Encapsulation of Curcumin by Methoxy Poly(ethylene glycol-*b*-aromatic anhydride) Micelles

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ABSTRACT: Suitable carrier systems for sustained release of curcumin were studied by using the self-assembled polymeric micelles. Poly(ethylene glycol) methyl ether and poly(aromatic anhydride) were used as the hydrophilic and hydrophobic blocks, respectively, in forming amphiphilic diblock copolymers. Four different types of polymers methoxy poly(ethylene glycol-*b*-1,3-bis(*p*-carboxyphenoxy)propane) ($mPEG_{5000}CPP$, $mPEG_{2000}CPP$), methoxy poly(ethylene glycol-*b*-1,6-bis(*p*-carboxyphenoxy)hexane) ($mPEG_{5000}CPH$, $mPEG_{2000}CPH$) were synthesized via melt condensation approach. Micelles were formed at very low polymer concentration with stable hydrophobic cores. The particle sizes of micelles remained stable during degradation period. All four different polymeric micelles showed low cytotoxicity toward human fibroblasts cells and can kill cancer cells in very low concentrations. High loading efficiency and drug content were observed in curcumin-loaded micelles. Curcumin showed mild initial burst (30% of drug loading in the first 24 h) when released from the micelles and its release was sustained for at least 18 days. These micelles, especially those of $mPEG_{5000}CPP$, show potential to serve as the delivery vehicles for sustained release of curcumin.
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Key words: amphiphilic diblock copolymers; poly (aromatic anhydride); polymeric micelles; drug release

INTRODUCTION

Curcumin, a yellow polyphenol extracted from the rhizome of turmeric (*Curcuma longa*),[1] has potent anticancer properties,[2–4] but its low solubility[5,6] in PBS limits its application.

Polymeric micelles[7,8] represent a class of micelles and are formed from block copolymers consisting of hydrophilic and hydrophobic monomer units[9] by a core-shell structure.[10] They can be used as efficient carriers for hydrophobic compounds, which exhibit poor solubility in water by themselves,[11] and can provide protection to compounds with low stability in a physiological environment.[12] Compared to other forms of nanosized carriers, polymeric micelles are more stable toward dilution in biological fluids.[12] Studies to date indicate that polymeric micelles can solubilize many hydrophobic anticancer drugs,[8,13] which extend their applicability in cancer chemotherapy[14,15] and produce no severe allergic reactions so far.

The most commonly used types of materials to form micelles were poly(ethylene glycol) methyl ether (mPEG)[16] and polyester.[17,18] mPEG is often used to build hydrophilic block[19] of micelle-forming copolymers, because it is biocompatible, highly soluble,[20] and hydrated in water.[21,22] Polyesters, such as poly(lactic-*co*-glycolic acid) (PLGA), are used for the hydrophobic block.[23] However, some drawbacks exist in PLGA, including nonlinear protein release, moisture-induced protein aggregation that result from the bulk erosion mode of degradation[24] and unstable sizes with obvious initial burst. Also, the local acidity[25] presents another problem for PLGA, as the pH value may lower to pH 3 after 35 days.[26]

To overcome these problems, polyanhydrides have been considered to be used as carriers of drugs to various organs of the human body such as brain, bone, blood vessels, and eyes.[27] This class of polymers is characterized by chemistry-dependent surface erosion and payload release, moderate pH microenvironments, and superior protein stabilization capabilities.[28,29] Also, polyanhydrides had been approved by Food and Drug Administration for human use, and it could be removed by human metabolism after degradation. Therefore, poly(sebacic anhydride) (PSA) was studied to be used as the hydrophobic block.[21,30,31] However, PEG-PSA had low-curcumin encapsulation efficiency (EE) and fast degradation and release rate in our own experiences (data not shown).

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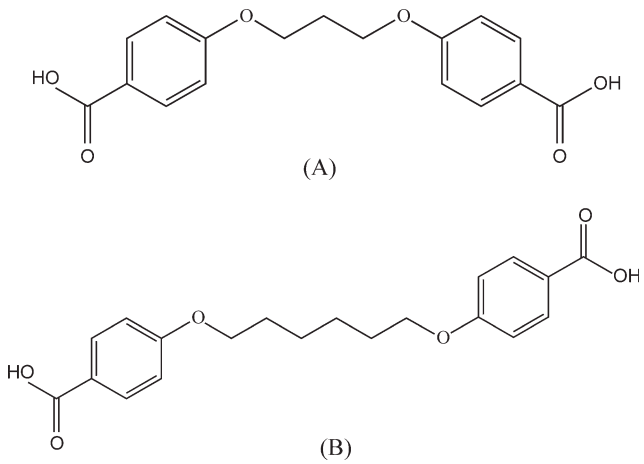

Figure 1 The chemical struture of (A) 1,3-bis(*p*-carboxyphenoxy)propane (CPP) and (B) 1,6-bis(pcarboxyphenoxy)-hexane (CPH).

Polyanhydrides of aromatic acids show better mechanical properties, stability,[32] and longer release and degradation time than aliphatic polyanhydrides.[33,34] In this study, poly(bis(*p*-carboxyphenoxy) alkane)s were chosen to be the hydrophobic component (Fig. 1). Prior studies also showed the low toxicity of these materials.[35,36] The effect of different backbones on erosion rates of this series was demonstrated by others when the number of methylene groups in the backbone increased from one to six, thus decreasing the reactivity of the anhydride linkage and rendering the polymer more hydrophobic, and the erosion rates underwent a decrease of three orders of magnitude.[37] In this study, we are aiming at making new types of micelles from mPEG-polyanhydrides of aromatic acids, which have more stable release rate with much improved loading efficiency, drug content, and longer duration period for the release of curcumin.

MATERIALS AND METHODS

Materials

Monomethoxyl poly(ethylene glycol) (mPEG) (M_n = 5000 and 2000) and curcumin and pyrene were purchased from Fluka. 1,3-Dibromopropane and 1,6-dibromohenxane were supplied by Acros. 1,4-Dioxane was obtained by Fisher Scientific. Succinic anhydride was from Merck. *p*-Hydroxybenzoic acid was purchased from Lancaster.

Succinic anhydride modification of mPEG

Monomethoxyl poly(ethylene glycol) $(mPEG)_{5000}$ (10 g) was dissolved in 50 mL dichloromethane together with 0.56 mL of triethylamine. In another bottle, 0.9 g of succinic anhydride was dissolved in 50 mL of 1,4-dioxane and was added 0.244 g of 4-dimethylaminopyridine. Combine the above solutions after both had dissolved completely, and then the mixture was put under nitrogen protection for 24 h at room temperature. Solvent was then removed by rotary evaporator. Tetrahydrofuran (10–15 mL) was then added. The solution was dialyzed for 24 h with deionized water (DI water) and lyophilized. Modification of $mPEG_{2000}$ was performed using the same method.

