Control of Thermo Reversible Gelation of Methylcellulose Using Polyethylene Glycol and Sodium Chloride for Sustained Delivery of Ophthalmic Drug

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ABSTRACT: The effect of molecular weight of polyethyleneglycol (PEG) and sodium chloride (NaCl) on the gelation temperature of methylcellulose (MC) was studied with the objective to develop a MC based formulation for sustained delivery of ophthalmic drug. The gelation temperature of 1% MC was 60 ± 0.40°C. It was found that the gelation temperature of MC was reduced with the addition of 10% PEG and extent of reduction of gelation temperature was depended on the molecular weight of PEG at same PEG concentration of 10%. The gelation temperature of MC was reduced by 10.4 to 5.9°C with the increasing molecular weight of PEG starting from 400 to 20,000 ($\overline{Mn}$) depending on the method of determination of gelation temperature. To reduce the gelation temperature of MC close to physiological temperature (37°C), 6% NaCl was added in the different MC-PEG combinations containing different molecular weight of PEG. It was observed that the drug release time increased from 5 to 8 h with the increase in molecular weight of PEG from 400 to 20,000 ($\overline{Mn}$) and this was due to the maximum viscosity and gel strength of MC-PEG20000-NaCl ternary combination. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 631–637, 2010

Key words: molecular weight; sustained delivery; ophthalmic drug; physiological temperature; viscosity; gel strength

INTRODUCTION

Hydrogels are three-dimensional network of hydrophilic polymers, which have the ability to imbibe a large quantity of water and biological fluids.[1,2] Hydrogels are increasingly finding applications in many areas, such as biomaterials, pharmaceutical devices, or controllable sensors.[3–7] Thermo reversible hydrogel is an aqueous polymeric solution, which gels upon heating and redissolve upon subsequent cooling. Many polymers show the thermo reversible physical gelation behavior[3] and Heyman first investigated the thermo reversible gelation of Methylcellulose (MC).[8]

In the aqueous polymeric solution, the polymer is completely hydrated and the polymer–polymer interaction is lower than the polymer–solvent interaction. When the critical temperature reaches, sufficient dehydration occurs and because of this dehydration the interaction between polymer molecules is increased than the polymer–solvent interaction and due to this reason polymer solution turns into gel.

MC is a derivative of cellulose, which is water soluble and it turns into gel at a particular temperature because of the hydrophobic interaction.[9,7] The MC gel is completely thermo reversible, which is gelled upon heating and liquefied upon cooling. The gel strength of pure methylcellulose solution depends on the degree of substitution and the molecular weight.[10] Methylcellulose is unique for its thermo reversibility; it undergoes swelling and erosion *in vivo*, so it is not necessary to remove the gel after complete release of drug from the applied area. MC is recognized by Food and Drug Administration and has multidisciplinary applications[11] and is highly biocompatible[12–14] and is used for ophthalmic drug delivery. Majumder and coworkers reported

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the use of MC for maximum precorneal residence of ofloxacine.[15] Hadded and Loucas patented the use of MC based formulation for dry eye syndromes.[16] Smith advocated the use of MC in ophthalmic compositions of carbonic anhydrase inhibitor.[17] Kumar et al. have used methylcellulose with carbopol to make an ophthalmic formulation. MC is used to increase the viscosity of the formulation.[18,19] Alenki and coworkers.[20] describes the formulation and evaluation of ophthalmic delivery system of ciprofloxacin from polyacrylic acid-hydroxyl propyl methyl cellulose (HPMC)-insulin gelling combination. They also used HPMC in combination with pluronic F-127 to reduce the concentration of pluronic required for *in situ* gelling property for controlled delivery of ciprofloxacin. Mueller and Deardroff have formulated an ophthalmic formulation for homatropine hydrobromide by using methylcellulose and it has been concluded that MC dose not create any eye irritation or damage, and 1% MC is standardized for that formulation.[21,22]

The gelation of MC depends on the different environmental conditions such as temperature,[23,24] solvent,[25] and pH.[26] The gelation temperature of MC can be altered by adding different additives like natural polymers, synthetic polymers and various salts.[27–29] The effect of salts on the gelation temperature of MC has been studied extensively. There is a competition between the MC and the salt for the water molecules and as the affinity of water molecules with the salt is more compare to the affinity of the same toward MC molecules thus the salt will help dehydration of MC molecules at lower temperature. Because of this reason the salt is capable to reduce the gelation temperature of MC solution.[25–31]

