In Situ Fast Gelling Formulation of Methyl Cellulose for *In Vitro* Ophthalmic Controlled Delivery of Ketorolac Tromethamine

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ABSTRACT: The development of an *in-situ* fast gelling vehicle for ophthalmic drug delivery based on methylcellulose (MC) was studied. The gelation temperature of 1 % MC was 59°C. Additives such as fructose and sodium citrate tribasic dihydrate (SC) were added in different proportions to reduce the gelation temperature of MC from 59°C to the physiological temperature i.e. 37°C. With the variation of fructose concentration from 2 to 10% at constant 1% MC concentration, the gel temperature was reduced from 59 to 54°C. To reduce the gelation temperature of MC further, SC was added in the system where the concentration of MC (1%) and fructose (10%) were kept constant. It was observed that with the variation of SC concentration from 1 to 5%, the sol–gel transition temperature was further lowered from 54 to 32°C. The gel temperature of all the combinations was measured by test tube tilting method and followed by UV–vis spectroscopy. The developed formulation corresponding to gelation temperature 32°C provided sustained release of Ketorolac Tromethamine (KT) over a 9 h period. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 1241–1246, 2009

Key words: citrate; fructose; *in situ* gelation; ketorolac tromethamine; methyl cellulose; sustained delivery

INTRODUCTION

The conventional liquid ophthalmic solution shows low bioavailability because of lacrimal secretion and nasolacrimal drainage which cause rapid precorneal elimination of drug. So, frequent instillation of concentrated solution of drug is required in order to achieve the desired therapeutic response.[1–5] To increase ocular bioavailability and duration of action, various ophthalmic vehicle such as viscous solutions, ointments, gels, suspensions, or polymeric inserts are used.[6] These vehicles have not been widely used because of some drawbacks, such as blurred vision (e.g. ointments) or lack of patient compliance from inserts.[7]

From patient acceptability point of view, a liquid dosage form that can control drug release and remain in contact with the cornea of the eye for extended period is ideal. Delivery system consists of phase transition polymers that are instilled in a liquid phase and shift to the gel phase should provide these properties. Several polymers show sol–gel transition due to changes in microenvironment, are investigated. Among them pluronics,[8] tetronics,[9] as well as methylcellulose[10,11] exhibit thermo reversible gelation i.e. their solutions show increase in viscosity upon increasing temperature. However, most of the systems require high concentration of polymers to reach sol–gel transition temperature close to eye temperature and is not well tolerated by the eye. To reduce the total polymer content and to improve gelling properties, Joshi et al.[12] introduced the concept of combination of polymers in the delivery system. Kumar et al. also developed an ocular drug delivery system based on the combination of carbopol and methyl cellulose which shows sol–gel transition in aqueous solution as the pH is raised above its pK_a of about 5.5.[13] Cohen et al.[14] have suggested that the use of alginate with high guluronic acid content to improve the gelling properties and to reduce the total polymer concentration to be introduced into the eye.[14] Li and Sung[15] developed and characterized a series of carbopol and pluronic based solution as the *in-situ* gelling vehicle for ophthalmic drug delivery. They prepared a mixture of 0.3% carbopol

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and 14% pluronic solution, which showed a significant enhancement in gel strength in the physiological conditions; this gel mixture was also found to be free flowing at pH 4.0 and temperature 25°C.

An ideal *in-situ* gelling delivery system should be a free flowing liquid with low viscosity at non-physiological condition (pH 4.0 and 25°C) to allow reproducible instillation in to the eye as drops and it should also undergo *in-situ* phase transition to form strong gel and sustained drug release at physiological conditions (pH 7.4 and 37°C).[16]

Methylcellulose (MC) shows good solubility in water at low temperature. MC molecules are linked to the water through intermolecular hydrogen bonding, and surrounding the methyl groups[17,18] cage-like water structure is formed. The presence of less intermolecular hydrogen bonding between MC molecules leads to higher gelation temperature at low concentration.[19] The gelation temperature can be reduced by adding salts and other additives.[20–22] According to the Hofmeister theory,[23] the addition of salt will affect the interactions between MC and water molecules.

Fructose is a monosaccharide, and also fructose increases the viscosity more rapidly and achieves a higher final viscosity than sucrose. Compared with other sugars and sugar alcohols, fructose has the highest solubility in water. It is an excellent humectant to retain moisture for a long period even at low humidity. So, fructose is used as additive for this study.

