Synthesis, Characterization, and Drug-Release Behavior of Novel PEGylated Bovine Serum Albumin as a Carrier for Anticancer Agents

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ABSTRACT: To develop a novel pH-sensitive PEGylated carrier for protein-based anticancer agents, we modified bovine serum albumin (BSA) with poly(ethylene glycol) citrate ester (PEG–CA) through amidation with its amino groups. Increasing the mixing ratio of albumin from 3 to 6 with respect to PEG–CA resulted in a 2-fold increase in the degree of albumin modification. Adriamycin (ADR)-loaded PEG–CA–BSA hydrogels and microparticles were prepared, and the cumulative amounts of ADR released from the PEG–CA–BSA hydrogels (phosphate-buffered saline, pH 7.4) showed that all the PEG-CA-$BSA_{(x)}$ (x represents degree of substitution of PEG to amino group of albumin, i.e. 26%, 28%, 31% and 49%) hydrogels had lower ADR release rates with a slight initial burst release. During the first 24 h, the cumulative releases were 15.5% for PEG–CA–$BSA_{(49)}$, 24% for PEG–CA–$BSA_{(31)}$, 31% for PEG–CA–$BSA_{(28)}$, and 38% for PEG–CA–$BSA_{(26)}$. Afterward, all the release rates slowed, and they were almost in the following order: PEG–CA–$BSA_{(26)}$ > PEG–CA–$BSA_{(28)}$ > PEG–CA–$BSA_{(31)}$ > PEG–CA–$BSA_{(49)}$. The release rates of ADR from the microparticles were dependent on the amount of glutaraldehyde. According to our findings, a higher PEG–CA/BSA molar ratio led to a reduced cumulative amount of ADR released from the hydrogels, whereas higher release rates were observed for microparticles with a lower amount of BSA in the conjugates in a pH-dependent manner. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2635–2643, 2011

Key words: biopolymers; drug delivery systems; nanotechnology

INTRODUCTION

Over the past 3 decades, a great deal of research has been devoted to altering the pharmacokinetic and biodistribution profiles of antineoplastic agents by means of various delivery systems such as liposomes,[1] nanoparticles,[2] polymeric micelles,[3,4] and emulsions.[5] Of these, nanoscopic systems have been shown to enhance drug accumulation at tumor sites and consequently reduce the distribution of drugs to healthy tissues.[6,7] Neoplastic tissues generally possess porous or leaky vasculature and poor lymphatic drainage; this results in enhanced permeation of colloidal particles across the endothelium as well as greater retention within tumors.[8–10]

Emulsions possess distinct advantages in comparison with other colloidal systems because of their better biocompatibility, acceptable stability, ability to solubilize large quantities of lipophilic compounds, and relative ease of manufacture on a large industrial scale.[11,12] However, the number of products on the market is relatively low, and this indicates their limited success. The physical instability (caused by the incorporation of drugs) of emulsions, their short blood circulation time, their short half-lives, their high plasma clearance, and their insufficient solubility for some drugs are thought to be the major factors preventing their broader use in drug delivery.[13] Recent studies, however, suggest that PEGylation of surfaces of colloids such as emulsions with a hydrophilic and flexible poly(ethylene glycol)(PEG) could be an alternative method for overcoming the aforementioned problems.[14–16] PEGylation is a process by which one or more chains of PEGs of different molecular masses are attached to target molecules (e.g., peptides, proteins, liposomes, and gene-based therapeutics). It was pioneered by Abuchowski and coworkers[17,18] at Rutgers University for the protection of proteins from biological destruction during delivery to targeted sites. PEGylation has been shown to improve the pharmacokinetic and pharmacodynamic properties of attached polypeptide drugs by acting like an outer barrier and protecting them from proteolytic enzyme degradation, rapid renal clearance, and adverse

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immunological effects.[19–22] Recent studies have also revealed that PEG–drug conjugates are more stable against pH and temperature changes than their non-PEGylated counterparts.[23–27] Moreover, the PEGylation of colloid systems (e.g., emulsions) has been shown to confer a number of clinical benefits, such as prolonged blood circulation time, increased half-life, decreased plasma clearance, and a distribution shift in favor of diseased tissues.[28,29]

The use of macromolecular, water-soluble carriers for anticancer drugs represents a promising approach in cancer therapy. In fact, low solubility in water appears to be an intrinsic property of many anticancer drugs. Thus, nanosized, macromolecular, water-soluble carriers are widely used for poorly soluble drugs to optimize their physicochemical characteristics. The release of drugs from the carrier system is a prerequisite of therapeutic activity for most macromolecular anticancer conjugates, whereas the incorporation of acid-sensitive spacers inside the carriers or between the drug and carrier enables the release of an active drug from the carrier into slightly acidic extracellular fluids or into endosomes or lysosomes of cancer cells.[30] Hence, the purpose of this study was to develop a novel pH-sensitive PEGylated carrier for anticancer agents. For this purpose, a novel PEG–protein conjugate was synthesized and characterized. Bovine serum albumin (BSA) was modified with poly(ethylene glycol) citrate ester (PEG–CA) through amidation with its amino groups with PEG–CA carboxyl groups. PEG–CA–BSA was loaded with adriamycin (ADR) and formulated as hydrogels or microparticles.