Joshi et al. have used four surfactants, namely, sodium *n*-decyl sulfate (SDeS), sodium n-hexadecyl sulfate (SHS), sodium *n*-dodecylsulfate (SDS), and triton X-100, to study thermal behavior and sol–gel transformation in dilute aqueous HPMC/surfactant mixtures. The influence of anionic surfactant, SDS on the gelation varied with SDS concentration where the sol–gel transition started at a higher temperature. SDeS and SHS resulted in "salt-in" effect of a different magnitude during gelation. Triton X-100 being a nonionic surfactant, showed a minor "salt-out" effect on the thermogelation process.[32]

Joshi et al. have also studied the effects of various inorganic salts and isotopic solvents on the thermal gelation behavior of HPMC in aqueous solutions by microdifferential scanning calorimetry and rheological measurements. They have found that salting out salts, such as NaCl, promoted the sol–gel transition of HPMC at a lower temperature. Rheological and microcalorimetric results indicated that the change in the thermodynamics of the gelation of the HPMC aqueous solution was determined by the salt types and concentration, and the effect of monovalent salts was found to be more cooperative than that of multivalent salts on the sol–gel transition.[33]

Glatter and coworkers have developed the self assembled hydrogelling emulsions by blending with internally self assembled particles (ISAsomes) with the methyl cellulose and k-carrageenan mixture[34] and also studied the effect of additives like k-carrageenan on the methyl cellulose gelation and concluded that a double thermal transition gel-sol-gel is possible.[35] Kundu and Pal Singh have studied the effect of various surfactant on the methyl cellulose gelation and concluded that the gel strength is depends on the surfactant character.[36]

A variety of *in situ* gelling systems based on biodegradable polyethylene glycol (PEG) have been used for injectable drug delivery system.[37] PEG belongs in the group of polyol and has the capacity to alter the physical properties of the hydrogel of MC. PEG is biocompatible and nontoxic.[38] PEG is one polymer that may be used as a scaffold, yet also rendered bioactivity. Here PEG is used to enhance the viscosity as well as to reduce the gelation temperature.

NaCl is used to reduce the gelation temperature of MC for the formulation of drug delivery.[30] The salt reduces the gelation temperature of MC because of salting out effect.[39] The aim of this experiment is not only to see the effect of molecular weight of PEG and NaCl concentration on the gelation temperature of MC but also to study the drug release property of MC-PEG-NaCl combinations.

The ophthalmic medication becomes more and more important because of the increase in environmental pollution and the population. One of the problems of conventional liquid ophthalmic solution is the low bioavailability of drug because of lacrimal secretion and nasolacrimal drainage, so frequent instillation of concentrated solution of drug is required to achieve the desired therapeutic response. To increase ocular bioavailability and duration of action, various ophthalmic vehicles such as viscous solutions, ointments, gels, suspensions, or polymeric inserts are used. There are some drawbacks with these vehicles, so from the patient acceptability point of view a liquid dosage form has been formulated that can control the drug release and remain in contact with the cornea of the eye for extended period of time.

The objective of the present work is to control the gelation temperature of MC by using different molecular weight of PEG and NaCl and also to study the sustained drug release property of MC-PEG-NaCl combinations.

MATERIALS AND METHODS

Materials

Methylcellulose (Metolose SM-400) was obtained from Shinetsu Chemical Co., Japan. Metolose is a derivative of cellulose and have 29.6 percent methoxyl content (Viscosity-5070mPa.s.). The sample was vacuum dried at 50°C for 7 h before use and kept in vacuum desiccators.

Sodium chloride, sodium bicarbonate, calcium chloride dihydrate, and PEG: 400, 600, 4000, 6000, 20,000 ($\overline{Mn}$) were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Ketorolac tromethamine (KT) is a gift sample from Sun Pharma, Baroda, Gujarat, India.

