

## Study on Poly(D,L-lactic) Microspheres Embedded in Calcium Alginate Hydrogel Beads as Dual Drug Delivery Systems

Dagen Zhong,<sup>1</sup> Zonghua Liu,<sup>1</sup> Shasha Xie,<sup>1</sup> Wei Zhang,<sup>1</sup> Yuanming Zhang,<sup>2</sup> Wei Xue<sup>1</sup>

<sup>1</sup>Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Department of Biomedical Engineering, Jinan University, Guangzhou, 510632, China

<sup>2</sup>Department of Chemistry, College of Life Science and Technology, Jinan University, Guangzhou, 510632, China

Correspondence to: Z. Liu liu-zonghua@hotmail.com and W. Xue weixue\_jnu@yahoo.com.cn

**ABSTRACT:** This work is to develop a novel dual drug delivery system that can simultaneously load and release 18 $\beta$ -glycyrrhetic acid (GA, a hydrophobic drug) and bovine serum albumin (BSA, hydrophilic model drug) in a single formulation. The system consists of poly(D,L-lactic) (PDLLA) microspheres embedded in calcium alginate hydrogel beads. The GA-loaded microspheres were first prepared and then dispersed in the aqueous solution of sodium alginate and BSA. The resulting suspension was dropped into aqueous calcium chloride solution to obtain the dual drug delivery system. The morphology of the microspheres and beads, the drug content and loading efficiency, the interaction between GA and PDLLA, and the drug release behaviors were studied. Scanning electron microscope (SEM) revealed that the PDLLA microspheres were homogeneously distributed in the beads. Differential scanning calorimetry (DSC) measurement suggested certain interaction between GA and PDLLA, and the crystal structure of GA was influenced by the polymer. The dual release *in vitro* showed a rapid BSA release but a sustained GA release in all the systems. Furthermore, the release rate of BSA was accelerated by increasing PDLLA/alginate ratio, while the release rate of GA was decreased, and the release of both hydrophobic and hydrophilic drugs could be adjusted by changing the ratio of PDLLA/alginate. © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 129: 767–772, 2013

**KEYWORDS:** dual-drug delivery systems; dual release; hydrogel beads; 18 $\beta$ -glycyrrhetic acid; BSA; alginate

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### INTRODUCTION

During the past few decades, the researchers developed various drug delivery systems to deliver different drugs or bioactive substances,<sup>1,2</sup> and some of them are already commercially available. However, the previous studies mostly focus on single drug delivery systems, which often could not satisfy the requirements in clinical therapies.<sup>3</sup> In addition, pharmaceutical and biomedical applications usually require multi-action of different drugs for multiple-purpose therapy. To overcome the shortcomings, combination therapies is considered advantageous, in which multiple drugs of better therapeutic effects are used in many cases of disease treatment to improve therapy efficacy,<sup>4,5</sup> and meanwhile their suboptimal doses are used to minimize toxicity or side effects of the therapeutic doses of these drugs.<sup>6</sup> Encapsulation of two different types (hydrophilic and/or hydrophobic) of drugs in a single vehicle is a challenging as well as an important aspect for smart drug delivery.<sup>7</sup> Thus, development of novel modes and systems for regulated multiple-drug delivery in a single formulation is of great importance in terms of advancement of future drug delivery systems.<sup>8,9</sup>

At present, various types of dual-drug delivery systems have been prepared using emulsion electrospun nanofibrous mats,<sup>10</sup> hydrogel,<sup>11</sup> hydrogel/micelle composite,<sup>12</sup> alginate beads embedded silk fibroin scaffold,<sup>13</sup> superparamagnetic iron oxide nanoparticles,<sup>14</sup> etc. Alginate is a well known biopolymer, which is biocompatible, nontoxic, nonimmunogenic, and biodegradable.<sup>15</sup> Poly (D,L-lactic) (PDLLA) is biocompatible and biodegradable, and has been approved by the FDA for certain human clinical uses.<sup>16</sup> Alginate and PDLLA have been widely used in drug delivery systems,<sup>17–19</sup> the former suitable for hydrophilic drugs and the latter for hydrophobic drugs.