Synthesis of 1,3-bis(*p*-carboxyphenoxy)propane (CPP), 1,6-bis(*p*-carboxyphenoxy)hexane (CPH), and their prepolymer

p-Hydroxybenzoic acid (13.8 g) and sodium hydroxide (8.0 g) were dissolved in 40 mL DI water, and 1,3-dibromopropane (5.15 mL) was added drop-wise into the mixture. The mixed solution was refluxed for further 3.5 h. Sodium hydroxide (5*M*, 10 mL) was added in the mixture to reflux for another 2 h. After cooling, precipitate was washed with methanol, then dissolved in 300 mL of DI water, heated to 60°C, and this solution was acidified by sulfuric acid to pH 2. The precipitate was filtrated, washed with acetone, and dried under vacuum at room temperature. CPH was obtained in the same manner if 1,3-dibromopropane was replaced with 1,6-dibromohexane.

CPP or CPH (5 g) was refluxed with acetic anhydride (100 mL) under nitrogen protection for 20 min at 150°C. The unreacted acetic anhydride was removed under vacuum at 60°C. The crude prepolymer was recrystallized from dichloromethane and then washed by anhydrous ethyl ether/petroleum ether mixture (1:1,v/v). Prepolymer was obtained under vacuum and stored at −20°C before used.

Synthesis amphiphilic copolymers

Prepolymers of succinic anhydride-modified $mPEG_{5000}$ 500 mg (or $mPEG_{2000}$ 200 mg) and 40 mg CPP (or CPH) were polymerized by melt condensation at 180°C under vacuum for one hour. The crude product was dissolved in dichloromethane and then purified by precipitation in iced ethyl ether. The precipitate was separated by filtration and washed with ethyl ether. $mPEG_{5000}$CPP, $mPEG_{5000}$CPH, $mPEG_{2000}$CPP, and $mPEG_{2000}$CPH were dried under vacuum at room temperature for 24 h. A generalized procedure for synthesis of these amphiphilic copolymers is presented in Figure 2.

Characterization of copolymers

^{1}H-NMR spectroscopy was performed on a 500 MHz NMR spectrometer (Varian Unityinova 500 NMR) at

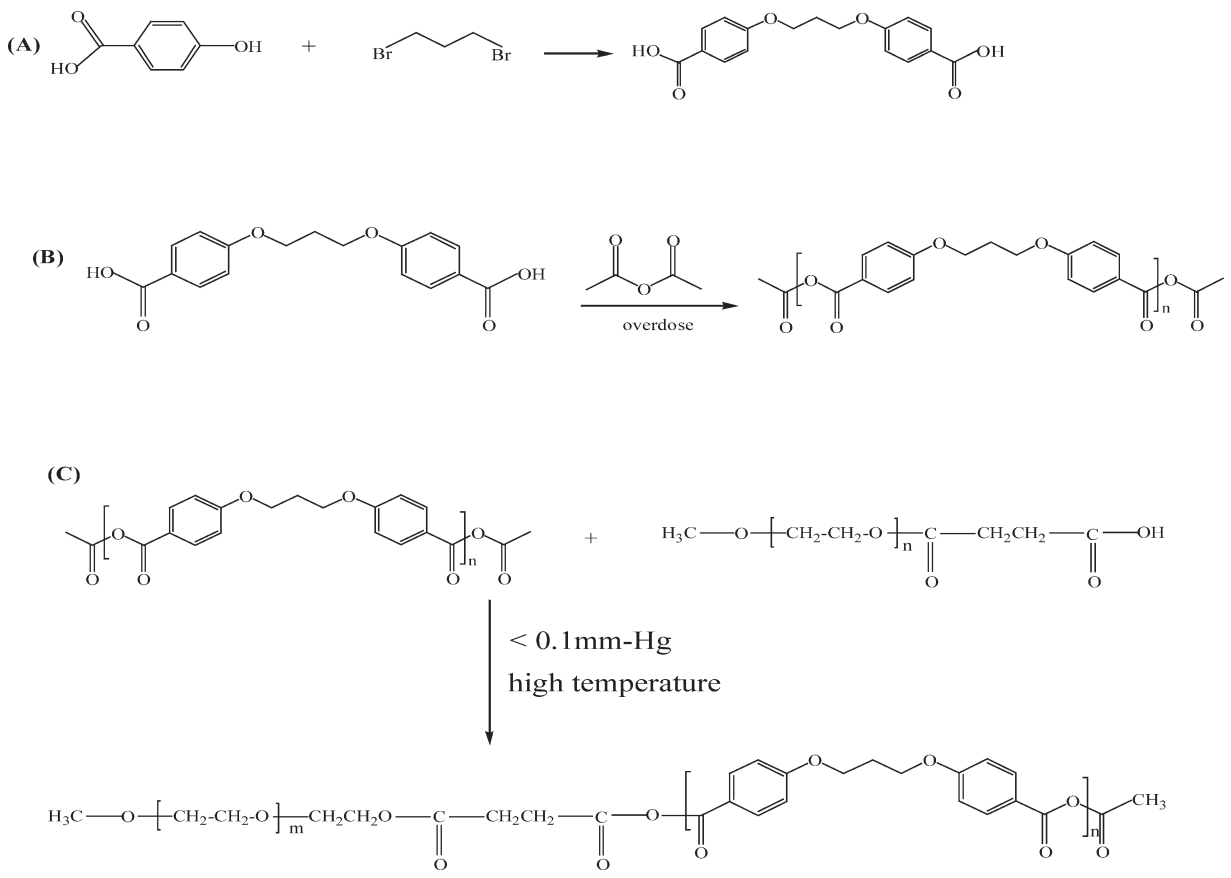

Figure 2 Synthesis scheme of (A) CPP, (B) prepolymer of CPP, and (C) amphiphilic copolymers.

room temperature with $CDCl_3$ to study molecular structure. Molecular weight and molecular weight distributions were determined by gel permeation chromatography (GPC, RI-2031, PU-2080, JASCO) and ^{1}H-NMR, which used ratio of $\delta = 3.35$ ppm (CH_3O—CH_2—) and $\delta = 6.94$ ppm (—C=CH—C) for the calculation of molecular weight. FTIR spectra were measured using Fourier transfer infrared (Perkin–Elmer system 2000) with KBr pellets.

Critical micellization concentration determination

The CMC of copolymers was determined with a fluorescence spectrophotometer (FL 2500, Hitachi), using pyrene as the fluorescence probe. Namely, apply 200 mg of copolymer into 5 mL of THF and sonicate for 10 min. Slowly add 20 mL of DI water into the polymer/THF solution. Mix the prepared solution in a magnetic mixer for 12 h. A polymer solution with a concentration of 10 mg/mL would be obtained. Dilute the polymer solution with the same factor into 16 vials. The pyrene solution is prepared by adding 5 mg of pyrene into 250 mL of THF. The prepared solution is then sonicated for 3 h and placed in a 4°C refrigerator for 1 day. The pyrene solution should avoid direct irradiation at all times when being prepared. Add 50 mL of deionized water into 1.0 mL of the previously prepared pyrene solution. Mix two solutions in equal volume and sonicate for 5 min (amplitude : 100%, pulse : 5 s). The concentration range of copolymer solution and pyrene mixture was from 1.53×10^{-4} to 5.0 mg/mL. The fluorescence excitation spectra were measured at emission wavelength of 378 nm. The ratio of fluorescence intensity at 336 and 340 nm (I340/I336) was calculated and plotted against the logarithm of the copolymer concentrations to determine the CMC.

