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Controlled co-delivery of hydrophilic and hydrophobic drugs from thermosensitive and crystallizable copolymer nanoparticles

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ABSTRACT: Functionalized amphiphilic block copolymers poly(*N*-isopropyl acrylamide)-*b*-poly(stearyl methacrylate) (PNIPAM-PSMA) are synthesized. Their self-assembled core-shell nanoparticles have the hydrophilic thermosensitive shell and hydrophobic crystallizable core. Nanoparticles exhibit volume phase transition at temperature of 38 °C and its poly(stearyl methacrylate) (PSMA) moiety could form nano size crystals to retain drugs, making them good carriers for drug co-delivery system. Thermosensitivity and crystallinity of nanoparticles are characterized with dynamic light scattering (DLS), differential scanning calorimetry (DSC), small-angle X-ray scattering (SAXS), and atomic force microscopy (AFM). The interactions and relationship between chemical structures of copolymer nanoparticles and loading drugs are discussed. Different loading techniques and combined loading of hydrophobic/hydrophilic drugs are studied. Nanoparticles show a good and controllable drug loading capacity (DL) of hydrophilic/hydrophobic drugs. The drugs release kinetics is analyzed with Fick's law and Weibull model. A general method for analyzing drug release kinetics from nanoparticles is proposed. Weibull model is well fitted and the parameters with definite physical meaning are analyzed. PNIPAM-PSMA nanoparticles show a quite different thermal response, temporal regulation, and sustained release effect of hydrophilic and hydrophobic drugs, suggesting a promising application in extended and controlled co-delivery system of multi-drug. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 44132.

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INTRODUCTION

Polymeric nanoparticle drug delivery systems have been received widespread attention over the past decades. They have been engineered for improving the solubility and stability of hydrophobic drugs or bioactive agents, efficient loading, targeted delivery, sustained/controlled release profiles, and prolong circulation time.[1–5] Amphiphilic block copolymers are of interest for drug delivery applications because they are capable of forming aggregates comprising of a hydrophobic core and hydrophilic shell in aqueous solutions. Hydrophobic drugs can be loaded in the core of block copolymer nanoparticles and are protected from hydrolysis and enzymatic degradation.[6] Hydrophilic blocks usually can form hydrogen bonds with the aqueous surroundings and form a hydration shell around. The size and morphology of nanoparticles formed by amphiphilic block copolymers can be varied by changing the composition, molecular weight, and block length ratios of hydrophobic and hydrophilic segments on the polymer chains.[7–9] Co-delivery of drugs with different solubility by same nanoparticle delivery device is becoming another promising application of amphiphilic block copolymers. Most present research on drug delivery systems are applied to load and delivery a single drug, which often could not satisfy the demands of combination therapy. Co-delivery of multiple drugs has some potential advantages, such as suppressed drug resistance, synergistic effect, and the ability to tune the relative dosage of various drugs to the level of a single delivery.[10] Therefore, it is necessary to develop co-delivery system of controlled release sequence, timing, doses, and duration of each drug, to achieve an effective combination therapy.[11] Amphiphilic copolymer core-shell nanoparticles have been investigated for drug co-delivery systems with improved function properties. Some strategies, such as forming nanoparticle-drug conjugates via complexation or electrostatic interactions, have been developed to co-delivery of drugs or bioactive agents.[12,13] When incorporating the desired functionalities to achieve targeted delivery, temporally regulated drug release, or co-delivery, maintaining the simplicity in design is a challenge in scale-up, and

Additional Supporting Information may be found in the online version of this article.

wide application. In our previous work, we developed a facile and nontoxic strategy for preparing amphiphilic block copolymers and self-assembled core-shell nanoparticles via thermally induced interfacial absorption in miniemulsion polymerization.[14] Poly(*N*-isopropyl acrylamide) (PNIPAM) formed the hydrophilic shell of nanoparticles, and its volume phase transition behavior could improve temporal control. Stearyl methacrylate (SMA) was used as hydrophobic monomer to synthesize comblike polymers and form nanocrystalline core, since it has pendent long alkyl side chain to form crystalline domain.[15–17] The nanoparticle had a hydrophilic and thermo-sensitivity shell, and a hydrophobic and crystalline core. It has a lot of potential in co-delivery of hydrophobic and hydrophilic drugs, temporally regulated, and controlled release applications.

Based on our previous studies, we prepared poly(*N*-isopropyl acrylamide)-*b*-poly(stearyl methacrylate) (PNIPAM-PSMA) polymeric nanoparticles with a volume phase transition temperature (VPTT) of 38 °C, which may be suitable for biomedical applications. The thermosensitivity and crystallinity of nanoparticles were characterized to study the interactions and relationship between chemical structures and loading drugs. There are very few reports on controlled loading capacity of both hydrophilic and hydrophobic drugs. In this work, we use ibuprofen as hydrophobic model drug and procaine as hydrophilic model drug to load into nanoparticles. Different loading techniques and drugs combined loading were conducted and compared. The nanoparticles showed a controllable loading capacity of both hydrophilic and hydrophobic drugs. Drugs were temporal controlled released or simultaneously released, and the relative velocity was changed with temperature. The release kinetics was analyzed with Fick's law of diffusion and empirical Weibull model. Parameters obtained from models were employed for comparing release profiles and different delivery systems. The Weibull model is adequately fitted the experimental release data. It has several parameters with definite physical meaning and was sensitive to the release kinetic data.[18] A general method for analyzing drug release kinetics from nanoparticles was proposed, which is useful in elucidating release mechanisms and helping the control of drug release.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAM, Acros Organics, Shanghai) and stearyl methacrylate (SMA, 95.5%, TCI, Shanghai) were purified by recrystallization in *n*-hexane and dried in a vacuum oven at room temperature. Potassium persulfate (KPS, Acros organics, Shanghai) was used as initiator. Sodium dodecyl sulfate (SDS, 99%, Acros Organics, Shanghai), procaine (98%, J&K, Shanghai), and ibuprofen (98%, J&K, Shanghai) were used as received. *n*-Hexane and other reagents were of analytical grade and used as received. Deionized water was applied for all of the polymerization and treatment process.

