Macroporous thermosensitive poly(HEMA-*co*-NIPAAm) hydrogels for controlled drug delivery application

Aplikasi termosensitif macroporous hydrogel poly(HEMA-*co*-NIPAAm) untuk kontrol pelepasan obat

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Abstract

Controlled delivery systems of a predetermined dose over a sustained period have been used to overcome the shortcomings of conventional dosage forms. This is because the controlled drug delivery system can provide sustained therapeutic level of drug concentration without toxicity and convenience for patients. It would be more beneficial and ideal if the drug could be delivered by a device that would respond to external environmental change. Therefore, the correct amount of drug would be released upon the stimulation of such a temperature change. The purpose of study is synthesis of macroporous thermal responsive poly(HEMA-*co*-NIPAAM) hydrogels by free radical polymerization for controlled drug delivery applications. Effect of varying water and HEMA-NIPAAm ratio in the monomer mixture were resulted interconnected macroporous structure. From the result, 5HEMA15NIPAAm was showed the most rapid responsiveness in swelling ratio, polymer volume fraction, swelling and deswelling kinetics. The high drug loading capacity was achieved at or below ambient temperature, whilst the release profile was revealed sustain release of conventional anti-inflammatory drug; prednisolone 21 hemisuccinate sodium salt. In general, incorporating appropriate amount of water and HEMA-NIPAAm ratio can improve the swelling properties, drug loading capacity and drug release profile, which can be use for sustained release of various medication.
Key words: macroporous, thermosensitive hydrogel, controlled drug delivery application

Abstrak

Sistem pelepasan terkendali dosis yang telah ditentukan selama periode yang berkelanjutan telah digunakan untuk mengatasi kekurangan dari metode konvensional. Hal ini karena sistem pelepasan obat terkontrol dapat memberikan tingkat berkelanjutan terapi konsentrasi obat, tanpa keracunan dan nyaman bagi pasien. Hal ini, akan lebih bermanfaat dan ideal jika obat dapat disampaikan oleh perangkat yang akan merespon perubahan lingkungan eksternal. Oleh karena itu, dosis obat yang benar akan dilepas dengan stimulasi seperti perubahan suhu. Tujuan penelitian adalah untuk mensintesis poli (-co HEMA-NIPAAm) hidrogel berpori yang responsif terhadap termal dengan metode free radical polymerization untuk aplikasi pelepasan obat terkontrol. Pengaruh dari jumlah air dan rasio HEMA-NIPAAm dalam campuran monomer menghasilkan struktur yang berpori. Dari hasil tersebut, 5HEMA15NIPAAm ini menunjukkan respon yang paling cepat di swelling rasio, polimer volume fraction, swelling dan deswelling kinetik. Drug loading capacity yang tinggi dicapai pada atau di bawah suhu kamar, sedangkan profil drug release dari obat anti-inflamasi konvensional; prednisolon 21 hemisuccinate sodium salt. Secara umum, penggunaan air dan rasio HEMA-NIPAAm yang tepat dapat

meningkatkan swelling properties, drug loading capacity dan profil drug release, yang dapat digunakan untuk pelepasan obat berkelanjutan dari berbagai macam obat.
Kata kunci: makroporus, hydrogel termosensitif, aplikasi di kontrol pelepasan obat

Introduction

Poly(2-hydroxyethylmethacrylate)/(PHEMA) hydrogels often appear opaque or translucent due to the presence of macropores in the materials (Chirila, *et al.,* 1993) which makes them chemically identical, but structurally distinctive to the transparent and homogeneous type of PHEMA that are commonly used for applications in which a combination of optical clarity and limited diffusive characteristics is required, such as contact lens and intraocular lens (Oxley, *et al.,* 1993). Their application has been demonstrated in the novel design of an artificial cornea and an orbit implant in which the porous PHEMA component allows host cells and tissue to grow into the device, thereby preventing extrusion of the implants (Chirila *et al.,* 1998), (Crawford, *et al.,* 2002), (Hicks, *et al.,* 2006). However, although they allow diffusion of various solutes, their transport properties are limited by effective mean pores, or mesh diameters within the polymer matrices. These materials have limited drug loading capacity (Li, *et al.,* 2008) and are not suitable for the delivery of macromolecules (Oxley, *et al.,* 1993)

