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The Influence of Temperature and Drug Concentrations Prednisolone in NIPAAm Copolymer

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Abstract

Controlled delivery systems would be more beneficial and ideal if the drug could be delivered with respond to external environmental change. It could be used to overcome the shortcomings of conventional dosage forms. Therefore, the correct amount of drug would be released upon the stimulation of such a temperature and concentration change. The purpose of study is to investigate the influence of temperature and drug concentration from poly(2-hydroxyethyl methacrylate and *N*-isopropylacrylamide)/poly(HEMA-NIPAAm). The macroporous structure 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, drug loading capacity and drug diffusion kinetics are influence by the porosity of hydrogels, temperature, and drug concentration.

1. Introduction

Poly(*N*-isopropylacrylamide)/PNIPAAm hydrogels are usually formed by the covalent crosslinking of PNIPAAm chains with a commercial crosslinking agent like *N, N'*-methylenebisacrylamide (mBAAm)[1]. According to the unique hydrophilic and hydrophobic change, many thermosensitive hydrogels are promising used as carriers in controlled drug delivery through responding to thermal stimulation by swelling and deswelling behavior to controlled delivery of hydrophilic drugs [2,3]. In this

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application, a fast response rate of the hydrogels to the temperature is needed. Further, the release rate can be modulated by the hydrophilic to hydrophobic change of PNIPAAm induced by temperature change. As we know that PNIPAAm has slow response rate to the temperature change because of the structure gel in the morphology. The mainly responsible for the formation of porous structure in dried state is a phase separation occurred depending on the synthesis parameters. Many studies have been investigated the formation process of macroporous networks derived from monomers 2-hydroxyethyl methacrylate[4-7]. It has been reported that poly(hydroxyethyl methacrylate)/PHEMA also has macroporous structure which influenced with amount of water[8,9]. However, when used in controlled release systems, it has been shown that an initial "burst release" occurs immediately after water hydration, making PHEMA potentially unsafe in drug delivery application[10]. To counter this problem, achieve a controlled release near human body temperature and macroporous structure, we modified the *N*-isopropylacrylamide (NIPAAm) properties with copolymerized with hydroxyethyl methacrylate (HEMA) which offers a promising alternative. Free radical polymerization mechanism gives flexibility properties of the hydrogels due to the abundance of co-monomers that can be polymerized with NIPAAm. Based on these ideas, a kind of fast responsive macroporous hydrogels were synthesized. The novel hydrogels is expected to be a good material for controlled drug delivery systems which have fast respond to the small temperature change.

2. Experiment

2.1. Hydrogel preparation

N-isopropylacrylamide (97%) from Polyscience (NIPAAm) and Ophthalmic grade 2-hydroxyethyl methacrylate from Bimax Inc USA (HEMA) were prepared by free-radical crosslinking copolymerization with 3.67% total mol monomer of mBAAm (Sigma-Aldrich Co, Australia) as a cross linker according to the formulae listed in Table 1. The solution was purged with nitrogen gas for 20 minute prior to addition of ammonium persulfate (APS) 25 wt% and *N,N,N',N'*-tetramethylethylenediamine (TEMED) from Sigma-Aldrich Co, Australia. The monomers mixtures were poured into cell culture mould 24 well and poured into two glass plates with 1mm gap. Each mould was filled with 1ml of monomer mixtures. All samples were polymerized at room temperature for 24 h. Following the process polymerization, the disc samples were removed from the mould and glass plates then immersed in deionised water. The purification was done for 2 weeks to remove residual monomers from the samples by using deionised water. They then remained in fresh deionised water for further process and some of the disk samples were further freeze dried in vacuum oven for 2 days.

2.2. Characterization

The interior morphology of the cross-section hydrogels was studied by a scanning electron microscope (SEM, ZEISS EVO 40XVP, Japan) at 10kV prior to gold coated. Equilibrium swelling ratio (ESR) of each specimen at various temperatures was calculated in order to examine the sample responsiveness to the temperature changes. ESR was determined using equation (1) where $W_{w,a}$ is the weight of sample in air and $W_{d,a}$ is the weight of the same polymer when it is dried.

$$ESR = \frac{W_{w,a} - W_{d,a}}{W_{d,a}} \tag{1}$$

Polymer volume fraction, Φ, was determined using equation (2) in order to determine polymer porosity quantitatively, where V_p represents volume of polymer after dehydration and V_{total} represents volume of the same hydrogel in an equilibrium swelling. V_p and V_{total} were calculated using Eq. (3) and (4) respectively, where ρ_w is the water density at the measured temperature.

