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Effect of PEG–salt mixture on the gelation temperature and morphology of MC gel for sustained delivery of drug

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ABSTRACT

Gelation temperature of MC was reduced from 59 °C to 54 °C with the addition of 10% PEG. Sodium tartrate (NaT) and sodium citrate (NaC) were added to the MC–PEG solution to further reduce the gelation temperature close to physiological temperature. Different techniques were used to measure the gelation temperature of all formulations. It was observed that NaC was more effective in reducing the gelation temperature of MC–PEG combination than NaT. Environmental scanning electron microscopy (ESEM) images of hydrogels containing NaC and NaT showed that NaC containing hydrogel having an interconnected microporous structure instead of the hollow rod like structure as in the case of NaT containing hydrogel. In vitro drug release studies showed that drug release time increased from 6 to 9 h by only changing the type of salt from NaT to NaC in MC–PEG combination.

1. Introduction

The most common method of application of a drug into the eye is to formulate the drug dosage in the form of an eye drop, but this method is not considered ideal for ocular delivery of drugs due to poor bioavailability of a drug arising from precorneal loss. This loss of the drug from the precorneal area is a net effect of a consistent lachrymal drainage of the drug. Therefore, frequent instillation of the drug is required. To avoid these problems, controlled drug delivery systems have gained interest. Recently, some devices such as ointments, gels, and polymeric inserts have been employed to increase bioavailability as well as the release time of the drug. The prospective applications of such approaches are, however, limited by several limitations including blurred vision, eye irritation, etc. To overcome these problems, thermo reversible hydrogel based ophthalmic formulations can be used as a vehicle for controlled delivery of drugs.

Hydrogels are hydrophilic polymer networks, which absorb enough quantity of water (Bain et al., 2010; Jeong, Kim, & Bae, 2002; Zan et al., 2006). Hydrogels can be prepared by physical or chemical crosslinking of polymers. Physically crosslinked hydrogels may show thermoreversible gelation behavior as polymer entanglement or secondary force including ionic, hydrogen bonding or hydrophobic forces is being responsible for gelation. Thermoreversible hydrogels are increasingly being used in biomedical applications due to their hydrophilicity and reversibility (Geng & Xiao, 2009; Hoffman, 2002).

Applications of thermoreversible hydrogels include many areas such as controlled drug delivery, pharmaceutical devices, and controllable sensors (Dinh, DeNuzzio, & Comfort, 1999; Eichenbaum, Kiser, Shah, Simon, & Needham, 1999; Guent, 1992; Hu, Chen, Wang, Zheng, & Li, 1998; Osada & Gong, 1998). The release of the drug is controlled either by the molecular diffusion of the drug molecules through the hydrogel or by the shrinking and swelling of the gel (Dinh et al., 1999; Eichenbaum et al., 1999).

Methylcellulose (MC) is a chemically modified polysaccharide made by partial substitution of —OH groups with methoxy moieties. This results in a polymer that is water-soluble at low temperature and displays lower critical solution temperature (LCST) phase behavior at elevated temperature. As such, aqueous solution of MC have long been employed and studied due to their ability to form gels as temperature is increased. Heymann first investigated the thermo reversibility of MC (Heymann, 1935). Currently, there is no consensus on the detailed mechanism of the gelation process, so a precise determination of the physical structure present in these hydrogels may lead the way to a new mechanistic understanding.

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The gelation of MC depends on temperature (Li et al., 2002; Wanka, Hoffman, & Ulbricht, 1990), pH of the medium (Siegel & Firestone, 1988) and type of solvent (Jeong et al., 2002). Different additives like natural polymers, synthetic polymers and various salts can alter the gelation temperature of MC (Iso & Yamamoto, 1970; Levy & Schwarz, 1958; Qiu & Park, 2001; Sarkar, 1979; Shimokawa, Saegusa, & Ishii, 2009). The effect of salts on the gelation temperature of MC has been studied extensively. During gelation there is a competition between the MC and the salt for the water molecules. Salt out salt has greater affinity for water molecules than the MC molecules, which helps in dehydration of MC molecules at lower temperature. As an outcome, the salt is capable of reducing the gelation temperature of the MC solution (Jeong et al., 2002; Kundu & Kundu, 2001; Sarkar, 1979; Siegel & Firestone, 1988; Xu, Wang, Tam, & Li, 2004).

Polyethylene glycol (PEG) belongs to the group of polyol and has the ability to reduce the gelation temperature of MC. It is biocompatible and non-toxic (Merrill & Salzman, 1983).

The effect of the molecular weight of PEG on the gelation temperature of MC has been studied earlier and it was observed that the molecular weight of PEG has a distinct effect on the gelation temperature of MC. The gelation temperature of MC was reduced from 60 °C to 54 °C and 52 °C when PEG (6×10^3) and PEG (2×10^3) were used along with MC, respectively (Bain et al., 2010). Previously, 6% sodium chloride (NaCl) was used to reduce the gelation temperature up to physiological temperature, i.e., 37 °C (Bain et al., 2010). Although, there are some formulations available in the market containing 6% NaCl, it is better to develop a MC–PEG based formulation with lower salt concentration. Therefore, our objective is to develop an ophthalmic formulation containing a salt of lower concentration.