EXPERIMENTAL

Materials

A PEG diacid (acid number = 175, 96–98%) with an average molecular weight of 600 (PEG_{600} diacid) was obtained from Fluka Chemical Corporation (Ronkonkoma, USA); and dried over Na_2SO_4. BSA was purchased from Sigma Chemical Co. (St. Louis, MO). Citric acid, thionyl chloride, and *N,N*-dicyclohexylcarbodiimide (DCC) were purchased from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were of the highest quality.

Preparation of PEG–CA

PEG–CA, as shown in Scheme 1, was prepared according to the literature.[31,32] Briefly, PEG_{600} diacid was converted into diacyl chloride. Anhydrous pyridine (0.2 mL, 2.48×10^{-3} mol) was added to a solution of citric acid (0.637 g, 1.66×10^{-3} mol) in 20 mL of anhydrous *N,N*-dimethylformamide (DMF), and this was stirred for 20 min. A solution of PEG_{600} diacyl chloride (1.057 g, 1.66×10^{-3} mol) in 10 mL of anhydrous DMF was added slowly at 0°C over 30 min. The mixture was stirred at 0°C for 1 h, then warmed to room temperature and stirred for 3 h, and finally warmed to 50°C and stirred for another 6 h. The mixture was cooled to room temperature, and solid materials were removed by filtration. The diethyl ether was added to the clear filtrate, and the resulting residue was separated by filtration. The residue was washed with dichloromethane, acetone, and toluene, dissolved in 10 mL of DMF, and reprecipitated in diethyl ether several times. The residue was poured into 5 mL of water at 25°C and placed into a cellophane membrane dialysis bag (cutoff = 2000), which was closed and transferred into a flask containing 100 mL of water; this was stirred for 24 h. The water was then replaced with 100 mL of fresh water, and this was stirred for another 24 h. The residue was then removed from the dialysis bag and placed *in vacuo* at 50°C for 24 h; the product was a reddish oil (yield = 68%).

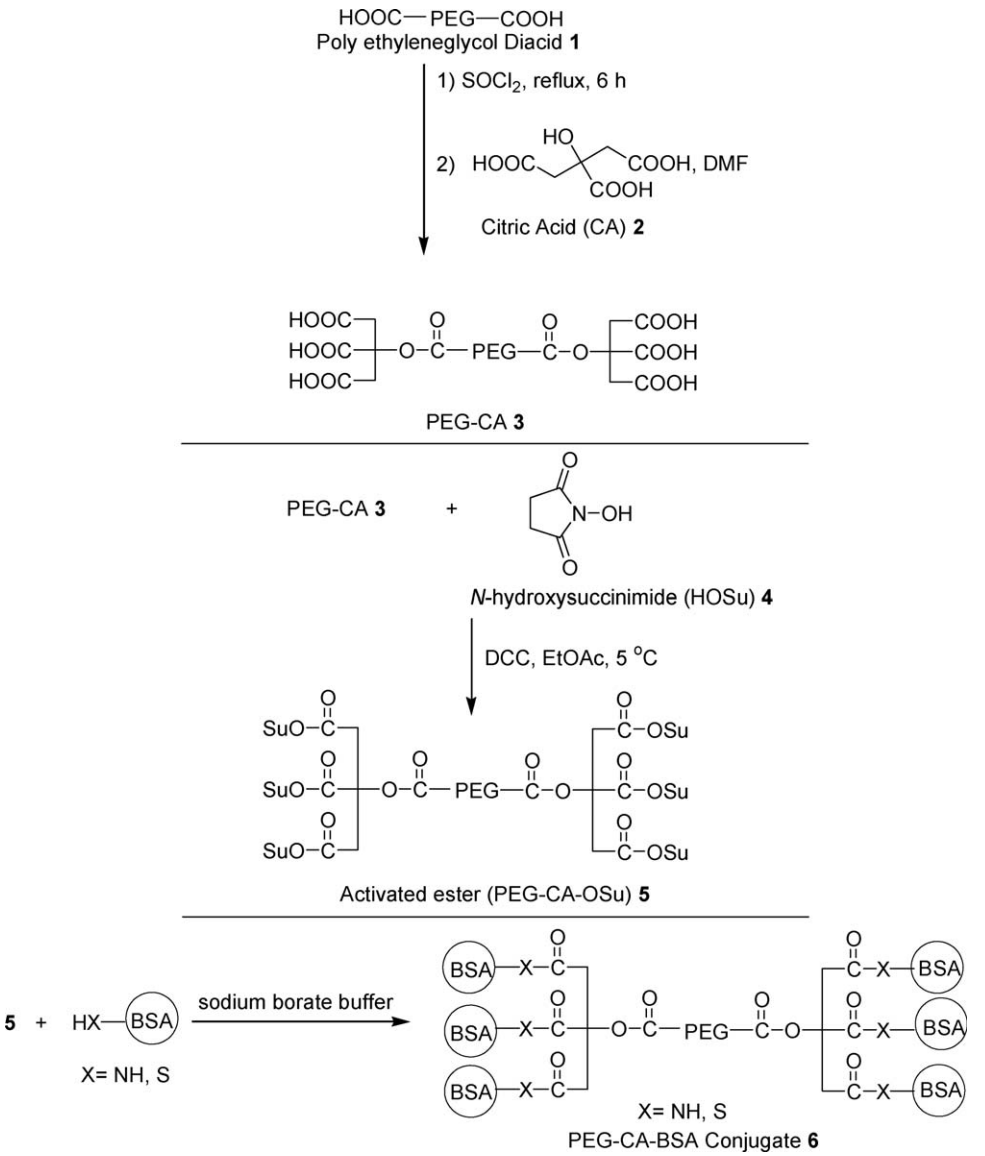
Fourier transform infrared (neat, λ, cm^{-1}): 3500–2650 (COOH), 1730–1748 (C=O), 1118, 1205 (C—O). ^{1}H-NMR (hexadeuterated dimethyl sulfoxide, ppm): 1.2 (t, 4H, —$CH_2CH_2OCH_2COO$) 2.5–2.8 (q, 2H, CH_2, citric acid), 3.6–3.9 (s, 4H, OCH_2CH_2O), 4.1–4.2 (2H, —$COCH_2O$; the integral ratio of aliphatic protons of PEG to citric acid end groups was 6).