Our interests in the macroporous PHEMA are extended to their applications as ophthalmic drug delivery systems in which a major challenge in hydrogel usage is to sustain the release for a long period of time and also to incorporate sufficient amounts of drugs in the hydrogel matrix (Hicks, *et al.,* 2002). In order to further increase the drug loading capacity and to improve the controlling ability of the release of the drugs, *N*-isopropylacrylamide (NIPAAm) is incorporated into the macroporous PHEMA hydrogels. The unique hydrophilic/hydrophobic change of the NIPAAm component at a temperature above the VPTT has promoted numerous efforts in preparing intelligent hydorgels for controlled drug delivery of hydrophilic drugs (Hoffman, 1987), (Suzuki and Tanaka, 1990), (Zhang, *et al.,* 2008). In this study, copolymers of HEMA and NIPAAm/poly(HEMA-*co*-NIPAAm) were produced in the presence of 70 and 80 wt% of water. The interior morphology of the produced hydrogels, the change in their swelling ratios and the polymer volume fractions in response to the temperature change were investigated. Swelling and de-swelling kinetics were studied to optimize the drug loading conditions. The loading capacity and drug diffusion profiles of the hydrogels were also examined.

Methodology

Chemicals and materials

Ophthalmic grade 2-hydroxyethyl methacrylate (HEMA) was purchased from Bimax Inc USA and was used as received. Monomer N-isopropylacrylamide (NIPAAm) (97%), crosslinking agent *N, N'*-methylenebisacrylamide (mBAAm) (99%), and initiators ammonium persulfate (APS) (98%) and *N,N,N',N'*-tetramethylethylenediamine (TEMED) (99.5%) were supplied by Sigma-Aldrich Co. Australia and used as received. Prednisolone 21-hemissucinate sodium salt powder was purchased from Sigma Chemical Co. Belgium and used as a model drug. Deionised water was used for all experiments in this study.

Preparation of polymer hydrogels

HEMA, NIPAAm and mBAAm were mixed with water according to the formulae listed in Table I. The solution was purged with nitrogen gas for 20 min prior to the addition of appropriate amounts of the APS solution and TEMED. The monomer mixtures were then dispensed into a mold to produce hydrogel discs for swelling behavior and drug loading capacity measurement. Hydrogel membranes of 1-2 mm thickness were also casted for drug diffusion experiments. The detailed casting methods can be found in the previous reports (Lou, *et al.,* 2005). All samples were cured at room temperature for 24 h. After the curing process, the samples were removed from the molds and stored in deionised water with daily water exchange for 2 weeks to remove residual monomers and impurities. Samples for morphology examination and drug loading characterization were freeze-dried prior to the measurements. Others were kept in deionised water for further process.

Interior morphology examination

The interior morphology of the produced hydrogels was examined using a Scanning Electron Microscope (SEM, ZEISS EVO 40XVP, Japan). Samples were coated with gold prior to the SEM examination.

Determination of equilibrium swelling ratio and polymer volume fraction

Equilibrium swelling ratio (ESR) was determined using equation (1) where W_s is the weight of a swollen polymer at equilibrium and W_d is the weight of the same polymer when it is dried.

$$ESR = \frac{W_s - W_d}{W_d} \quad \text{.....(1)}$$

The measurement was carried out at 10, 22, 30, 40, 50 and 60°C respectively in order to examine the sample responsiveness to the temperature changes. The reported ESRs were average values of five measurements for each sample at each temperature. The temperature dependence of the ESR is shown in Figure 2.

