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Drug release behavior from *in situ* gelatinized thermosensitive nanogel aqueous dispersions

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ABSTRACT

In situ gellable thermosensitive poly(*N*-isopropylacrylamide-*co*-acrylamide) (designated as PNIP/AAm) nanogel aqueous dispersions were prepared through precipitation polymerization. Their thermosensitive volume phase transition and *in situ* gel-forming behavior were investigated. 5-Fluorouracil (5-Fu) and bovine serum albumin (BSA) were used as model drugs. The drug-loading properties of PNIP/AAm nanogel particles and release behavior from *in situ* gelatinized PNIP/AAm nanogel aqueous dispersions were investigated. The prepared PNIP/AAm nanogel particles and aqueous dispersions show good thermosensitivity. The presence of the drugs in the systems has no significant influence upon the thermosensitivity of the systems. In addition, the amount of cross-linker used in the preparation of the PNIP/AAm nanogel has little influence upon the drug-loading capability of PNIP/AAm nanogel particles and also on the release behavior from gelatinized dispersions. It was found that the drug-loading efficacy and entrapment efficiency of PNIP/AAm nanogel particles for low molecular weight 5-Fu was higher than that for biomacromolecular BSA. Furthermore, the cumulative release ratios of 5-Fu from *in situ* gelatinized PNIP/AAm nanogel aqueous dispersions were distinctly higher than that of BSA. These results imply potential application of prepared thermosensitive nanogel dispersions as embolizing and tissue engineering materials.

1. Introduction

Thermosensitive hydrogels usually exhibit a significant volume phase change at a certain temperature called as volume phase transition temperature (VPTT) (Tanaka, 1978; Pelton, 2000). For example, hydrogels based on poly(*N*-isopropylacrylamide) (PNIPAAm) are in swollen status below the VPTT and in shrunken status above the VPTT. Taking the advantage of temperature-responsivity of the PNIPAAm, drug loading and release ability may be intelligently triggered by a temperature change (Li and D'Emanuele, 2001; Zhang et al., 2002). Nanogels are kinds of colloids with an average diameter below 1000 nm and usually disperse well in aqueous media (Ogawa et al., 2003). As a nano-based drug carrier, the advantages of nanogel particles include easy administration, the possibility of enhanced therapeutic efficacy, reduced side effects through more precise delivery, passively targeting to the tumour site (Matsumura and Maeda, 1986; Siepmann et al., 2004). Similar to the VPTT of conventional hydrogels, concentrated thermosensitive nanogel dispersions show characteristic sol–gel transitions at specific temperatures (Zhao et al., 2001; Hu and Xia, 2004; Wang et al., 2007). This characteristic is valuable for the preparation of injectable materials, for example, *in situ*-formed gels such as embolizing and tissue engineering materials. In other words, drugs can be easily incorporated into the nanogel dispersion in the sol status, and then being injected to a specific site. Subsequently, the drug-loaded nanogel dispersion turns into a semi-solid gel and release occurs therefrom at the target site. This system takes advantages of the absence of organic solvents and has no requirement for a surgical procedure to form a drug-loaded gel in the body.

In recent years, some micron-sized thermosensitive gels (usually called microgels) have been reported as drug carriers. Zhang et al. (2005) investigated a biodegradable and thermosensitive microgel as proteins carrier. The result showed that the model proteins could be encapsulated into the microgels at 4 °C and released at 37 °C. Huo and co-workers reported temperature and pH dual sensitive poly(*N*-isopropylacrylamide-*co*-acrylic acid) microgels. The adsorption of bovine serum albumin (BSA) onto the microgels was strongly dependent on the content of acrylic acid and on the pH of the suspension (Huo et al., 2006). Huang et al. (2004) reported controlled drug release from hydrogel nanoparticle networks. However, little attention has been paid to the drug loading and release of gelatinized thermosensitive nanogel aqueous dispersions.