Sodium citrate tribasic dihydrate is used to relieve discomfort from bacterial infections and reduces the incidence of corneal ulcerations. So, sodium citrate (SC) is used for development of *in-situ* fast gelling formulation based on MC.

The objective of the present work is to develop a temperature triggered *in-situ* gelling ophthalmic delivery system of Ketorolac Tromethamine (KT), which is used to relieve from itching and swelling of the eyes caused by seasonal allergies and also to prevent and treat inflammation of the eyes. A combination of MC, fructose and SC are investigated as vehicle for the formulation of eye drops of KT that would gel when instilled into the eye and provide controlled release of the drug during treatment in ocular infections.

MATERIALS AND METHOD

Materials

A cellulose derivative, methylcellulose (29.6% methoxyl content, viscosity-4000 cps) with the trade name Metolose SM 4000 was obtained from Shinetsu Chemical Co. Ltd, Japan. Fructose and sodium citrate tribasic dihydrate were purchased from Sisco Research Laboratories Pvt Ltd, Mumbai, India. Ketorolac Tromethamine (KT) was collected from Sun Pharma, Baroda, Gujarat, India. The reagents were vacuum dried at 50°C for 7 h before use and kept in a vacuum desiccators. Sodium chloride, sodium bicarbonate, and calcium chloride dihydrate were purchased from E. Merck, India Pvt. Ltd., Mumbai, India. All the chemicals are analytical grade.

Sample preparation

MC solution (1%) was prepared by dispersing the MC in water with continuous stirring until homogenous dispersion and kept in a refrigerator for 48 h to get transparent solution. The MC-KT solution was prepared by same method as MC solution with 0.3% KT. The binary and ternary *in situ* gelling systems with and without KT were developed by incorporation of SC (1–5%) and/or fructose (2–10%) into the MC and MC-KT solutions. All the sample solutions were prepared with deionized double distilled water.

Viscosity

The viscosity of the solution was measured with the Brookfield Viscometer (LVDV-II + PRO, USA) equipped with temperature controller for viscosity measurement. The sample was placed in the sample container for 5 min so that it reaches the constant temperature. The viscosity of the samples are measured at different temperature.

Gel studies

The reversible sol–gel transition temperature was measured by following the test tube tilting method in a constant temperature bath and was confirmed with UV–vis spectroscopy system (Agilent 8453 Spectrophotometer, HP, USA) equipped with temperature controller for the turbidity measurement. The sample was placed in the cell, which was covered with a plastic cap to prevent evaporation of solvent. Deionized double distilled water was used as the reference. The absorbance was measured at a wavelength of 500 nm through a thermal cycle of 20–70°C at a scanning rate of 1°C/min. The absorbance was converted to transmittance according to Lambert–Beer's Law.[24,25] Three times, of UV, reading has been taken for each sample but the readings are same for the three different runs.

In vitro 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 $CaCl_2.2\ H_2O$ and distilled water qs to 100 g].[7] The dialysis membrane, previously soaked overnight in the dissolution medium, was tied at 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. One milliliter of formulation was accurately pipetted and placed over the dialysis membrane. Aliquots, each of 1 mL starting from one minute after the gelling system were exposed to the dissolution medium, was 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 DISCUSSION

The gelation temperature of 1% MC solution is observed at (59 ± 0.58)°C. Figure 1 shows the effect of fructose concentration on the gelation temperature of MC. When MC concentration is kept constant at 1% and the concentration of fructose is varied from 2 to 10%, the reversible sol–gel transition temperature is decreased to (54 ± 0.76)°C at 10% fructose concentration. Fructose depress the gelation temperature of MC due to their greater affinity for water than MC, thus dehydrating the MC molecules and accelerate gel formation.

It has been observed that the variation of salt (SC) concentration (1–5%) diminishes the gelation temperature from (59 ± 0.58)°C to 35°C, which is clearly depicted in Figure 2. On the addition of SC, the water molecules will be placed themselves around the citrate anions of the salt. This will reduce the intermolecular hydrogen bond formation between water and hydroxyl group of methyl cellulose.[19] This cause depletion of water layer leading to enhance hydrophobe–hydrophobe interaction, which ultimately leads to lowering the gelation temperature.