The dialysis membrane (LA390, average flat width-25.27 mm, average diameter-15.9 mm and capacity ~1.99 mL/cm) was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

METHOD

Sample preparation

The MC solution (1 wt %) was prepared by dispersing the required amount in distilled water with continuous stirring until completely dissolved. Then the solution was kept in refrigerator for 48 h to get a transparent MC solution. For preparation of binary and ternary solutions of MC-PEG, MC-PEG-NaCl, required amount of PEG and NaCl were added to the MC solution. For the drug delivery, required amount (0.5%) of KT was used to prepare the stock solution with continuous stirring until complete solution and then the solution was kept in refrigerator at about 10–15°C.

Gel studies

The reversible sol–gel transition temperature was measured with the test tube tilting method (TTM). To measure the gelation temperature by this process, the solution is kept in a 20 mL sealed glass tube and is kept in a constant temperature bath. When the solution is completely turned into gel the solution becomes turbid and does not flow with the tilting of the test tube and the temperature is called the gelation temperature. This process has been repeated 2–3 times and again the gelation temperature is confirmed by viscosity measurement, UV-spectroscopy, rheological studies.

The viscosity of the solutions was measured with the Brookfield Viscometer (LVDV-II Þ PRO) equipped with temperature controller for viscosity measurement and also to measure the gelation temperature. The sample was placed in the sample container for 5 min so that it reaches the constant temperature. The viscosity of the samples was measured at different temperature. The gel temperature was also measured with UV. An UV–Vis spectroscopy system (Agilent 8453 Spectrophotometer) equipped with temperature controller was employed for the turbidity measurement. The absorbance was measured at a wavelength of 500 nm. The sample was heated from 20°C to 70°C at a scanning rate of 1°C/min.

Rheological measurement

Rheological characterization of the MC-PEG gels was done using an Advanced Rheometer AR 2000 (TA Instrument). The experiments were performed by using cone and plate geometry on the peltier plate. The cone diameter was 4 cm, (4°) cone and 60-cm plate was used to measure the dynamic viscoelastic functions, such as the shear storage modulus (G') and loss modulus (G'') as function of time and temperature. The temperature ramp experiment was done from 30°C to 75°C at the heating rate of 2°C/min using very low frequency, e.g., 1 Hz. The MC solution was first transferred on the peltier plate to remove the air gap with the cone. The sample was loaded at 25°C and then immediately heated to 25°C, where it was kept for 1 min to equilibrate with the temperature. The data obtained were analyzed with the help of "Rheology Advantage Data Analysis" software, version V5.2.19.

In vitro drug release studies

The *in vitro* release of KT from prepared formulations was studied through dialysis membrane using a Franz diffusion cell. The dissolution medium used was artificial tear fluid [composition: 0.67 g NaCl, 0.20 g $NaHCO_3$, 0.008 g $CaCl_2$ $2H_2O$ and distilled water qs to 100 g[19,40]]. The dialysis membrane, previously soaked overnight in the dissolution medium, was tied to one end of the specifically designed glass cylinder. The cylinder was suspended in 50 mL of dissolution medium maintained at 37°C so that the membrane just touches the medium surface and the stirring rate was maintained at 50 rpm. 1 mL of formulation was accurately pippeted and placed over the dialysis membrane. Aliquots, each of 1 mL starting from zero hour, were withdrawn at hourly interval and replaced by an equal amount of artificial tear fluid. The aliquots were analyzed by UV spectrophotometer at 323 nm.

RESULTS AND DISCUSSIONS

The gelation temperature of MC and all the combinations of MC, PEG and NaCl is initially measured by using TTM. Table I shows the gelation temperature of MC and all the combinations of MC-PEG by using different methods. The gelation temperature of

TABLE I
Gelation Temperatures of 1% MC Solution by the TTM (Test Tube Tilting Method), UV–Vis Spectroscopy, Viscosity Measurement and by G' Measurement

Method used to measure the gelation temperature, °C	MC	PEG400	PEG600	PEG4000	PEG6000	PEG20000
TTM	60	58	57	54.5	54	52
UV	63	62	61.5	59.5	58.5	57.1
Viscosity	56.4	55.5	51	46.5	46	46
Rheometer	62.9	57.7	56.3	55.9	55.7	53.7