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In the present work, hydrophilic acrylamide (AAm) was used as a comonomer to prepare aqueous dispersion of thermosensitive poly(*N*-isopropylacrylamide-*co*-acrylamide) (designated as PNIP/AAm) nanogels. The *in situ* gelation of nanogel aqueous dispersions in the body of rats was investigated. Two kinds of drugs (5-fluorouracil and bovine serum albumin) were used as model drugs and their release behavior from *in situ* gelatinized nanogel aqueous dispersions was investigated in this work.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAAm, Acros) and *N*,*N*′-methylenebisacrylamide (MBAAm, Tianjin Kermel Co.) as a cross-linker were crystallized from *n*-hexane and methanol, respectively. Acrylamide (AAm, Tianjin Kermel Co.), sodium *n*-dodecyl sulfate (SDS) and other reagents were of analytic grade and used as received. 5-Fluorouracil (5-Fu, 99.8%, Nantong General Pharmaceutical Factory) was used after being dried in vacuum and bovine serum albumin (BSA, Amresco) was used as received. Milli-Q ultrapure water was used throughout all experiments. Phosphate-buffered saline solution (PBS, pH 7.4, ionic strength 0.2 mol/L) was prepared according to the standard method.

Wistar rats (♂, 190 ± 25 g) were obtained from the Laboratory Animal Center of Hubei Province, China. Animal experiments were approved by the Institutional Animal Care and Use Committee of this Center.

2.2. Preparation of PNIP/AAm nanogels

Thermosensitive PNIP/AAm nanogels were prepared through precipitation polymerization according to a procedure described elsewhere (Pelton and Chibante, 1986; Wang et al., 2007). The molar ratio of NIPAAm to AAm was 95/5. MBAAm, potassium persulfate and SDS were used as a cross-linker, initiator and surfactant, respectively. The polymerization was performed in aqueous solution for 4.5 h under a N_2 atmosphere at 70 °C with stirring. Two kinds of nanogels with varied crosslinking degree were obtained by using different amounts of MBAAm. The molar ratio of MBAAm to total monomers was 1% and 3% and the resulting products were designated as PNIP/AAm-1 and PNIP/AAm-2, respectively. PNIP/AAm nanogel aqueous dispersions were further dialyzed and subsequently lyophilized to collect xerogel for further measurements.

2.3. Dynamic light scattering (DLS)

The freeze-dried PNIP/AAm nanogel powder (0.5 mg/mL) was dispersed in PBS containing 5-Fu (2 mg/mL) or BSA (2 mg/mL). The hydrodynamic diameters and polydispersion index (PDI) of the nanogel particles in PBS were measured at temperatures ranging from 20 to 50 °C by dynamic light scattering (Nano-ZS 90, Malvern) equipped with a He–Ne laser (λ = 633 nm). All samples were maintained at a designed temperature for 5 min before testing. The swelling ratio (SR) of the nanogels was calculated by the equation (Crowther and Vincent, 1998):

$$\mathrm{SR} = \frac{V_{\mathrm{swollen}}}{V_{\mathrm{shrunken}}} = \left(\frac{D_{20}}{D_{50}}\right)^3 \quad (1)$$

Here, V_{swollen} and V_{shrunken} are the volumes of nanogel particles in swollen status at 20 °C and in shrunken status at 50 °C, respectively. D_{20} and D_{50} are the average diameters of the nanogels particles at 20 and 50 °C, respectively.

2.4. Preparation of PNIP/AAm nanogel dispersions and the gelation behavior therein

The freeze-dried PNIP/AAm nanogel powder (8.3%, w/v) was dispersed in PBS and allowed to stand overnight at room temperature. The phase transition temperatures of the prepared PNIP/AAm nanogel dispersions were determined by vial inverting combined with a visual method (Jeong et al., 1999; Wang et al., 2007) at temperatures ranging from 25 to 45 °C.

The hair on Wistar rats' back was removed by a chemical depilatory and then by a razor. The area of bareness was about 5 cm^2. A sterilized PNIP/AAm nanogel aqueous dispersion (0.5 mL) was injected subcutaneously into rats by a syringe with a 20-gauge needle after anesthetizing the rat with carbon dioxide. At designated time intervals after injection, the injection site was cut open and the *in situ* formed gel was investigated.