$$\phi = \frac{V_p}{V_{total}} \tag{2}$$

$$V_p = \frac{W_{d,a} - W_{d,w}}{\rho_w} \tag{3}$$

$$V_{total} = \frac{W_{w,a} - W_{w,w}}{\rho_w} \tag{4}$$

Swelling kinetics of the produced hydrogels was investigated at ambient temperature (22 °C) using conventional gravimetric method. In brief, a hydrogel polymer of known dry weight, $W_{d,a}$, was put into water and taken out at a chosen time point to record the weight, W_t. The equilibrium swelling weight, $W_{w,a}$, also was recorded. The percentage water uptake capacity, W_u, then was calculated using equation 5. Similarly, the deswelling kinetics of the hydrogels at 37 °C was investigated. In this experiment, an equilibrium hydrogel at 22 °C was put into water at preferred temperature and taken out for weighing at regular time intervals. Equation 5 then was used to determine the water retention capacity (W_r) respectively.

$$W_u / W_r = \frac{W_t - W_{d,a}}{W_{w,a} - W_{d,a}} \times 100 \tag{5}$$

Prednisolone 21-hemisuccinate was used as a model drug to examine the smart controlled release profile of fast response macroporous thermosensitive HEMA-co-NIPAAm. A stock solution of 0.5, 1 and 2 wt% prednisolone 21-hemmisuccinate was prepared by weighing drug powder and dissolving into deionised water in volumetric flask. Each swollen hydrogels was shrunk in 22 and 37 °C for 2 days in deionised water to achieve equilibrium condition prior to drug release.

Table 1. Chemical composition of macroporous thermosensitive hydrogels

Sample Codes	HEMA	NIPAAm	EMA	mBAAm	Water	APS (25 wt%)	TEMED
	mmol	mmol	mmol	mmol	g	μL	μL
20NIPAAm	0	17.7	0	0.65	8	50	20
5HEMA15NIPAAm	3.8	13.3	0	0.63	8	50	20
10HEMA10NIPAAm	7.7	8.8	0	0.61	8	50	20
15HEMA5NIPAAm	11.5	4.4	0	0.58	8	50	20
20HEMA	15.4	0	0	0.57	8	50	20

The in vitro release experiments were carried out in diffusion cell containing 2 chambers and done at 22 and 37 °C in incubator orbital shaker at 50 rpm speed, respectively. Prednisolone 21-hemisuccinate release experiments were conducted by fill one chamber with drug solution at same temperature and the other with deionised water. At a predetermined time interval, each chamber volume was withdrawn 250 μL solution to monitor both concentrations. The concentration was measured using a UV-Visible spectrometer (UV/VIS 918, GBC Scientific Equipment, Australia) at fixed wavelength of 247 nm.

3. Results and Discussion

The result of macroporous structure of thermo-sensitive hydrogels are shown in Figure 1. The appearances of the hydrogels are opaque which indicates with pores structure network. Homogenous networks derived from free radical polymerization with effect of cross-linking[11] and initiator[12]. In 80 percentage amount of water, when HEMA copolymer with NIPAAm resulted less porous network, compare to 20 HEMA. It is well known that phase separation during the polymerization process is a basic condition to produce porous structure[13]. The polymerization started after the initiator and accelerator added into the solution, however, the role of solvent especially water is significant to become more extensive and resulted in the porous structures of the resultant hydrogels.
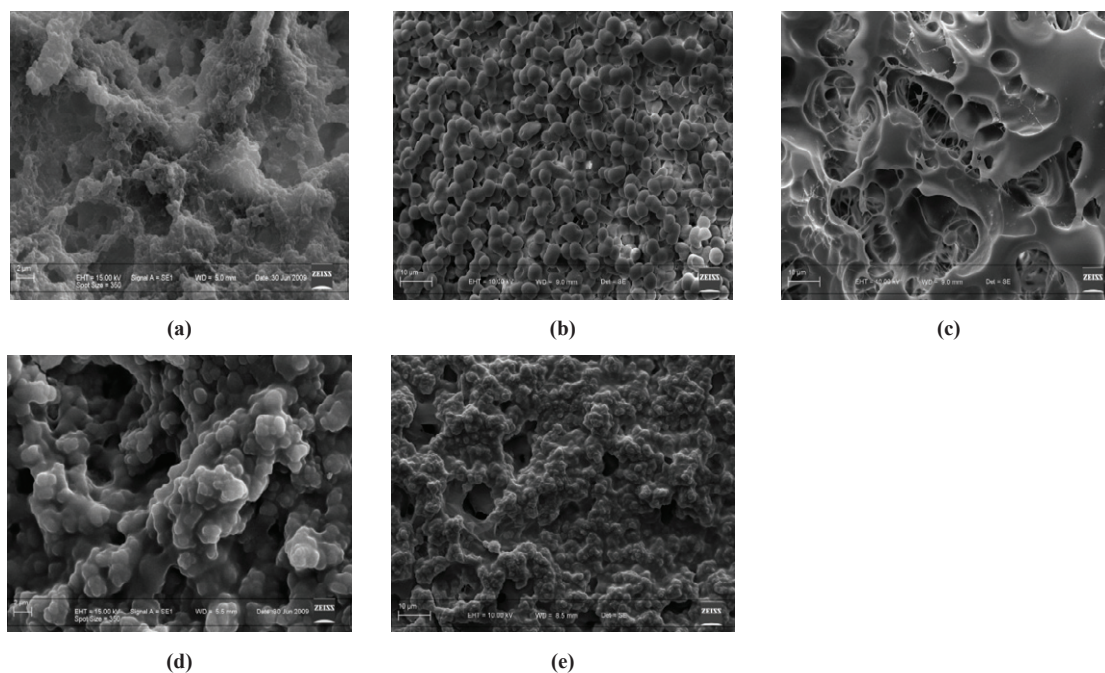