Synthesis of Nanoparticles

Block polymer nanoparticles were prepared by miniemulsion polymerization.[14] Monomers SMA (3.38 g) and NIPAM (1.13g) were mixed with *n*-Hexane (5 g) to obtain oil phase. This oil phase was stirred and dissolved at 50 °C. Then oil phase was added to the aqueous solution containing surfactant SDS (0.12 g). The miniemulsion is obtained by ultrasonic dispersing the mixture for 20 min, using a pulsed sequence (10 s sonication followed by 5 s break) with a 560 W duty cycle (SCIENTZ, JY92) under magnetic agitation in an ice bath. The polymerization was carried out in a jacketed reactor and purged with nitrogen. After the temperature was raised to 70 °C, an aqueous solution of KPS (0.02 g) was fed into the reactor to start the polymerization. The polymerization was continued for 6 h under stirring at 300 rpm. The purified copolymer was obtained as follows: copolymer solution was subjected to centrifugation at 10,000 rpm for 10 min to get precipitation, and then redispersed in deionized water; the centrifugation was carried out three times to remove NIPAM monomer and PNIPAM homopolymer; precipitation was dissolved in THF (tetrahydrofuran) and then precipitated in toluene to remove SMA monomer and poly(stearyl methacrylate) (PSMA) homopolymer; eventual precipitation was dried in a vacuum oven overnight.

Characterization

Differential Scanning Calorimetry (DSC Q200, TA Instruments, USA) was used to measure the volume phase transition temperature of nanoparticles. Samples, at a concentration of 5 wt %, were heated in the range from 10 to 60 °C at a rate of 2 °C/min. Sample analysis was performed in hermetically sealed alumina pans and under a nitrogen flow of 60 mL/min. VPTT was designated by the maximum temperature of the endothermic peak. The scans were performed in triplicates.

The particle size and size distributions (Polydispersity Index, PDI) were measured by dynamic light scattering (DLS, Zetasizer 3000 HSA, Malvern Instruments, UK) at different temperature. The PDI is a dimensionless number that describes the heterogeneity of the sample and it can range from 0 (monodisperse) to 1 (polydisperse). The nanoparticles were diluted in deionized water before analysis. Five measurements were performed and averaged for each sample.

Chemical structures of copolymers nanoparticles were studied by ^{1}H-NMR on an Agilent DD2-600 nuclear magnetic resonance (NMR) instrument using $CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal standard.

Atomic force microscopy (AFM) analysis was performed on a Veeco Nanoscope MultiMode scanning probe microscopy (SPM) in a tapping mode. The nanoparticles dispersed in deionized water were deposited on glass slides and dried at different temperature for analysis.

Synchrotron radiation small-angle X-ray scattering (SAXS) experiments were performed on the BL16B beam line of Shanghai Synchrotron Radiation Facility (SSRF), Shanghai, China. The distance from sample to detector is 5,130 mm and the X-ray wavelength is λ=0.124 nm. The 2D SAXS data were converted to 1D intensity $I(q)$ as a function of the scattering vector q [$(q=(4\pi/\lambda)\sin(\theta/2)$], where θ is the scattering angle. The nanoparticles dispersed in deionized water (5 wt %) were loaded into a liquid sample holder for SAXS analysis, and the temperatures of samples were controlled by a Linkam heating-cooling stage.

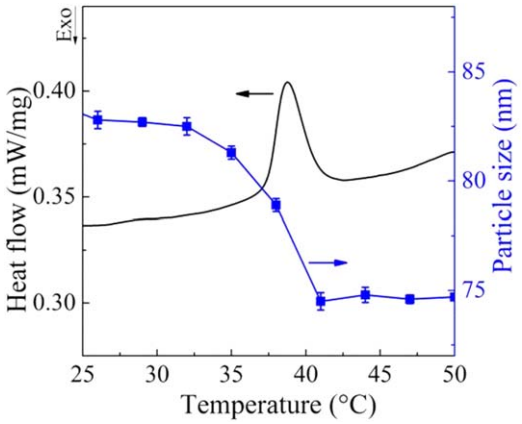

Figure 1. DSC curve and Z-average size of copolymer nanoparticles (Error bars indicate standard deviation for each sample. Where not shown, error bars are smaller than the data symbols). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Drug Loading

Drug concentration in phosphate-buffered saline (PBS) aqueous solution is much larger than that in nanoparticles. In addition, there are multiple interactions between drugs and polymers, such as hydrophobic interactions, hydrogen bonds or electrostatic interactions. Polymeric nanoparticles can retain drugs. Therefore, drugs would be more inclined to diffuse into nanoparticles to reach dissolution equilibrium. The copolymer nanoparticles were ultrasonic re-dispersed in PBS aqueous solution (phosphate buffered solution, pH 7.4) or in ethanol aqueous solution (0.4 mole fraction) of procaine with a concentration of 2 mg/mL. After continuously stirring for 6 h at desired temperature, the nanoparticles were loaded with procaine by diffusion and adsorption and then centrifuged at10,000 rpm for 10 min to get the drug-loaded nanoparticles. This centrifugation was carried out three times and the supernatant was analyzed using ultraviolet–visible spectrophotometer (SHIMADZU UV-1800). The precipitate was washed with deionized water and then freeze-dried. The ibuprofen was loaded in the same way. Absorption maximum wavelength at 290 nm and 263 nm were respectively used for the quantitative determination of procaine and ibuprofen by UV spectrophotometer. Ultraviolet-visible absorption spectra of procaine and ibuprofen are shown in Supporting Information Figure S1. ^{1}H-NMR measurements were used to confirm the incorporation of drugs into nanoparticles.