Preparation of micelles

About 15.0 mg of copolymer sample in 1 mL of tetrahydrofuran was added drop-wise into 10 mL of DI water in 5 min under sonication. The solution was stirred over night, freeze dried, and dissolved back in phosphate buffered saline (PBS, pH = 7.4). Preparation of curcumin-loaded micelles was done in the similar way by adding 1.500, 1.125, and 0.750 mg of curcumin to the copolymer solution to get products of drug/polymer ratio of 0.100, 0.075, and 0.050, respectively.

Characterization of micelles

Dynamic laser scattering (photon LPA3100, Otsuka) was used to measure micelles size with a

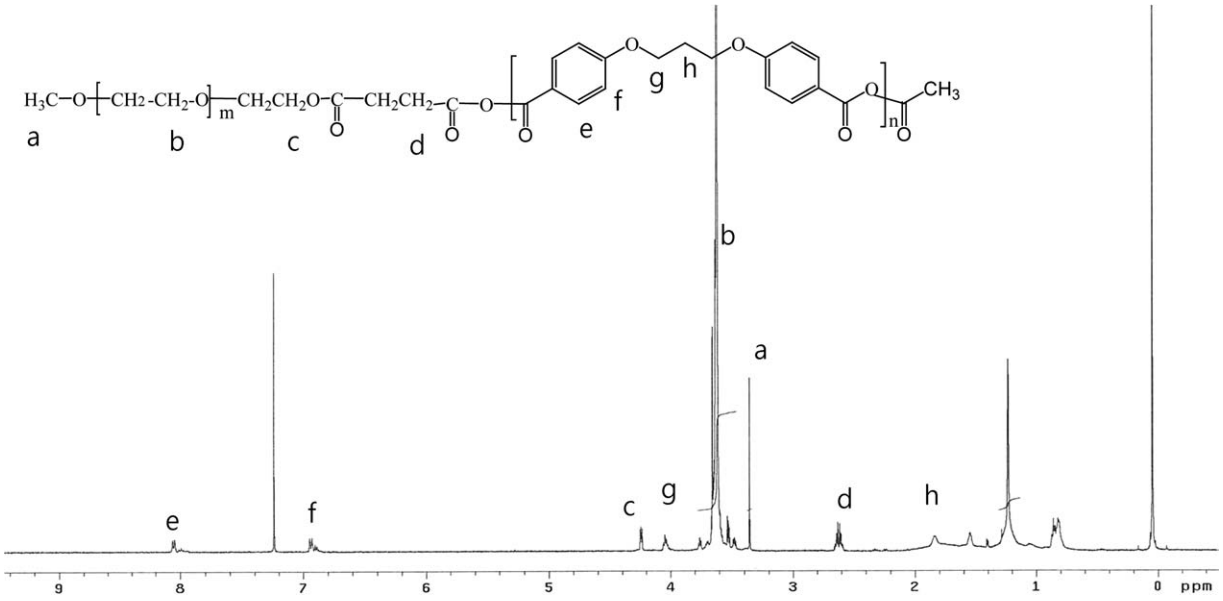

Figure 3 ^{1}H NMR spectrum of $mPEG_{2000}CPP$.

photocorrelator at room temperature. ^{1}H-NMR spectroscopy was used on a 500 MHz NMR spectrometer (Varian Unityinova 500 NMR) at room temperature with D_2O. pH value was measured by a pH meter (Shindengen).

The morphology of these micelles was observed by scanning electron microscope (SEM) and transmission electron microscope (TEM). SEM was used to study the surface, and the dried tablets were coated with gold before imaging. The TEM specimens for micelles and curcumin-loaded micelles were observed under a JEM-2100 (HT) instrument. Samples were prepared by placing a drop of micelles solution on the Formvar-coated copper TEM grid and then dyed by phosphatotungstic acid (0.5 wt %).

Evaluation of DL and EE

High-performance liquid chromatography (HPLC, Hitachi) was used to quantify the drug loading (DL). The mobile phase was a mixture of acetonitrile/0.01 *M* acetic acid solution (70 : 30 v/v). UV wavelength was 420 nm and flow rate was 1 mL/min in 35°C.[38] A Lichrospher 100 RP-18 column was used. The DL and encapsulation efficiency (EE) were calculated according to the following equations.

$$\text{DL } (\%) = \frac{\text{weight of drug in micelles}}{\text{weight of drug} - \text{loaded micelles}} \times 100\%$$

$$\text{EE } (\%) = \frac{\text{weight of drug in micelles}}{\text{weight of drug originally provided}} \times 100\%$$

In vitro curcumin release

The drug release experiments were performed at 37°C in an orbital shaker incubator (Hotech instruments Corp.) at 100 rpm. Lyophilized curcumin-loaded micelles were resuspended in 10 mL phosphate buffer solution (pH = 7.4). After dissolved completely, the solution was divided into 12 microcentrifugation tubes. At certain time intervals, one Eppendorf tube was centrifuged for 5 min at 8000 rpm (HERMLE) at 4°C. A portion of supernatant (0.2 mL) was added to 0.2 mL of acetonitrile. The mixture was then filtrated by 0.22-μm syringe filter (Millipore). The amount of curcumin in the supernatant calculated after curcumin in the sample solution was quantified by HPLC using the conditions described before. It was determined that no micelles were left in the supernatant after centrifugation and that released curcumin never exceeded its solubility limit. So the measured curcumin concentration would give accurate assessment of release profiles.

Toxicity test

Cytotoxicity of the polymeric materials, with or without curcumin, was evaluated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method. Shortly, 5×10^4 human fibroblast cells/well in a 48-well plate were used to test micelle's toxicity and 1×10^3 HeLa cells/well in a 96-well plate were used to test curcumin-loaded micelle's toxicity toward cancer cells.

After cell adhesion for 1 day, testing samples were added to the wells and incubated for 48 h. MTT solution at 2.5mg/mL was used for the test. MTT is a

TABLE I
The Molecular Weight of Polymers

	M_n[a]	M_w[a]	PDI[b]	M_n[c]
$mPEG_{5000}COOH$	4646	4822	1.04	
$mPEG_{2000}COOH$	2076	2193	1.06	
CPPA	402	423	1.05	464
CPHA	634	783	1.24	654
$mPEG_{-5000}CPP$	4891	5057	1.04	5500
$mPEG_{-2000}CPP$	2200	2553	1.16	2500
$mPEG_{-5000}CPH$	4892	5049	1.04	5900
$mPEG_{-2000}CPH$	2435	2829	1.16	2400

[a] Determined by GPC measurement in THF.
[b] Polydispersion (M_n/M_w).
[c] Calculated by ^{1}H-NMR.

yellow tetrazole, which is reduced to purple formazan by mitochondria in living cells. Optical density measured at 570 nm would give data on the number of living cells.