1% MC (SM-400) is observed at 60 ± 0.40°C by TTM. Here different molecular weights of PEG are used to observe the effect of molecular weight of PEG on the gelation temperature of MC (1 wt %) keeping the concentration of PEG at 10 wt %. It has been observed that with the increase of molecular weight of PEG from 400 to 20,000 ($\overline{Mn}$), the gelation temperature is reduced from 60 ± 0.40–52 ± 0.45°C by TTM. PEG is responsible in reducing the gelation temperature, because PEG accelerates the formation of physical cross-linking of MC molecules by inducing the micro phase separation.[41] Takeuchi et al.[41] has been reported that there is no effect of molecular weight of PEG on the gelation temperature of MC but in our case we have found that with the increase of molecular weight of PEG the gelation temperature of MC decreases from 60 ± 0.40°C to 52 ± 0.45°C.

As the TTM method is a crude method to determine the gelation temperature, so measurement of viscosity by Viscometer, measurement of cloud point by UV-Vis spectroscopy and measurement of G' by Rheometer are used to get the accurate gelation temperature of all the MC-PEG combinations. Table I shows a comparison of gelation temperatures measured by above four methods. It is clear from Table I that although the gelation temperatures of all the MC-PEG combinations measured by four methods are not close to each other but the decreasing trend of gelation temperature with increasing molecular weight of PEG are same in all the cases. So, it is confirmed from Table I that the molecular weight of PEG has some effect on the gelation temperature of MC. Figure 1 shows that the change of viscosity with temperature for all the combinations of MC-PEG at 6 rpm. The sharp change in viscosity shows the gelation temperature of all the aforementioned combinations and tabulated in Table I. It is observed that the gelation temperature of MC decreases with increasing molecular weight of PEG from 55.5 to 46°C.

Figure 2 shows that the derivative of absorbance changes with temperature for all the combinations of MC-PEG individually. During gelation the solution becomes turbid and with the increase in the temperature the derivative of absorbance increases. The highest derivative of absorbance depicts the gelation temperature and tabulated in Table I. This sharp increase of absorbance is due to the the formation of clusters of MC-PEG molecules and this formation of clusters accelerates with the increase of molecular weight of PEG. So, it has been concluded that as the molecular weight of PEG increases the gelation temperature of the methylcellulose decreases from 62 to 57.1°C. The higher the molecular weight of PEG the higher will be the water absorbing power and because of this reason the effect of molecular weight of PEG is more pronounced and PEG is capable to reduce the gelation temperature.

Rheological studies of MC–PEG gels is done to determine the gelation temperature as it has been reported earlier[41] that measurement of G' is the most accurate method to determine the gelation temperature and to measure the gel strength as the gel strength has pronounced effect on the sustained release properties of drugs. The sharp increase in G' indicates the gelation temperature.[42] From the Figure 3, it is clear that with the increase in molecular weight of PEG, the gelation temperature of MC decreases from 57.7 to 53.7°C and each curve of the rheology follows a similar pattern. During heating, MC solution absorbs heat to destroy the cage like structures among MC chains for the formation of hydrophobic aggregates. Figure 3 also shows that

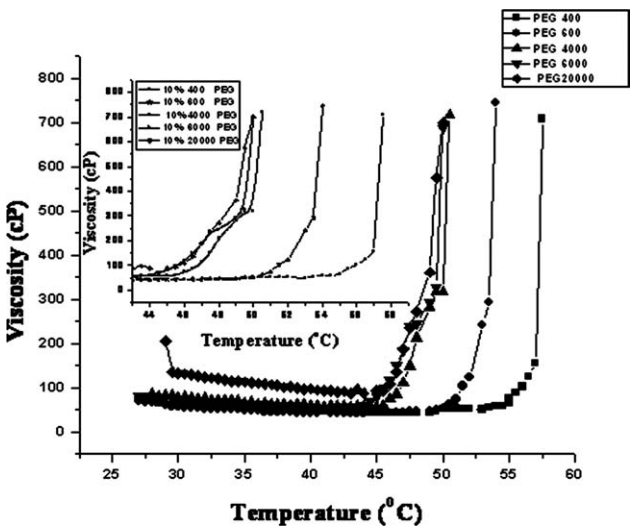

Figure 1 Effect of temperature on the viscosity of MC solution containing different molecular weight of PEG at (6 rpm).