Drug Release

The drug release behaviors were determined using dialysis technique. Drug-loaded nanoparticles (50 mg) were dispersed in 20 mL PBS aqueous solution (pH 7.4), and introduced into a dialysis tube (Spectra/Por2, 12,000 to 14,000 molecular-weight cut-off, Spectrum Medical Industries, Inc., CA, USA) immersed into 500 mL PBS aqueous solution at the desired temperature. At each time interval, 5 mL released solvent was taken out for UV spectrophotometer measuring the maximum wavelength absorbance of the drug. Withdrawn solvent was replaced with the same volume of fresh PBS aqueous solution. As drug concentration in nanoparticles is much larger than that in the pure PBS aqueous solution, drug would be constantly diffused into PBS aqueous solution to reach saturation. In addition, the saturation could not be achieved because the large amount of PBS aqueous solution and it will be partly replaced with fresh PBS aqueous solution. Drugs would be constantly diffused into PBS aqueous solution. Accumulated release rate was calculated as follows:

$$R = \frac{V \cdot C_i + v\sum C_{(i-1)}}{m} \times 100\% \quad (1)$$

where V is the total volume of dialysate and v is sampling volume; C_i is the measured drug molality at each time; and m is the total mass of loaded drug.

Drug release experiments were conducted for about 10 h until the concentration stabilized. All experiments were done in triplicate to determine reproducibility of the data. Using the absorbance values obtained from the spectrophotometer, the amount of drug released was determined as a function of time.

RESULTS AND DISCUSSION

Preparation and Characterization of Thermosensitive and Crystallizable Copolymer Nanoparticles

PNIPAM is used to conduct miniemulsion polymerization of hydrophobic and hydrophilic monomers, forming core-shell polymer nanoparticles in a simple and facile way. SMA is used to form the hydrophobic and crystalline core. The solubility and thermosensitivity of PNIPAM are utilized to produce interfacial adsorption and incorporation into oil phase. By volume phase transition behavior of PNIPAM oligomers and interfacial absorption, copolymer is generated and self-assembled into core-shell nanoparticles *in situ*. Since the PNIPAM chains undergo a coil-to-globule transition near its VPTT, the particle size will change with the temperature variation. Thermosensitivity of nanoparticles was determined by DSC and DLS (Figure 1). The change of Z-average size with temperature shows that copolymer nanoparticles exhibited a sudden drop in particle size between 35 °C to 40 °C, which is coincident with temperature range of endothermic melting peak in DSC. Thermosensitive polymer PNIPAM will undergo a coil-to-globule transition in response to changes in temperature. Homopolymer

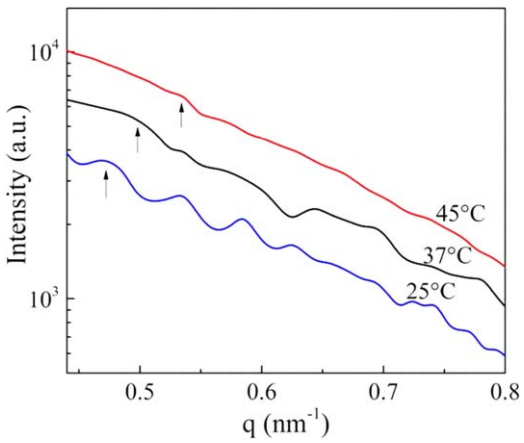

Figure 2. SAXS profiles for 5 wt % aqueous solution of copolymer at various temperatures. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

