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In vitro cytotoxicity and drug release properties of pH- and temperature-sensitive core–shell hydrogel microspheres

Liwei Ma, Mingzhu Liu*, Hongliang Liu, Jun Chen, Dapeng Cui

State Key Laboratory of Applied Organic Chemistry, Department of Chemistry, Lanzhou University, Lanzhou 730000, PR China

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ABSTRACT

A simple method has been developed to prepare smart P(*N*,*N*-diethylacrylamide-co-methacrylic acid) (P(DEA-co-MAA)) microspheres that consist of well-defined temperature-sensitive cores and pH sensitive shells. The microgels have been prepared by surfactant-free emulsion polymerization using water as the solvent. The core–shell hydrogel microspheres have been characterized by Fourier transform infrared (FTIR) spectroscopy, UV spectrometry, dynamic light scattering (DLS) and transmission electron micrograph (TEM). Preliminary characterization of the biocompatibility of hydrogel microspheres has been done by the cytotoxicity assays using the HeLa human breast cancer cell line as probes. The in vitro drug release indicates that drug release rate, encapsulation efficiency (EE) and release kinetics depend upon the pH value and copolymer composition. According to this study, the hydrogel microspheres based on P(DEA-co-MAA) could serve as suitable candidate for drug site-specific carrier in intestine.

1. Introduction

Stimuli-responsive hydrogel microspheres are microgel particles that show an ability to change their physical–chemical properties and colloidal properties in response to environmental stimuli, such as temperature, pH, magnetic, light, etc. Environmental stimuli-responsive microgel particles are considered to be more effective than macroscopic gels for many potential applications, because microgel particles have a much higher interfacial area per unit mass of hydrogel, which results in much greater exchange rates. Furthermore, microgel particles are readily packed in columns or used internally in the body. Recently, functional microspheres have been widely investigated (Chen et al., 2002; Lee et al., 2005; Kima et al., 2004; Kimura et al., 2003) and a widespread attention has been paid to environmental stimuli-responsive polymeric hydrogel microspheres due to their potential applications in numerous fields (Jones and Lyon, 2000, 2003), such as controlled drug delivery (Leobandung et al., 2003; Ichikawa and Fukumori, 2000; Vihola et al., 2002), chemical separations (Kawaguchi and Fujimoto, 1998), sensors (Panchapakesan et al., 2001), catalysis (Bergbreiter et al., 1998), and enzyme immobilization (Guiseppi-Elie et al., 2001). In addition, pH- or temperature-responsive microspheres could be used in more extended domains, because temperature and pH are important environment factors in biomedical and other system.

Microgels have attracted many interests in biomedical applications owing to their core–shell structures and properties (Irene et al., 2002). Core–shell microcapsules, in particular, consisting of aqueous, oil or polymer cores surrounded by a polymer shell, may provide unique opportunities to control drug release rates (Lee et al., 2002; Yang et al., 2003). For example, particle size and shell thickness have been shown to strongly affect the release rates (Berkland et al., 2004). Further, polymer chemistry and the mechanisms of degradation and erosion of the shell- and core-forming materials add a tunable parameter to such a delivery system. Meanwhile, core–shell type microgels, which contain a hydrophobic core and a hydrophilic thermosensitive shell, have become attractive for scientists since such systems may combine the characteristics of both the core and the shell. Microparticles and nanoparticles based on core–shell structures are advantageous in terms of their long circulation in the body in addition to drug solubility, stability and high level of drug encapsulation. As the representative one, poly(*N*,*N*-diethylacrylamide) (PDEA) is a typical temperature-sensitive polymer, which exhibits a lower critical solution temperature (LCST) at about 28–30 °C (Marilia and Ruth, 2005; Panayiotou et al., 2007). Below the LCST, the polymer chains are soluble in water due to the formation of hydrogen bonds between the water molecules and the amide side chains. When the temperature increases, the polymer undergoes a volume phase transition. Water is expelled from the microgel interior, thus causing a drastic decrease in volume above the LCST of the polymer. The 'smart' microgels can be tuned specifically in order to generate fast and targeted swelling responses to multiple external stimuli, such as both temperature and pH. As it mentioned above, surprisingly

* Corresponding author. Tel.: +86 931 8912387; fax: +86 931 8912582.
E-mail address: mzliu@lzu.edu.cn (M. Liu).