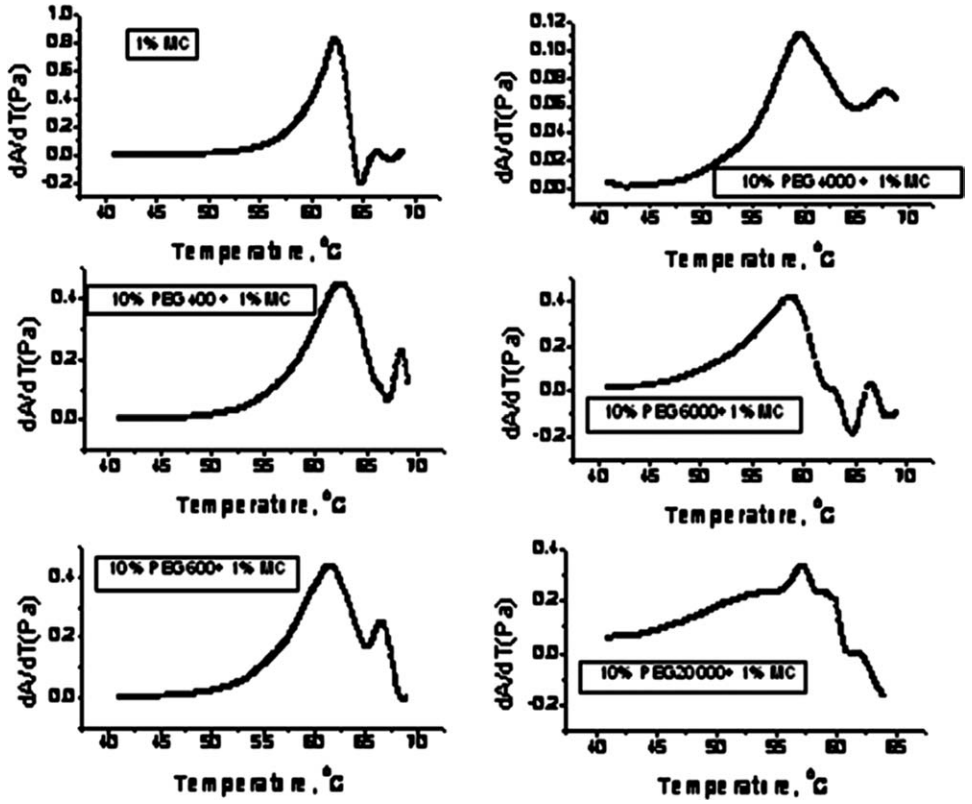

Figure 2 Effect of temperature on the first derivative of absorbance (dA/dT) of MC and MC-PEG solutions (1% MC and 10% PEG of different molecular weight) during heating at a scanning rate of 1°C/min at the wavelength of 500 nm.

