

Biodegradable Microspheres with Poly(*N*-isopropylacrylamide) Enriched Surface: Thermo-responsibility, Biodegradation and Drug Release^{*}

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Abstract Microspheres with thermo-responsive surface were fabricated by PCL-*b*-PEO-*b*-PNIPAM triblock copolymers. Thermo-responsive morphological changes of PCL-*b*-PEO-*b*-PNIPAM microspheres immersed in aqueous solution at temperatures above the LCST (e.g. 37 °C) were observed from porous surface structure to compact surface layer. Enzymatic degradation and *in vitro* drug release results showed that the thermo-responsive surface layer greatly influenced the degradation of microspheres as well as the drug release behavior from microspheres. With the copolymerization of PNIPAM block into PCL-*b*-PEO copolymers, the drug release could be well regulated by changing temperatures and microspheres composition, which revealed the great potentials of microspheres with thermo-responsive surface for controlled drug release.

Keywords: Microspheres; Poly(*N*-isopropylacrylamide); Thermo-sensitive; Biodegradation.

INTRODUCTION

Polymeric microspheres have been widely utilized as favorable microcarriers for the delivery of hydrophilic drugs^[1, 2], hydrophobic drugs^[3], and bioactive agents^[4] in terms of both efficiency of drug loading and effectiveness of treatment at target sites. With polymeric microspheres as drug carriers, the controlled drug release is of great importance for maintain the therapeutic drug levels over a specified time period. The short half-life and poor bioavailability of some drugs can be overcome by implanting the drug-loaded microspheres into the target tissues thus minimizing the absorption into the systemic circulation. Reduced drug plasma levels could minimize the incidence and severity of adverse side effects.

Microspheres could be fabricated by several methods utilizing emulsion systems^[5–9], such as oil-in-water, oil-in-oil and water-in-oil-in-water, as well as by spray drying^[10]. Among various techniques, oil-in-water (O/W) emulsion method is considered to be the most common one. Despite its limited ability to encapsulate hydrophilic drugs, this relatively simple method enables the effective entrapment of various hydrophobic drugs. And the morphology of microspheres could be adjusted effectively by changing fabrication parameters, such as the concentration of polymer solution (oil phase), stirring rate or temperature^[11].

Due to the biodegradability in human body and the plasticity in fabrication, aliphatic polyesters such as

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polylactide (PLA), polyglycolide (PGA), poly(ϵ -caprolactone) (PCL) and their copolymers have been selected to fabricate microspheres as drug carriers. However, these microspheres have inherent limitations that their bulk and surface properties cannot be varied beyond copolymer composition and molecular weight, and they have no chemical functionality to enhance drug incorporation and release^[12]. Consequently, many methods have been reported to modify the aliphatic polyester microspheres through physical and chemical methods^[13–21]. Among them, chemical modification, which is utilized to design and synthesize biodegradable polymers with versatile architectures and functional groups, has shown great potentials to precisely adjust the surface and bulk properties of microspheres. By introducing different functional groups, hydrophilic block or functionalized block chains into biodegradable polymer backbone chains, the surface properties of biodegradable microspheres could be adjusted^[22].

Poly(*N*-isopropylacrylamide) (PNIPAM), which undergoes a fast and reversible phase transition in aqueous solution at a lower critical solution temperature (LCST, around 32 °C), has been widely studied as a potential candidate for controlled drug release^[13, 23–26]. When PNIPAM contained copolymers used as drug carries, the drug release behavior could be regulated by external temperature stimulation.

The PCL-*b*-PEO-*b*-PNIPAM triblock copolymers, used in this study, were synthesized in our early study. By modifying PCL with hydrophilic PEO and thermo-responsive PNIPAM blocks, the hydrophilicity of the copolymer increased and a thermo-responsive block was introduced. Therefore, amphiphilic triblock copolymers PCL-*b*-PEO-*b*-PNIPAM with a thermo-responsive PNIPAM block were used to fabricate microspheres by emulsion solvent evaporation method. The influence of PNIPAM blocks on the surface properties and enzymatic degradation of microspheres as well as the drug release behavior from microspheres were well studied. It is expected that the thermo-responsive PNIPAM blocks would affect the surface properties of the microspheres. The controllable surface properties and the morphology of the microspheres would have great influence on the enzymatic degradation and the drug release behavior.