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functionalized PDEA-based microgels have not been investigated for this purpose to date.

Four methods have been reported for the preparation of microgel particles, namely emulsion polymerization (Wu et al., 1994), anionic copolymerization (Antonietti et al., 1995), crosslinking of neighbouring polymer chains, and inverse micro-emulsion polymerization (Neyret and Vincent, 1997). Emulsion polymerization can be performed in the presence of a surfactant (Chai et al., 2003; Xiao et al., 2004) or as surfactant-free emulsion polymerization (SFEP), also called precipitation polymerization (Öle Kiminta et al., 1995; Wu and Zhou, 1996). SFEP can be used to prepare microgels with diameters between 100 and 1000 nm. Another advantage of SFEP is also that the final microgel does not contain any surfactant. In contrast to previous studies, our present work is aim to prepare the core–shell type pH- and thermosensitive P(*N*,*N*-diethylacrylamide-co-methacrylic acid) hydrogel microspheres by SFEP technique to give controlled nano-size and core–shell structures in water solution, and bovine serum albumin (BSA) is considered as a model drug. The microspheres had been prepared together with a characterization of the morphology, the phase transition, and the biocompatibility of these structures in comparison to PDEA microspheres. In vitro release studies have been performed in pH 1.2 buffer solution and pH 7.4 buffer solution, and also have been compared to release behavior of PDEA microspheres. Release data have been analyzed in terms of Fickian equation and diffusion parameters.

2. Materials and methods

2.1. Materials

The monomer *N*,*N*-diethylacrylamide (DEA) was synthesized according to the literature (Shibanuma et al., 2000). Methacrylic acid (MAA) (Shanghai Chemical Co., Ltd., China) was distilled under vacuum before using. Dubelcco's modified eagle's medium (DMEM) was obtained from GIBCO Invitrogen Corporation. Ammonium persulfate (APS) (Tianjin chemical company, China) was of analytical grade and used without further purification. *N*,*N*′-methylenebisacrylamide (NNMBA) (Shanghai Chemical Co., Ltd., China) was purified by recrystallization in ethanol. Bovine serum albumin (BSA) was purchased from Sangon Co., Ltd. (Shanghai, China). Methyl thiazoyl tetrazolium bromide (MTT) was purchased from Sigma Co. Dimethyl sulfoxide (DMSO) (Shanghai Chemical Co., Ltd., China) and other reagents were analytical grade and used without further purification. Double distilled water was used for preparing all the solutions of the swelling and deswelling experiment.

2.2. Synthesis of core–shell type microspheres

Microspheres were prepared by SFEP according to references (Leung et al., 2004; Panayiotou et al., 2007). In particular, DEA (1.0 g) and MAA (25 μL) monomer and crosslinker (NNMBA) (0.03 g) were dissolved in 35 mL water in a round-bottomed flask equipped with a condenser, a nitrogen inlet, and a stirrer. The mixture was degassed to remove oxygen and put under nitrogen atmosphere. After thorough mixing, 5 mL APS water solution (6 mg/mL) was added to the reactive system, and then the temperature of the flask was raised to 70 °C to initiate the polymerization for 2 h. Similarly, P(DEA-co-MAA) copolymers microgel with different compositions, P(DEA-co-MAA) (DEA:MAA = 1 g:50 μL, 1 g:75 μL and 1 g:100 μL) were synthesized. Afterwards, the hydrogel particles were purified by dialysis (MW cutoff, 14,000 Da) for 7 days. The dialyzed solution of hydrogel microspheres was frozen in liquid nitrogen and lyophilized (Christ, Germany) to obtain dried microgel products for subsequent use.

To investigate the influence of the charges on the LCST and drug release profile for these microspheres, the pure PDEA microgels were prepared by same method.

2.3. FTIR characterization

The samples were analyzed using the Fourier transform infrared (FTIR) spectroscopy (Nicolet NEXUS 670 FTIR Spectrometer, USA) in the region of 4000–400 cm^{-1}. Prior to the measurement, the samples were dried under vacuum until reaching to constant weight. The dried samples were pressed into the powder, mixed with KBr powder, and then compressed to make a pellet for FTIR characterization.