Synthesis of succinimidyl carbonate (PEG–CA–OSu)

Anhydrous ethyl acetate (150 mL), PEG–CA (15.0 g), *N*-hydroxysuccinimide (HOSu; 0.86 g), and DCC (1.55 g) were placed into a glass reactor equipped with magnetic agitation and controlled heat. The mixture rapidly heated to form a solution, and then it was allowed to rest for 24 h at 30 ± 1°C. At the end of this period, the reaction mixture was filtered, and the residue was washed with cold ethyl acetate; then, it was placed *in vacuo* until it reached a constant weight. The residue was then dissolved in benzene (3 times the weight of the residue), and then the same volume of petroleum ether was added; the residue was cooled and filtered two more times. The product (PEG–CA–OSu) was kept in the flask under a nitrogen blanket and was used in the PEGylation reaction with BSA (Scheme 1).

PEGylation of BSA with PEG–CA–OSu

An accurately weighed amount of six-arm activated PEG–CA–OSu was added to a solution of 50 mg/mL BSA in a sodium borate buffer (200 mmol, pH 9.4), as shown in Scheme 1. The reaction mixture was stirred at 4°C for 2 h. After the pH was adjusted to 3, the unreacted PEG was removed by repeated ultrafiltration with 1 mmol of HCl. The PEGylated BSA was collected and dried *in vacuo*. The molar ratio of multifunctional PEG (PEG–CA) to BSA and the degree of modification are given in Table I. The degree of protein modification was determined by



Scheme 1

the quantitative analysis of its terminal carboxyl groups by titration with phenolphthalein as the indicator (Table I). As shown in Scheme 1, both amide and thioester bonds were formed upon the reaction of BSA and activated ester **5**: the ratio of amide bonds exceeded the ratio of thioester bonds.

Determination of the swelling ratio of the PEGylated BSA hydrogels

Dynamic swelling measurements were performed by means of gravimetric measurements. The hydrogel samples were placed into a beaker and were suspended in 500 mL of distilled water. The hydrogel samples were removed from the distilled water at different intervals (1, 2, 3, 4, 5, and 6 h) and were quickly blotted free of surface water with filter paper. The samples were weighed and then returned to the distilled water. The dynamic swelling experiments were performed in distilled water, and the increase in the mass was followed as a function of time. The equilibrium swelling ratio (ESR) of polymers was determined after 6 h via the weighing of the swollen gels in a borate buffer solution (pH = 9) before and after vacuum drying:

$$\mathrm{ESR} = \frac{\text{Weight of the swollen gel} - \text{Weight of the gel}}{\text{Weight of the dry gel}} \times 100$$

Morphological study of the PEG–CA–BSA hydrogels

The interior morphology of the swollen PEG–CA–BSA hydrogels at room temperature was studied

TABLE I
Degree of BSA Modification

Sample	BSA/PEG–CA (mol/mol)	Degree of substitution of PEG versus amino groups of albumin (%)[a]	Swelling ratio (%)	Thioester bond content in the conjugate (%)[b]
PEG–CA–$BSA_{(26)}$	3	26	1986	9
PEG–CA–$BSA_{(28)}$	4	28	1742	11.5
PEG–CA–$BSA_{(31)}$	5	31	1540	12
PEG–CA–$BSA_{(49)}$	6	49	1225	14.5

[a] Determined by ^{1}H-NMR spectroscopy.
[b] Determined by ^{13}C-NMR and Fourier transform infrared spectroscopy.

with scanning electron microscopy, SEM, LEO 440i - Zeiss (Oberkochen, Germany). The hydrogel specimens were immersed in distilled water for 24 h, and the swollen hydrogels were removed from the solution, quickly frozen in liquid nitrogen, and then freeze-dried in a Christ Alpha (Osterode, Germany) 1-4 freeze drier *in vacuo* at −50°C for 3 days until the samples became completely dry before morphological observation. The freeze-dried hydrogels were mounted onto aluminum stubs with double-sided carbon tape and were sputter-coated with gold for 40 s. The SEM examination was conducted at 25 kV and 12 nA.

Drug loading in the PEG–CA–BSA hydrogels

ADR-loaded hydrogels were prepared by 24-h incubation of 100 mg of dry hydrogels in 10 mL of an ADR solution in distilled water (5 mg/mL). After the removal of the immersed hydrogels after 24 h, the solution on the hydrogel surface was blotted with wet filter paper. These hydrogels were dried at 60°C *in vacuo* for at least 48 h until no weight change was observed. The difference in weight between the ADR-loaded hydrogel and the original hydrogel was the initial amount of loaded ADR. All release studies were carried out in triplicate. The results are presented in the terms of the cumulative release as a function of time.