With the objective to develop an ideal ophthalmic formulation containing a lower concentration of salt, measurement of the gelation temperature and drug release property of MC–PEG-6000 is studied with two different salts, sodium citrate (NaC) and sodium tartrate (NaT).

2. Materials and methods

2.1. Materials

Methylcellulose (MC, MetoloseSM-4000) was obtained from Shinetsu Chemical Co., Japan. The methoxyl content of MC was 29.6%. 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, sodium citrate, sodium tartrate and PEG of weight average molecular weight (M_w) of 6×10^3 Da with polydispersity index 2.5 were purchased from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Ketorolac tromethamine (KT) was a gift sample from Sun Pharma, Baroda, Gujarat, India. The dialysis membrane (LA390, average flat width-25.27 mm, average diameter around 15.9 mm and capacity 1.99 mL/cm, cut-off molecular weight 12,000–14,000) was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

2.2. Sample preparation for measurement of gelation temperature and drug release kinetics

Methylcellulose (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 obtain a transparent solution (Neely, 1960, 1963) and used as stock solution throughout the experiment. The solution of MC–PEG was prepared by dissolving 10% PEG (w/v) in MC solution by stirring using a magnetic stirrer. After total dissolution of MC and PEG, the whole solution was kept in the refrigerator for 24 h to get transparent binary solution of MC–PEG. The binary and ternary in situ gelling systems with and without PEG were developed by incorporation of 3% NaC or NaT with continuous stirring with MC and MC–PEG solutions. Then all solutions were kept in a refrigerator at 10–15 °C for one day. 0.5 wt% of KT was used to prepare the stock solution for drug delivery application. Deionized double distilled water was used to prepare all solutions. Throughout the experiment, 1% MC solution was used as a stock solution.

2.3. Measurement of gelation temperature

The reversible sol–gel transition temperature was measured by the test tube tilting method (TTM). To measure the gelation temperature by this process, the solution was sealed in a 20 mL glass tube and placed in a controlled temperature bath. The temperature of the bath was increased at a very slow rate. At a certain temperature the solution was completely converted into gel. The gel became turbid and did not flow with the tilting of the test tube. This characteristic temperature is called the gelation temperature. The same process has been repeated 2–3 times to get an accurate value. The gelation temperature was also confirmed by measurement of viscosity, UV–vis spectroscopy, and rheological studies.

2.4. UV–vis spectroscopic study

The turbidity measurement was performed on a UV–vis spectrophotometer (Agilent 8453 Spectrophotometer equipped with a temperature controller). The sample was placed in the cell and covered with a Teflon cap to prevent evaporation of solvent. Deionized double distilled water was used as the reference. The absorbance was measured at 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 (Xu & Li, 2005; Zheng, Li, Hu, & Zhao, 2004).

2.5. Viscosity measurement

The viscosity and gelation temperature of the solutions were measured with viscotester HAAKE, V-550 equipped with a temperature controller. The sample was placed in the sample container and kept idle for 5 min so that it reached a constant temperature. The viscosity of the solutions was measured at 10 rpm.

2.6. Rheological measurement

Rheological characterization of the MC–PEG gels was done using an Advanced Rheometer AR 2000 (TA Instrument, USA). 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 a 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 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.

2.7. Morphological studies

All hydrogel samples were lyophilized carefully to maintain their three-dimensional porous structure. Lyophilized hydrogel samples were immersed in liquid nitrogen, and the vitrified samples carefully cut with a cold knife. Cut samples were mounted, sputter coated with gold, and their morphology investigated by