Fig. 1. SEM images of homopolymer HEMA and copolymer HEMA and NIPAAm hydrogels prepared: (a) 20NIPAAm; (b) 20HEMA; (c) 15 HEMA5NIPAAm; (d) 10HEMA10NIPAAm; dan (e) 5HEMA15NIPAAm

The equilibrium swelling ratio of the hydrogels at various temperatures is shown in Figure 2a. significant change in ESR was observed in the range of 10 and 50 ºC among which 5HEMA15NIPAAm shows the greatest change of ESR, from approximately 9 to approximately 1. This phenomenon also has been noticed by Tuncel and Huang and their co-workers[14,15] and tentatively explained by a theory of equilibrium macrosyneresis and microsyneresis[16]. Swelling kinetics was observed for all hydrogels

within 30 hours and the results are displayed in Figure 2b. Interestingly, the fastest swelling kinetic rate was demonstrated in homopolymers 20HEMA and 20NIPAAm. The swelling kinetics, which shows how fast the hydrogels can uptake the water in response to certain temperature changes due to the transformation from hydrophobic to hydrophilic chains in the hydrogel. Whilst the faster swelling can be affected to the presence of greater pores, respectively. As they respond quickly to the change in temperature, the surface of the materials might have changed their porosity (shrank) in the early stage of swelling, therefore preventing further swelling[17]. At 37 °C (Figure 2c), the water retention in the copolymers made in 80 wt% water was lower, indicating that the higher is the porosity, the lower is the water retention. The decrease in water retention of copolymers was faster due to the skin effect as a consequence of the initial fast shrinking of the hydrogel surface that prevents the diffusion of water out from the hydrogel matrix.
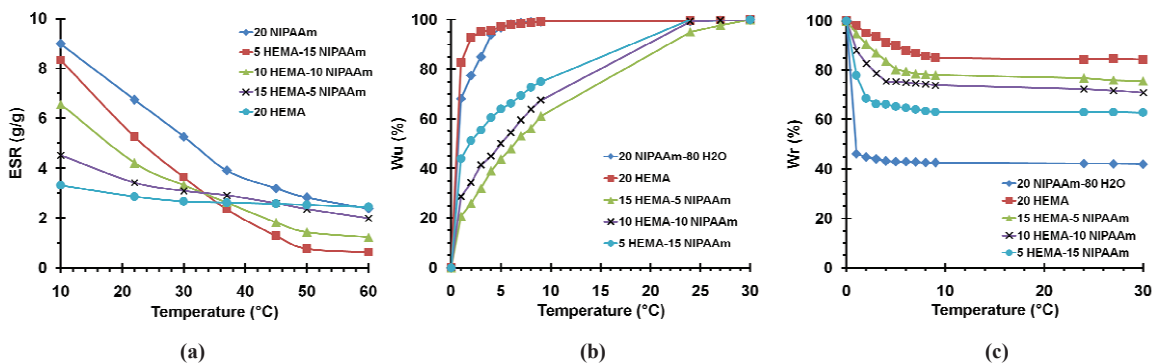

Fig. 2. Properties of hydrogels prepared: (a) equilibrium swelling ratio; (b) water up-take; (c) water retention

Polymer fraction of the copolymers also changed rapidly with the change in temperature, Table 2. At 22 °C, an increase in the polymer volume fraction value was demonstrated in the order of 20NIPAAm, 5HEMA15NIPAAm, 10HEMA10NIPAAm, and 5HEMA15NIPAAM. For the hydrogels made of the same water concentration, the higher is the NIPAAm, the lower is the value of polymer volume fraction, i.e., the higher is the porosity, which is consistent with the observations made in SEM.