Preparation of the PEG–CA–BSA microparticles

ADR-loaded PEG–CA–BSA microparticles were prepared by emulsion crosslinking with glutaraldehyde (GA) according to the previously reported method.[33,34] Dried PEG–CA–BSA (5% w/v) was dissolved in a 2% aqueous acetic acid solution, and the mixture was stirred overnight to obtain a uniform bubble-free solution. ADR (20% w/w with respect to the dry weight of the polymer) was added to the polymer solution, which was stirred until a homogeneous solution formed. This solution was emulsified into a light, liquid paraffin in the presence of 0.5% Span 80 at 400 rpm for 15 min. Then, a mixture of different quantities of GA (Table II) and 1 mL of 5*N* HCl was added slowly to the solution, and stirring was continued for 2 h. The microspheres were separated by filtration, washed with *n*-hexane, dried at 50°C for 24 h, and stored in a desiccator.

SEM images were taken of drug-loaded microparticles sputtered with gold and placed on a copper stub. Scanning was performed with a Leo model 440i scanning electron microscope. The thickness of the gold layer was approximately 15 nm. The size and size distribution of the microparticles were

TABLE II
ADR-Loaded Microparticles with Different Degrees of BSA Modification

Formulation code	Sample	Crosslinker amount (%)	Particle size (μm)	Encapsulation efficiency (%)
F1	PEG–CA–$BSA_{(26)}$	0.5	327.65	79.56
F2	PEG–CA–$BSA_{(28)}$	0.5	165.45	82.43
F3	PEG–CA–$BSA_{(31)}$	0.5	173.5	86.65
F4	PEG–CA–$BSA_{(49)}$	0.5	157.6	89.5
F5	PEG–CA–$BSA_{(26)}$	1	275	82.54
F6	PEG–CA–$BSA_{(28)}$	1	145.7	88.9
F7	PEG–CA–$BSA_{(31)}$	1	156.6	90.65
F8	PEG–CA–$BSA_{(49)}$	1	137.5	92.34
F9	PEG–CA–$BSA_{(26)}$	1.5	195.4	87.5
F10	PEG–CA–$BSA_{(28)}$	1.5	132.6	89.54
F11	PEG–CA–$BSA_{(31)}$	1.5	119.76	91.43
F12	PEG–CA–$BSA_{(49)}$	1.5	98.32	95.56

recorded with a laser diffraction particle size analyzer SALD 2101, Shimadzu Co. (Kyoto, Japan). Calculated values of the volume mean particle size of the microspheres are included in Table II. The drug-loading efficiency of the microparticles was defined as the ratio of the amount of the entrapped drug to the amount of the drug used for microparticle preparation.

Drug release from the PEG–CA–BSA matrices

In vitro drug release from different formulations of PEG–CA–BSA matrices was investigated at pH 3 (ionic strength = 0.1) and pH 7.4 (0.05*M* potassium dihydrogen phosphate, ionic strength = 0.09). Drug-loaded PEG–CA–BSA hydrogels or microspheres (100 mg) were immersed in 50-mL buffered solutions at 37°C. To estimate the drug release, 5 mL of the release medium was withdrawn periodically, and the amount of the drug released into the medium was quantified spectrophotometrically (UV-160, Shimadzu) at 479 nm. For each sample, all studies were performed in triplicate, and average values were recorded for data analysis.

RESULTS AND DISCUSSION

Synthesis of the multifunctional PEG–BSA conjugate

Recent studies have shown that increasing the number of carboxylic acids within the PEG complex has a direct effect on the reactivity of the PEG–protein amide bond toward hydrolysis or aminolysis.[35] A higher number of active carboxylic groups within a single PEG complex can yield a higher number of amide bonds, and this, in turn, can increase the reactivity of the PEG–protein conjugate toward hydrolysis or aminolysis.[35] In this article, the PEG diacid was converted into a PEG–CA complex (Scheme 1) containing six carboxylic acid groups within the PEG complex instead of the two carboxylic acid groups in the PEG diacid. Then, the PEG–CA complex was converted into active PEG–CA–OSu through the reaction of PEG–CA with more than 6 equiv of HOSu in the presence of DCC (Scheme 2). The resulting PEG–CA–OSu active complex had six active carbonate bonds per PEG complex. The PEG–CA–OSu active complex was then reacted with BSA to afford the PEG–CA–BSA conjugate, as shown in Scheme 1. IR and ^{1}H-NMR confirmed the formation of the amide bonds. Along with the formation of the amide bonds, the IR and NMR data also revealed the formation of thioester bonds, which could have resulted from the reaction of the cysteine residue in BSA with the active PEG–CA–OSu complex at pH 9.4.

Degree of albumin modification

The degree of albumin modification was influenced by changes in the molar ratio of albumin to activated PEG–CA. As deduced from Table I which compares PEG-CA-BSA$_{(26)}$, PEG-CA-BSA$_{(28)}$, PEG-CA-BSA$_{(31)}$ and PEG-CA-BSA$_{(49)}$, increasing the mixing ratio of albumin from 3 to 6 with respect to PEG–CA resulted in a 2-fold increase in the degree of albumin modification. The relatively high degree of modification was probably due to the presence of six carboxylic acid groups in each PEG–CA conjugate.