Polymer volume fraction, defined as the ratio of the volume of the dry gel and the volume of the fully hydrated hydrogel at a particular temperature, was also determined at the various temperatures to estimate the porosity change in response to the temperature change (Table II). The experimental details can be found in our previous report (Lou, *et al.,* 2004), (Lou, *et al.,* 2007), (Lou, *et al.,* 2005).

Swelling and deswelling kinetics

Swelling kinetics of the produced hydrogels was investigated at an ambient temperature (22 °C) using the conventional gravimetric method. In brief, a hydrogel polymer of known dry weight was put into water and taken out at chosen time points to record the weight. The equilibrium swelling weight was also recorded. The water uptake capacity was then calculated using equation (2),

$$W_u = \frac{W_t - W_d}{W_{es} - W_d} \times 100 \quad \text{.....(2)}$$

where W_u is the percentage water uptake capacity, W_t the weight of a hydrogel at time t, W_{es} the weight of the same hydrogel at equilibrium swelling, and W_d the weight of the dry gel. The plot of W_u against time t is displayed in Figure 3a.

Similarly the deswelling kinetics of the hydrogels, at both 37 °C and 50 °C, was investigated. In this experiment, an equilibrium hydrogel at 22 °C was put into water at a preferred temperature and taken out for weighing at regular time intervals. Equation (2) was then used to determine the water retention capacity, W_r, using the measured W_d, W_{es} and W_t. The change of W_r in time t is plotted in Figure 3b-c.

Drug loading and diffusion experiments

Prednisolone 21 hemisuccinate sodium salt stock solutions of 1 and 2 wt% were prepared for drug loading capacity measurement. The loading was carried out through a simple diffusion procedure at the selected temperature. In brief, a freeze-dried sample was weighed and kept in a drug solution that was approximateky 2 times of its ESR until it became fully swollen. The residue drugs in the remaining solution were determined using a UV-Vis spectrometer (UV/VIS 918, GBC Scientific Equipment Australia) (Lou *et al.,* 2004), (Lou *et al.,* 2007). The loading capacity was determined using the weight percentage of drugs in dry polymers (Table 2).

Drug diffusion experiments were carried out using a diffusion cell consisting of two 25 cm^3 compartments that was separated by a circular hydrogel membrane of 22 mm in diameter. Both compartments of the diffusion cell were first filled with deionised water and kept at the selected temperature for 24 hours, thus allowing the hydrogel membrane to reach its equilibration. After that, water in one compartment was removed and refilled with prednisolone 21-hemmisuccinate sodium salt solution (1 wt%) of the same temperature. The changes of drug concentrations in both compartments were then monitored regularly for 15 days. Drug diffusion profiles were established using the time dependence of the cumulative amounts of drugs in the water compartment. The effect of temperature on the diffusion profile was also investigated (Figure 4a-b).

Results and Discussion

Interior morphology of produced hydrogels

Seven HEMA and NIPAAm based polymer and copolymer hydrogels were prepared in this study (Table 1). Whilst five of them, 20NIPAAm, 5HEMA15NIPAAm, 10HEMA10NIPAAm, 15HEMA5NIPAAm and 20HEMA, were produced in the presence of 80 wt% water, the other two, 30HEMA and 10HEMA20NIPPAm, were produced in the presence of 70 wt% of water. Numerical numbers in the sample codes represent the weight percentages of monomers used in the polymerization. For instance, 10HEMA20NIPAAm represents a polymer that was produced from 10 wt% HEMA, 20 wt% NIPAAM and 70 wt% water.