2.4. Morphological properties of microspheres

Ten milligram of microspheres were dispersed in 5 mL of deionized water and sonicated in an ultrasonic bath for 1 min. Transmission electron micrograph (TEM) observation was carried out at 100 kV with a JEM-1200EX/S transmission electron microscope (TEM) (Hitachi, Japan). The micellar solution was negatively stained with 0.01% phosphotungstic acid and placed on a copper grid coated with former film.

2.5. LCST measurement

For this purpose, the optical density of the aqueous solution (containing between 0.3 and 1.0 g/L of microgels) was monitored as a function of the temperature at 500 nm using the Lambda 35 UV-spectrophotometer (PerkinElmer Co., USA) equipped with a TB-85 THERMO BATH (Shimadzu) thermostat. The solutions were heated from 23 to 45 °C at a heating rate of 0.5 °C interval. At each temperature, the samples were stabilized for 10 min before measurements. The critical temperature was taken as the point of inflection (approximated at half height) of the resulting turbidity curves. Microgels were first dissolved in phosphate buffer solution (pH 7.4), and ionic strength of the buffer was adjusted to 0.105 mol/L by the addition of NaCl.

2.6. pH dependent particle size measurements

Particle size and size distribution of particles were measured by dynamic light scattering (DLS) at the scattering angle of 90° in different buffer solutions (pH 1.2 and pH 7.4, respectively) at room temperature using 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation). About 5 mg of dried microspheres after lyophilized were transferred to the 25 mL buffer solution, and dispersed by ultrasound for 1 min to avoid the agglomeration of particles during measurements.

2.7. In vitro drug release experiments

2.7.1. Preparation of calibration curves

In this procedure, the absorbance of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations was measured on the UV–vis spectrophotometer (Lamda 35, PerkinElmer Co., USA) and calibration graph was constructed. The concentration of the drug in the sample solution was read from the graph as the concentration corresponding to the absorbance of the solution. Three calibration graphs of BSA were made to determine the amount of drug released from the drug-loaded hydrogels in different medium (distilled water, pH 1.2 buffer and pH 7.4 buffer).

2.7.2. Drug loading

The samples (10 mg) were added to 10 mL BSA aqueous solution (5 mg/mL), incubated for 24 h at room temperature and centrifuged for 5 min. The UV absorbance of the supernatant was measured at 278 nm to determine the amount of supernatant BSA. The obtained amount was used for calculation of the BSA content. The encapsulation efficiency (EE) of BSA was calculated according to Eq. (1):

$$\mathrm{EE}\,(\%) = \frac{D_a}{D_t} \quad (1)$$

where D_a is the actual amount of the drug in microspheres and D_t is the theoretical amount of the drug in the microspheres.

2.7.3. Drug release from hydrogel microspheres

In vitro drug release experiments were performed according to references (Li et al., 2008; Liu et al., 2008a). One hundred milligrams of BSA-loaded PDEA-co-PMAA microspheres were resuspended in 10 mL different solutions (pH 1.2 and pH 7.4 buffer solution, respectively) and placed in a dialysis membrane bag (3 kDa cut off). Then, it was tied, and sunk into 50 mL of homogeneous solution. The entire system was kept at 37 °C with continuously magnetic stirring. After a predetermined period, 3 mL of the saline medium was drawn out from release system for analysis, and 3 mL of fresh medium was added into the release system. The released BSA amount was determined by UV analysis with a calibration curve as described above. The release percent of BSA was calculated by Eq. (2):

$$\text{Drug release}\,(\%) = \frac{M_t}{M_\infty} \times 100 \quad (2)$$

where M_t is the amount of BSA released from the hydrogels at time t and M_∞ is the complete releasing amount of BSA, respectively.