Morphology of the PEG–CA–BSA hydrogels

The morphology of the freeze-dried, swollen hydrogel samples as a function of the BSA feed ratio is shown in Figure 1. As shown in Figure 1, from PEG–CA–BSA$_{(26)}$ to PEG–CA–BSA$_{(49)}$, the pore size decreased from approximately 4 μm to approximately 200 nm, and the network became denser and more compact. The reduction in the pore size with an increase in the BSA content (from a molar ratio of 3 to 6) could be attributed to the increased number of PEGylated bonds in the PEG–CA–BSA conjugate.

Water content of the PEGylated BSA

In this study, we prepared a newly synthesized family of PEG–BSA conjugates as novel hydrogel structures that may be useful as protein carriers or matrices for the controlled release of hydrophilic or hydrophobic drugs. These polymers are bioartificial polymeric materials. The binding of water molecules makes PEGylated compounds function as if they are 5–10 times larger than a corresponding soluble protein of a similar molecular mass. The PEG polymer, along with the associated water molecules, acts like a shield and protects the attached drug from enzyme degradation, rapid renal clearance, and interactions with cell-surface proteins.[36] The PEG–CA–BSA hydrogels reported in this study had high water contents (Table I) and should be useful as matrices for the controlled release of sensitive drugs. The swelling ratios of PEG–CA–BSA are shown in Table I. During the swelling step, each of the four conjugates (PI–PIV) underwent significant water uptake, which led to considerable volume expansion. When the molar ratio of BSA to PEG–CA was increased from 3 to 6, the swelling ratio of the resulting hydrogels decreased from 1986 to 1225%. Figure 2 shows the effect of the BSA/PEG–CA molar ratio on the swelling ratios of the PEG–CA–BSA hydrogels at room temperature. The data show that an increase in the BSA content led to a decrease in the swelling of the corresponding PEG–CA–BSA hydrogels. The dependence of the swelling ratio of the BSA–PEG–CA hydrogels on the BSA content is in a

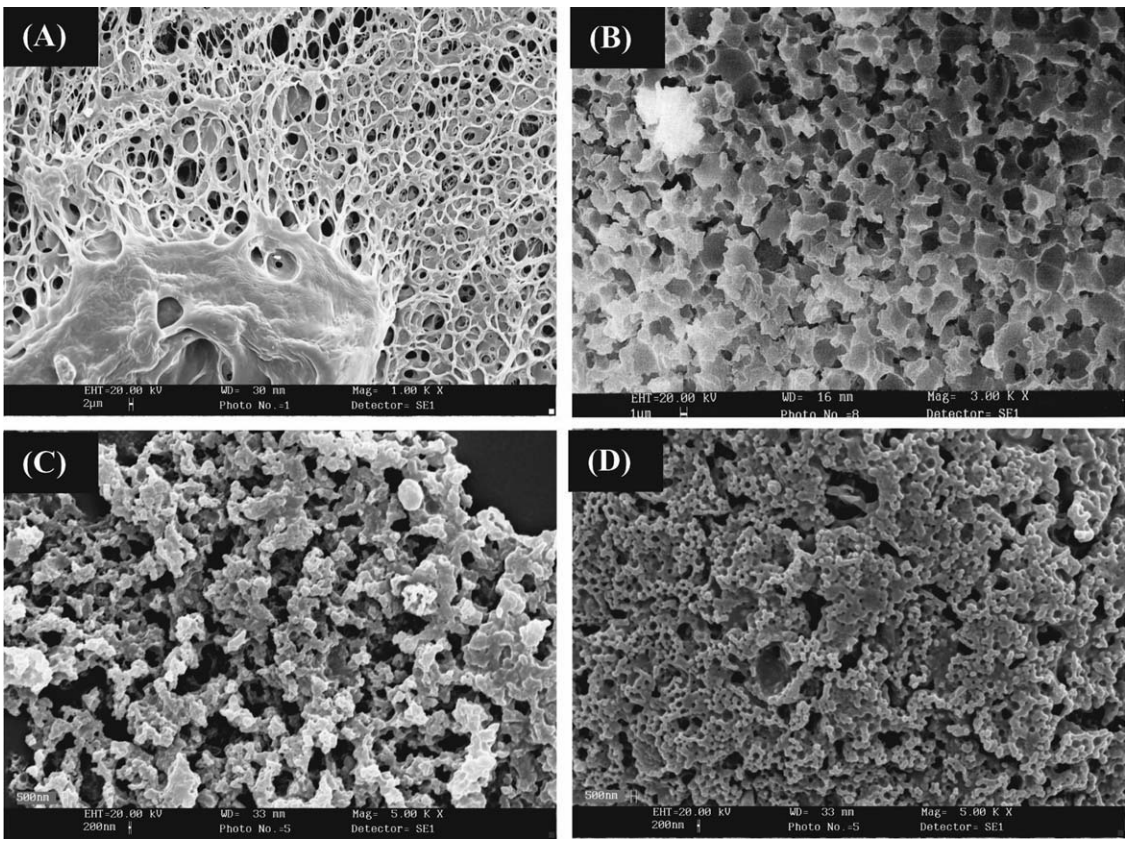


Figure 1 SEM images of the BSA–PEG–CA hydrogels: (A) PEG–CA–BSA(26), (B) PEG–CA–BSA(28), (C) PEG–CA–BSA(31), and (D) PEG–CA–BSA(49).