EXPERIMENTAL

Materials

PCL-*b*-PEO and PCL-*b*-PEO-*b*-PNIPAM copolymers were synthesized according to our previous work^[22]. Ibuprofen (Alfa), poly(vinyl alcohol) (PVA, average molecular weight 65000, Aldrich) and dichloromethane were used without further purification.

Copolymer Characterization

The chemical structures of the copolymers were characterized by ¹H-NMR spectrometer (Bruker AV 400 Germany). The molecular weight and its distribution (M_w/M_n) of the copolymers were determined by gel permeation chromatography (GPC) with a Waters 515 HPLC pump, a Waters 2414 refractive index detector and a combination of Styragels HT-2, HT-3 and HT-4, where tetrahydrofuran (THF) was used as eluent at a flow rate of 1.0 mL/min at 40 °C. Polystyrenes with narrow distributed molecular weights were used as standards for calibration. The lower critical solution temperature (LCST) of the copolymers was determined by measuring the optical transmittance of the copolymers aqueous solution (0.1 mg/L) at varied temperature with an increasing rate of 0.5 K/min.

Preparation and Characterization of Microspheres

Microspheres were prepared by emulsion evaporation method. Briefly, 0.5 g of copolymers in 10 mL of dichloromethane was dispersed in an aqueous phase consisting 200 mL of a 0.2% (*W/V*) PVA solution under stirring at 300 r/min with the temperature at (22 ± 1) °C. After stirring for 2 h, microspheres were collected by filtration and washed with distilled water before freeze-dried.

The morphological characteristics of microspheres were observed with a field emission-scanning electron microscope (SEM). For the sample preparation, microspheres were incubated in distilled water at 22 °C and/or 37 °C for 2 h and then lyophilized. The freeze-dried microspheres were spread on the sticky surface of an aluminum stub and they were coated with platinum/palladium for 60 s using an ion sputter at 1.333×10^{-5} –

6.67×10^{-6} MPa and 10 mA of ion current. The microspheres were cut into halves in liquid nitrogen and then subjected to SEM for their internal morphology inspection.

Enzymatic Degradation of Microspheres

Enzymatic degradation of microspheres was carried out in phosphate buffer solution (PBS, pH = 7.4) containing 0.5 mg/mL *Pseudomonas cepacia* lipase at 22 °C and/or 37 °C. Briefly, 0.1 g of microspheres was added into a vial containing 4 mL of PBS with 0.5 mg/mL of lipase. The vial was incubated at 22 °C and/or 37 °C in a shaker at a speed of 100 r/min. After a certain time, the microspheres were collected and lyophilized. The weight loss was analyzed by weighting method and the surface morphology was analyzed by SEM.

Drug Loading and in vitro Release

The ibuprofen was loaded into the polymeric microspheres during microspheres preparation. The preparation of ibuprofen-loaded microspheres was similar to that of the blank microspheres except that the dichloromethane solution of copolymer was instead by a dichloromethane solution of copolymers and ibuprofen (33.9 wt% of copolymers). To determinate the drug loading content (DLC), the filtrates containing the non-capsulated drugs during drug-loaded microsphere preparation were collected, passed through a 0.20 μ m of filter and analyzed by UV absorbance at 273 nm using a Shimadzu UV-2450 UV-Vis spectrophotometer. The amount of the non-capsulated drug was cumulated by using a standard calibration curve experimentally obtained with the aqueous solution of ibuprofen. Then, the amount of non-capsulated drug subtracted from the initial amount of drug gave the amount of drug encapsulated in microspheres. The DLC were determined by using the following equation.

$$\text{DLC (\%)} = \frac{W_{\text{encapsulated drug}}}{W_{\text{drug-loaded microsphere}}} \times 100$$