RESULTS AND DISCUSSION

Characterization of copolymers

The molecular structures of copolymers were characterized by ^{1}H-NMR. For 1,3-bis(*p*-carboxyphenoxy) propane anhydride, the peaks at 7.96 (e) and 6.94 (f) ppm are the aromatic protons to anhydride bonds, and peaks at 4.10 (g) and 1.92 (h) ppm represent the methene protons of CPP segments. The sharp peak at 3.64 (b) ppm is attributed to methylene protons of mPEG (see Fig. 3). The molecular weights were measured by GPC and ^{1}H-NMR as shown in Table I. In FTIR spectra, modified $mPEG_{5000}COOH$ and $mPEG_{2000}COOH$ both showed the peak at 1730 cm^{-1}, which corresponds to carboxyl group (see Fig. 4).

Characterization of micelles

Micelles size measured at room temperature were shown in Table II. It is shown that the size did not change much before or after lyophilization and redissolving. Also, the size showed mild decrease after curcumin incorporation. Loaded more curcumin, the size decreased slightly because of the more hydrophobic force, only micelles made by $mPEG_{2000}CPH$ exhibited the largest sizes due to its instability. The distribution of sizes was rather narrow and uniform.

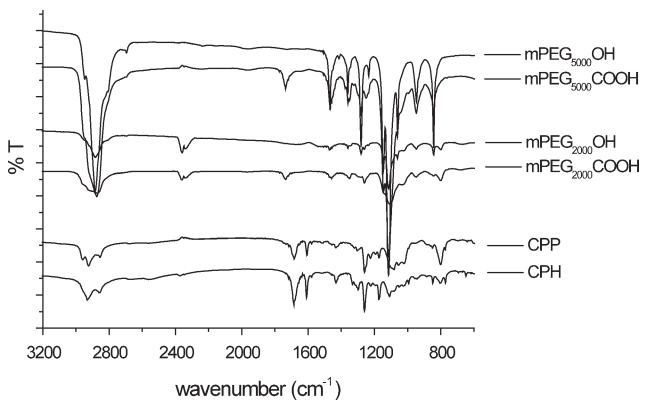

Figure 4 FT-IR spectra of $mPEG_{5000}OH$, $mPEG_{5000}COOH$, $mPEG_{2000}OH$, $mPEG_{2000}COOH$, CPP, and CPH.

After redissolving in PBS, the size stability of the micelles was observed for 30 days at 37°C in an orbital shaker. Micelles composed of $mPEG_{5000}CPP$ maintained an average size smaller than 200 nm during that period of time and have the best stability of the micelles tested. All the other micelles have relatively the same size stability, though $mPEG_{2000}$ series of polymers had larger sizes than those of $mPEG_{5000}$, series (see Fig. 5). We took 200 nm as the basis for evaluation, because micelles larger than this size are easily recognized and removed by immune systems.

Acidification of the surrounding environment due to degradation of biomaterials may cause harmful effect to the local tissues. We monitored the pH values of the micelles solution for 30 days. The results were shown in Figure 6: $mPEG_{5000}$ series showed only small decline in pH, to between 6.90 and 7.00, while $mPEG_{2000}$ series exhibited larger pH decrease. This can be attributed to the fact that for the same weight/volume concentration, $mPEG_{5000}$ series polymers contain less diacid residues. However, all four types of micelles did not acidify the medium too much, and so damages caused by local acidity can be avoided.

^{1}H-NMR was used to analyze the surface chemical structure of micelles using D_2O as the solvent. As shown in Figure 7, the peaks related to CPP and CPH all disappeared, including 2.20, 6.94, and 7.96 ppm. On the other hand, the characteristics peak of mPEG, 3.60 ppm, remained, when compared with the spectrum observed in $CDCl_3$. This suggests that CPP and CPH, the hydrophobic segments, were indeed embedded in the core, confirming the core-shell structure of the self-assembled micelles.

Micelles morphology was examined by SEM. Spherical particles were observed, with no obvious aggregation (see Fig. 8). The sizes of the particles were about 200 nm. It can also be observed in Figure 8 that the micelle of $mPEG_{5000}CPP$ were more uniform in sizes and also smaller, while the $mPEG_{2000}$ series were larger with some aggregated particles. The same trend also appeared in curcumin-loaded micelles. As shown in Figure 9, the images of micelles and drug-loaded micelles under TEM also gave evidence of spherical morphology with core-shell structures.

CMC can be obtained by the plot of intensity ratio I340/I336 and the logarithm of copolymer concentration. CMC values for the four materials were all

TABLE II
The Sizes of Micelles Before and After Freeze-Drying Process (n = 3)

Polymer	Drug/ Polymer (D/P)	Particle size (nm)	Polydispersity (PD) ($\times 10^{-1}$)	Resuspension particle size (nm)	Polydispersity (PD) ($\times 10^{-1}$)
$mPEG_{5000}CPP$	0.100	140.6 ± 5.6	1.49 ± 0.1	145.9 ± 5.0	1.29 ± 0.1
	0.075	152.6 ± 3.9	1.59 ± 0.1	157.1 ± 5.8	1.61 ± 0.1
	0.050	153.4 ± 6.4	1.74 ± 0.1	157.8 ± 4.3	1.73 ± 0.2
	0.000	179.5 ± 6.8	1.90 ± 0.1	169.2 ± 11.5	2.19 ± 0.1
$mPEG_{5000}CPH$	0.100	143.5 ± 2.9	1.56 ± 0.1	140.2 ± 2.9	1.28 ± 0.1
	0.075	155.3 ± 3.4	1.41 ± 0.2	159.4 ± 5.7	1.68 ± 0.0
	0.050	152.1 ± 2.2	1.40 ± 0.2	146.6 ± 3.2	1.29 ± 0.1
	0.000	196.0 ± 6.9	2.57 ± 0.1	197.0 ± 5.5	2.96 ± 0.1
$mPEG_{2000}CPP$	0.100	158.2 ± 5.7	1.39 ± 0.2	155.2 ± 4.4	1.27 ± 0.1
	0.075	167.9 ± 4.1	1.49 ± 0.0	160.3 ± 5.3	1.47 ± 0.3
	0.050	170.4 ± 1.7	1.39 ± 0.2	179.7 ± 4.4	1.50 ± 0.1
	0.000	182.0 ± 1.2	1.92 ± 0.2	195.2 ± 3.5	2.43 ± 0.1
$mPEG_{2000}CPH$	0.100	203.8 ± 4.4	1.25 ± 0.1	206.7 ± 5.8	1.12 ± 0.1
	0.075	210.0 ± 3.8	1.21 ± 0.2	218.9 ± 9.3	1.39 ± 0.3
	0.050	201.3 ± 4.2	1.20 ± 0.1	196.7 ± 8.3	1.30 ± 0.2
	0.000	165.5 ± 2.0	2.71 ± 0.1	172.5 ± 4.6	2.87 ± 0.1

under 10 mg/L, The standard free energy for micellization (ΔG) is usually calculated from

$$\Delta G = \mathrm{RT} \ln(X_{\mathrm{CMC}})$$

where R is the universal gas constant, T is the absolute temperature, and X_{cmc} is the corresponding CMC expressed as mole fraction.[21] ΔG obtained in this study was all smaller than 0, that is, the four copolymers can self-assemble to form micelles spontaneously at a very low concentrations (see Table III).

