONTAINING KETOROLAC TROMETHAMINE (AVERAGE OF THREE RUNS)

F.	Swelling at 3 h	Zero order		First order		Higuchi		Korsmeyer-peppas		
	k ± s.d	r^2 ± s.d	k ± s.d	r^2 ± s.d	k ± s.d	r^2 ± s.d	k ± s.d	r^2 ± s.d	k ± s.d	n ± s.d
F1	0.0396 ± 0.0002	0.9534 ± 0.0010	0.5786 ± 0.0019	0.9860 ± 0.0009	0.0068 ± 0	0.9943 ± 0.0002	7.7445 ± 0.0199	0.9977 ± 0.0001	0.3122 ± 0.0178	0.7698 ± 0.0087
F2	0.0315 ± 0.0006	0.9289 ± 0.0009	0.4365 ± 0.0004	0.9502 ± 0.0013	0.0075 ± 0.0007	0.9947 ± 0.0002	6.8602 ± 0.0097	0.9942 ± 0.0005	0.2309 ± 0.0324	0.7749 ± 0.0149
F3	0.0305 ± 0.0004	0.9728 ± 0.0007	0.3432 ± 0.0010	0.9777 ± 0.0029	0.0038 ± 0	0.9812 ± 0.0005	5.8989 ± 0.0149	0.9992 ± 0	0.0973 ± 0.0485	0.7746 ± 0.0257
F4	0.0303 ± 0.0004	0.9545 ± 0.0043	0.2789 ± 0.0009	0.9516 ± 0.0114	0.0034 ± 0	0.9941 ± 0.0018	5.386 ± 0.0110	0.9988 ± 0.0007	0.2831 ± 0.035	0.6824 ± 0.016
F5	0.0293 ± 0.0005	0.9608 ± 0.0017	0.2784 ± 0.0005	0.9588 ± 0.0061	0.0032 ± 0	0.9922 ± 0.0003	5.3521 ± 0.0128	0.9982 ± 0.0007	0.1465 ± 0.0463	0.7382 ± 0.0208
F6	0.0239 ± 0.0004	0.9529 ± 0.0016	0.2420 ± 0.0003	0.9767 ± 0.0018	0.0030 ± 0	0.9924 ± 0.0006	5.1001 ± 0.0042	0.9954 ± 0.0012	0.2951 ± 0.0272	0.6878 ± 0.0616

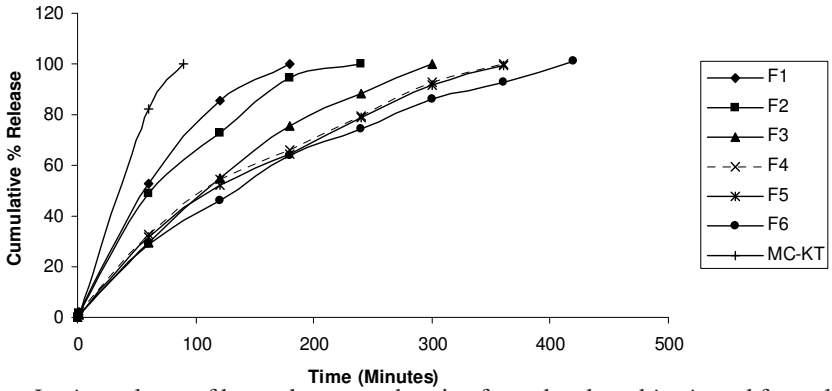

Fig. 2. *In vitro* release of ketorolac tromethamine from developed *in situ* gel formulations and MC-KT solution in artificial tear fluid at 37 ºC (average of three runs)

The different kinetic equations (zero order, first order and Higuchi's equation) are used to interpret the release pattern from *in situ*-gel system. It is found that the *in vitro* drug release was best explained by Higuchi's equation, as the plots showed the highest linearity ($r^2 > 0.9812 \pm 0.0005$) for all the formulations followed by first order and zero order kinetics. From the Table-2 it is clear that when swelling rate decreases from F1, F2 and F3, drug release rate also be decreased due to increasing viscosity. Result shows that viscosity of F6 is greater than other caused slower swelling and drug release rate. From the result it has been observed that the drug release rate is decreased by addition of HPMC.

All the kinetic data are fitted to the Korsmeyer-Peppas equation $M_t/M_\infty = kt^n$. where M_t/M_∞ is the fraction of drug released at time t; k is a constant related to structural and geometrical characteristics of formulation as release rate and 'n' is the release exponent indicative of the drug release mechanism. More acceptable linearity ($r^2 > 0.9942 \pm 0.0005$) is observed and the values of release exponent 'n' varied from 0.6824 ± 0.016 to 0.7749 ± 0.0149, which appeared to indicate an anomalous, non-Fickian drug diffusion, *i.e*., coupling of the diffusion and erosion mechanism indicated that the drug release is controlled by more than one process.

Conclusion

Ketorolac tromethamine was successfully formulated as thermotriggered *in situ* gel forming eye drops using methyl cellulose as gelling agent in combination with NaCl. The formulations were liquid at 25 ºC and undergo rapid gelation at 37 ºC. The gel formed *in situ* afforded sustained drug release. The developed formulations are viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and decreased frequency of administration resulting in better patient acceptance.

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