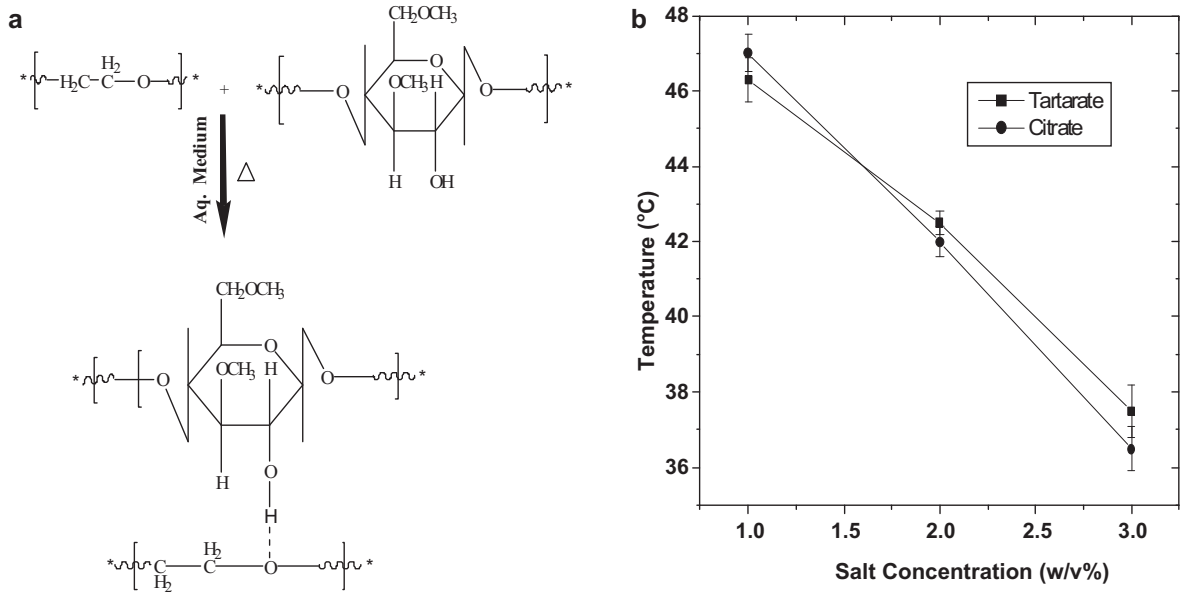

Fig. 1. (a) Schematic representation of interaction between MC and PEG chains during gelation. (b) Effect of weight percentage of salts (NaT and NaC) on the gelation temperature of MC–PEG combination.

scanning electron microscopy using a FEI Quanta 200 ESEM microscope.

2.8. In vitro release studies

The in vitro release of KT from prepared formulations was studied through a 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 $CaCl_2{\cdot}2H_2O$ and distilled water qs to 100 g (Bain, Bhowmik, Ghosh, & Chattopadhyay, 2009)]. 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 pipetted out and placed over the dialysis membrane. Aliquots, each of 1 mL starting from zero hour, were withdrawn at an hourly interval and replaced by an equal amount of artificial tear fluid. The aliquots were analyzed by UV–vis spectrophotometer at 323 nm.

3. Results and discussion

3.1. Measurement of gelation temperature by TTM

The gelation temperature of all the ternary solution (MC–PEG–salt) was initially measured by using TTM. PEG is responsible for reducing the gelation temperature of MC and this reduction is due to the presence of strong hydrogen bonding between these two polymer molecules. Fig. 1a shows the schematic representation of interactions present between MC and PEG molecules. As it is not possible to reduce the gelation temperature of MC up to physiological temperature (37 °C) by addition of polyethylene glycol (PEG) alone, so sodium tartrate (NaT) and sodium citrate (NaC) are used. Our aim is to select a salt, which is more efficient in reducing the gelation temperature of MC–PEG combination to avoid the high salt concentration in the drug formulation.

The gelation temperature of all combinations of MC–PEG–salts is measured by TTM. It is observed that the variation of NaT and NaC concentration (1–3 wt%) diminishes the gelation temperature of MC from 59 °C to 37 °C and 36 °C, respectively, and is shown in Fig. 1b. When NaC and NaT are added to the MC–PEG combinations, the water molecules will be placed around the citrate and tartrate ions of the salt resulting in suppression of the intermolecular hydrogen bond formation between water and hydroxyl groups of methylcellulose. Such depletion of the water layer around hydroxyl groups of methylcellulose leads to an enhancement of hydrophobic–hydrophobic interaction responsible for gelation, and hence, resulting in reduction of the gelation temperature.

According to the Hofmeister series: $citrate^{3-} > SO_4^{2-} = tartrate^{2-} > HPO_4^{2-} > CrO_4^{2-} > acetate^{-} > HCO_3^{-} > Cl^{-} > NO_3^{-} > ClO_3^{-}$ (Rossetto, de Souza, & Pandolfelli, 2008) citrate ion is more effective than the tartrate ion in reducing the gelation temperature.

According to the Hofmeister theory, the addition of a salt will affect the water structure through the interactions between ions and water molecules. Citrate and tartrate ion, which are categorized as a salt-out ion, exhibits strong interactions with water molecules. Therefore, these ions tend to compete with MC chains for water molecules, and they can attract more water molecules to surround

Table 1
Measurement of gelation temperature (°C) by various techniques.

Method used to measure the gelation temperature (°C)	1% MC (SM-4000) + 10% PEG (6×10^3)					
	Na-tartrate			Na-citrate		
	1%	2%	3%	1%	2%	3%
TTM	46	42	37	47	42	36
UV	50	43	39	48	42	35
Viscosity	49	44	38	46	41	36
Rheometer	45	41	39	40	39	37

them due to their strong hydration abilities, leading to a poorer solubility of MC in water. As a result, at the same temperature, there are more hydrophobic MC aggregates in a salted MC solution than a salt-free one. Thus upon heating it will then be easier to meet the requirement for the critical number of hydrophobic aggregates for a gel to be formed, so that the gelation occurs at a lower temperature in the presence of a salt-out salt.

Above results follow the same pattern, i.e., NaC is comparatively more effective in reducing the gelation temperature of MC–PEG combination than NaT.