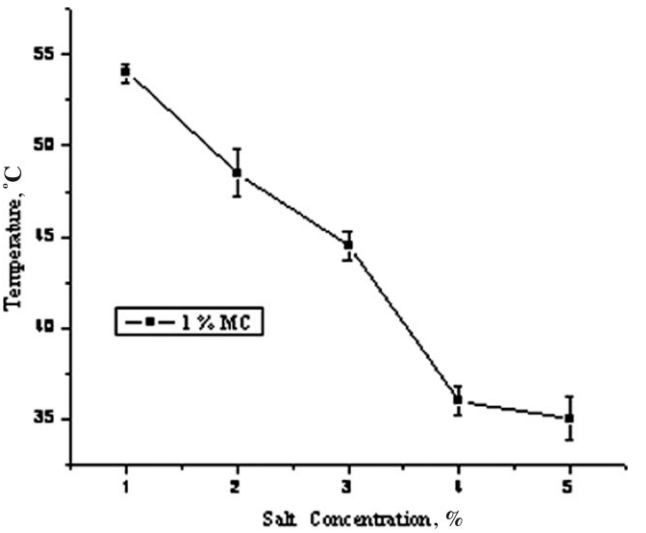

Figure 2 Variation of gelation temperature (°C) with (%) concentration of sodium citrate where MC concentration was kept constant at 1%.

In the ternary system, fructose and salt are used as additives. When fructose alone is added to MC solution, the sol–gel transition temperature is decreased, but the decrease is amplified by the simultaneous addition of citrate salt. In the binary system, fructose (10%) with 1% MC shows gel at (54 ± 0.76)°C. Therefore, in the ternary system, the 10% fructose is kept constant and the salt concentration is varied from 1 to 5%. As shown in Figure 3 when

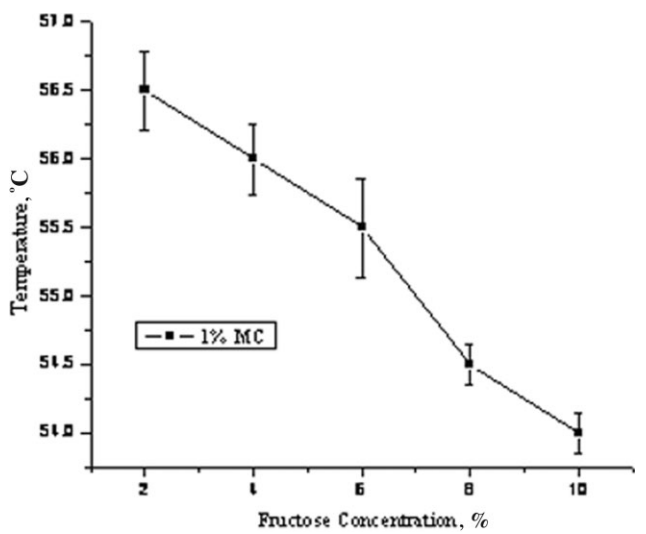

Figure 1 Variation of gelation temperature (°C) with (%) concentration of fructose where (1%) MC concentration was kept constant at 1%.

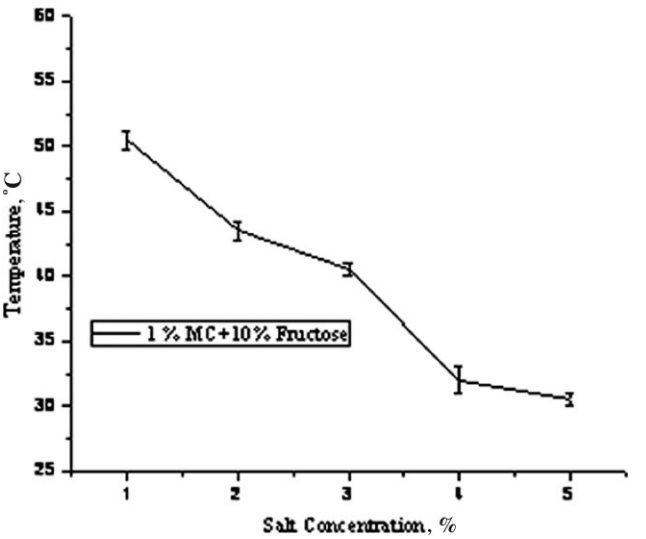

Figure 3 Variation of gel temperature (°C) with (%) concentration of sodium citrate on the MC(1%) – fructose (10%) solution.