good agreement with the morphological images (Fig. 1) and average pore size data. The morphological data showed that an increase in the content of BSA led to a compact network structure because of a higher crosslinking level of the corresponding hydrogel; this caused a reduction in the swollen or water-uptake capacity because of the decreased pore volume.[37]

In vitro ADR release from the PEG–CA–BSA hydrogels

Loading capacity

The ADR loading capacity in PEG–CA–BSA hydrogels was 0.376, 0.265, 0.201, and 0.189 for PEG–CA–$BSA_{(49)}$, PEG–CA–$BSA_{(31)}$, PEG–CA–$BSA_{(28)}$, and PEG–CA–$BSA_{(26)}$, respectively. The highest loading capacity was found for PEG–CA–$BSA_{(49)}$. The differences in the loading capacity of the hydrogels could be attributed to the higher PEGylation of BSA in PEG–CA–$BSA_{(49)}$ versus the other hydrogels. This formulation, possessing more amino groups on albumin, could produce stronger interactions with ADR carboxylic acid group.

Cumulative release of ADR from the hydrogels at pH 7.4

The cumulative amounts of ADR released from the PEG–CA–BSA hydrogels at pH 7.4 (phosphate-buffered saline) are shown in Figure 3(A). The data show that all PEG–CA–BSA hydrogels had lower ADR release rates. The interaction between the BSA

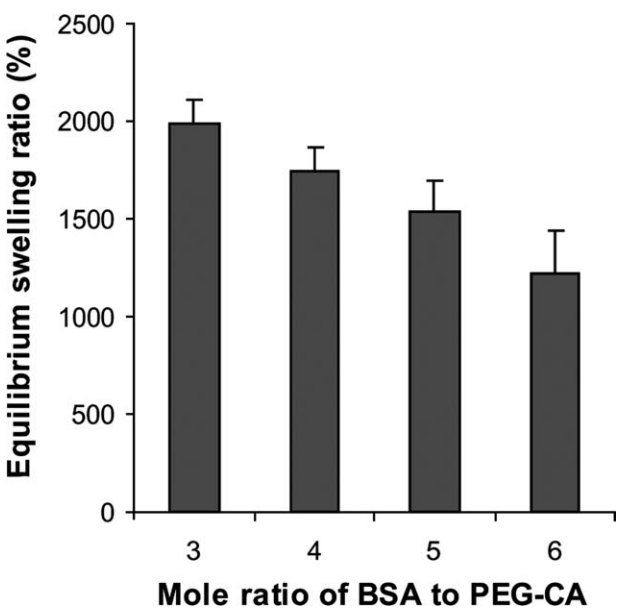


Figure 2 ESRs of the PEG–CA–BSA hydrogels at room temperature.

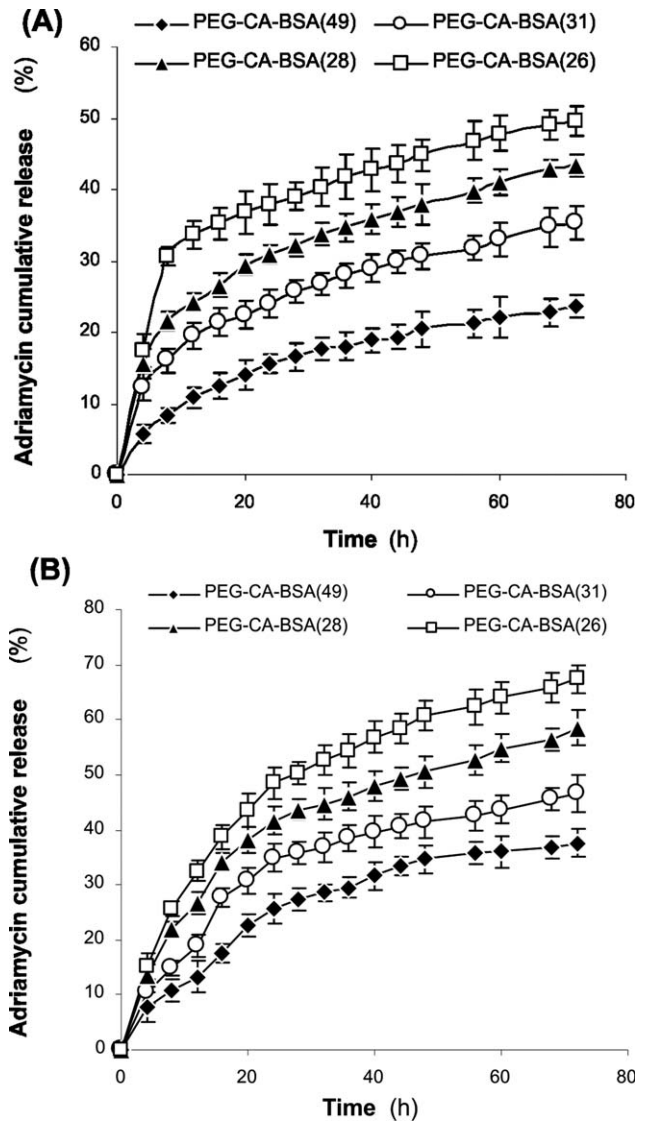


Figure 3 Cumulative release of ADR from the PEG–CA–BSA hydrogels at 37°C: (A) pH 7.4 and (B) pH 3.