EE and curcumin content

Encapsulation efficiency (EE) of curcumin was evaluated from the ratio of curcumin in micelles and the initial curcumin provided for loading. Each formulation was tested in three repeats. As shown in Figure 10, curcumin EE can reach ~ 75.0%, when the D/P ratio was low. $mPEG_{2000}CPP$ had higher EE, but the difference is not significant. At higher D/P ratio, for example, D/P = 0.100, the EE values were lower, with curcumin content approached 6.75%. The four polymeric micelles had similar drug content at the same D/P ratio.

Curcumin is a poor water-soluble compound, and the solubility[39] of curcumin in PBS at 37°C is 2.99 × 10^{-8} mol/L. In this study, curcumin encapsulation by polymeric micelles greatly enhanced its solubility up to 10^4-fold. Further attempts to increase D/P ratio or curcumin content resulted in turbid solution, which was caused by nonencapsulated curcumin and unstable micelles. The current micelles formulations were shown to alleviate the solubility problem of this drug.

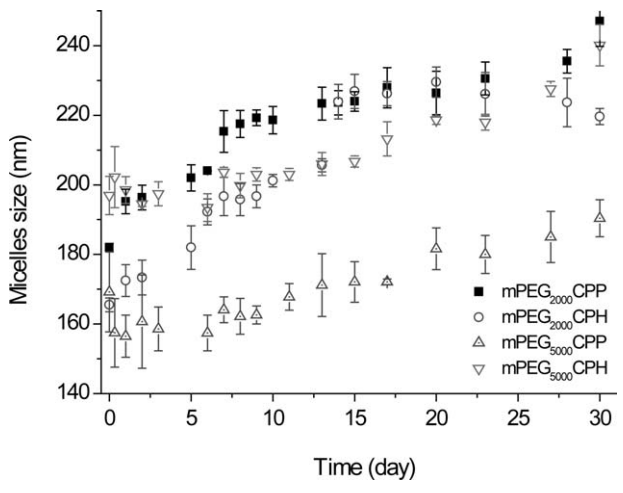

Figure 5 The size variation of micelles during a 30-day period.

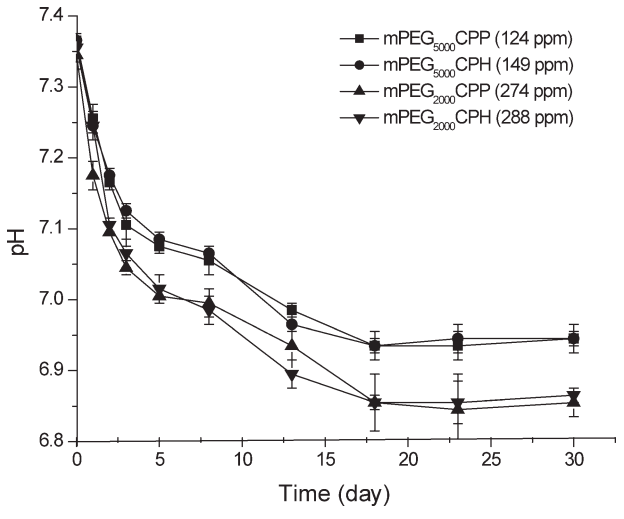

Figure 6 The pH profiles of micellar solution (in PBS) during a 30-day period.

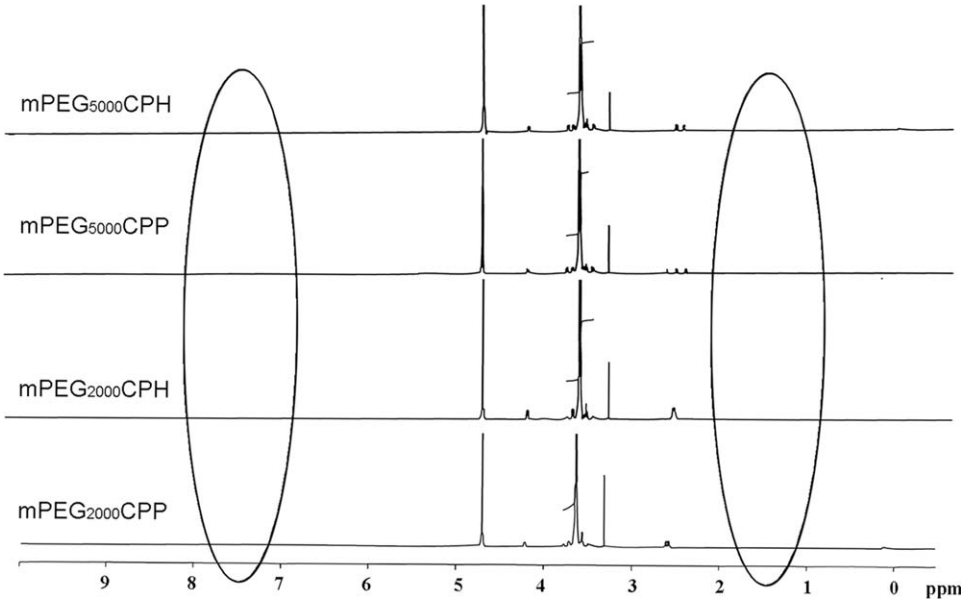

Figure 7 ^{1}H-NMR spectra of the four kinds of micelles dissolved in D_2O.

Curcumin release

The release profiles of four types of polymeric micelles carrying curcumin at three different ratios were observed. After 24 h, with the exception of $mPEG_{5000}CPP$, micelles, other micelles released more than 40% of their initial drug content and showed to have obvious initial burst phenomenon. After the following 18 days of drug release, it was found that almost 75% or more drug were released in these four types of polymeric micelles. $mPEG_{2000}CPP$ was the most unstable one, while $mPEG_{5000}CPP$ was quite stable (Fig. 11). Comparing these micelles, the length of mPEG segment influences the drug release behavior greatly; longer mPEG blocks [comparing Fig. 11(a–c) and (b–d)] render the micelles with slower and more stable drug release rates, perhaps due to their ability to stabilize the micelles, at least in the first few days of the release. On the other hand, the different hydrophobic blocks CPP or CPH used in the micelles [comparing Fig. 11(a,b) and 11(c,d)] do not show significant effects on drug release rates.