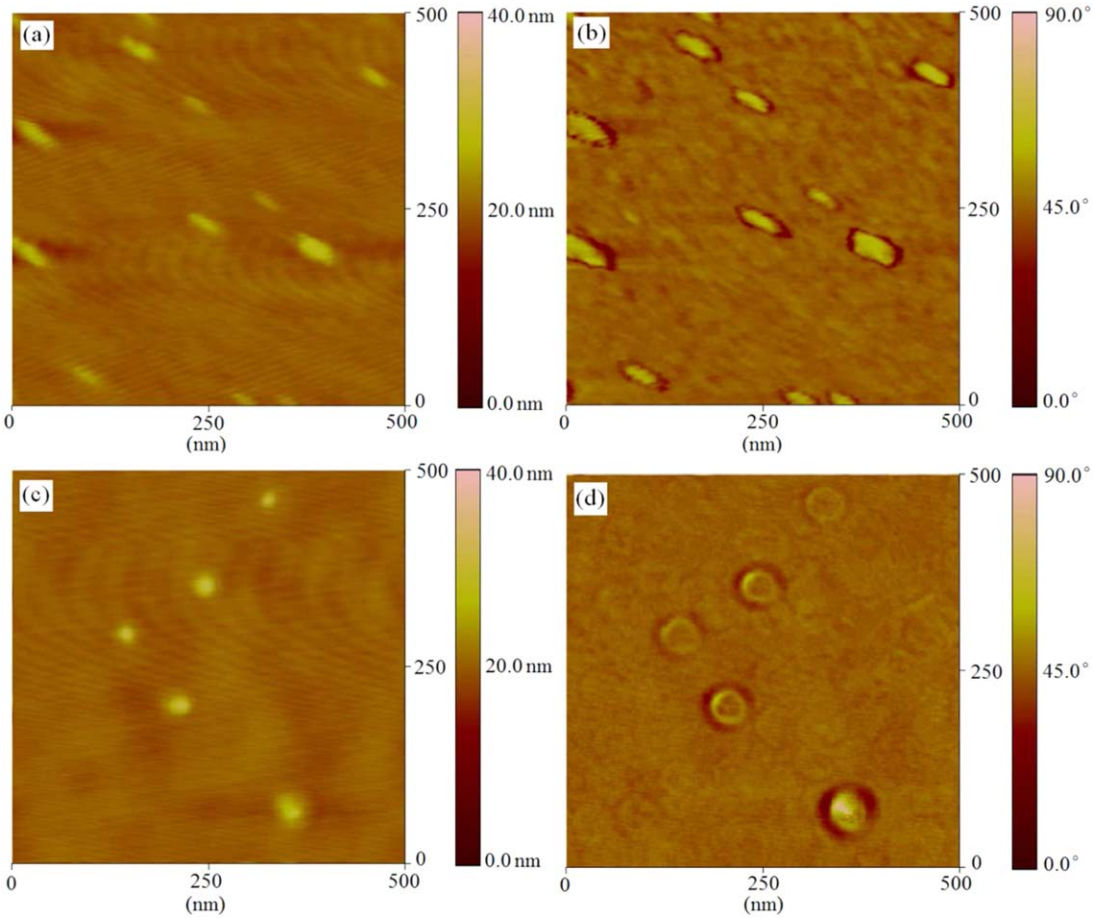

Figure 3. AFM images of copolymer nanoparticles at various temperatures: (a) top view height image at 25 °C; (b) phase image at 25 °C; (c) top view height image at 45 °C; and (d) phase image at 45 °C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

PNIPAM possesses a VPTT of 32 °C. The VPTT of PNIPAM/PSMA copolymer was increased to 37∼38 °C, by the terminal hydrophilic sulfate radical group of chain and the hydrophobic hydration caused by spatial effect of PSMA. The temperature above VPTT causes a dehydration of the hydrophilic and hydrophobic polymer chains. Thus, polymer chains would shrink and

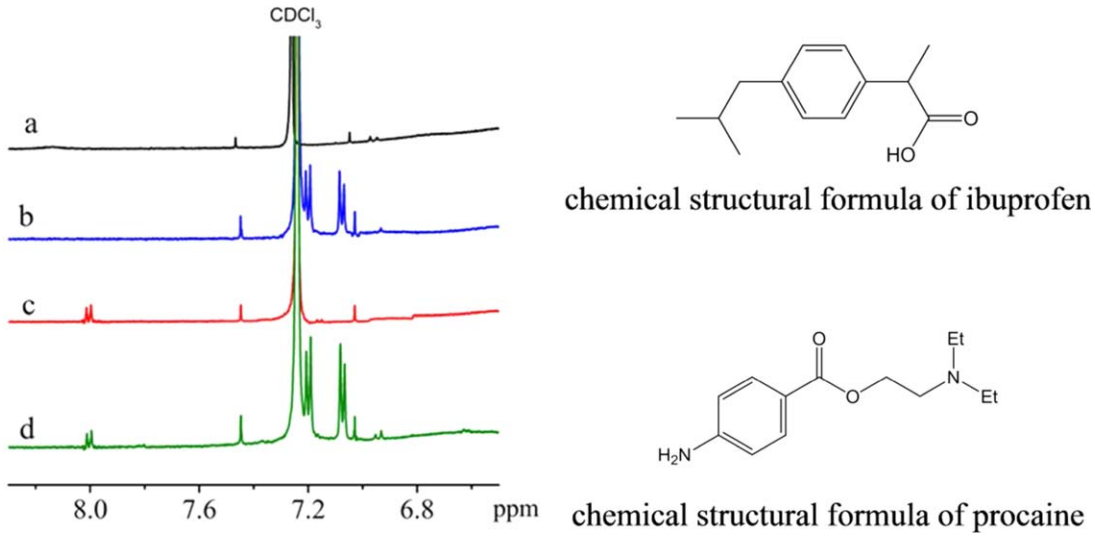

Figure 4. ^{1}H-NMR spectra of copolymer and drug loaded copolymer nanoparticles: (a) copolymer nanoparticles; (b) ibuprofen loaded nanoparticles; (c) procaine loaded nanoparticles; and (d) drug combinations loaded nanoparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

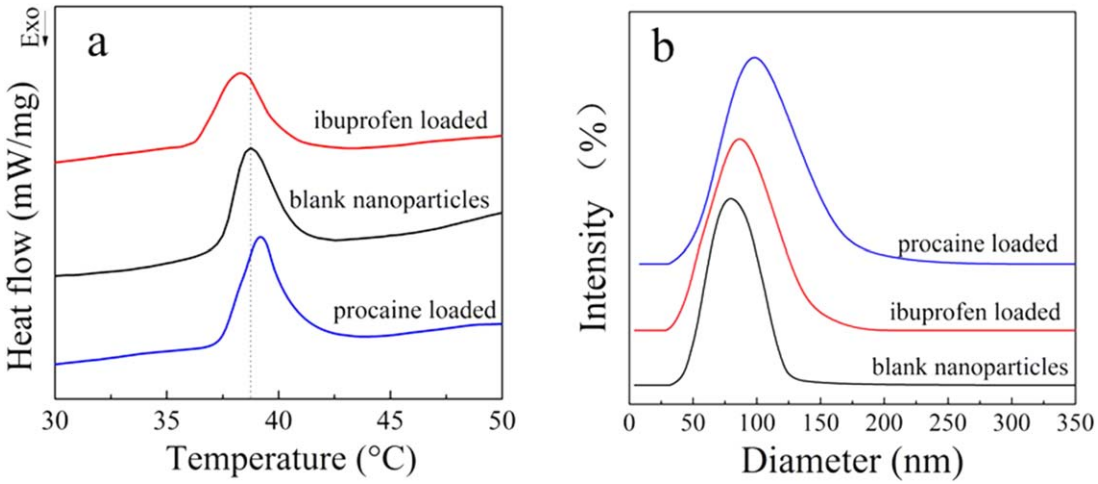

Figure 5. DSC heating curves (a) and DLS size evolution (b) of blank and drug loaded PNIPAM-PSMA nanoparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

particle size suddenly decreased. The endothermic peaks of copolymer would include both contributions of melting of PSMA unit and volume phase transition of PNIPAM unit, because the melting temperature of PSMA segment and volume phase transition temperature of PNIPAM segment are in the similar temperature range and overlapped.[14] The pendent long alkyl side chain of SMA can form crystalline domain, making polymer phase transition. O'Leary *et al.* indicated that PSMA exhibit a 'jump' in gas permeability at the T_m of the side-chain lengths, which is mainly caused by a switch in the side-chain morphology from crystalline to amorphous upon melting.[16,17] The crystalline domain has certain mechanical strength, hardness, and lower permeability. Those properties are suitable for controlled drug release.