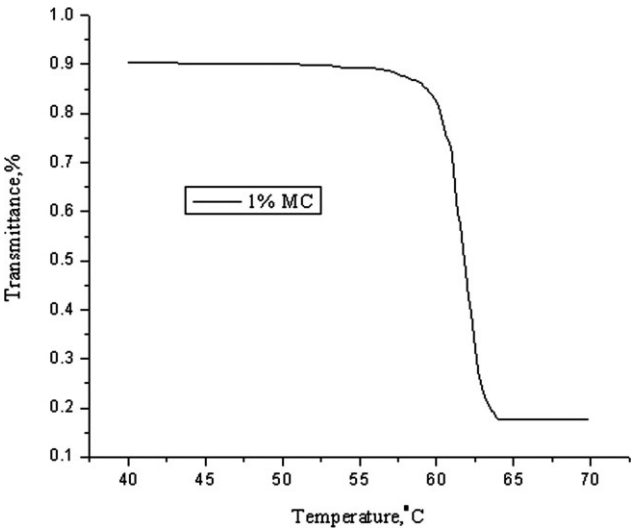

Figure 4 Optical transmittance of 1% MC solution at the wavelength of 500 nm during heating at a scanning rate of 1°C/min.

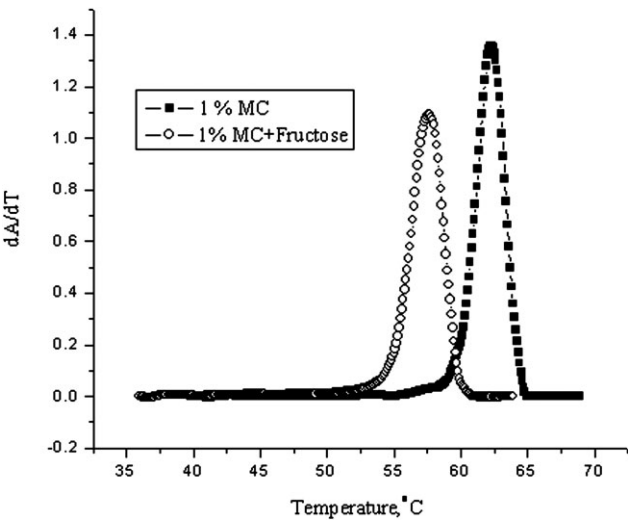

Figure 5 First derivative of absorbance ($\partial A/\partial T$) of 1% MC solution and 1% MC-10% fructose solution at the wavelength of 500 nm during heating at a scanning rate of 1°C/min.

the salt concentration is varied up to 5%, the gel temperature is reduced from 54°C to (31 ± 0.5)°C.

The test tube tilting method is a crude method to determine the gelation temperature. So UV is used to get the accurate gelation temperature. The Figure 4 shows the transmittance of 1% MC solution. At 35°C, MC solution is transparent and clear which gives 90% transmittance but as the temperature increases the transmittance of the solution decreases, this is due to the effect of gelation. During the gelation, a morphological change has been occurred. The MC random coils rearranged themselves to the intermolecular hydrophobic aggregates.[25] The transmittance decreases during gelation due to scattering of light by the aggregates of MC.

The sol–gel transition is due to the micro phase separation of the solution. Jonson et al.[26] defined the cloud point as the temperature at which the phase separation occurs for 1% polymer solution. Cloud point is extensively used as the gelation temperature of the polymer solution. The absorbance increases with the increase in temperature due to initiation of gel formation.

Figure 5 shows the change of derivative of absorbance (dA/dT) of the pure 1% MC solution as well as 1% MC-10% fructose solution with respect to the temperature. The derivative rises sharply after about 57°C and 52°C respectively as can be found in Figure 5. This sharp increase of the absorbance is due to the formation of hydrophobic clusters of the MC molecules.[25] The highest derivative of absorbance shows the gelation temperature of the solution. The gelation temperature of 1% MC and 1%MC-10% fructose solutions are 62°C and 55°C respectively according to the Figure 5.

Figure 6 shows the change of the derivative of absorbance of the MC-Fructose solution (1% MC and 10% fructose) containing different concentration of salt. It is clear from the Figure 6 that the gelation temperature i.e. the highest derivative of the absorbance decreases with the increasing concentration of SC from 52 to 27°C.