Cytotoxicity of micelles

Cytotoxicity of methoxy poly(ethylene glycol-*b*-aromatic anhydride) micelles was studied using human fibroblasts via MTT assay. All observed cell viability data from MTT test were greater than 90% (see

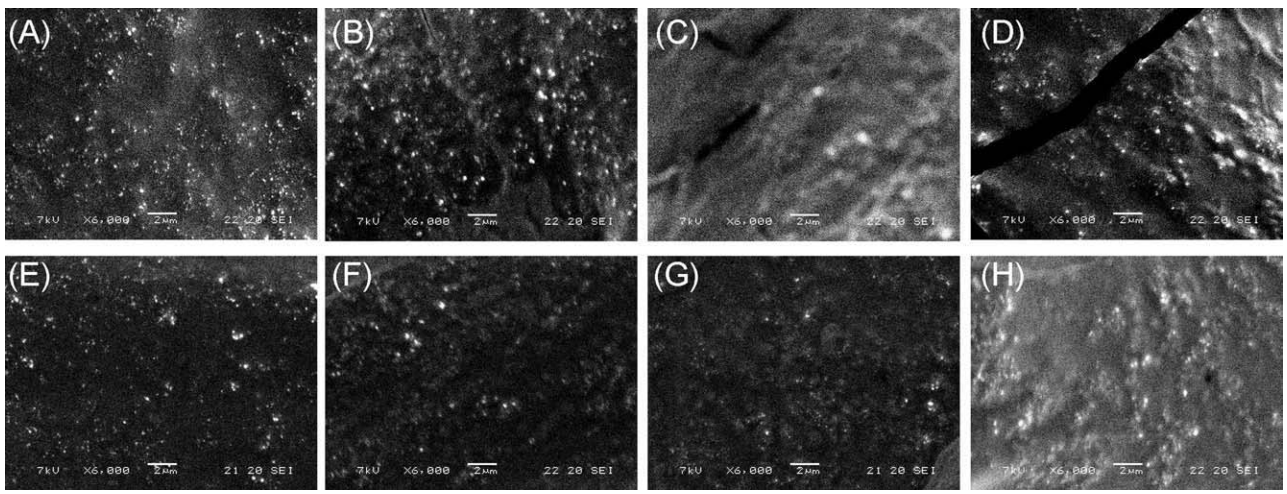

Figure 8 The SEM microphotographs of polymeric micelles: (A) $mPEG_{5000}CPP$, (B) $mPEG_{5000}CPH$, (C) $mPEG_{2000}CPP$, and (D) $mPEG_{2000}CPH$; and curcumin-loaded micelles: (E) $mPEG_{5000}CPP$, (F) $mPEG_{5000}CPH$, (G) $mPEG_{2000}CPP$, and (H) $mPEG_{2000}CPH$.

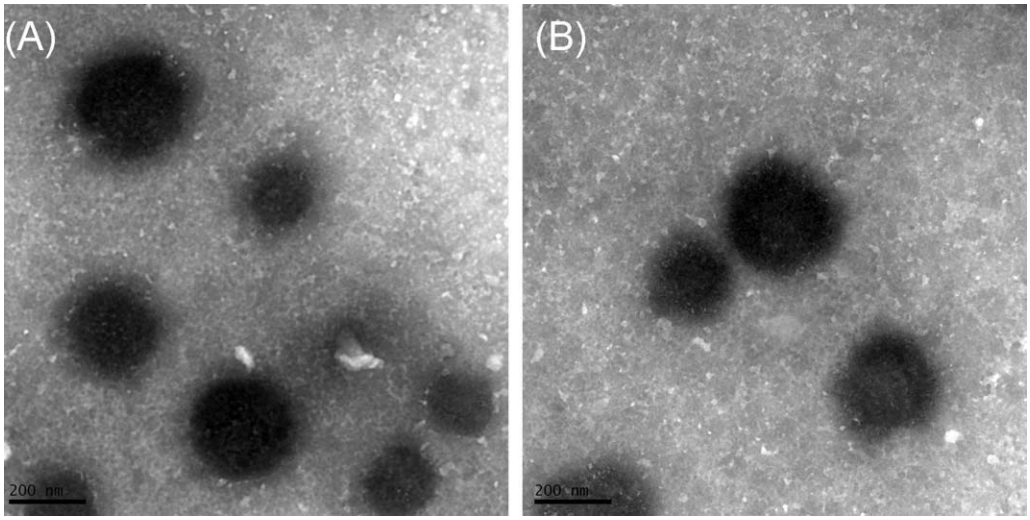

Figure 9 The TEM microphotographs of (A) $mPEG_{5000}CPP$ polymeric micelles and (B) $mPEG_{5000}CPP$ curcumin-loaded micelles. Scale bars are both 200 nm.

Fig. 12). This clearly demonstrated that the materials were of low cytotoxicity.

In addition, the curcumin-loaded micelles had the toxicity toward HeLa cancer cells, the 50% inhibiting concentration (IC_{50}) were shown less than 10 ppm of curcumin concentration (see Fig. 13). Clinically, curcumin often used dimethyl sulfoxide as cosolvent, the IC_{50} value is about 3.5 ppm. These results were similar to ours. This means drug carried by mPEG-poly(anhydride) micelles does not lose its activity against cancer cells.

CONCLUSIONS

Novel types of amphiphilic mPEG-poly(anhydride) polymers were prepared by melt-condensation. The four different polymers ($mPEG_{5000}CPP$, $mPEG_{5000}CPH$, $mPEG_{2000}CPP$, and $mPEG_{2000}CPH$) formed nanomicelles via self-assembly at very low concentration. The pH of the solution surrounding the micelles decreased a little during 30 days' degradation, thus can avoid the damages caused by the acidification of surrounding tissues as seen in many polyester biomaterials.

Curcumin was incorporated into these micelles with EE around 75%, depending on loading ratio. Drug content was around 6.8%, and, as a result, the solubility of curcumin was raised more than 10^4-fold. Stability of micellar sizes was good at 37°C for at least 15 days, and the steady release of curcumin for more than 10 days was achieved *in vitro* for the $mPEG_{5000}CPP$ group. Compared to the published results,[40–43] the polymeric micelles in this study provided more stable and extended period of release for curcumin. All four different polymeric micelles showed low cytotoxicity toward human fibroblasts cells and can kill HeLa cancer cells in low concentration. Therefore, we expect that the nanomicelles of methoxy poly(ethylene glycol-*b*-aromatic anhydride) to be useful as drug carriers for the delivery of various hydrophobic drugs.