2.8. Cytotoxicity test

The hydrogel samples with varied size and weight were sterilized by γ (^{60}Co) radial. Thereafter, the hydrogel samples were immersed in ultra purified water for 24 h. During this period, the water was refreshed every several hours. For the cytotoxicity test, 200 μL of HeLa cells in DMEM, with a concentration of 5.0×10^4 cells/mL was firstly added to each well in a 24-well plate and incubated for 24 h in an incubator (37 °C, 5% CO_2). Subsequently, the culture medium was changed to 200 μL of DMEM containing the hydrogel sample with particular weight and incubated for another 48 h. Then DMEM with the hydrogel was substituted by fresh DMEM, and 30 μL of MTT solution (5 mg/mL) was added to the HeLa cells. After incubation for 4 h, 200 μL of DMSO was added and the mixture was shaken at room temperature. The optical density (OD) was measured at 570 nm with a Microplate Reader, Model 550 (BIO-RAD, USA). The cell viable rate was calculated as follows:

$$\text{viable rate}\,(\%) = \frac{\mathrm{OD}_{\text{treated}}}{\mathrm{OD}_{\text{control}}} \times 100 \quad (3)$$

where $\mathrm{OD}_{\text{control}}$ is obtained in the absence of hydrogel and $\mathrm{OD}_{\text{treated}}$ is obtained in the presence of hydrogel, respectively.

3. Results and discussion

3.1. Synthesis and characterization of P(DEA-co-MAA) microspheres

Colloidal microgel particles with core–shell poly(DEA-co-MAA) were prepared by a single-step SFEP reaction. Since the reaction was heated above the LCST of PDEA, the PDEA became hydrophobic and phase-separated during the polymerization. The more hydrophilic monomer MAA polymerization was expected to take place at the periphery to the PDEA particles while the temperature raised. Once the crosslinked PMAA chains formed, it was expected that they would adhere to the PDEA particles as a result of their hydrophobicity at temperature above 28 °C. The formed first MAA layer would further trap the monomer and crosslinker from the water phase, so that the polymerization would continue and the shell would grow. Using this approach, it was able to prepare core–shell microgel as stable colloids with about nanometer size.

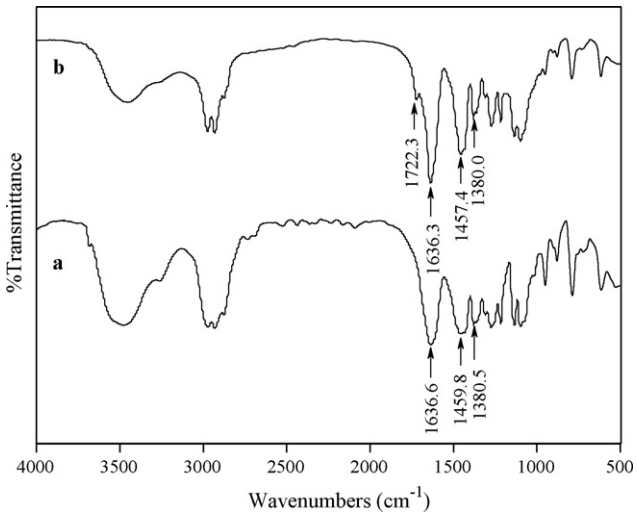

Fig. 1. FTIR spectra of PDEA (a) and P(DEA-co-MAA) (b) hydrogel microspheres.

The copolymerization was confirmed by comparing the FTIR spectra of PDEA with that of the P(DEA-co-MAA). FTIR spectra of the normal PDEA and P(DEA-co-PMAA) microgels samples are shown in Fig. 1, which shows that the spectra of all the microgels are similar. Each spectrum exhibits a broad band at about 1636 cm^{-1} consisting of C═O stretch of PDEA. One can also observe two typical bands of C—H vibration with almost the same intensity at about 1457 and 1380 cm^{-1} in each spectrum, which belong to the bands of $-CH_2-$ and $-CH_3$ groups. The main difference observed is the presence of carbonyl stretching vibration absorption band at 1722 cm^{-1} in the FTIR spectra of the copolymer, which corresponds to the carbonyl group of PMAA chains. Therefore, it can be concluded that P(DEA-co-MAA) microgel is successfully prepared.