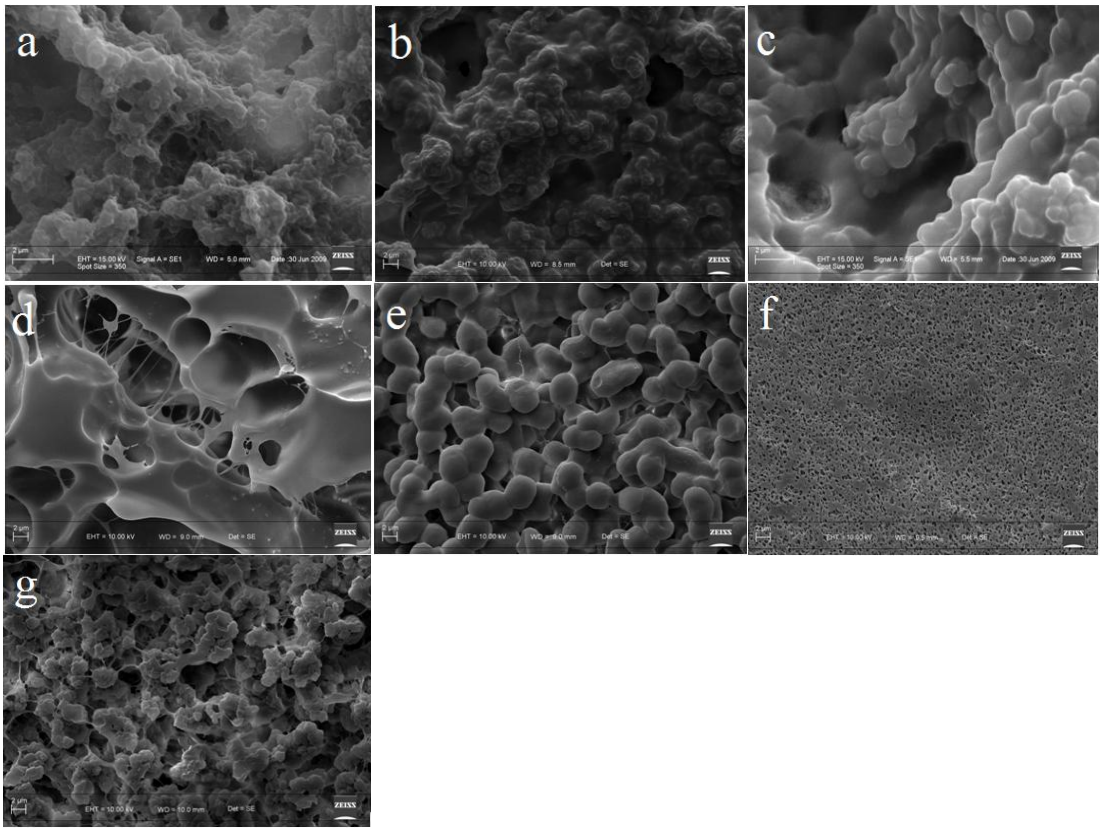

Figure 1. (a) SEM micrographs of 20NIPAAm, (b) 5HEMA15NIPAAm, (c) 10HEMA10NIPAAm, (d)15HEMA5NIPAAm, (e) 20HEMA, (f) 30HEMA (g)10HEMA20NIPAAm.

All polymers appeared opaque when they were fully hydrated, indicating a porous polymer network. Changes in the sample morphology were well demonstrated in their SEM micrographs (Figure 1). Hydrogels made from 80 wt% water (Figure 1a-e) were more porous than those made from 70 wt% water (Figure 1f-g), indicating a clear influence of the water contents to the phase separation process (Chirila, *et al.,* 1993), (Okay, 2000). Pores and the polymer textures were well defined and evenly distributed in 20HEMA, 30HEMA and 10HEMA20NIPAAm (Figure 1e-g), but more random in others (Figure 1a-c). Polymer textures in 15HEMA5NIPAAm were quite different from others (Figure 1d). The results demonstrated that the porous structure of the hydrogel polymers can be well tuned by adjusting the chemical composition in the formula.

Equilibrium swelling ratio and polymer volume fraction

The temperature effect on the ESR of the hydrogel polymers are shown in Figure 2. Apart from 20HEMA and 30HEMA (data not displayed), all copolymers including the homopolymer of 20NIPAAm, were apparently responsive to the change of temperature. Incorporation of NIPAAM into HEMA has indeed enhanced the thermo sensitivity of the hydrogels. The change in ESR was clearly dependent on the content of NIPAAm in each copolymer. The most rapid change of ESR was demonstrated by 5HEMA15NIPAAm for which the ESR value decreased from 8.4 g/g at 10 °C to less than 0.6 g/g at 60 °C. The change in ESR of the copolymer containing 30 wt% water was less significant indicating that the porosity of the hydrogel also affects the swelling capacity.