TABLE III
CMC and ΔG Value of Copolymers

Copolymer	CMC (mg/L)	ΔG (kJ/mol)
$mPEG_{5000}CPP$	8.13	−43.21
$mPEG_{2000}CPP$	7.29	−41.53
$mPEG_{5000}CPH$	8.19	−43.37
$mPEG_{2000}CPH$	7.31	−41.42

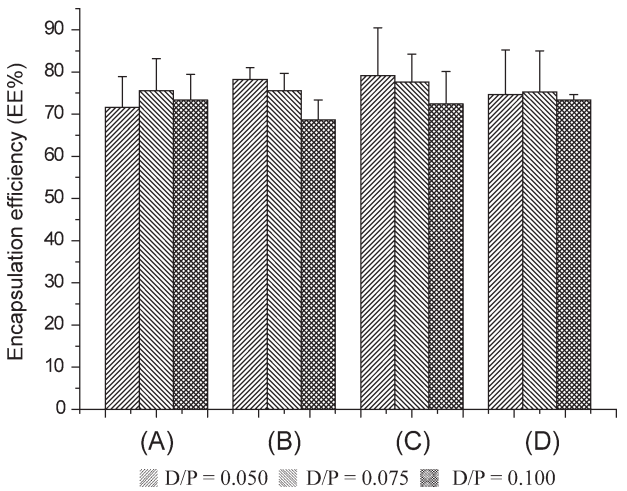

Figure 10 Encapsulation efficiency of curcumin-loaded micelles. (A) $mPEG_{5000}CPP$, (B) $mPEG_{5000}CPH$, (C) $mPEG_{2000}CPP$, and (D) $mPEG_{2000}CPH$.

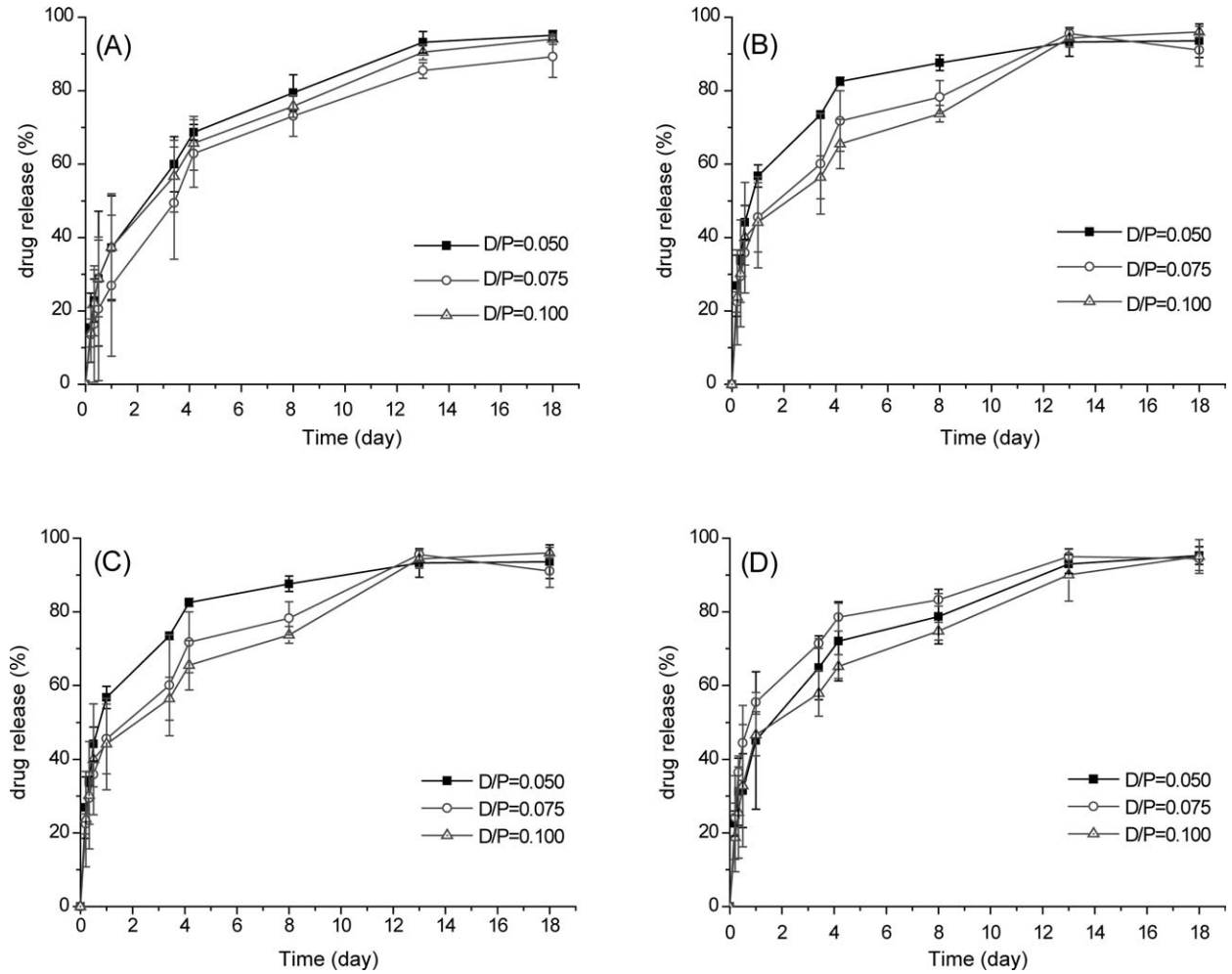

Figure 11 In vitro drug release profiles of micelles in pH = 7.40 PBS at 37°C. (A) $mPEG_{5000}CPP$, (B) $mPEG_{5000}CPH$, (C) $mPEG_{2000}CPP$, and (D) $mPEG_{2000}CPH$ ($n = 3$).

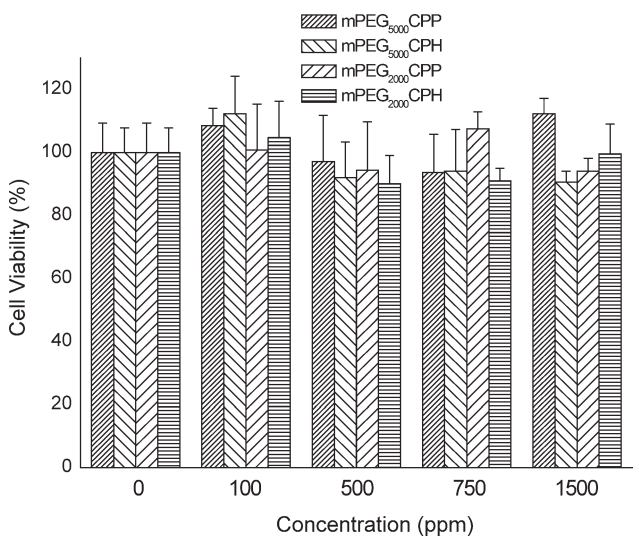

Figure 12 Cytotoxicity test (MTT assay) of different drug-free polymeric micelles at various concentrations.