However, as the test tube tilting method (TTM) is a crude method to determine the gelation temperature, it demands further confirmation of the as-measured gelation temperature. Therefore, different techniques such as test tube tilting method, UV–vis spectroscopy, viscometry and rheometry are used to substantiate the results obtained from TTM of all the solutions containing MC–PEG–salt.

3.2. UV–vis spectroscopic studies

Gelation temperature of all combinations of MC–PEG–salt ternary system is measured by different methods and has been tabulated in Table 1. Fig. 2 shows changes of the derivative of absorbance with respect to temperature (dA/dT). The peak temperature is defined as gelation temperature. As the temperature

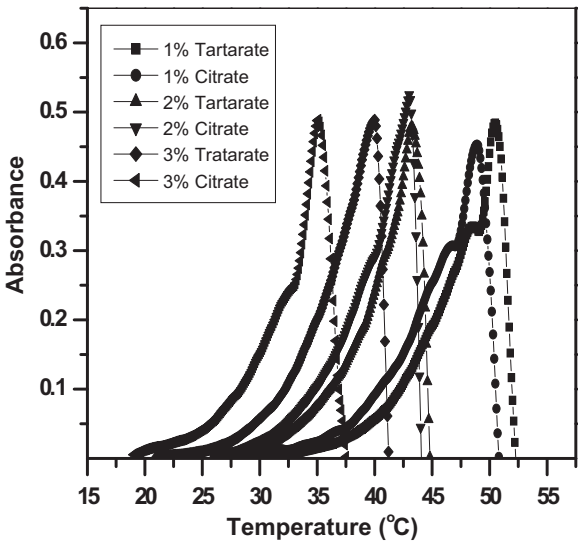

Fig. 2. Effect of temperature on the first derivative of absorbance (dA/dT) of MC–PEG–ST (1–3%) and MC–PEG–NaC (1–3%) during heating at a scanning rate of 1 °C/min at the wavelength of 500 nm.