Electron microscopy has the advantage that a visual assessment can be made of the shape and size of the microspheres. TEM photos of PDEA and P(DEA-co-MAA) hydrogel microspheres are shown in Fig. 2. From MS1, MS2, MS3, and MS4, it can be observed that a uniform thin layer with a thickness exists on the outer surface of microspheres, and the thin layer has coated the whole sphere. The TEM images clearly indicate the core–shell morphology of the P(DEA-co-MAA) microspheres, where the dark spherical area presents the PDEA core and the light corona around the core shows the crosslinked PMAA shell. It is worth noting that the shell of PMAA has a quite regular spherical shape, which is different from the morphology of PDEA microspheres prepared by the conventional method. Moreover, the thickness of the PMAA shell increases with the increase of the MAA content in the system. However, when MAA content is up to 100 μL, core–shell borderline becomes indistinct. This indicates that the core–shell structure of PDEA networks could be controlled by changing the MAA content in the polymerization procedure.

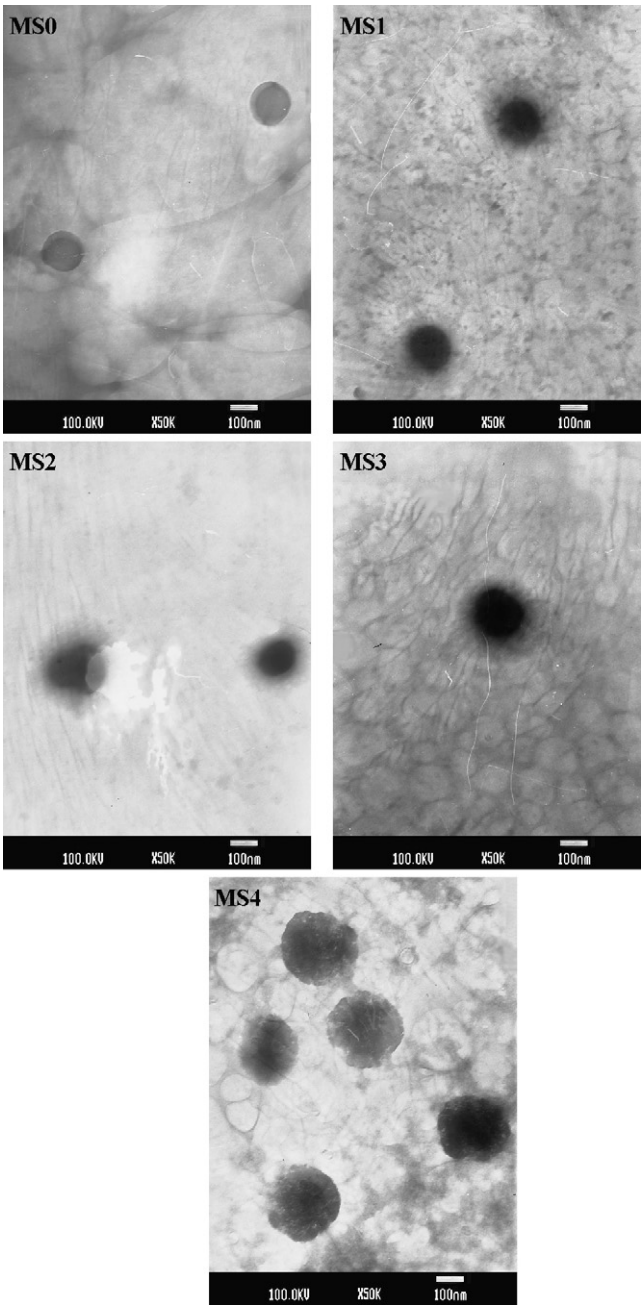

Fig. 2. TEM photos of PDEA and P(DEA-co-MAA) hydrogel microspheres.