component of the PEG–CA–BSA hydrogels and ADR may be partially responsible for this slower release phenomenon. All hydrogel samples had a slight initial burst release. During the first 24 h, the cumulative releases were 15.5% for PEG–CA–$BSA_{(49)}$, 24% for PEG–CA–$BSA_{(31)}$, 31% for PEG–CA–$BSA_{(28)}$, and 38% for PEG–CA–$BSA_{(26)}$. After this initial release period, all the release rates slowed, and they were almost in the following order: PEG–CA–$BSA_{(26)}$ > PEG–CA–$BSA_{(28)}$ > PEG–CA–$BSA_{(31)}$ > PEG–CA–$BSA_{(49)}$. As shown in Figure 3(A), a higher PEG–CA/BSA molar ratio led to a reduction of the cumulative amount of ADR released from the hydrogels. These results can be rationalized in terms of the number of PEG–CA chains bound per BSA molecule. With a higher degree of modification of BSA, the distance between the BSA molecules would be smaller. The shortening of the distance between the BSA molecules could decrease the porosity of the hydrogel (Fig. 1). The decrease in the porosity of the hydrogel reduced the release rate of the drug from the hydrogel.

Cumulative release at pH 3.0

The pH sensitivity of the PEG–CA–BSA hydrogels was also reflected in their ADR release, as shown in Figure 3(B). The data show the cumulative release of ADR from PEG–CA–BSA hydrogels at pH 3.0. The ADR release profiles at pH 3.0 were very similar to those at pH 7.4, and the main differences were the amounts of ADR released cumulatively over a fixed period and the release duration. All the hydrogels released higher amounts of ADR at pH 3.0 versus pH 7.4. For instance, after 24 h PEG–CA–$BSA_{(49)}$, PEG–CA–$BSA_{(31)}$, PEG–CA–$BSA_{(31)}$, and PEG–CA–$BSA_{(26)}$ had ADR release rates of 25.6%, 34.86%, 41.64%, and 48.5%, respectively, at pH 3. In an endocytic process, macromolecules can internalize, enter a cell as small vesicles, and lead to the formation of endosomes, which subsequently fuse with lysosomes containing a variety of enzymes effective in the environment with a lower pH.[38] The efficiency of lysosomotropic drug delivery depends on the proper choice of the polymer carrier, the drug, and the type of linkage in the carrier or between the drug and carrier.[39–41]

Preparation and characterization of the PEG–CA–BSA microparticles

Mean particle size of the microparticles

ADR-loaded PEG–CA–BSA microparticles were prepared by emulsion crosslinking with GA as a crosslinking agent. The newly formed microparticles were all spherical and had smooth surfaces, as revealed by SEM images shown in Figure 4. The size and size distribution of the microparticles were recorded with a laser light diffraction technique. The calculated values of the volume mean particle size of the microspheres are included in Table II. These data show that with the amount of the crosslinking agent increasing, smaller microparticles (probably due to the formation of a more rigid network) were produced. Besides, with an increase in the ratio of BSA within the PEGylated polymer structure, a decrease in the size of the microparticles was observed.

Encapsulation efficiency and drug release from the microparticles

In vitro drug-release studies were performed in simulated gastric fluid media and then in simulated intestinal fluid media (without enzymes). The

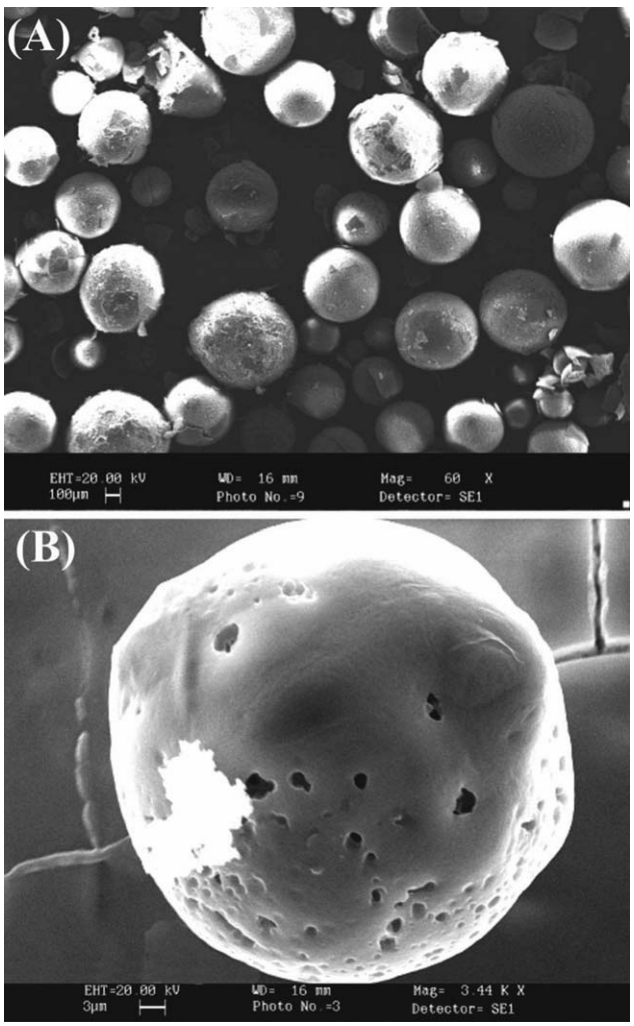


Figure 4 SEM images of the microparticles: (A) a group of the particles and (B) a single particle.