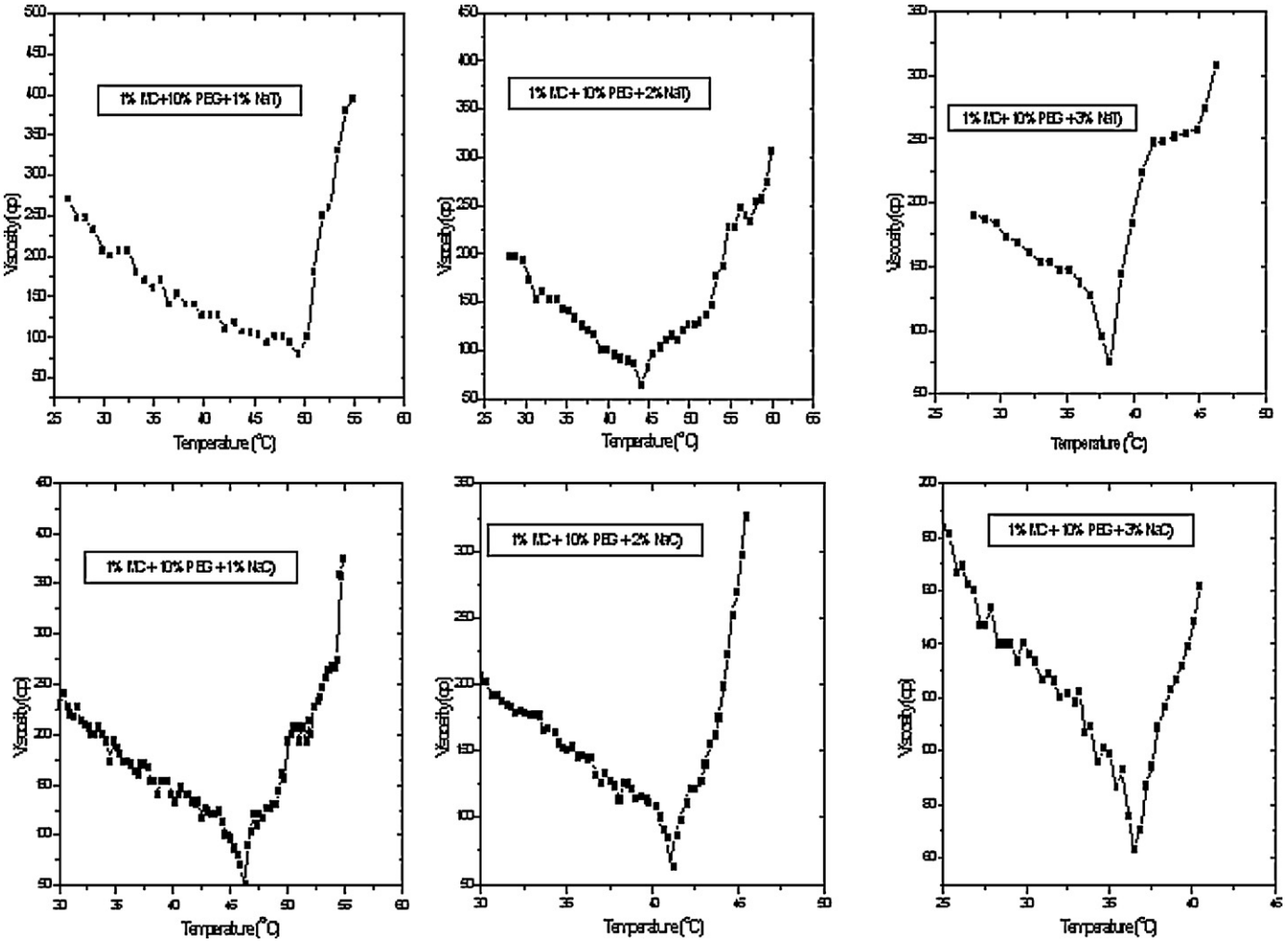

Fig. 3. Effect of salts (NaT and NaC) on the viscosity and gelation temperature of MC–PEG binary solution.

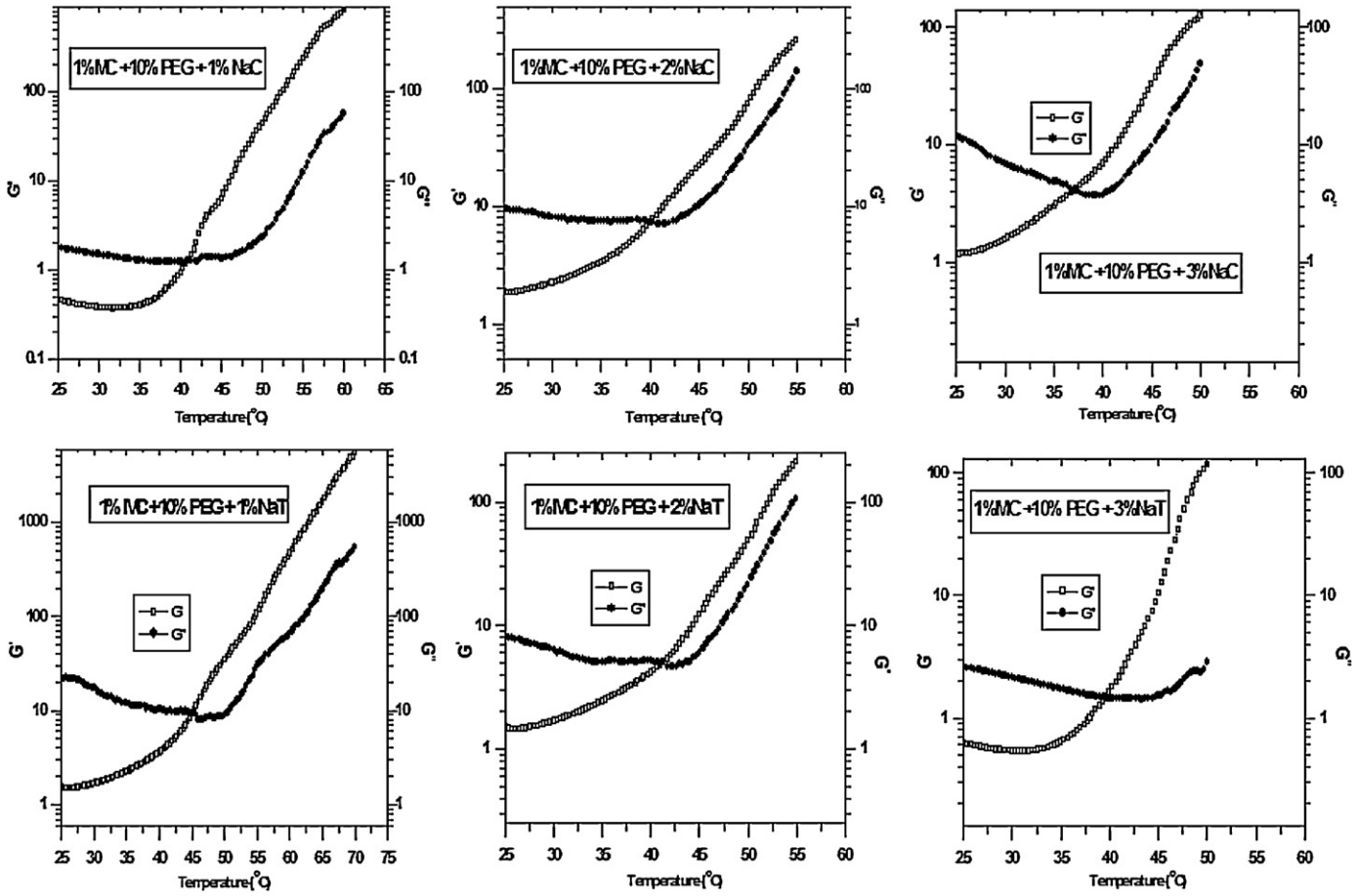

Fig. 4. Change of storage modulus (G') of MC–PEG solutions containing different concentration of NaT and NaC as a function of temperature in a heating process at scanning rate of 2 °C/min.

increases turbidity of the solution increases and this is due to formation of clusters containing molecules of MC and PEG. During heating, the dA/dT remains initially low but after a certain temperature, it rises sharply and this is due to the formation of physical crosslinking in-between molecules of these two polymers MC and PEG. The extent of physical crosslinking is enhanced by the presence of salt out salts. From Fig. 2 it is clear that when 3% NaT and NaC are used; the peak is obtained at 40 °C and 35 °C, respectively, which means NaC is more effective in reducing the gelation temperature of MC–PEG combination. With the addition of salt, polymer–polymer interactions supersede interactions between polymer and water molecules and thus help in the reduction of gelation temperature.