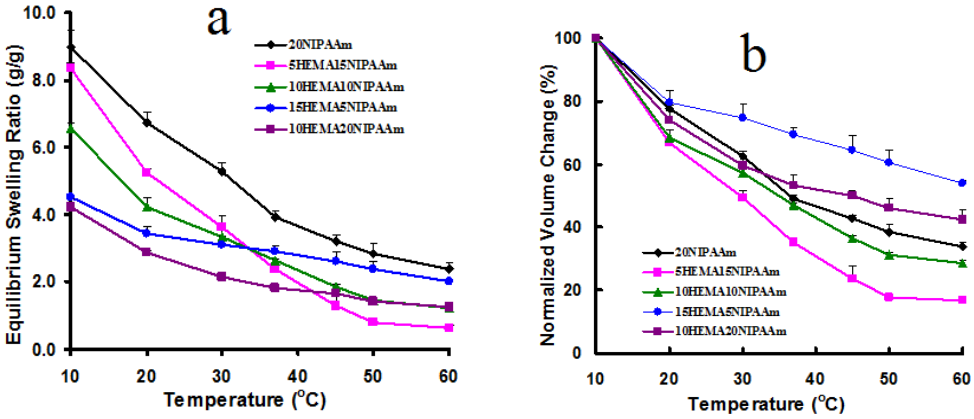

Figure 2. The equilibrium swelling ratio (a) and the normalized volume change (b) of hydrogels at various temperatures.

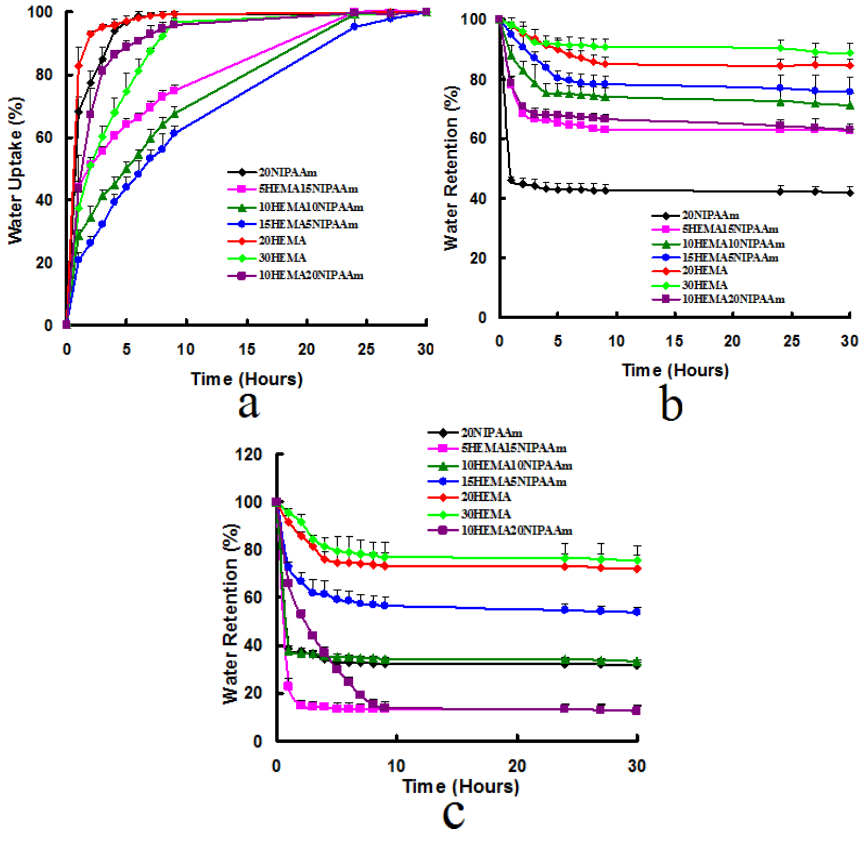

Figure 3. (a) Swelling kinetics at 22 °C, Deswelling kinetics at 37 °C, and (c) Deswelling kinetics at 50 °C.