In vitro release of ibuprofen from drug-loaded microspheres was studied at different temperatures as a function of time. Briefly, 2 mL of PBS containing 15 mg of drug-loaded microspheres was cased in dialysis bag and immersed into 150 mL PBS at 22 °C and/or 37 °C, respectively. At regular time intervals, aliquots of 10 mL were withdrawn and the volume of solution was held constant by adding 50 mL fresh PBS solution after each sampling. According to standard curve of ibuprofen in the PBS solution, the amount of ibuprofen released from microspheres was measured using UV absorbance at 222 nm. The cumulative release was calculated using the following equation:

$$\text{Cumulative release (\%)} = \frac{150c_n + 10 \sum c_{n-1}}{W_0} \times 100$$

where W_0 (mg) was the weight of ibuprofen in the microspheres, c_n and c_{n-1} (mg/mL) were the concentration of ibuprofen in aqueous solution which was withdrawn for n and $n-1$ ($n > 0$) times, respectively. The release of ibuprofen from microspheres in the present of lipase was performed similarly except phosphate buffer solution was instead by the solution of lipase.

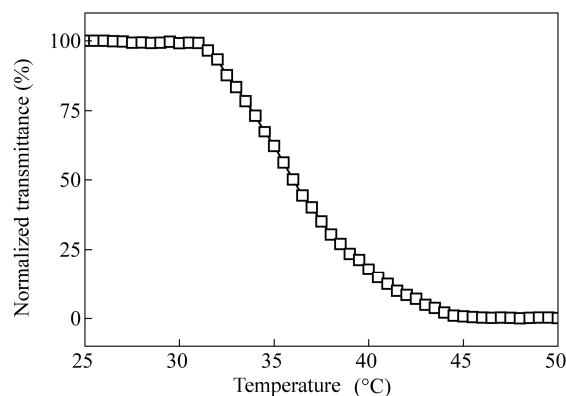
RESULTS AND DISCUSSION

Synthesis and Characterization of Copolymers

PCL-*b*-PEO copolymers were synthesized by anionic ring-opened polymerization of EO and CL monomers and PCL-*b*-PEO-*b*-PNIPAM copolymers were synthesized *via* RAFT polymerization of NIPAM with dithioester-capped PCL-*b*-PEO as macromolecular chain transfer agent. The molecular weight and the chemical composition could be well controlled by the feed ratio of the monomers, as discussed in our previous works. Detailed characterization of the copolymers is listed in Table 1. Similar to PNIPAM homopolymer, the PCL-*b*-PEO-*b*-PNIPAM triblock copolymers show phase transition at a lower critical solution temperature (LCST) of 36.0 °C, as shown in Fig. 1.

Table 1. Characterization of copolymers

Copolymers	M_n^a	Block length ^a			M_w/M_n^b	LCST (°C)
		PCL	PEO	PNIPAM		
PCL- <i>b</i> -PEO	8800	8000	800	0	1.25	–
PCL- <i>b</i> -PEO- <i>b</i> -PNIPAM	9500	5000	800	3700	1.34	36.0

^a Calculated by NMR data; ^b Determined by GPC**Fig. 1** Optical transmittance of aqueous solutions of PCL-*b*-PEO-*b*-PNIPAM at 500 nm upon heating at 0.5 K/min (The copolymer concentrations were 0.1 mg/mL.)

Thermo-responsive Surface

As microcarriers, surface properties of the microspheres are one of the most essential factors in the field of tissue engineering and controlled drug release. Many methods were reported to modify the microcarriers by bonding functional groups onto the surface through physical and chemical methods. Herein, thermo-responsive PNIPAM chains were introduced to modify the microspheres.

Figure 2 shows the XPS profile of PCL-*b*-PEO-*b*-PNIPAM microspheres and the corresponding copolymer powder. The peak of N_{1s} of PCL-*b*-PEO-*b*-PNIPAM microspheres is significantly stronger than that of the corresponding copolymer powder, indicating a higher nitrogen content on the surface of microspheres. The higher nitrogen content on the microspheres surface is due to the PNIPAM chain enriched surface.