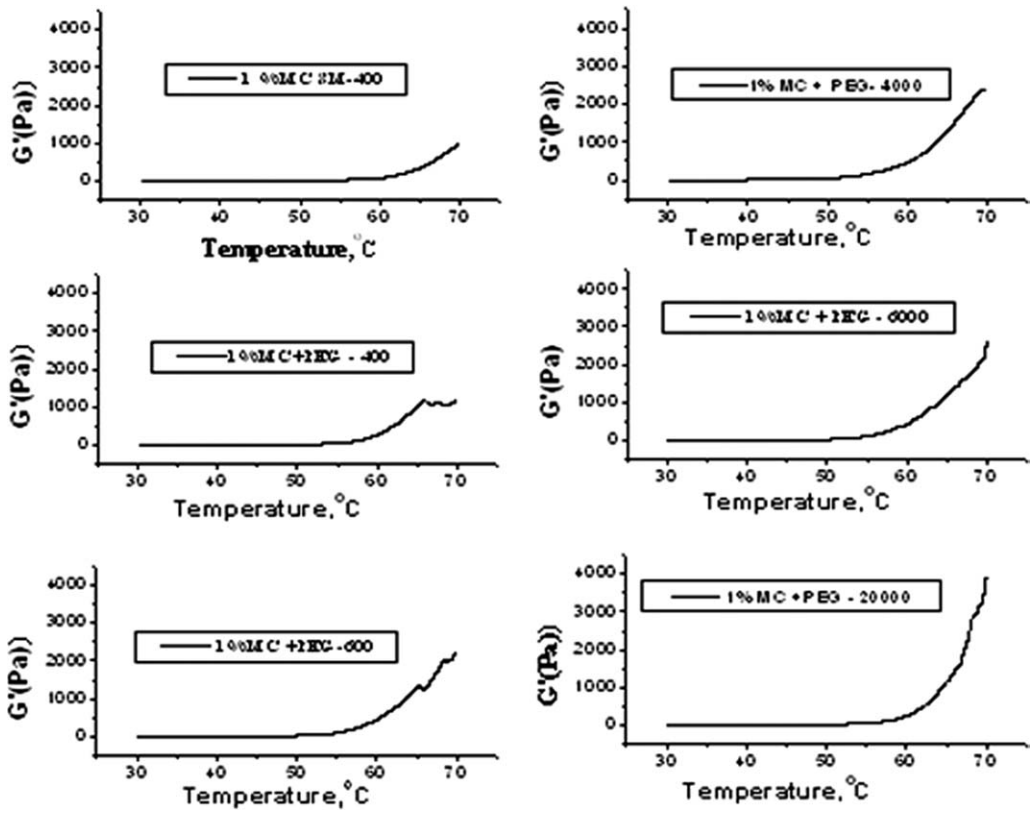

Figure 3 Change of storage modulus G' of MC and MC-PEG solutions (1% MC & 10% PEG of different molecular weight) as a function of temperature in a heating process at scanning rate of 2°C/min.

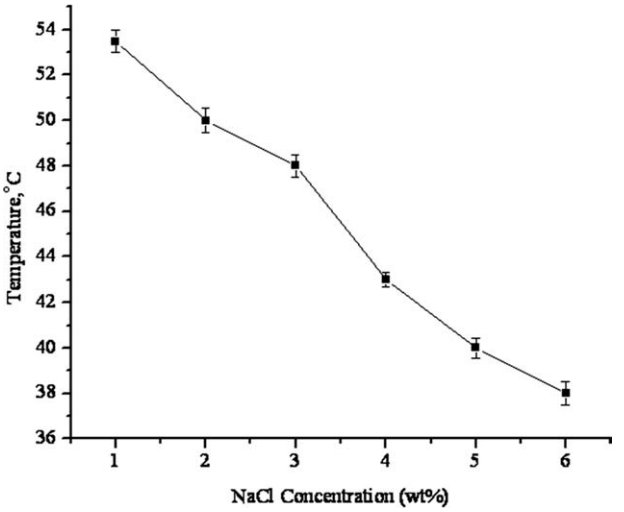

Figure 4 Effect of different weight percentage of NaCl on the gelation temperature of MC.

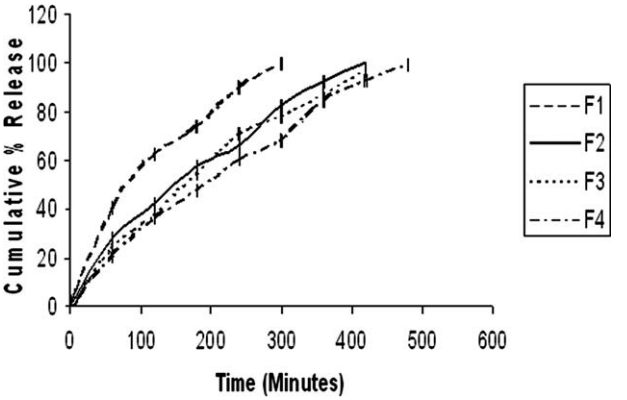

Figure 5 *In vitro* release of ketorolac tromethamine from *in situ* gelling formulation containing different molecular weight of PEG (F_1 - 1% MC+PEG 400, F_2 - 1% MC+PEG4000, F_3 - 1% MC+PEG6000, F_4 - 1% MC+PEG20000).

the value of G', i.e., storage modulus is maximum in case of MC-PEG20000 combination.