As reviewed by Quaglia,<sup>20</sup> PDLLA microspheres or poly(lactide-co-glycolide) nanoparticles, etc. were integrated in tissue engineering scaffolds (e.g., alginate-based scaffold) as a hybrid delivery system for the controlled release of single drug. It is often difficult for the single drug therapy to obtain the best effect in the clinical treatment. In this work, we present a dual-drug delivery system of PDLLA microspheres embedded in calcium alginate hydrogel beads. Two different types (hydrophilic and hydrophobic) of drugs, BSA and GA were used as model drugs.

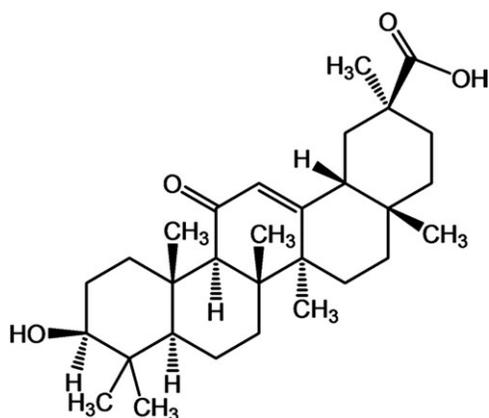


Figure 1. Structure of 18 $\beta$ -glycyrrhetic acid.

BSA is an often applied material to model highly water-soluble protein-type drugs. However, GA is a hydrophobic drug with a very low solubility in water (Figure 1). It has been shown to possess many beneficial pharmacological activities, such as anti-inflammatory activity, interferon inducibility, antiallergenic, direct and indirect antiviral activity, etc.<sup>21</sup> The GA-loaded microspheres were first prepared and then dispersed in the aqueous solution of sodium alginate and BSA. The resulting suspension was dropped into aqueous calcium chloride solution to obtain the dual drug delivery system. The surface morphology and cross section of the dual drug-loaded beads were observed using SEM. The compatibility between GA and PDLLA was detected using DSC. The drug content, loading efficiency, and release behaviors were also studied. This dual carrier system to deliver multiple drugs may find broad utility in complicated clinical syndrome which needs several drugs to treat.

## MATERIALS AND METHODS

### Materials

Sodium alginate (low viscosity, 250 cps for 2% solution at 25°C) was purchased from Sigma-Aldrich (St. Louis, MO). Poly (D,L-lactic) ( $M_w = 100$  kDa) was purchased from Jinan Daigang Biological Technology (Shandong, China). 18 $\beta$ -glycyrrhetic acid and poly(vinyl alcohol) (PVA-1798) were purchased from Aladdin Chemistry (Shanghai, China). BSA was purchased from Beijing Dingguo Biotechnology (Beijing, China). BCA Protein Assay Kit was purchased from Thermo Scientific (Rockford, IL). Methanol,

water, and phosphoric acid were of HPLC grade and all other reagents were of analytical grade.

### Preparation of GA-Loaded PDLLA Microspheres

GA-loaded PDLLA microspheres were prepared by a solvent evaporation method as described previously.<sup>22</sup> GA (0.6 g) and PDLLA powder (2 g) were dissolved in methylene dichloride (62 g). The resulting homogeneous solution was added to 340 mL of aqueous solution of PVA (1%, w/v) while stirred at 1000 rpm for 1 h. The resulting oil-in-water emulsion was further stirred for 5 h at 42°C to completely evaporate the organic solvent, and the formed microspheres were collected by centrifugation. The microspheres were washed five times with water and subsequently freeze dried. The GA-loading efficiency of PDLLA microspheres was determined according to the method reported by Patomchaiwivat et al.<sup>23</sup> The concentration of GA was determined at 255 nm by high performance liquid chromatography (HPLC) (Agilent Technologies USA).

### Preparation of the Alginate Hydrogel Beads

The beads were prepared by extrusion through a syringe needle into a CaCl<sub>2</sub> solution. A schematic representation for the preparation process is shown in Figure 2. First, BSA was dissolved in the alginate solution (3%, w/v). Then, the GA-loaded PDLLA microspheres were directly suspended in the BSA/alginate solution (3%, w/v) and vigorously stirred for ~8 h to produce a well-dispersed suspension. The suspension was dropped into a CaCl<sub>2</sub> solution (9%, w/v) with gentle stirring. The hydrogel beads were allowed to crosslink with Ca<sup>2+</sup> immediately. Subsequently, the beads in the solution were incubated for 60 min at room temperature. The resultant beads were rinsed thrice with distilled water to remove unreacted Ca<sup>2+</sup> on their surface and then freeze-dried. The preparation formula of the beads are summarized in Table I.