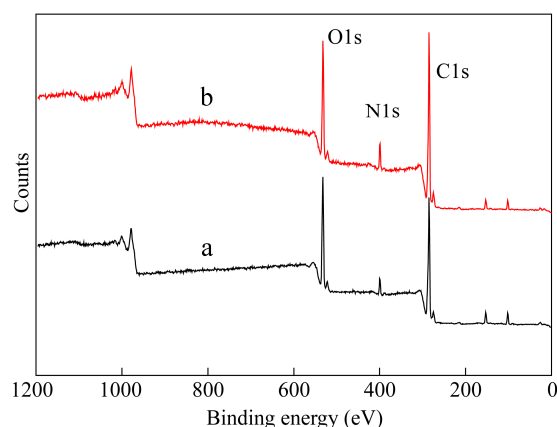
**Fig. 2** XPS profiles of (a) PCL-*b*-PEO-*b*-PNIPAM copolymer powder and (b) PCL-*b*-PEO-*b*-PNIPAM microspheres

Figure 3 shows the surface morphology and internal morphology of microspheres fabricated by PCL-*b*-PEO and PCL-*b*-PEO-*b*-PNIPAM. The PCL-*b*-PEO microspheres show a relatively smooth surface while the PCL-*b*-PEO-*b*-PNIPAM microspheres show a porous surface. The PCL-*b*-PEO microspheres have a symmetrical internal structure while the PCL-*b*-PEO-*b*-PNIPAM microspheres have an asymmetric structure.

These differences of morphology are attributed to the hydrophilicity of PNIPAM chain at 22 °C (the temperature at which microspheres were fabricated) and the o/w fabrication method of microspheres. For PCL-*b*-PEO-*b*-PNIPAM microspheres, hydrophilic PNIPAM blocks tend to be located at the interface between dispersed polymer solution and the continuous aqueous phase and therefore tend to be located at the surface of the result microspheres. Water penetrates into PNIPAM chains and the short hydrophilic PEO chains, which results in a swollen out-layer wrapping the microspheres. After freeze-drying, the water-penetrated morphology is fixed and results in a porous surface and a symmetrical internal structure. For PCL-*b*-PEO microspheres, the hydrophilic PEO chains are relatively short as compared to hydrophobic PCL chain. Therefore, a relatively smooth surface and an asymmetric internal structure are formed for PCL-*b*-PEO microspheres.

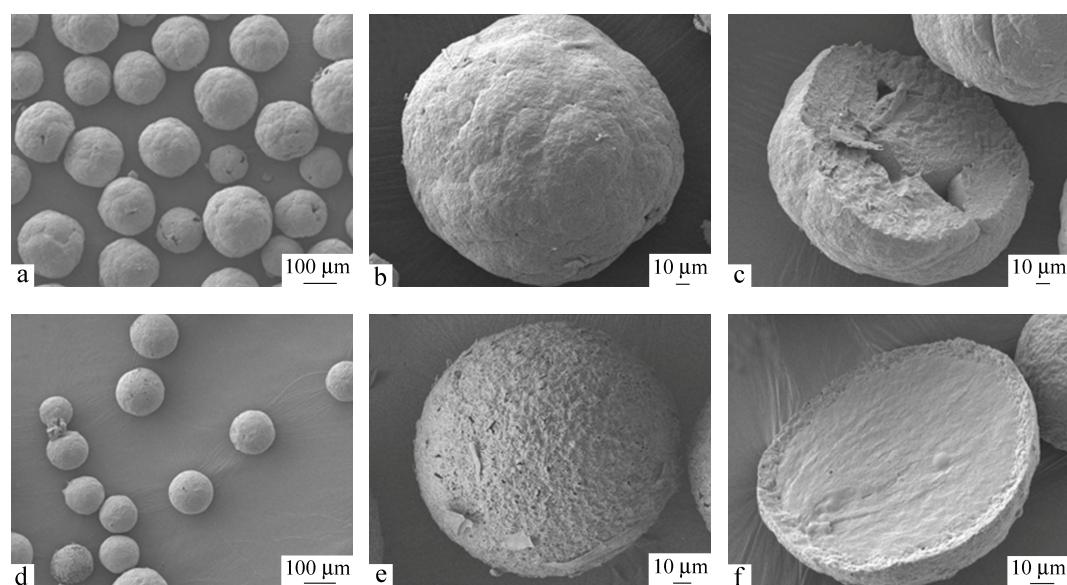


Fig. 3 SEM micrographs of microspheres fabricated by PCL-*b*-PEO (a–c) and PCL-*b*-PEO-*b*-PNIPAM (d–f) copolymers

When the microspheres are thermostatic in aqueous solution at a higher temperature above the LCST (*e.g.* 37 °C), the surface morphology of PCL-*b*-PEO-*b*-PNIPAM microspheres obviously changes compared with that observed at 22 °C. The porous surface of microspheres looks like to melt to form a relatively smooth surface at 37 °C which is above LCST (Figs. 4c and 4d). In contrast, no significant change is observed for PCL-*b*-PEO microspheres (Figs. 4a and 4b). The change in surface morphology of PCL-*b*-PEO-*b*-PNIPAM microspheres is due to the phase transition of PNIPAM block chains in microspheres with temperature below or above LCST.