With the addition of citrate ion, the absorbance shifts to the left (i.e. lower temperature). The higher the SC concentration the more the curve shifts toward left. This indicates that the addition of citrate makes the turbidity appear at a lower temperature upon heating, meaning that SC displays a typical

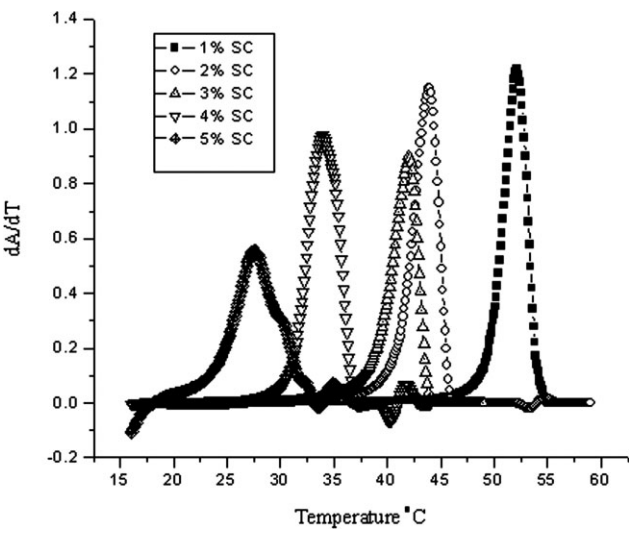

Figure 6 First derivative of absorbance ($\partial A/\partial T$) of MC-Fructose solution (1%MC and 10% Fruc) containing different concentration of SC at the wavelength of 500 nm during heating at a scanning rate of 1°C/min.

TABLE I
Gelation Temperature Measured with Test Tube tilting Method and UV

Process	1%MC	1%MC + 10% Fruc	1%MC + 10% Fruc + 1%SC	1%MC + 10% Fruc + 2%SC	1%MC + 10% Fruc + 3%SC	1%MC + 10% Fruc + 4%SC	1%MC + 10% Fruc + 5%SC
TTM	59 ± 0.58	54 ± 0.76	51 ± 0.76	44 ± 0.76	41 ± 0.5	33 ± 1.04	31 ± 0.5
UV	62	55	52	44	42	35	27

TTM, Test tube tilting method; UV, Ultraviolet visible spectroscopy.

salt out effect. We know that citrate ion tends to have a stronger interaction with water molecules. Thus, the salt destroys some of the original hydrogen-bonding network formed by water and this effect is similar to increasing temperature. The competition for water molecules from SC and the salt-induced destruction of the hydrogen bonds between the MC chains and water cause the decrease of MC solubility in water.

From the Table I, it has been concluded that there is some minor difference in gelation temperature observed by the test tube tilting method and UV method. This is due to the manual error or eye estimation error.

In vitro release kinetics

The drug release properties of the 1%MC-10% fructose-5% SC and 1%MC-5% SC are studied. The cumulative percent release of KT as a function of time for different formulations (MC-KT solution containing SC without and with fructose named as Keto-1 and Keto-2 respectively) has been shown in Figure 7. In case of Keto-1, the drug released about 100% after 5 h whereas Keto-2 shows about 100% of drug release after 9 h. This results indicate that the incorporation of fructose increases the duration of drug release from gel system. The viscosity of the above two formulations are measured and is depicted in the Figure 8. From the figures, it is clear that the viscosity of the Keto-2 (58.7 cps) is nearly about three times higher than that of Keto-1 (20.2 cps) at physiological temperature (37°C). This increase in viscosity is due to the presence of fructose.

When the formulations come in contact with the lacrimal fluid at 37°C and gelation occurs, a prehydrated matrix system is formed in which water penetration and hydration are the rate limiting step of drug release. If water penetration is faster, hydration and drug release will be faster. So, sustained drug release will not be achieved. Keto-1 formulation i.e. 1%MC-5% SC without fructose forms less viscous gel results in faster drug release because water penetration is more in prehydrated matrix that enhance drug diffusion and erosion of gel. In case of Keto-2, water penetration is slower because fructose increases the viscosity of gel matrix more rapidly compared to Keto-1. Also fructose acts as an excellent humectant to retain water in the prehydrated gel matrix thus increases the duration of drug release.