3.2. pH-sensitive characteristics of microspheres

Variation of hydrodynamic sizes of core–shell microspheres as a function of pH value is investigated with dynamic light scattering. Table 1 shows that heightening pH of the P(DEA-co-MAA) microgel dispersion from 1.2 to 7.4 significantly increases the hydrodynamic size (Dv) of the microgels in diameter. The results suggest that the change of pH has obvious influence on the particle size of P(DEA-co-MAA) microspheres, and the increase of the size is attributed to the expansion of the PMAA shell. Moreover, contrast TEM, the decrease of hydrodynamic size at pH 1.2 is due to the formation of hydrogen bonds among carboxyl groups, and the formed hydrogen bonded complex would restrict the movement or relaxation of network chains. As a result, the lower swelling ratio and small hydrodynamic size are exhibited at a low pH due to the compact hydrogel network. Reversely, in an alkaline medium, the free carboxyl groups in the microspheres were ionized, which lead to the break of hydrogen bonds and the generation of the electrostatic repulsion among polymer chains. Therefore, a higher swelling ratio could be obtained at high pH, and hydrodynamic size increases. These results evidently demonstrate that PMAA shells are pH-sensitive, and the thickness of the shell could be easily altered with the change of pH of the dispersing medium. Moreover, as shown in Table 1, the diameter of the entire core–shell microspheres in pH 7.4 medium measured from the TEM images are in good agreement with data measured by DLS.

Table 1
Effect of pH on the hydrodynamic diameter (Dv) of PDEA and P(DEA-co-MAA) core–shell microspheres.

Sample ID	DEA (g):MAA (μl)	Average diameter (nm)	
		pH 1.2	pH 7.4
MS0	1:0	132.4	135.1
MS1	1:25	75.4	153.1
MS2	1:50	86.7	179.9
MS3	1:75	93.3	197.5
MS4	1:100	94.1	247.2

3.3. Temperature-sensitive characteristics of microspheres

It is well known that the PDEA chains assume an extended coil conformation at temperatures below the LCST (Shibanuma et al., 2000). In PDEA system, such a conformation is energetically favored by lower enthalpy due to the hydrogen bonding between the amide groups of the polymer and hydrogen of water (Liu et al., 2008b). So the free energy of polymer chains at temperatures below the LCST is dominated by lower enthalpy and higher entropy due to the more available degrees of freedom. However, by increasing temperature, the thermal motion of the polymer chains is enhanced. The attractive forces induced by the increased hydrophobic interactions also drive the chains to collapse and entangle with each other and hydrated water is excluded to become free water. During this process, the entropy of the polymer chains decreases and the entropy of the hydrated water surrounding the polymer chains of the hydrogel increases. But, as a whole, the total entropy of the hydrogel system, including the polymer chains and the surrounding water, increases (Zhang et al., 2002). However, a phase transition occurs only when the entropic gain at the critical temperature (LCST) offsets the increased enthalpy due to the break of hydrogen bonds. In the present system, when the amount of PMAA increases, more hydrogen bonds will be formed, and naturally higher enthalpy is needed to break the hydrogen bonds. As a result, only higher temperature can be entropic compensation. Therefore, the LCST shifts to higher temperatures by increasing amount of PMAA. Fig. 3 shows that the LCST of the microgels increases with the increase of PMAA content. This is attributed to the hydrophilic nature of PMAA units. An increase of PMAA will improve the hydrophilicity of the whole microgel network and increase the LCST of the microgels. Furthermore, from Fig. 3, it can be seen that the phase transition direction of microspheres becomes more broaden. The phenomenon could be explained that, with the number of MAA increasing, the effect of hydrogen bonds between carboxyl groups becomes stronger, which hinders the speed and extent of the PDEA chains contracting.

3.4. In vitro release

Bovine serum albumin (BSA) is a natural biocompatibility and nontoxic material, but the protein drugs would be destroyed or inactivated in acidic environment of the stomach. Therefore, resistance to acid, enzyme and time-controlled release are necessary for a viable BSA drug carrier. BSA molecules on the existence of

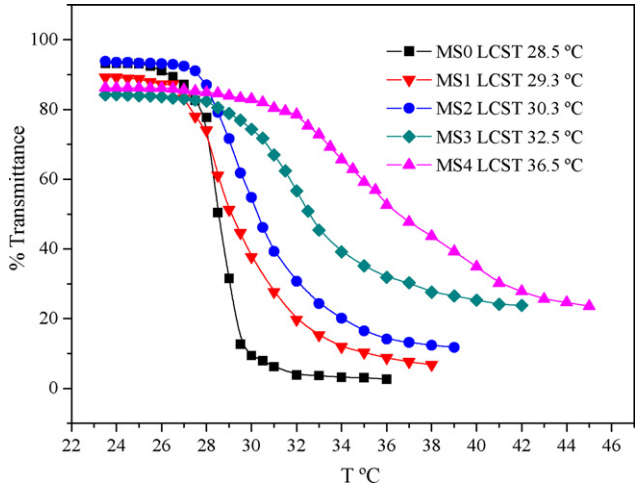

Fig. 3. Plot of transmittance of PDEA and P(DEA-co-MAA) microgel solutions at a function of temperature at 500 nm.