3.3. Viscosity studies

Fig. 3 shows the change of viscosity with temperatures for all the combinations of MC–PEG–salts at 10 shear rate. The sharp change in viscosity marks the gelation temperature of all the above-mentioned combinations and the results are tabulated in Table 1. It is observed that the gelation temperature of MC–PEG decreases with an increase in salt concentration. When the NaC and NaT are used the gelation temperature is reduced from 59 °C to 36 °C and 38 °C, respectively, and it is clear that NaC is more effective than NaT in reducing the gelation temperature.

3.4. Rheological studies

It is likely that the mechanical properties of these solutions are highly dependent on inter and intra molecular hydrogen bonds between —OH groups of MC and PEG molecules. Measurement of G' is an important method to determine the gelation temperature as reported earlier (Bain et al., 2009, 2012; Takeuchi et al., 2003). Gel strength is an important factor in the field of sustained release of drug, so it is an essential method, which evaluates the gelation temperature as well as the gel strength. The polymer solution moves toward gel during heating and so G' increases. The sharp increase in G' indicates the gelation temperature (Bain et al., 2009). From Fig. 4, it is clear that with the addition of NaT the gelation temperature of MC shifted from 59 °C to 39 °C but NaC is capable of reducing the gelation temperature further to 37 °C. Each curve of the rheology follows a similar pattern. During heating, MC solution absorbs heat to destroy the cage like structures between MC chains and water molecules and thus helps in the formation of hydrophobic aggregates. The presence of salt helps in the destruction of the cage like structure between MC and water molecules and thus enhances the formation of hydrophobic aggregates at lower temperature that means gelation takes place at a lower temperature.

It is clear from Table 1 that although the gelation temperatures of all the MC–PEG–salt combinations measured by the above three methods are not close to each other but the decreasing trend of gelation temperature with different salt concentrations are the same in all the cases. So, it is substantiated from Table 1 that NaC is more effective than NaT in reducing the gelation temperature of MC–PEG salt combinations.

3.5. Morphological studies

In order to have an idea about the surface topography and cross-sectional structure of lyophilized pure 1% MC, MC–PEG–NaC and MC–PEG–NaT hydrogel samples are investigated by scanning