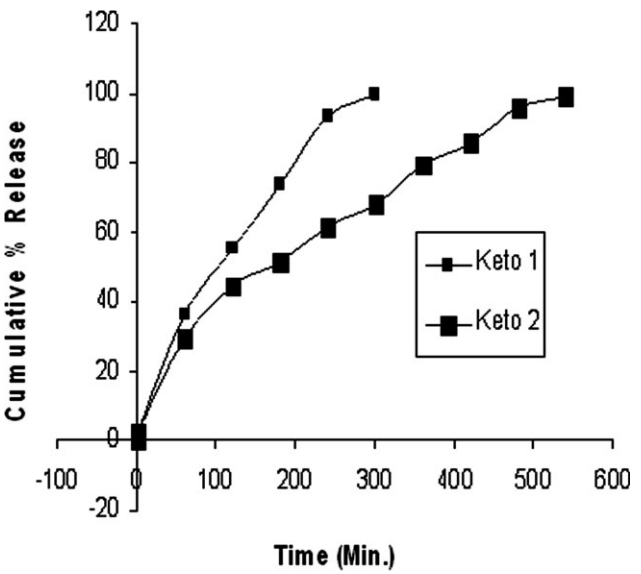

Figure 7 *In vitro* release of ketorolac tromethamine from *in situ* gelling systems.

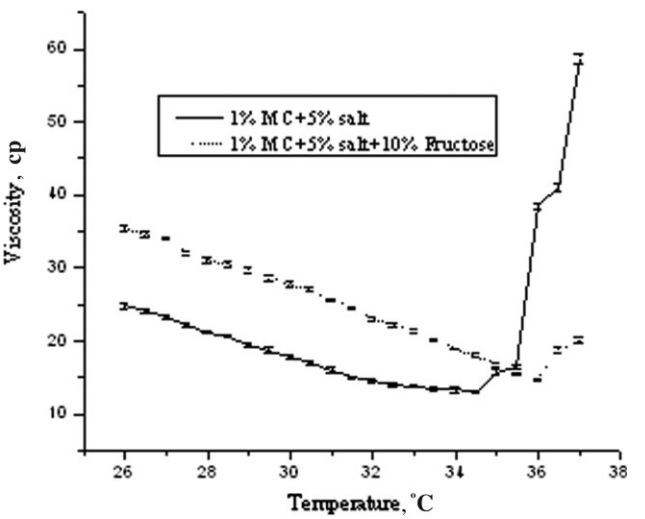

Figure 8 Effect of temperature on the viscosity of the two formulations (Keto-1 & Keto-2).

TABLE II
Release Kinetics of KT from in-situ-gel Formulations (Keto-1 and Keto-2)

Formulation	Composition (%W/V)				Zero order	First order	Higuchi	Korsmeyer-Peppas	
	KT	MC	SC	Fructose	r^2	r^2	r^2	r^2	n
Keto-1	0.3	1	5	–	0.9479	0.8222	0.9947	0.9986	0.59
Keto-2	0.3	1	5	10	0.9641	0.8047	0.9885	0.9984	0.75

The different kinetic equations (Zero order, First order, and Higuchi's equation) are applied 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 (correlation coefficient $r^2 > 0.9885$) for all the formulations given in the Table II. Therefore all the data are also fitted to the Korsmeyer-Peppas Equation $M_t/M_{¥} = kt^n$, where $M_t/M_{¥}$ is the fraction of drug released at time t; k is a constant incorporating structural and geometrical characteristics of formulation and 'n' is the release exponent indicative of the drug release mechanism. More acceptable linearity ($r^2 > 0.9984 \pm 0.0027$) is observed and the values of release exponent 'n' is varied from 0.59 ± 0.06 to 0.75 ± 0.05, which appear to indicate a coupling of the diffusion and erosion mechanism so-called anomalous diffusion and indicated that the drug release was controlled by more than one process.

CONCLUSION

KT is successfully formulated as an *in-situ* gelling system using MC in combination with fructose and SC. Fructose and SC were added in different proportions to reduce the gelation temperature from (59 ± 0.58)°C to 32°C. It has been found that the presence of fructose increases the duration of drug release from 5 to 9 h. This is due to the viscosity enhancing and water retention property of fructose. Thus, this formulation can be viewed as viable alternative to conventional eye drops by virtue of its ability to enhance precorneal residence time and consequently ocular bioavailability.

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