2.5. Adsorption of model drugs on the PNIP/AAm nanogel particles

PBS-containing drugs were prepared by dissolving 25 mg of 5-Fu or BSA in 50 mL of PBS. Then 0.5 g of PNIP/AAm nanogel xerogel powder was dispersed therein with sonication for 10 min. Similarly, PNIP/AAm nanogel aqueous dispersions without drug were prepared. A part of the dispersion was incubated at 4 °C for 72 h and then centrifuged (Hermle Z323K, 15,000 rpm) at 4 °C for 60 min. Drug-loaded PNIP/AAm nanogel particles were separated from the dispersion. The other dispersion was incubated at 37 °C for 72 h and then centrifuged (15,000 rpm) at 37 °C for 3 min. The obtained supernatant was used to detect the free drug content. The amount of 5-Fu in the supernatant was determined by absorbance measurements at 295 nm using a UV spectrophotometer (UV-2102 PC, UNICO). The amount of BSA in the supernatant was measured by absorbance measurements at 595 nm through binding of Coomassie Brilliant Blue to BSA using a Bio-Rad protein assay method (Bradford, 1976). All the absorbance measurements were performed (in triplicate) by using the supernatant of dispersions of drug-free PNIP/AAm nanogel particles as a blank. The drug-loading efficacy (DL) and entrapment efficiency (EE) of PNIP/AAm nanogel particles were calculated as follows:

$$\mathrm{DL} = \frac{(C_0 - C_1)}{C_2} \times 100\% \quad (2)$$

$$\mathrm{EE} = \frac{(C_0 - C_1)}{C_0} \times 100\% \quad (3)$$

Here, C_0 and C_1 are the concentration of the drug in the PNIP/AAm nanogel dispersions and in the supernatant after incubation, respectively. C_2 is the concentration of the PNIP/AAm nanogel particles in the dispersions.

2.6. Drug release from in situ gelatinized PNIP/AAm nanogel dispersions

Homogeneous aqueous PNIP/AAm nanogel dispersions containing 8.3% (w/v) of the nanogels and 1 mg/mL of drug were prepared. The samples were allowed to stand in a refrigerator at 4 °C overnight. One milliliter of the nanogel aqueous dispersion with drug was added in a flat-bottom test tube and then placed in a water bath at 37 °C for 5 min. The nanogel dispersions became semi-solid gels. Each sample was prepared in triplicate. The sample without drug was prepared as a blank using similar method. The cylindrical gel was carefully separated from the test tube by using a needle. Ten milliliters of PBS (pre-warmed at 37 °C) were added as a receiving medium in the test tube, resulting in a suspended gel. One milliliter of the receiving medium was removed from the test

Table 1
The parameters of PNIP/AAm nanogel particles at 20 °C

Sample	Diameter (nm)	PDI	Swelling ratio (SR)
PNIP/AAm-1	229	0.032	21.8
PNIP/AAm-2	231	0.069	14.2

tube at designed time intervals. Simultaneously, 1 mL of fresh PBS was replenished into the test tube. The amount of released drug was determined by absorbance measurements described above.

3. Results and discussion

3.1. Volume phase transitions and in situ gelation behavior of PNIP/AAm nanogel dispersions

Table 1 lists basic characteristic parameters of the thermosensitive PNIP/AAm nanogels. The sizes of the PNIP/AAm nanogel particles are about 230 nm and nearly monodisperse. The SR of PNIP/AAm-1 with less cross-linker content is higher than that of PNIP/AAm-2. This may be attributed to the lower crosslinking density of PNIP/AAm-1, resulting in more water absorption into a more flexible polymer network (Senff and Richtering, 2000; Wang et al., 2007). This significant characteristic will be further discussed in the section of drug loading and release.