Figure 2 shows SAXS profiles for 5 wt % aqueous solution of copolymer at various temperatures and series of reflections were observed. The first-order reflection peak is at 0.468 nm^{-1} and the main series of multiple scattering peak spacings progress in the following ratios 1:1/2:1/3:1/4:. . ., indicating the crystalline side-chains pack is similar to a lamellar system.[19,20] The order structures of copolymer are weakened with raising temperatures and the reflections even vanished for copolymer at 45 °C. The *d*-spacing ($d=2\pi/q$) is 13.4 nm at 25 °C, which is slightly larger than that at 37 °C (12.6 nm). At 45 °C, a very weak peak is observed and *d*-spacing shifted to value of 11.9 nm. The decrease in *d*-spacing is due to the changes of packing structures.[21] The vanishing of reflections shows the melting of crystalline lamellae.

AFM images of copolymer nanoparticles are shown in Figure 3. The transitions are special because the melting temperature of PSMA and VPTT of PNIPAM are in the similar temperature range. PNIPAM moiety is swelling and could dissolve in water and PSMA moiety crystallized into ordered domain at 25 °C, showing as lamellae [Figure 3(a,b)]. When heating, PNIPAM moiety will curl up and PSMA moiety is turning to amorphous state, showing as nanoparticles [Figure 3(c,d)]. The core-shell structure is obvious in phase images. Transmission electron microscopic (TEM) images of copolymer nanoparticles at various temperatures (Supporting Information Figure S2) confirmed morphology transformation with temperature. The crystallinity of PSMA reflects permeability for the polymer. PSMA moiety will turn to an amorphous state and show favorable permeability above the melting temperature. In addition, PNIPAM chains undergo a coil-to-globule transition upon its VPTT. Thus, rising temperature could switch on the pathway to delivering drugs in nanoparticles.

Controlled Loading of Hydrophilic and Hydrophobic Drugs

The degree of drug loading depends on the loading technique and drug interaction with core forming blocks.[22,23] In addition to being incorporated in micelle cores by direct mixing of a drug powder and micellar solution, some complex techniques have been developed for loading drugs into micelles, e.g., oil-in-water emulsion method, solvent evaporation method, etc.[8,24] However, the drug loading capacity (DL) depends to a high extent on the interactions between polymers and incorporated drug. The high drug loading capacity (DL) and entrapment efficiency (EE) can be achieved through effective multiple interactions such as hydrophobic interactions, hydrogen bonds, electrostatic interactions or ion coordination. Polymeric micelles can exhibit a high drug loading capacity for both hydrophobic and hydrophilic agents following appropriate design of hydrophobic blocks and the side functional groups. Hydrophobic drugs usually can be efficiently encapsulated in the hydrophobic core of polymer micelles by hydrophobic interactions.[25,26] If the interaction between the shell-forming block and the drug is favorable, even if only slightly, the drug can be present in the outer shell.[23] NIPAM has amide group and can form hydrogen bonds and electrostatic interactions with hydrophilic drugs. Hydrophobic drugs can be encapsulated in SMA due to its hydrophobic long alkyl side chain. Those make nanoparticles potential to controlled loading of both hydrophilic and hydrophobic drugs.

Up to now, there are few reports on the controlled loading of hydrophilic and hydrophobic drugs. In this research, we have chosen ibuprofen and procaine as hydrophobic and hydrophilic model drugs respectively, to demonstrate the loading of drugs.

These two drugs are typical and widely used. ^{1}H-NMR measurements were used to confirm the incorporation of drugs into nanoparticles. ^{1}H-NMR spectra in $CDCl_3$ for individual drugs and drug combination loaded copolymers are shown in Figure 4 (partial enlargement). All of the spectra show the prominent resonance peaks representative of copolymer. The drugs are identified by aromatic protons of benzene rings. Ibuprofen has benzene rings with isobutyl (electron-donating group). The protons of benzene rings show a chemical shift to high filed of 7.09 and 7.20 ppm [Figure 4(b)]. The signal at 7.95 ppm corresponds to protons of benzene rings in procaine, which are attached with electron-withdrawing ester linkages and shifted to low field [Figure 4(c)]. Protons of benzene rings of both procaine and ibuprofen show their characteristic peaks in Figure 4(d), which is indicative of drugs incorporation into nanoparticles.

Loaded drugs can interact with hydrophilic/hydrophobic chains of copolymer nanoparticles, thus affect the phase transition temperature, structure, and size of nanoparticles. Their interactions also reflect the compatibility between copolymers and drugs. Figure 5 shows DSC heating curves and DLS size evolution of blank and drug loaded PNIPAM-PSMA nanoparticles. The melting peak at 38.7 °C of blank nanoparticles is observed, which is contributed by melting of PSMA segment and volume phase transition of PNIPAM segment. Melting temperature of ibuprofen loaded nanoparticles is reduced to 38.1 °C, because ibuprofen is loaded in hydrophobic core and the crystallization of the PSMA moiety is destroyed to some extent. Its crystallinity is decreased. Therefore, the melting enthalpy and melting temperature of ibuprofen-loaded nanoparticles are decreased. However, procaine loaded nanoparticles have slightly higher melting temperature, and the melting enthalpy are increased. This is mainly because hydrophilic procaine is loaded in shell, with almost no effect to the crystallization of PSMA moiety in core. On the other hand, hydrophilicity of procaine would strengthen hydrogen-bonding interaction between PNIPAM moiety and water, thereby increasing the melting enthalpy and melting temperature of procaine loaded nanoparticles. However, the influence of drugs on thermal property of polymer is weak, because quite small amount of drug is loaded. Figure 5(b) shows procaine has influence on size of nanoparticles. This is due to the diameter DLS measured is average hydrodynamic diameter, containing hydration layer. As procaine strengthens hydrogen-bonding interaction between PNIPAM moiety and water, it enlarged the hydration layer, making both particle size and size distribution increased. The particle size and size distribution of ibuprofen loaded nanoparticles is increased also. This is because ibuprofen weakens the crystallization of PSMA moiety in core, reducing the compactness and orderliness of crystal, and increasing the size.