Enzymatic Degradation

When biodegradable copolymers are utilized as drug carries, the degradation rate and the surface properties changes caused by degradation have great influences on the drug release behavior. As denoted by the XPS and surface morphological results, the microspheres fabricated by PCL-*b*-PEO-*b*-PNIPAM have a thermo-responsive surface due to the enriched PNIPAM chains on the surface of microspheres. Herein, to investigate the influence of thermo-responsive surface layer on the biodegradation of microspheres, the enzymatic degradation of microspheres fabricated by PCL-*b*-PEO-*b*-PNIPAM and PCL-*b*-PEO (as control) copolymers are examined.

Figure 4 shows the surface morphological changes of PCL-*b*-PEO-*b*-PNIPAM and PCL-*b*-PEO microspheres after enzymatic degradation for 4 h. Sunflower-like pleat structure is observed on the surface of microspheres after enzymatic degradation for the PCL-*b*-PEO microspheres fabricated at 22 °C or 37 °C. By comparison, many large holes are observed at the surface of PCL-*b*-PEO-*b*-PNIPAM microspheres after enzymatic degradation. These different changes of surface morphology for the microspheres could be considered due to the different structure of microspheres.

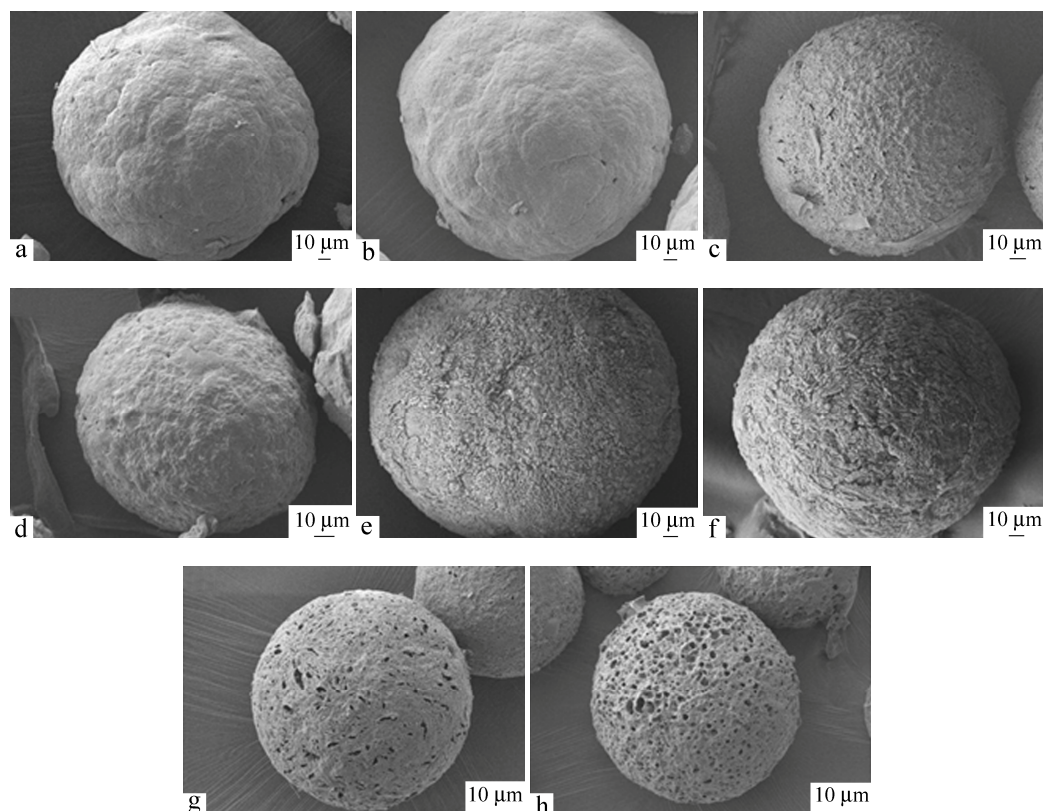


Fig. 4 SEM micrographs of PCL-*b*-PEO microspheres at 22 °C (a) and 37 °C (b) and PCL-*b*-PEO-*b*-PNIPAM microspheres at 22 °C (c) and 37 °C (d), and the corresponding surface morphology of these microspheres after enzymatic degradation for 4 h (e, f, g, h)

Figure 5 shows the weight loss of microspheres in the course of enzymatic degradation. It was found that both the thermo-responsive PNIPAM layer and degradation temperature have great influences on the biodegradation rate of microspheres. Both the microspheres no matter fabricated by PCL-*b*-PEO or PCL-*b*-PEO-*b*-PNIPAM copolymers showed a higher