Fig. 1 shows the influence of 5-Fu and BSA bound into the PNIP/AAm-1 nanogel dispersions on the hydrodynamic diameters and temperature sensitivity. As expected, the diameters of nanogel particles decreased with an increase of the temperature, thereby exhibiting typical temperature-sensitivity. In addition, the temperature-sensitivity of PNIP/AAm nanogels seems to be not interfered by the presence of the drugs in the system. In comparison with the presence of 5-Fu, BSA induces a slight increase of the diameter of nanogel particles, possibly because of macromolecular BSA (66 KD) absorbed on the surface of nanogel particles by hydrogen bonding. The turning points of the curves in Fig. 1 correspond to the volume phase transition temperature (VPTT). The VPTT of both PNIP/AAm-1 and PNIP/AAm-2 is around 35–36 °C, near to body temperature. As shown in Fig. 2, gel forming of PNIP/AAm nanogel aqueous dispersions originates from the volume phase transition of PNIP/AAm nanogel particles in this range of temperature (Wang et al., 2007).

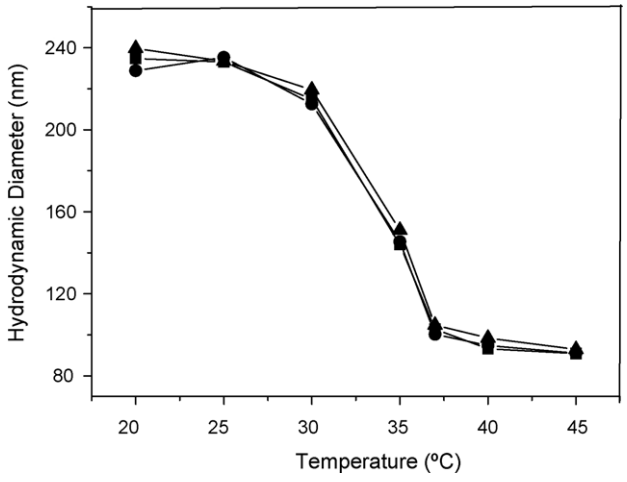

Fig. 1. Effect of drugs on the hydrodynamic diameter as a function of temperature for PNIP/AAm-1 nanogel particles. The concentration of the nanogels in the aqueous media was 0.5 mg/mL. (■) Blank. (●) 5-Fu. (▲) BSA.

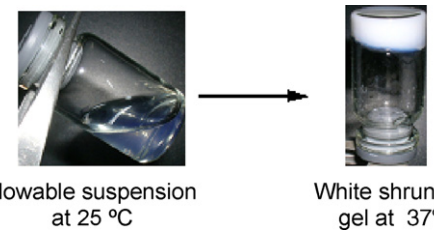

Fig. 2. Sol–gel phase transition of PNIP/AAm-1 dispersion (8.3%, w/v in PBS) above the VPTT.

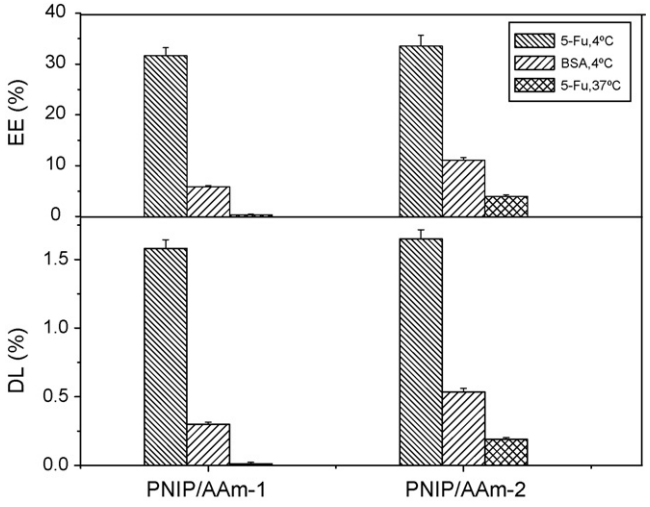

Fig. 3. The DL and EE of 5-Fu at 4 and 37 °C, and that of BSA at 4 °C onto PNIP/AAm nanogel particles. The concentration of the nanogels in PBS solution was 10 mg/mL and the drug concentration was 0.5 mg/mL.