Table 2
Data of EE (%) of PDEA and P(DEA-co-MAA) microspheres.

Sample ID	MS0	MS1	MS2	MS3	MS4
EE (%)	38.7	58.2	63.9	67.8	70.02

a large number of carboxyl and amino, which can form hydrogen bonds and electrostatic interaction with carboxyl group on the MAA. Table 2 shows that the MAA is joined as a result of an increase of the adsorption of BSA, and PDEA copolymer microspheres significantly increase the rate of drug-embedded. Along with increase of MAA, the amount of carboxyl on microgels increases, which enhances the interaction of BSA and MAA. As a result, the EE (%) of drug increases by increasing amount of MAA. From the practical application, we chose the sample MS4 to carry out drug release experiments and cytotoxicity test, which have highest EE (%) and its LCST is closed to the physiological temperature better.

3.4.1. BSA release behaviors in pH 1.2 and pH 7.4 buffer solutions

The in vitro release studies have been performed in pH 1.2 and pH 7.4 buffer solutions. Fig. 4 depicts the release profile of BSA from drug-loaded microspheres at various time intervals in different buffer solutions (pH 1.2 and 7.4) at 37 °C. From Fig. 4, it could be seen that, the BSA release percentage of MS4 is extremely small (nearly 13%) in pH 1.2 buffer solution within 2 h, while the release percentage of MS0 reaches up to nearly 49% within same time. Moreover, the drug release percentage of MS4 in pH 1.2 buffer solution is significantly lower than in the alkaline environment of the release rate. The reason is that the release depends on the swelling rate of MAA accession. For the MS0, without pH-sensitivity group, drug release rate does not influence by pH value of the solution. For the MS4, as discussed previously, the swelling ratio of the microgels in an acidic medium is lower than that in a weakly basic medium. Because of MAA accession, not only does the EE (%) increase, the drug release rate in the stomach also has reduced. Thus, in a more practical point of view, these microgel systems can bypass the acidity of gastric fluid without liberating substantial amounts of the loaded drug and accomplish purpose in controlled release for drug side-specific in intestine.

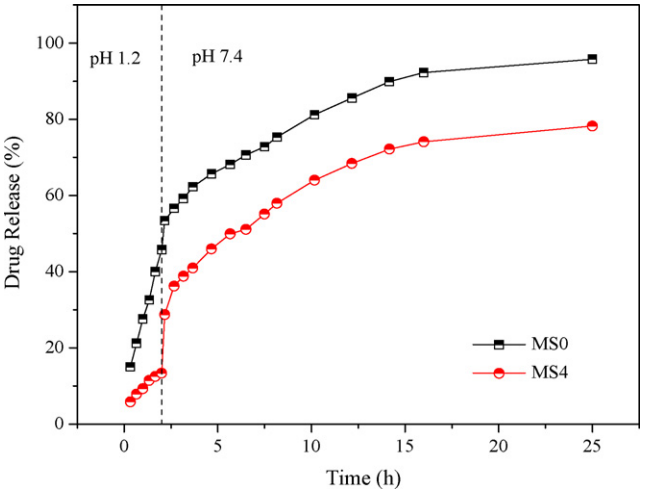

Fig. 4. BSA release behavior from MS0 and MS4 hydrogel microspheres microgels at 37 °C.

Table 3
Data obtained from Peppas model fitting for the polymer coated optimized formulations (sample: MS4).