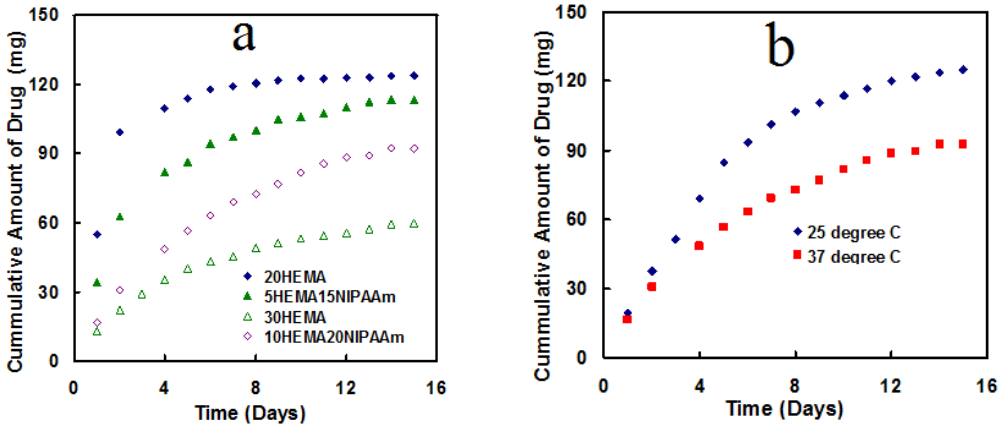

Figure 4. (a) Drug diffusion profiles of different hydrogels (37 °C) and (b) drug diffusion profiles of 10HEMA20NIPAAm at various temperatures.

Polymer fraction of the copolymers also changed rapidly with the change in temperature (Table II). At 22 °C, an increase in the polymer volume fraction value was demonstrated in the order of 20NIPAAm, 5HEMA15NIPAAm, 10HEMA10NIPAAm, 5HEMA15NIPAAM, 20HEMA, 10HEMA20NIPAAm and 30HEMA. This is consistent with the observations made in SEM (Figure 1). This order changed with the change in temperature. When comparing the normalized polymer volume fraction values of the hydrogels (Figure 2b), 5HEMA15NIPAAm demonstrated the most significant change from 100% to less than 20% in response to the temperature change from 10 °C to above 50 °C, which is greater than the change in 20NIPAAM (100% to slightly lower than 40%).

Swelling and deswelling kinetics

Complete swelling was observed for all polymers within 30 hours (Figure 3a). Faster swelling was demonstrated in polymers including 20HEMA, 20NIPAAm and 10HEMA20NIPAAm and 30HEMA. Swelling of copolymers 5HEMA15NIPAAm, 10HEMA10NIPAAm and 15HEMA5NIPAAm was slower, which is probably due to the less even porous structure in these materials (Figure 1). It should be noted that prior to the swelling kinetics experiments, the hydrogel samples were pre-dried at 50 °C. The drying process may have affected the internal structure of the samples which in turn would influence the swelling kinetics.

After having reached the equilibrium swelling at room temperature, the hydrogels were quickly transferred to a hot water environment of 37 °C and 50 °C respectively. Water retention of the hydrogels at various time points of the experiment is displayed in Figures 3b-c. For 20HEMA and 30HEMA, approximately 90% water was retained at 37 °C, and 80% at 50 °C. The water retention rate of copolymers changed more dramatically, especially at 50 °C. Water contained in 10HEMA20NIPAAm and 5HEMA15NIPAAM was less than 20% (Figure 3c) at 50 °C. Most copolymers reduced their water content to the lowest value within two hours, indicating a rapid response to the temperature change. However 10HEMA20NIPAAm took about 10 hours to reach equilibration which is slower than even the two homopolymers.