degradation rate at 37 °C than at 22 °C. This is due to the higher activity of lipase at 37 °C compared to 22 °C. Compared to the control PCL-*b*-PEO microspheres, the PCL-*b*-PEO-*b*-PNIPAM microspheres have a higher degradation rate at 22 °C (below the LCST). This is due to the loosely porous surface of PCL-*b*-PEO-*b*-PNIPAM microspheres, which is advantaged for the absorption and diffusion of lipases into the microspheres and results in a faster degradation rate. However, at 37 °C which is above the LCST, the degradation rate of PCL-*b*-PEO-*b*-PNIPAM microspheres is slower than that of the control PCL-*b*-PEO microspheres. As revealed by SEM, porous surface looks like to melt to form a relatively compact layer on the surface of PCL-*b*-PEO-*b*-PNIPAM microspheres when the temperature soars to higher than LCST. The compact layer blocked the diffusion of lipases into the inner of microspheres. Because the degradable PCL chains only located in the inner of microspheres, the percent of PCL chains that could be biodegraded for PCL-*b*-PEO-*b*-PNIPAM microspheres is smaller than that of PCL-*b*-PEO microspheres, thus results in a slower degradation rate than PCL-*b*-PEO microspheres at 37 °C.

Drug Loading and Controlled Release

Ibuprofen was loaded in the microspheres during microspheres preparation. The values of DLC are 15.6% and 16.2% for PCL-*b*-PEO and PCL-*b*-PEO-*b*-PNIPAM microspheres, respectively. As the DSC results shown in Fig. 6, there is no melting endotherm of ibuprofen-loaded microspheres near 78 °C which is the melting point of ibuprofen, indicating that the drug was amorphously dispersed in the polymer matrix.

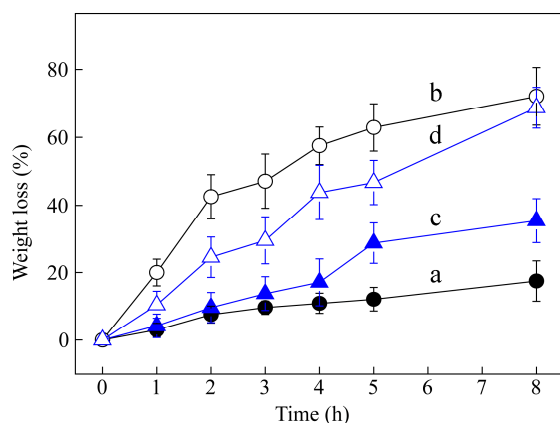


Fig. 5 Weight loss during enzymatic degradation for the microspheres fabricated by PCL-*b*-PEO microspheres at 22 °C (a) and 37 °C (b) or by PCL-*b*-PEO-*b*-PNIPAM at 22 °C (c) and 37 °C (d)