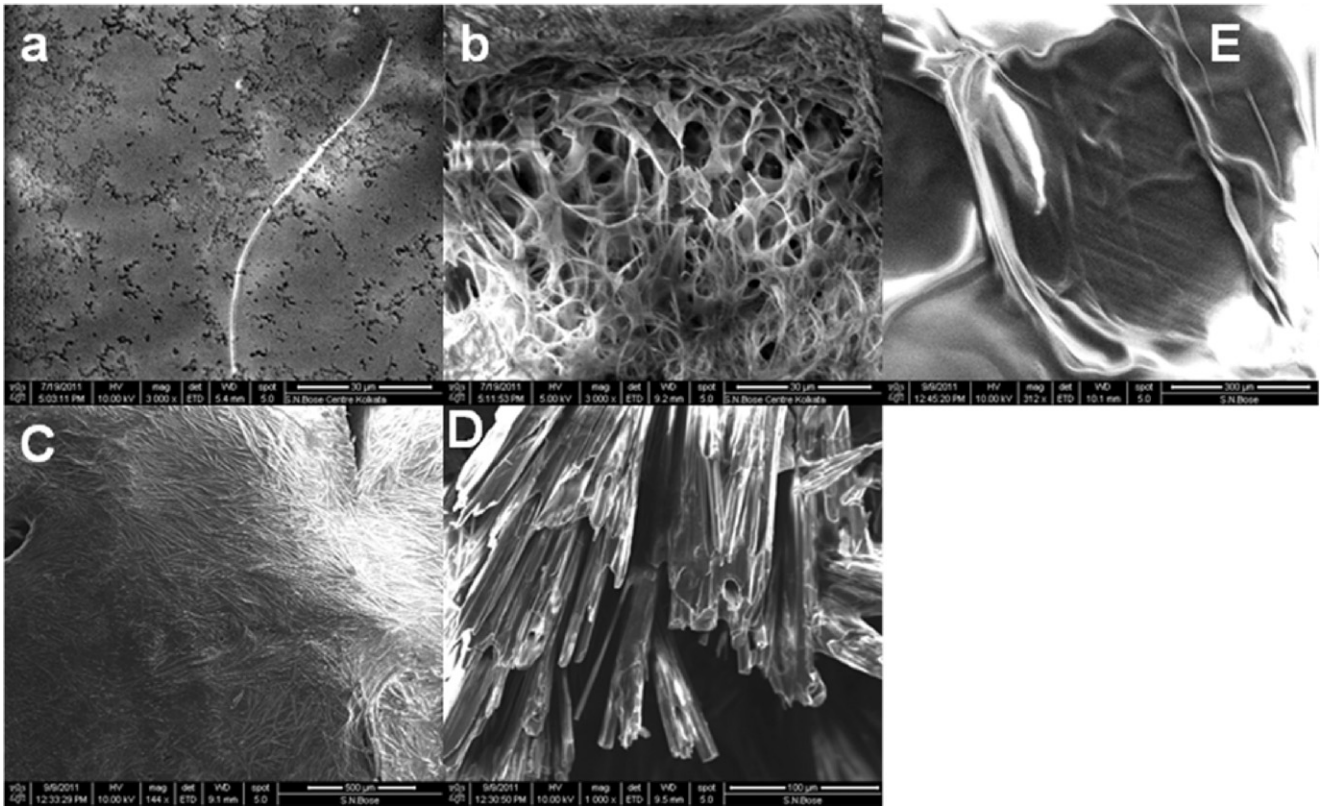

Fig. 5. The surface (a, c) and cross-sectional (b, d) ESEM images of freeze dried hydrogel samples (MC–10% PEG–3% NaC and MC–10% PEG–3% NaT), and the surface morphology of 1% MC hydrogel (e).

electron microscopy (SEM) (Omidian, Park, & Rocca, 2007) and shown in Fig. 5a,c and b,d, respectively. Fig. 5e depicts the absence of the polymeric networks in the MC hydrogel. It is quite effortless to conclude from both the cross-sectional images that the NaC containing hydrogel demonstrated a regular microporous network indicating superior interaction between the components than that of the NaT containing hydrogel. Xu and Li (2005) stated that ionic strength and anion charge had a significant role on the gelation temperature. The higher anion charge density and greater ionic strength of $C_6H_5O_7^{3-}$ compared to $C_4H_4O_6^{2-}$ results in better interaction with water molecules, thus dipping intermolecular hydrogen bonding between water molecules and MC chains, which directs greater hydrophobic interaction between MC chains leading to more rapid onset of gelation. It is proposed that there exists a competitive attraction between anions and MC chains for water molecules. According to Hofmeister series citrate ion is more effective in destroying the cage-like structures between MC and water molecules, increasing the hydrophobicity of MC in water than tartrate. From the cross-sectional images, it can be concluded that the NaC containing solution gives a uniform microporous structure along with excellent pore interconnectivity whereas the NaT containing solution offers fibrous construction of several micrometer range and comparatively poor pore connectivity. It is fairly predictable that the reasonable pore connectivity in the NaC containing solution is likely due to the greater interactions between $C_6H_5O_7^{3-}$ and water molecules and thus the hydrophobic interactions between PEG and MC chains increases.

3.6. In vitro drug release studies

The cumulative percentage release of ketorolac tromethamine as a function of time from different formulations has been shown in Fig. 6. It has been observed that the addition of PEG in MC solution containing NaC or NaT sustains the drug release process from the gel matrices. When the formulation comes in contact with the artificial tear fluid at 37 °C, gelation occurs and a prehydrated gel matrix is formed in which water penetration and hydration becomes the rate-limiting step of the drug release process. If water penetration is faster, hydration and drug release will be faster, i.e., sustained drug release will not be achieved. Here four formulations (F1: 1% MC + 3% NaT + 0.5% KT, F2: 1% MC + 3% NaT + 0.5% KT, F3: 1% MC + 10% PEG + 3% NaT + 0.5% KT, F4: 1% MC + 10% PEG + 3% NaC + 0.5% KT) are used to study the in vitro drug release property. Among the above formulations, F4 containing 1% MC + 10% PEG + 3% NaC + 0.5% KT shows the highest drug release time. The reason behind the increase of drug release time can be argued to be due to the presence of PEG, which is responsible for increasing the viscosity as well as the gel strength. From the formulation F4, a more sustained release of drug has been achieved as observed up to 9 h. This phenomenon is attributable to the presence of NaC and PEG, which leads to the lowest gelation temperature as well as an increase in viscosity and hence slower drug diffusion. Therefore, F4 appears to be the best formulation of the developed in situ gelling formulations of ketorolac tromethamine for ocular delivery.

3.7. In vitro release kinetics

The drug release studies of the 1% MC–3% NaT (F1), 1% MC–3% NaC (F2) 1% MC–10% PEG (6×10^3)–3% NaT (F3), 1% MC–10% PEG (6×10^3)–3% NaC (F4), formulations show that F4 exhibits longer drug release time than F1, F2, and F3, respectively. To know the release pattern from in situ gel systems, data are treated according to different kinetic equations: Zero order (Eq. (1), cumulative percentage of drug release versus time), First order (Eq. (2), log cumulative percentage of drug remaining versus time) and

Table 2
Release kinetics of KT from in situ gel formulations: 1% MC–3% NaT (F1), 1% MC–3% NaC (F2), 1% MC–10% PEG (6×10^3)–3% NaT (F3), and 1% MC–10% PEG (6×10^3)–3% NaC (F4).