Drug loading capacity and diffusion profiles

Drug loading capacity for each hydrogel was calculated using the weight of drugs loaded and dry weight of polymers used for loading (Table III). It is clear that hydrogels made in the presence of 80% water have a greater loading capacity than those made in 70% water since the former was more porous than the latter. This is consistent with our previous

Table III. Drug loading capacity

Samples Codes	Drug Loading Capacity[a)]			
	10 °C		22 °C	
20NIPAAm	-	-	-	-
5HEMA15NIPAAm	8.3	17.5	-	10.4
10HEMA10NIPAAm	-	-	-	9.08
15HEMA5NIPAAm	-	-	-	7.42
20HEMA	-	-	-	8.51
10HEMA20NIPAAm	4.4	12.6	-	5.41
30HEMA	-	-	-	4.96

[a)] The first number in each culmn was obtained from 1 wt% drug loading solution and the second was from 2 wt% drug loading soluion.

experimental results (Lou, et al., 2004). Among all hydrogels investigated, 5HEMA15NIPAAm showed the highest loading capacity. Using a 2 wt% drug stock solution, the drug loading capacity of 5HEMA15NIPAAM changed from 10.4 wt% to 17.5 wt% when the temperature was reduced from 22 °C to 10 °C. This is due to the temperature effect on the ESR. The same temperature effect was observed on 10HEMA20NIPAAM (Table 3). The drug loading capacity of these hydrogels was also influenced by the drug stock concentration. For instance, when a 1 wt% drug stock solution was used at 10 °C, the loading capacity for 5HEMA15NIPAAM and 10HEMA20NIPAAM was reduced to 8.3 and 4.4 wt% respectively. The simple diffusion process that can be carried out below ambient temperature to achieve high drug loading is a significant advancement in the production of such formulations.

Two pairs of hydrogel polymers, 20HEMA and 5HEMA15NIPAAm, and 30HEMA and 10HEMA20NIPAAm, were selected for the drug diffusion experiments using the same drug stock solution. The diffusion profiles at 37 °C are displayed in Figure 4a. A descending trend in the release rate is shown in the order of 20HEMA, 5HEMA15NIPAAm, 10HEMA20NIPAAm and 30HEMA, with which the porosity also decreases as reflected in the change of polymer volume fraction (Table 2). For the 10HEMA20NIPAAm copolymers, the drug release rate at 37 °C was much slower than that at 22 °C (Figure 4b). This is due to the deswelling and shrinkage of polymer matrix of the thermal sensitive hydrogels.

Conclusion

Poly(HEMA-*co*-NIPAAm) hydrogels were prepared in the presence of varying amounts of water. Macroporous structures of the produced hydrogels were well demonstrated in their SEM micrographs, in which both the pore geometry and polymer density changed dramatically with the change in water content and the HEMA/NIPAAm ratio in the monomer mixture. Both the equilibrium swelling ratio and the polymer volume fraction of the copolymers were sensitive to the temperature change, with 5HEMA15NIPAAm showing the most rapid responsiveness. Swelling and deswelling kinetics of these hydrogels were dependent on the content of NIPAAM as well as on the porosity of the materials. Drug loading capacity was also dependent on the polymer composition and the temperature. The high swelling ratio of the copolymers at, or below, ambient temperatures is highly desirable for improved drug loading capacity with less concerning about drug instability. The diffusion of drugs through the hydrogels polymers was mainly controlled by the porosity of the hydrogels at the diffusion temperature which was affected by the thermal sensitivity of the hydrogel polymers. In conclusion, incorporating appropriate amounts of thermal sensitive NIPAAm into porous HEMA hydrogels can improve the drug loading capacity and condition, whilst optimizing the

release profiles of drugs from the hydrogels. The produced macroporus thermal sensitive hydrogels can be used for sustained release of various medications.

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