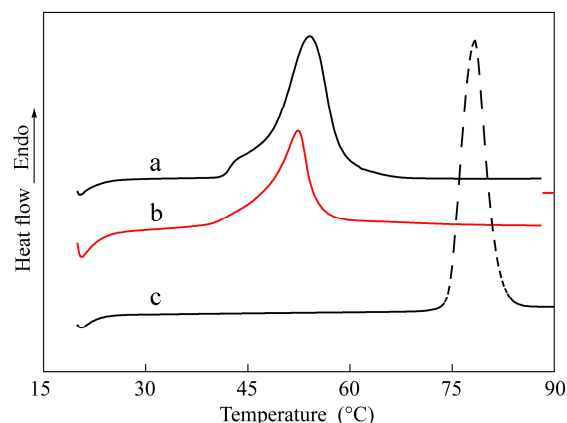


Fig. 6 DSC results of ibuprofen (c) and ibuprofen-loaded microspheres fabricated by PCL-*b*-PEO (a) and PCL-*b*-PEO-*b*-PNIPAM (b) copolymers

Figure 7 shows the release behavior of ibuprofen from the microspheres at temperatures below and above the LCST (e.g. 22 °C and 37 °C). It was found that the release of ibuprofen is dependent on both temperature and the composition of microspheres. As shown in Fig. 7, at temperatures below LCST (e.g. 22 °C), the drug release is faster from PCL-*b*-PEO-*b*-PNIPAM microspheres than from PCL-*b*-PEO microspheres. This is because of the porous structure on the surface of PCL-*b*-PEO-*b*-PNIPAM microspheres as shown in Fig. 4(c). However, the release behavior turned around when the temperature raised to above the LCST (e.g. 37 °C). It was found that the release of drug from PCL-*b*-PEO-*b*-PNIPAM microspheres is much slower than PCL-*b*-PEO microspheres at 37 °C. Furthermore, the drug release rate is much slower at 37 °C than at 22 °C for PCL-*b*-PEO-*b*-PNIPAM microspheres while the situation is contrary for PCL-*b*-PEO microspheres. These phenomena could be explained by the thermo-responsive surface of microspheres fabricated by PCL-*b*-PEO-*b*-PNIPAM. Generally, the drug release rate is faster at a higher temperature because of the higher solubility and higher diffusivity of drug. Consequently, the release of ibuprofen from PCL-*b*-PEO microspheres is much faster at 37 °C than at 22 °C. However, for the microspheres fabricated by PCL-*b*-PEO-*b*-PNIPAM copolymers, PNIPAM chains on the surface layer of microspheres collapsed by dehydration at the temperature above LCST, resulting in a compact layer on the surface of microspheres (Figs. 4c and 4d). The dense surface layer blocks the release of drug from microspheres and results in a slower release rate at temperature above LCST (e.g. 37 °C).

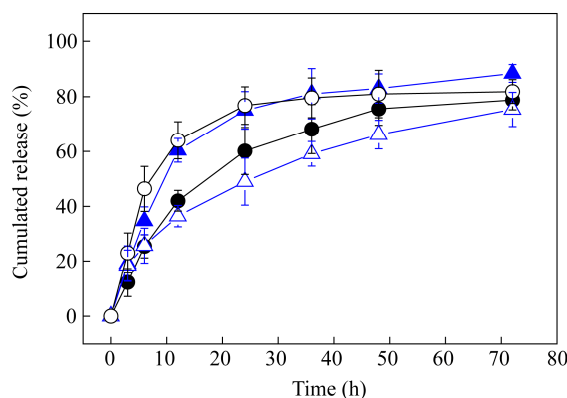


Fig. 7 Release of Ibuprofen from PCL-*b*-PEO microspheres and PCL-*b*-PEO-*b*-PNIPAM microspheres: (●) PCL-*b*-PEO, 22 °C, (○) PCL-*b*-PEO, 37 °C, (▲) PCL-*b*-PEO-*b*-PNIPAM, 22 °C and (△) PCL-*b*-PEO-*b*-PNIPAM, 37 °C

CONCLUSIONS

Biodegradable microspheres with PNIPAM enriched surface were prepared by PCL-*b*-PEO-*b*-PNIPAM triblock copolymers *via* emulsion evaporation method. These microspheres showed thermo-responsive surface morphology, which greatly influenced the enzymatic degradation of microspheres and the drug release behavior from microspheres. The *in vitro* release of ibuprofen from microspheres could be regulated by changing temperatures and microspheres composition, indicating the great potentials of these microspheres with thermo-responsive surface for drug controlled release.

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