Hydrophilic and hydrophobic drugs were single or combined loaded into nanoparticles to examine drugs loading capacity. Unloaded free drugs were removed by repeated centrifugation and washing. The UV/Vis spectra of supernatant were measured. The drug loading (DL) and the entrapment efficiency (EE) were calculated and shown in Table I. The nanoparticles show quite different capability of loading hydrophilic and hydrophobic drugs under different conditions. Both temperature increasing and ethanol aqueous solution could significantly increase the loading capacity of ibuprofen. The core was formed of PSMA, which possessed good properties in oil-absorption and retention; it was easy for ibuprofen loading at high temperature. Moreover, PSMA moiety in amorphous state shows favorable permeability and ethanol increases intermiscibility with hydrophobic drugs. However, the loading capacity of procaine is slightly decreased with temperature increasing or ethanol solution, due to the weakening of hydrogen bonds and electrostatic interactions with hydrophilic drugs. Combined loading of hydrophilic and hydrophobic drugs were carried out to study potential application in combination drug therapy. Combined loading shows little effect on loading capacity of ibuprofen or procaine. The loading capacity is only slightly decreased to that in the single loading. This suggests that the loading capacity of ibuprofen or procaine mainly depends on respective interaction between polymer and drug. These results demonstrate that temperature below VPTT and water solution are beneficial to hydrophilic drug loading, and temperature above VPTT and ethanol solution are conducive to hydrophobic drug loading.

Controlled Release of Hydrophilic and Hydrophobic Drugs and Kinetic Analysis

The release behaviors of model drugs were observed at temperatures above and below the VPTT of the copolymer. Both individual and combined drug release behaviors were studied. A general equation of diffusion based on the Fick's law is theoretically described as:

$$\frac{dC_e}{dt} = A(C_i - C_e) \tag{2}$$

$$A = \frac{PS}{V} = \frac{3P}{R} = \frac{3D}{Rh} \tag{3}$$

where P is the permeability, which can be converted into a diffusion coefficient (D) by means of multiplying the permeability with the length of diffusion path (h); S and V are the surface area and volume of nanoparticle; C_i and C_e represent the concentration of drug in nanoparticles and aqueous solution.[27,28]

The nanoparticles were assumed spherical with an average diameter. The drug concentration inside the nanoparticle is constant (saturated) and the drug concentration in the bulk C_e is much less. In this case, the concentration difference in eq. (2) can be replaced by $C_i - C_e = C_s$, where C_s denotes the concentration of a saturated solution. By integrating eq. (2), the concentration of drug in solution becomes

$$C_e(t) = C_s(1 - e^{-\frac{ps}{V}t}) \tag{4}$$

Equation (4) has a classical exponential form throughout the time course of the process approaching the plateau level C_s asymptotically.

Weibull model [eq. (5)] is an empirical model, which has been widely applied to release data of both rapid and extended release drug delivery systems.[29,30]

$$\text{Weibull Model} \ln[-\ln(1-F)] = \beta \ln(t - T_i) - \ln\alpha \tag{5}$$

where F is accumulated fraction of the drug; α, β, and T_i are scale parameter, shape parameter, and location parameter.

It can be written as

Table I. Drugs Loading Capacity of Nanoparticles at Different Temperature and Medium

Drug		Concentration (mg/mL)	DL[b] (%) PBS (25 °C)	DL[b] (%) PBS (45 °C)	DL[b] (%) Ethanol aqueous solution[a] (45 °C)	EE[c] (%) PBS (25 °C)	EE[c] (%) PBS (45 °C)	EE[c] (%) Ethanol aqueous solution[a] (45 °C)
Single loading	Ibuprofen	2	8.92	33.08	57.99	10.37	38.47	67.44
	Procaine	2	9.73	7.29	6.39	11.31	8.47	7.43
Combined loading	Ibuprofen	2	8.11	30.74	56.17	9.43	35.75	65.32
	Procaine	2	10.64	7.09	6.01	12.37	8.24	6.98

[a] Ethanol aqueous solution of 0.4 mole fraction.
[b] Drug loading (DL) $= \frac{C_{\text{initial drug}} - C_{\text{free drug}}}{C_{\text{copolymer}}} \times 100\%$.
[c] Entrapment efficiency (EE) $= \frac{C_{\text{initial drug}} - C_{\text{free drug}}}{C_{\text{initial drug}}} \times 100\%$.

$$F = 1 - e^{-\frac{(t-T_i)^{\beta}}{\alpha}} \tag{6}$$

Weibull model describe the release curve in terms of applicable parameters. The location parameter T_i, shows the delayed time of drugs release from nanoparticles. The shape parameter β, characterizes the curve as either exponential (β = 1), S-shaped with upward curvature (β > 1), or as curve with steeper initial slope than consistent with the exponential (β < 1). Scale parameter (α) shows the curve stretch in the x (time) direction. When $T_i = 0$, β = 1, the Weibull model [eq. (6)] characterizes the curve as typical exponential. It would correspond to the Fick's law of diffusion [eq. (4)]. Thus the diffusion coefficient, D, is given by

$$F = 1 - e^{-\frac{1}{\alpha}t} \sim 1 - e^{-\frac{Ps}{V}t} \Rightarrow \frac{1}{\alpha} = \frac{PS}{V} \tag{7}$$

$$D = \frac{hV}{\alpha S} = \frac{Rh}{3\alpha} \tag{8}$$

The diffusion coefficient was calculated to adequately characterize the release kinetic properties of the drugs. However, because the onset of this state is defined by constant concentration inside the nanoparticle and complete dissolution, and some other factors influencing the release rate (i.e., hydration, swelling, erosion, or dissolution of polymer) are not included, eq. (8) can only be used for permeability estimation. Although the Fick's law of diffusion [eq. (2)] has been used widely, it has been proven inadequate in modeling either S-shaped experimental data or data with a steeper initial slope. The Weibull model [eq. (6)] is applied empirically but more successfully in all types of release curves.[31] The time-dependent release data and Weibull model fitting curves of are displayed in Figures 6 and 7. The parameters calculated by Weibull model, determination coefficients (R^2), and diffusion coefficient (D) are summarized in Table II.