Formulations	Zero order	First order	Higuchi	Korsmeyer–Peppas	
	r^2	r^2	r^2	r^2	N
F1	0.906	0.982	0.986	0.999	0.344
F2	0.910	0.978	0.981	0.987	0.272
F3	0.950	0.834	0.992	0.984	0.373
F4	0.964	0.838	0.990	0.973	0.427

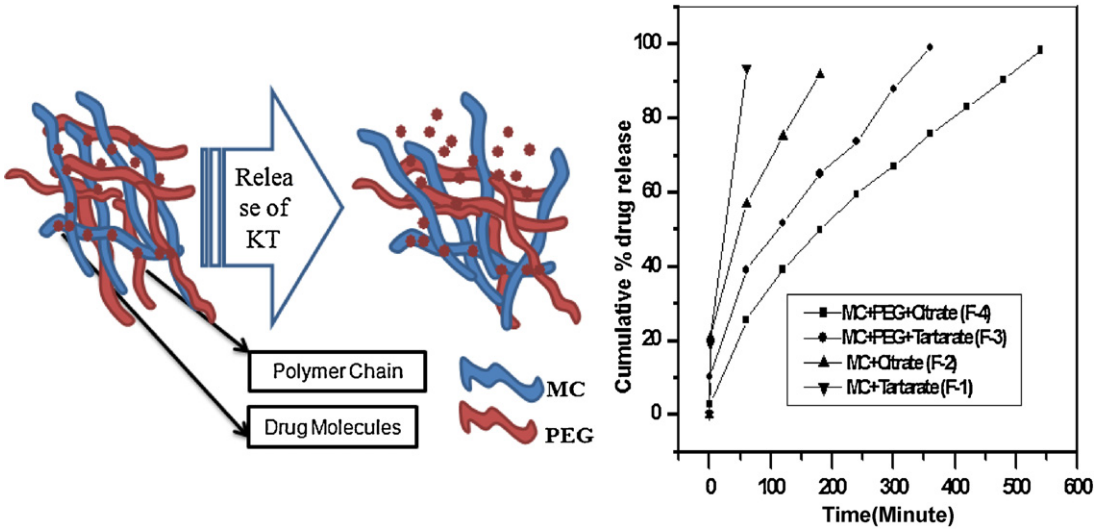

Fig. 6. Schematic representation of release and in vitro release kinetics of KT from in situ gelling formulations based on MC [F1: 1% MC + 3% NaT, F2: 1% MC + 3% NaC, F3: 1% MC + 10% PEG + 3% NaT, F4: 1% MC + 10% PEG + 3% NaC].

Higuchi's equation (Eq. (3), cumulative percentage of drug release versus square root of time).

$$\frac{dq}{dt} = K_0 \tag{1}$$

$$\frac{dq}{dt} = K_1(C_s - C_t) \tag{2}$$

$$Q = K_{HG}t^{0.5} \tag{3}$$

The cumulative percentage release of Ketorolac tromethamine as a function of square root time has been shown in Fig. 6. For MC–tartrate and MC–citrate solution, about 100% KT is released within 2 h and 3 h from MC–tartrate and MC–citrate solutions, respectively. It is also observed that addition of PEG in MC–citrate and MC–tartrate solution increases the drug release time, i.e., sustain drug release. From the cross-sectional images, it is clear that the NaC containing solution gives a uniform microporous structure along with excellent pore interconnectivity whereas the NaT containing solution offers fibrous construction of several micrometer range and comparatively poor pore connectivity. Therefore, MC–PEG–citrate (F4) exhibit longer drug release time than other formulations due to the presence of micro porous structure, where the drug molecules are entrapped.

From the kinetic studies (Table 2), it is found that the drug release is best explained by Higuchi's equation, as the plots showed the highest linearity ($r^2 > 0.981$). All data are fitted to the Korsmeyer–Peppas equation and more acceptable linearity ($r^2 > 0.973$) is observed. The release exponent 'n' varied from 0.272 to 0.427, which indicates drug release is controlled by Fickian diffusion.

4. Conclusion

The rationale of the present study was to develop ophthalmic formulations containing lower salt concentration. Ophthalmic formulations were developed by using an ion sensitive polymer MC and PEG as a viscosity-modifying agent. Two different salts such as NaT and NaC were used to reduce the gelation temperature of MC–PEG combination close to physiological temperature. It was found that NaC is more effective than NaT in reducing the gelation temperature of the MC–PEG solution. In vitro drug release studies showed that the incorporation of polymeric excipients such as polyethylene glycol could increase the release time of drug. It was also found that drug release time increased from 6 h to 9 h by changing only the nature of salt from NaT to NaC. This increase in drug release time was probably due to the change in morphology of gel structure from hollow fibrous to interconnected microporous structure. All the chemicals used to prepare above formulations were inexpensive and easily available. The developed formulation also promised to reduce the frequency of drug administration, thus improving patient compliance.

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