Gelation of PNIP/AAm nanogel aqueous dispersions (Fig. 2) exhibits a significant potential application in the fields of embolizing and planting materials. In the present work, *in situ* gel forming of PNIP/AAm nanogel aqueous dispersions *in vivo* was performed using Wistar rats. Sterilized PNIP/AAm nanogel dispersions (0.5 mL) were injected subcutaneously into the rats. At designed time intervals, the site of injection was cut open for observation. It was found that the nanogel dispersion turns into a white gel and that the shape

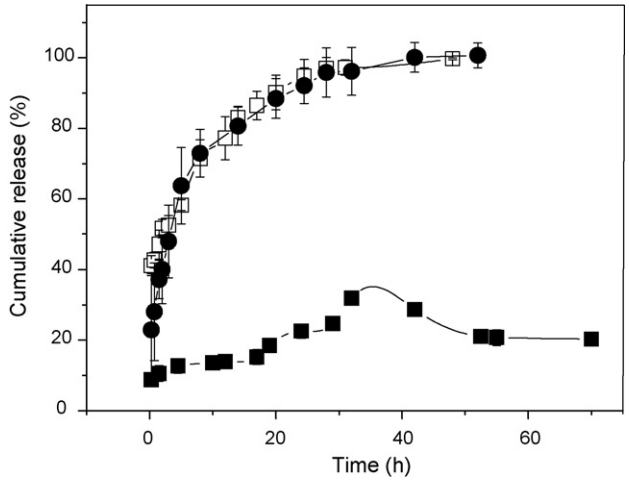

Fig. 4. Drug release from the drug-loaded *in situ* gellable nanogel dispersion at 37 °C *in vitro*. The concentration of the nanogels in the PBS solution was 8.3% (w/v). (□) PNIP/AAm-1 containing 5-Fu. (●) PNIP/AAm-2 containing 5-Fu. (■) PNIP/AAm-1 containing BSA.

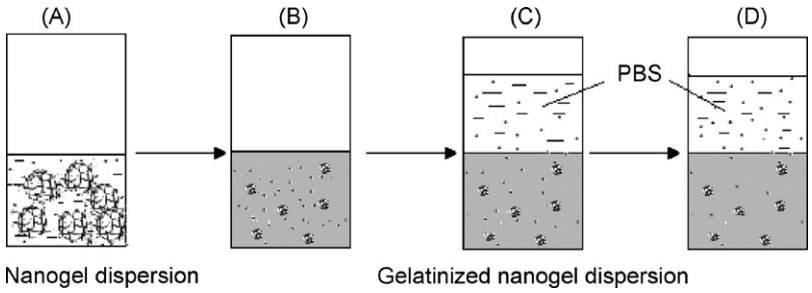

Fig. 5. Schematic illustration of drug release from gelatinized nanogel dispersions. (A) Dispersion containing swollen nanogel particles and drug at 4 °C. (B) Gelatinized dispersion containing drug-loaded shrunken nanogel particles at 37 °C and partial drug released from nanogel particles. (C) Free drug released from gelatinized nanogel dispersions at 37 °C. (D) drug released from shrunken nanogel particles at 37 °C.

of the gel was irregular at the first day after injection, which may be due to the subcutaneous spreading of the nanogel dispersion. At the 7th day after injection, the white gel was enwrapped by surrounding tissue and was turned into an integrated bean-like shape. The gel was strong enough to be easily taken out using forceps even at the 30th day after injection. All rats survive well before sacrifice, indicating good biocompatibility of the nanogel dispersions. (The pictures of subcutaneous *in situ* gel forming are not shown herein.)