encapsulation efficiency was within the range of 84.5–95.6%(Table II). The dependence on the amount of the crosslinker used during *in vitro* release is displayed in Figure 5(B) for three formulations: F1, F5, and F9. The release rates depended on the amount of GA used during crosslinking. The release was slower for formulations in which higher amounts of GA were used versus those formulations in which lower amounts of GA were present in the matrix. This could be due to the fact that with a higher degree of crosslinking, the free volume of the matrix decreased, and this hindered the transport of drug molecules through the matrix. As shown in Figure 5(B), for formulation F1, the release was faster versus formulation F5, which contained a larger amount of GA. A similar trend was observed between formulations F5 and F9. To study the effect of the BSA molar ratio of PEGylated BSA on drug-release rates, we chose four formulations: F9, F10, F11, and F12. The results are displayed in Figure 5(A). The release rates were slower for formulations containing larger amounts of BSA. The release rate decreased with an increasing amount of BSA in the conjugates. Drug-release rates were higher for microparticles with lower amounts of BSA in the conjugate versus those having larger amounts of BSA. Similar trends were seen between formulations F1–F4 and F5–F8. This means that the drug-release rate was affected by the BSA molar ratio, that is, the composition of the polymer. ADR release from the microparticles was slightly faster for formulations with a lower BSA molar ratio (a BSA/PEG–CA ratio of 3) in the matrix versus formulations with a higher BSA molar ratio (a BSA/PEG–CA ratio of 1 : 6).

CONCLUSIONS

It has been well documented that nanoparticles prepared from water-soluble polymers can be efficiently loaded with certain anticancer drugs, and the loaded

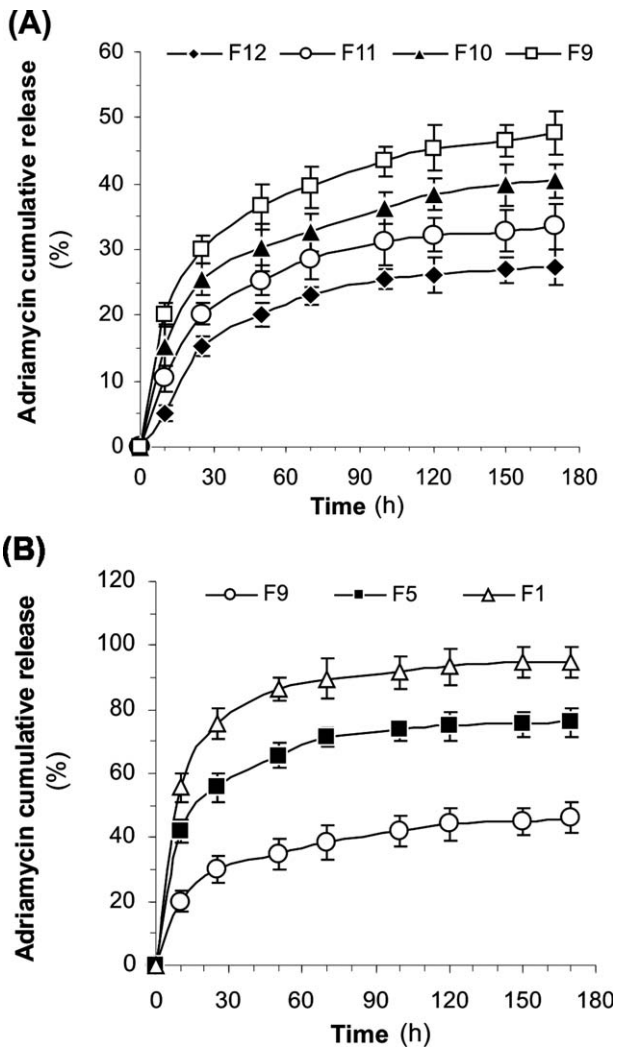


Figure 5 Cumulative release of ADR from the PEG–CA–BSA microparticles with different amounts of the crosslinker at 37°C: (A) pH 7.4 and (B) pH 3.

drugs can accumulate in tumors. Accordingly, we have exploited a novel PEGylated derivative of BSA (PEG–CA–BSA) as a delivery system for carrying ADR in the forms of hydrogels and microparticles. The hydrogels displayed a porous structure that improved the drug-loading efficiency as well as the drug-release longevity. Increasing the degree of BSA substitution revealed that the reduced pore size of the hydrogels led to a denser and more compact architecture. The microparticles possessed a spherical morphology with smooth surfaces. The drug-loading efficiency of the prepared matrices was affected by the amount of BSA in the PEGylated conjugate and by the amount of GA used to prepare the microparticle formulations. The release of ADR was dependent on the drug-loading percentage, the amount of the polymer used in the matrix, and the BSA ratio of the PEGylated conjugates. A higher PEG–CA/BSA molar ratio resulted in a reduction of the cumulative amount of ADR released from the hydrogels in a pH-dependent manner. In conclusion, this PEGylated BSA could be successfully exploited as a delivery system for the transport of anticancer agents to targeted cells and tissues.

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