Release of control samples from individual pure drugs were performed at 37 °C (Figure 6). It shows that both ibuprofen and procaine have a sharp initial burst release phenomenon in PBS solution, and the cumulative drug percentage within 2 h is more than 80%. This burst release behavior is greatly improved by drug-loaded nanoparticles. The drugs sustained release for more than 24 h and the cumulative drug percentage within 2 h is reduced to about 20%. It was observed that, at all three

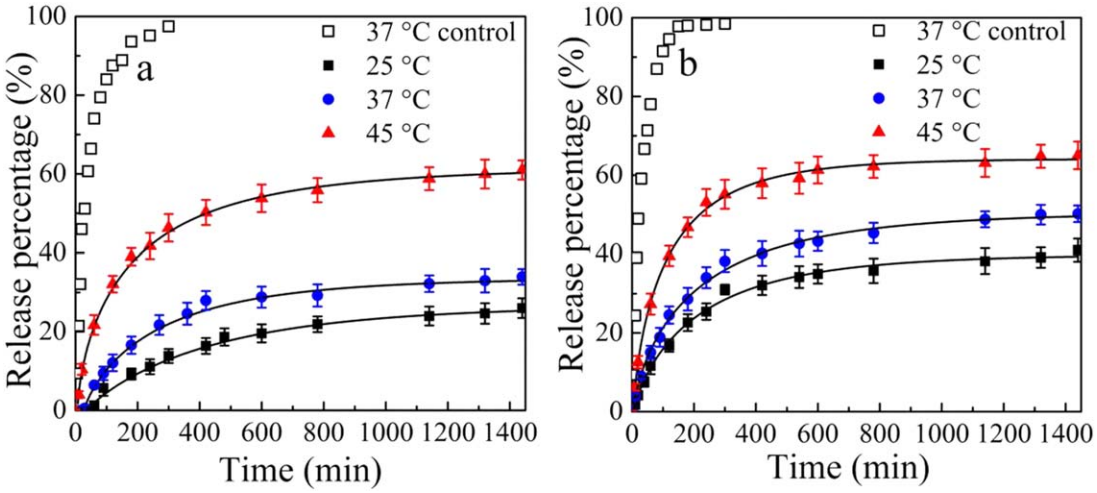

Figure 6. Profiles of individual drugs release from nanoparticles in PBS solutions at pH 7.4 (a) Ibuprofen and (b) Procaine. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

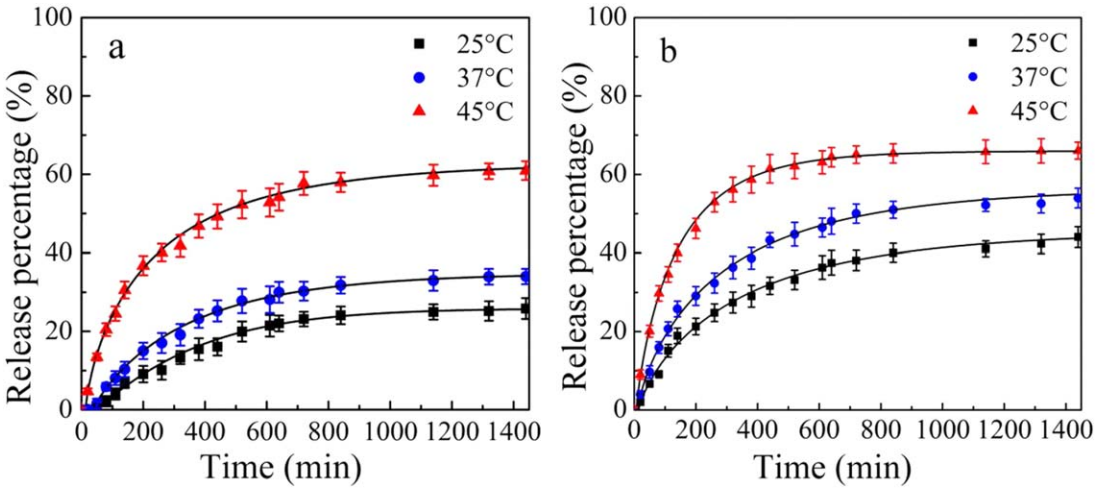

Figure 7. Profiles of combined drugs release from nanoparticles in PBS solutions at pH 7.4 [(a) Ibuprofen and (b) Procaine]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