3.2. Drug-loading capacity of PNIP/AAm nanogel particles

Fig. 3 shows the DL and EE of 5-Fu and BSA in PNIP/AAm nanogel particles at 4 and 37 °C, respectively. Under the same conditions, the DL and EE of PNIP/AAm-1 was slightly less than that of PNIP/AAm-2, possibly due to their different swelling ratios and crosslinking degrees (as shown in Table 1). PNIP/AAm-1 prepared using a low content of cross-linker possesses a polymer network structure of low density, resulting in easy diffusion and effusion of the drug. In addition, the DL and EE of 5-Fu in nanogel particles was remarkably higher than that of BSA, which may be a result of the lower capturing ability of polymer network to biomacromolecular BSA. Furthermore, little absorption of drug in nanogel particles was found at 37 °C in comparison with that at 4 °C. A possible explanation involves the thermosensitivity of the PNIP/AAm nanogel particles. As mentioned above, PNIP/AAm nanogel particles in a swollen status exhibit a favorable drug absorption at 4 °C, but in the shrunken status at 37 °C drugs are being squeezed out (together with water) from the polymer network. This essentially relates to the mechanism of drug loading and drug releasing in PNIP/AAm nanogels.

3.3. Drug release from in situ gelatinized PNIP/AAm nanogel dispersions

As discussed above, PNIP/AAm nanogel aqueous dispersions containing 8.3% (w/v) of nanogel can form gels *in situ* at 37 °C. Fig. 4 shows the release profile of 5-Fu and BSA from *in situ* gelatinized PNIP/AAm nanogel dispersions at 37 °C. The results indicate that no significant difference was found for the release of 5-Fu from the two kinds of nanogel dispersions. A burst release of 5-Fu at the initial period of release was observed, which was followed by a sustained release. The release equilibrium was reached after ca. 30 h. This release behavior reflects a characteristic feature of the gelatinized thermosensitive nanogel dispersions, *i.e.* the burst release can be attributed to the release of free drug in the aqueous phase of the nanogel dispersions and the sustained release from shrunken nanogel particles. In addition, the release rates of 5-Fu were remarkably faster than that of BSA. Similar to the drug-loading process discussed above, biomacromolecular BSA escapes less easily from the polymer network as compared with small molecular 5-Fu. The decrease of the release rates of BSA at the subsequent time period may be due to the adsorbing of BSA onto the hydrophobic surface of the thermosensitive nanogel above the VPTT (Kawaguchi et al., 1992).

Based on the discussion above, we propose a mechanism for drug release from the *in situ* gelatinized thermosensitive nanogel dispersions as shown in Fig. 5. At lower temperatures (below the VPTT), thermosensitive nanogel particles are in the swollen status. In this case, drug is entrapped together with water in the nanogel particles by diffusion (Fig. 5A). At more elevated temperatures (above the VPTT), the nanogel particles attain a shrunken status due to the deswelling, leading to a portion of loading drug effused (together with expelled water) into the aqueous phase. In this case, the nanogel dispersion will be in a gel status (Fig. 5B). After the receiving medium was added on the gel, the free drug and drug released from the nanogel particles will release to the receiving medium by diffusion (Fig. 5C). Finally, the residual drug in the nanogel particles releases gradually into the receiving medium (Fig. 5D).

4. Conclusion

The prepared PNIP/AAm nanogels show remarkable thermosensitivities. Their VPTT is around 35 °C. The presence of the drug in nanogel particles has no significant influence on the thermosensitivity and hydrodynamic diameter of the nanogel particles. The PNIP/AAm nanogel dispersions with 8.3% (w/v) of the nanogel can be gelatinized *in situ* in the body of rats. Taking advantage of this characteristic, drug can be absorbed into the swollen nanogel particles by diffusion at lower temperature and released from the shrunken nanogel particles at body temperature. The drug-loading efficacy and entrapment efficiency of PNIP/AAm nanogel particles for low molecular weight 5-Fu was higher than that for biomacromolecular BSA. The cumulative release of 5-Fu from *in situ* gellable PNIP/AAm nanogel dispersions was distinctly higher than that of BSA. Based on these studies, a mechanism for drug release from the *in situ* gellable PNIP/AAm nanogel dispersions is proposed.

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