Table 2. Polymer volume fraction of hydrogels at various temperatures

Samples Codes	Polymer Volume Fraction (v/v)						
	10 °C	22 °C	30 °C	37 °C	45 °C	50 °C	60 °C
20NIPAAm	0.095	0.123	0.152	0.195	0.224	0.251	0.285
5HEMA15NIPAAm	0.097	0.146	0.198	0.277	0.423	0.556	0.591
10HEMA10NIPAAm	0.116	0.170	0.204	0.249	0.319	0.376	0.411
15HEMA5NIPAAm	0.158	0.199	0.213	0.226	0.247	0.265	0.298
20HEMA	0.196	0.220	0.232	0.236	0.237	0.241	0.247

Figure 3 shows the effect of temperature and drug concentration in 5HEMA10NIPAAm hydrogels. At 22 °C, a fast diffusion was seen, however increasing the temperature to 37 °C resulted in a reduced diffusion rate, respectively. The drug diffusion through the hydrogels was faster when the temperature was low due to the expansion of the pores. Meanwhile, increasing the temperature of diffusion kinetics experiments resulted in slow drug diffusion because the pore structure network collapsed and entrapped drug molecules.
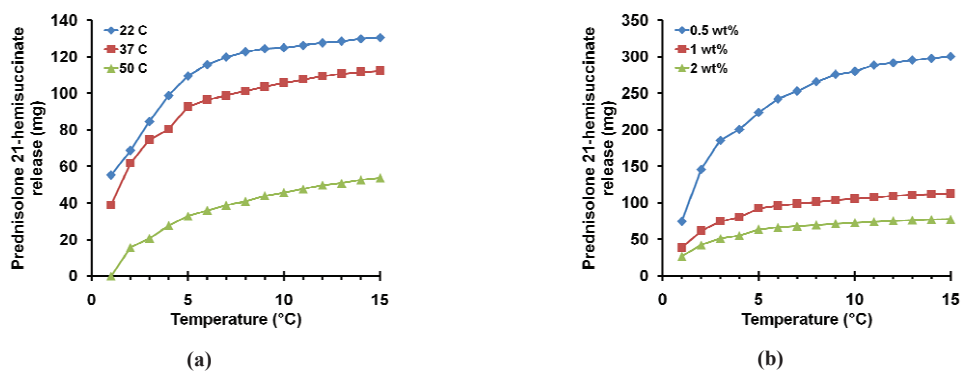
Fig. 3. Diffusion kinetic of 5HEMA15NIPAAm: (a) at various temperatures and (b) at various concentrations

The drug loading capacity of each hydrogel is presented in Table 2. The effect of temperature on drug loading was even more significant. For instance, using a 2 wt% drug stock solution, the loading capacity of 5HEMA15NIPAAm increased from 10.4 to 17.5wt% when the temperature was decreased from 22 °C to 10 °C. The significantly increased drug loading capacity at lower temperature indicates that the hydrogels contain greater pore volumes at the lower temperature due to their thermosensitive nature. The increased pore volumes cause the increased mass uptake of drugs into hydrogels as they provide more spaces for molecules to occupy during the diffusion[18,19].

Table 3. Drug loading capacity of selected hydrogels at various temperatures

Samples Codes	Drug Loading Capacity		
	10 °C		22 °C
	1 wt% (drug solution)	2 wt% (drug solution)	2 wt% (drug solution)
5HEMA15NIPAAm	8.3	17.5	10.4
10HEMA10NIPAAm	-	-	9.08
15HEMA5NIPAAm	-	-	7.42
20HEMA	-	-	8.51

4. Conclusion

This study demonstrates that drug loading capacity and drug diffusion kinetics are influenced by the porosity of hydrogels, temperature, and drug concentration. In general, the more porously structured hydrogels showed high drug loading capacity and faster diffusion kinetics because more drug molecules can reside in the porous hydrogel matrix and diffusion is easier through a more porous structure. Since the hydrogels investigated were positively thermosensitive, (i.e., the lower is the temperature, the higher is the porosity), the drug loading could be conducted at a temperature below body temperature, thus a higher drug content could be achieved and the release at an elevated temperature could be retarded due to the shrinkage of pores. Increasing drug concentration also resulted in higher drug loading.

Acknowledgement

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