Sample ID		$k \times 10^2$	n	R^2
MS0	pH 1.2 pH 7.4	0.99	0.6201	0.9864
MS4	pH 1.2 pH 7.4	0.32 1.21	0.4737 0.4908	0.9919 0.9904

3.4.2. BSA release kinetics

Release results were also analyzed using the empirical equation by Peppas (Ritger and Peppas, 1987). The initial drug release data was analyzed as a function of the time for $0 \leq (M_t/M_\infty) \leq 0.6$. The data were fitted to the following Eq. (4) to estimate the release kinetic parameter:

$$\frac{M_t}{M_\infty} = kt^n \quad (4)$$

where M_t and M_∞ are the amounts of drug released at time, t, and at equilibrium, respectively; n is a diffusion exponent and k is the proportionality constant characteristic of the drug–polymer system. For $n = 0.5$, the diffusion and release of the drug from the polymer matrix follow a Fickian diffusion. If $n > 0.5$, an anomalous or non-Fickian type drug diffusion occurs. Finally, for $n = 1$, a completely non-Fickian or Case II release kinetics is operative (Ramesh Babu et al., 2008; Gao et al., 2009). These data with the values of correlation coefficient (R) are presented in Table 3. In Table 3, the results indicated that the MS4 microspheres in pH 1.2 and 7.4 present an n value between 0.4737 and 0.4908, respectively, which suggest that the drug release mechanism of microspheres is similar Fickian diffusion type, but drug release mechanism of MS0 microspheres is non-Fickian drug diffusion type. It is evident from Table 3 that the correlation coefficient (R) values approached unity, suggesting a best fit to the Fickian model.

3.5. Cytotoxicity test

It is known that the cytotoxicity of the biomaterials is extremely important for their future applications. In this study, the cytotoxicity of MS0 and MS4 is investigated by MTT assay. Cytotoxicity study of the MS0 and MS4 microgels with different concentrations is displayed in Fig. 5. The results demonstrate that there is no significant decrease in cell viability when the concentration of MS0 or MS4 is between 20 and 140 mg/mL. After the 30 h cytotoxicity test, the cells in the P(DEA-co-MAA) microspheres extracts has no morphological change and the cell confluency is nearly 100%. The degree of floating cells is 0% during the test period. The results indicate that

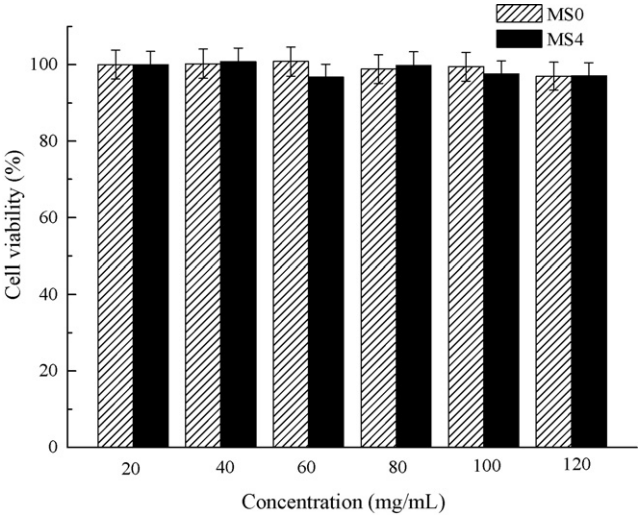

Fig. 5. Cytotoxicity study of the MS0 and MS4 microgels with different concentrations.

the P(DEA-co-MAA) microspheres pass the qualitative cytotoxicity test, and they have no apparent cytotoxicity.

4. Conclusions

In this work, a series of temperature- and pH-responsive P(DEA-co-MAA) microspheres of nanometer sized with core–shell structure have been prepared by SFEP method. These microspheres exhibited temperature-induced phase transition behavior over small changes. By increasing MAA content in the copolymers, their LCST increased, and LCST could reach the body's physiological temperature when the amount of MAA was 100 μL. The polar character of the MAA units changes from strongly hydrophobic when it was protonated at acidic conditions to highly hydrophilic upon ionization, the change of the polarity and solubility led to the microgels were sensitive to environmental pH changes. The cytotoxicity of all prepared microgels was low, and they had no apparent cytotoxicity. The sharp tunable phase transitions of dual temperature and pH-responses of core–shell microspheres around neutral pH may be exploitable in drug delivery and hyperthermia applications.

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