temperatures, both ibuprofen and procaine continued to release from nanoparticles within 24 h, showing a better sustained release effect of nanoparticles. It also shows the temperature has big effect on the release behavior. The release rate at 45 °C was suddenly much faster. Applicable parameters, describe by Weibull model, can clearly reflect the release property and its mechanism. Weibull model is well fitted for the drug release mechanisms and all the determination coefficients (R^2) are greater than 0.99. Scale parameter (α) shows the curve stretch in time. Release behavior at 25 °C stretch much longer, indicating that the sustained release is greatly extended at temperature below VPTT. There is slightly burst release at 45 °C. The shape parameter characterizes the release behavior as one same curve shape, which is steeper initial slope than consistent with the exponential ($\beta < 1$). The location parameter T_i, shows the nanoparticles could delay the release of the drugs and the delayed time can be reduced with temperature rising. T_i reduced from 37.70 min to 6.21 min for ibuprofen, and reduced from 6.42 min to 3.71 min for procaine. This is because the rise of temperature changes structure form of copolymer and accelerates diffusion velocity. Ibuprofen is lipophilic and tends to be loaded in core. It undergoes diffusion resistance in the core and shell. While procaine is hydrophilic and mainly retained in shell, the release is only briefly delayed (less than 10 min). When combined release, the delayed time is more than 30 min difference between ibuprofen and procaine at 25 °C. Moreover, the time difference is decreased with temperature rising. These implement temporal control of different drugs. The diffusion coefficients show the release rate of ibuprofen at 45 °C is almost three times larger than 37 °C and six times larger than 25 °C. This is due to the phase transition of PNIPAM moiety and the melting of PSMA moiety. There are striking changes signified that the temperature has a good control over the release of ibuprofen. However, the effect of temperature on the release of procaine is not so significant. As the procaine is hydrophilic and mainly retained in shell, the diffusion coefficient at 37 °C is slightly increased than that at 25 °C. However, the collapse of PNIPAM chains at 45 °C still doubles the diffusion coefficient of procaine release. Combined release of drugs shows that both ibuprofen and procaine keeps their own individual original release characters and rules. However, combined release is little slower than individual release. This should be caused by molecular interactions between drugs and thus to increase diffusion resistances.

A schematic diagram of release behavior of combined drugs from core-shell nanoparticles is proposed (Figure 8). These results demonstrate that, at low temperature, crystalline PSMA possesses good properties in retention of ibuprofen while

Table II. Parameters, Determination Coefficients, and Diffusion Coefficient Obtained for Drugs Release from Nanoparticles at Different Temperature

	Individual						Combined					
	Ibuprofen			Procaine			Ibuprofen			Procaine		
Parameters	25 °C	37 °C	45 °C	25 °C	37 °C	45 °C	25 °C	37 °C	45 °C	25 °C	37 °C	45 °C
α	173.60	81.83	32.60	69.64	50.32	33.17	307.69	173.91	55.34	100.60	80.32	51.98
β	0.89	0.80	0.68	0.79	0.73	0.73	0.94	0.90	0.74	0.79	0.78	0.81
T_i	37.70	28.11	6.21	6.42	4.43	3.71	45.47	36.42	13.47	14.40	11.06	5.58
R^2	0.9916	0.9919	0.9989	0.9946	0.9957	0.9985	0.9987	0.9969	0.9980	0.9993	0.9970	0.9951
D ($\times 10^{14}$ (cm^2/s))	3.23	6.85	17.18	8.05	11.13	16.89	1.82	3.22	10.13	5.57	6.98	10.78

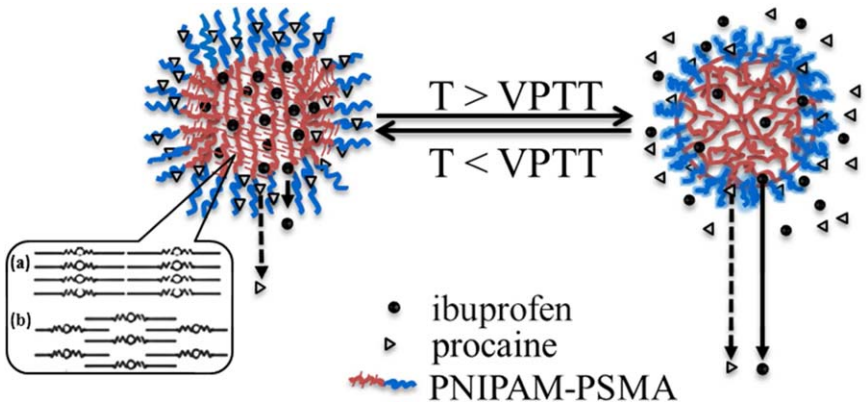

Figure 8. Schematic of release behavior of combined drugs from core-shell nanoparticles (end-to-end (a) and interdigitating (b) side-chain packing for crystalline PSMA; the length of arrow line indicates drug release rate). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

procaine in shell is easier to diffuse. Thus, release rate of hydrophilic procaine is much faster than that of hydrophobic ibuprofen. When temperature is above phase transition temperature, drugs release rates are both increased, and release rate of ibuprofen is almost the same as or even faster than procaine. It can be concluded that temperature can greatly change the relative release rate of hydrophilic and hydrophobic drugs.

CONCLUSIONS

The PNIPAM-PSMA polymeric nanoparticles have been successfully prepared via thermally induced interfacial absorption in miniemulsion. The thermo-sensitivity of PNIPAM moiety in the shell and crystallizability of PSMA moiety in the core were clearly detected. Nanoparticle size exhibited a sudden drop at a temperature about 38 °C and PSMA moiety could form nano size crystals to retain drugs. Nanoparticles showed good loading capacity of hydrophilic procaine and hydrophobic ibuprofen. Several loading techniques and drugs combined loading have been conducted. The loading capacity of ibuprofen or procaine mainly depends on respective interaction between polymer and drugs. It showed that temperature below VPTT and water solution are beneficial to hydrophilic drug loading, and temperature above VPTT and ethanol aqueous solution are conducive to hydrophobic drug loading. Fick's law of diffusion and Weibull model are combined to analyze the release kinetic and mechanism of drugs from PNIPAM-PSMA nanoparticles, which is adequately fit to the release data. The release behavior from nanoparticles showed a good sustained release effect more than 24 h, thermo-sensitive and temporally regulated. Combined release of ibuprofen and procaine were successfully performed. The relative release rate of hydrophilic procaine and hydrophobic ibuprofen can be controlled by temperature. The PNIPAM-PSMA nanoparticles show great potential in controlled release and co-delivery of hydrophilic and hydrophobic drugs.

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