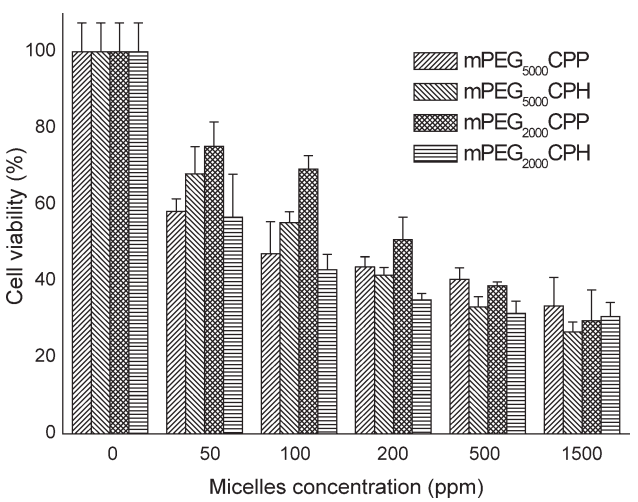

Figure 13 Cytotoxicity test (MTT assay) results of different curcumin-loaded micelles at various micelle concentrations.

References

1. Shi, M. X.; Cai, Q. F.; Yao, L. M.; Mao, Y. B.; Ming, Y. L.; Ouyang, G. L. Cell Biol Int 2006, 30, 221.
2. Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. J Pharm Biomed Anal 1997, 15, 1867.
3. Roy, S. C. M.; Siddiqi, M.; Bhattacharya, R. K. Asian Pacif J Cancer Prev 2002, 3, 61.
4. Rashmi, R.; Kumar, T. R. S.; Karunagaran, D. FEBS Lett 2003, 538, 19.
5. Tonnesen, H. H.; Masson, M.; Loftsson, T. Int J Pharm 2002, 244, 127.
6. Ireson, C. R.; Jones, D. J. L.; Orr, S.; Coughtrie, M. W. H.; Boocock, D. J.; Williams, M. L.; Farmer, P. B.; Steward, W. P.; Gescher, A. J Cancer Epidemiol Biomarker Prev 2002, 11, 105.
7. Jones, M. C.; Leroux, J. C. Eur J Pharm Biopharm 1999, 48, 101.
8. Kwon, G. S.; Okano, T. Adv Drug Del Rev 1996, 21, 107.
9. Torchilin, V. P. Cell Mol Life Sci 2004, 61, 2549.
10. Ramzi, A.; Rijcken, C. J. F.; Veldhuis, T. F. J.; Schwahn, D.; Hennink, W. E.; van Nostrum, C. F. J Phys Chem B 2008, 112, 784.
11. Lee, S. C.; Kim, C.; Kwon, I. C.; Chung, H.; Jeong, S. Y. J Control Release 2003, 89, 437.
12. Francis, M. F.; Cristea, M.; Winnik, F. M. Pure Appl Chem 2004, 76, 1321.
13. Gaucher, G.; Dufresne, M. H.; Sant, V. P.; Kang, N.; Maysinger, D.; Leroux, J. C. J Control. Release 2005, 109, 169.
14. Bae, Y.; Diezi, T. A.; Zhao, A.; Kwon, G. S. J Control Release 2007, 122, 324.
15. Gao, Z. G.; Fain, H. D.; Rapoport, N. J Control Release 2005, 102, 203.
16. Torchilin, V. P. Adv Drug Delivery Rev 2002, 54, 235.
17. Yoo, H. S.; Park, T. G. J Control Release 2001, 70, 63.
18. Riley, T.; Govender, T.; Stolnik, S.; Xiong, C. D.; Garnett, M. C.; Illum, L.; Davis, S. S. Colloid Surf B-Biointerfaces 1999, 16, 147.
19. Riley, T.; Stolnik, S.; Heald, C. R.; Xing, C. D.; Garnett, M. C.; Illum, L.; Davis, S. S.; Purkiss, S. C.; Barlow, R. J.; Gellert, P. R. Langmuir 2001, 17, 3168.
20. Photos, P. J.; Bacakova, L.; Discher, B.; Bates, F. S.; Discher, D. E. J Control Release 2003, 90, 323.
21. Zhang, N.; Guo, S. R.; Li, H. Q.; Liu, L.; Li, Z. H.; Gu, J. R. Macromol Chem Phys 2006, 207, 1359.
22. Veronese, F. M. Biomaterials 2001, 22, 405.
23. Jeong, B.; Bae, Y. H.; Kim, S. W. Colloid Surf B-Biointerfaces 1999, 16, 185.
24. Weiner, A. A.; Bock, E. A.; Gipson, M. E.; Shastri, V. P. Biomaterials 2008, 29, 2400.
25. Weiner, A. A.; Shuck, D. M.; Bush, J. R.; Shastri, V. P. Biomaterials 2007, 28, 5259.
26. Park, T. G.; Lu, W. Q.; Crotts, G. J Control Release 1995, 33, 211.
27. Kumar, N.; Langer, R. S.; Domb, A. J. Adv Drug Del Rev 2002, 54, 889.
28. Ulery, B. D.; Phanse, Y.; Sinha, A.; Wannemuehler, M. J.; Narasimhan, B.; Bellaire, B. H. Pharm Res 2009, 26, 683.
29. Lee, W. C.; Chu, I. M. J Biomed Mater Res B-Appl Biomater 2008, 84, 138.
30. Wan, S. R.; Huang, J. S.; Yan, H. S.; Liu, K. L. J Mater Chem 2006, 16, 298.
31. Tang, B. C.; Dawson, M.; Lai, S. K.; Wang, Y. Y.; Suk, J. S.; Yang, M.; Zeitlin, P.; Boyle, M. P.; Fu, J.; Hanes, J. Proc Nat Acad Sci USA 2009, 106, 19268.
32. Conix, A. J Polym Sci 1958, 29, 343.
33. Nagata, M.; Ioka, E. Eur Polym J 2006, 42, 2617.
34. Domb, A. J. Macromolecules 1992, 25, 12.
35. Katti, D. S.; Lakshmi, S.; Langer, R.; Laurencin, C. T. Adv Drug Del Rev 2002, 54, 933.
36. Brem, H.; Mahaley, S.; Vick, N. A.; Black, K. L.; Schold, S. C.; Burger, P. C.; Friedman, A. H.; Ciric, I. S.; Eller, T. W.; Cozzens, J. W.; Kenealy, J. N. J Neurosurg 1991, 74, 441.
37. Leong, K. W.; Brott, B. C.; Langer, R. J Biomed Mater Res 1985, 19, 941.
38. Liu, A. C.; Lou, H. X.; Zhao, L. X.; Fan, P. H. J Pharm Biomed Anal 2006, 40, 720.
39. Letchford, K.; Liggins, R.; Burt, H. J Pharm Sci 2008, 97, 1179.
40. Bisht, S.; Soni, S.; Ravi, R.; Karikar, C.; Maitra A.; Maitra, A. J Nanobiotechnol 2007, 5, 3.
41. Ma, Z. S.; Haddadi, A.; Molavi, O.; Lavasanifar, A.; Lai, R.; Samuel, J. J Biomed Mater Res A 2008, 86, 300.
42. Sahu, A.; Goswami P.; Bora U. J Biomater Appl Published online 5 March 2010.
43. Sahu, A.; Bora, U.; Kasoju, N.; Goswami, P. Acta Biomater 2008, 4, 1752.