As we are unable to reduce the gelation temperature of MC up to physiological temperature (37°C) by only adding PEG, so 6% NaCl[43] is added to all the above mentioned combinations of MC-PEG except MC-PEG600 formulation.

It has been observed that the variation of salt (NaCl) concentration (1–6 wt %) diminishes the gelation temperature of MC from 60 to 38°C and has been depicted on the Figure 4. When the NaCl is added the water molecules will be placed around the Cl^- ions of the salt.[42] This will reduce the intermolecular hydrogen bond formation between water and hydroxyl group of methylcellulose and this depletion of water layer leads to enhance the hydrophobic–hydrophobic interaction and because of this the gelation temperature is reduced. 6% NaCl is added to all the combinations of MC-PEG to study the drug release property.

In vitro drug release studies

The cumulative percentage release of ketorolac tromethamine as a function of time profiles from different formulations has been shown in Figure 5. It has been observed that addition of PEG in MC solution containing NaCl sustains the drug release process from the gel matrices. When the formulation comes in contact with the artificial tear fluid at 37°C then gelation occurs and a prehydrated gel matrix is formed in which water penetration and hydration becomes the rate-limiting step of drug release process. 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, F3, and F4, with increase in the average molecular weight of PEG from 400 to 20,000 ($\overline{Mn}$), drug release time increases from 5 to 8 h. The reason behind the extension of drug release time with corresponding increase in molecular weight of PEG seems to be the increase in viscosity and the gel strength as a result of slower penetration of solvent into the core region of the gel matrix. It is mentioned earlier that the value of G', i.e., storage modulus which is a direct measure of gel strength, increases with the increase in molecular weight of PEG and is maximum in case of MC-PEG20000 ($\overline{Mn}$). From the formulation F4, more sustained effect on drug release has been achieved as observed up to 8 h. This phenomenon might occur because of increase in average molecular weight of PEG, which leads to the increase in viscosity, hence slower drug diffusion. So, F4 appears to be the best formulation of the developed *in situ* gelling formulations of ketorolac tromethamine for ocular delivery.

TABLE II
Release Kinetics of KT from *in situ*-gel Formulations (F_1, F_2, F_3, F_4)

	Zero-order		First-order		Higuchi		Korsmeyer-peppas		
Formulation	r^2	k	r^2	k	r^2	k	r^2	k	n
F1	0.9644	0.3366	0.9561	0.0039	0.9920	5.8425	0.9985	0.4457	0.6375
F2	0.9728	0.2384	0.9369	0.0032	0.9843	4.9457	0.9989	0.1122	0.7272
F3	0.9723	0.2341	0.8837	0.0028	0.9812	4.8493	0.9939	0.1107	0.7196
F4	0.9775	0.2112	0.9393	0.0025	0.9794	4.7038	0.9976	0.0191	0.7443

In vitro release kinetics

The different kinetic equations (Zero-order, First-order, and Higuchi's equation) are applied to interpret the release pattern from *in situ* gel formulations. From the Table II, it is found that the *in vitro* drug release is best explained by Higuchi's equation, as the plots showed the highest linearity $r^2 > 0.9794$ followed by zero order and first order kinetics. From the result shown in Table 3 it is clear that the slowest release rate (k) is observed by the best formulation of F4 with respect to the drug release, hence, increase in viscosity decreases the rate of drug release.

All the kinetic data were 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 $r^2 > 0.9939$ of the four formulations were observed. The release exponent "n" observed to be in between 0.6375 and 0.7443 for all the formulations shown in Table II. The value of release exponent, which appeared to indicate anomalous non-Fickian drug diffusion, i.e., the drug release is controlled by coupling of diffusion and erosion mechanism.[44]

CONCLUSIONS

PEG of different molecular weight reduces the gelation temperature of MC. The 6% NaCl is effective in reducing the gelation temperature of MC-PEG combinations close to physiological temperature (37°C). It has been observed that the drug release time increases from 5 to 8 h with the increase in molecular weight of PEG from 400 to 20,000 ($\overline{Mn}$) and this is because of the maximum viscosity and the gel strength of MC-PEG20000-NaCl ternary system. Thus, MC-PEG20000-NaCl combination can be considered as a viable alternative to commercial eye drops by virtue of its ability to retain drug for longer time.

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