### Morphology Observation

The surface morphology of the PDLLA microspheres and the beads was observed using SEM (PHILIPS, ESEM XL 30, Holland). The samples were sputtered with gold and scanned at an accelerating voltage of 20 kV. To reveal the internal morphology, the wet beads were cross-sectioned and freeze-dried, and then observed with SEM.

### Determination of Drug Content and Loading Efficiency

Accurately weighed sample of GA-loaded microspheres was dissolved in 1 mL of methylene dichloride followed by the addition of 9 mL of ethanol to precipitate the polymer.<sup>23</sup> The resulting suspension was then centrifuged at 6000 rpm for 20 min, and 1 mL of supernatant was taken and analyzed by HPLC.

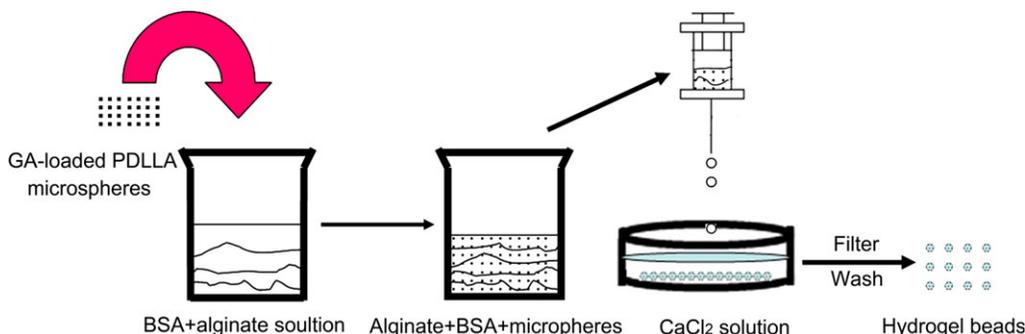


Figure 2. Schematic of the preparing procedure of the hydrogel beads. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

**Table I.** Compositions of the Samples Used for the *In Vitro* Release Experiment

Samples	Compositions
A	PDLLA microspheres (GA-load)
B	alginate and BSA
C	alginate, BSA and GA-loaded PDLLA microspheres (the mass ratio of BSA to GA is fixed at 1:0.5 when adding the microspheres into the beads)
D	alginate, BSA and GA-loaded PDLLA microspheres (the mass ratio of BSA to GA is fixed at 1:1 when adding the microspheres into the beads)
E	alginate, BSA and GA-loaded PDLLA microspheres (the mass ratio of BSA to GA is fixed at 1:1.5 when adding the microspheres into the beads)

Accurately weighed sample of alginate beads was dissolved in 5% sodium citrate (w/v) aqueous solution for 24 h with magnetic stirring. After centrifugation at 6000 rpm for 20 min, the BSA concentration in the supernatant was determined using a colorimetric method (Micro BCA protein assay, Pierce Biotechnologies), and the absorbance values at 570 nm were determined by an enzyme-labeled instrument (ELX800, Bio-Tek, USA).

The drug content and loading efficiency were calculated by eqs. (1) and (2), respectively. Each experiment was carried out in triplicate and their mean values are reported.

$$\text{Drug Content (\%)} = \frac{\text{Mass of drug in microspheres (beads)}}{\text{Mass of drug loaded microspheres (beads)}} \times 100 \quad (1)$$

$$\text{Loading efficiency (\%)} = \frac{\text{Mass of drug in microspheres (beads)}}{\text{Theoretical mass of drug in microspheres (beads)}} \times 100 \quad (2)$$

### Differential Scanning Calorimetry

DSC experiments were performed on a Netzsch DSC 204F1 system (Netzsch Instruments, Germany). Accurately weighed sample was placed in pierced aluminum pans with perforated lids. Heat scanning was performed at 10°C/min in the temperature range between 30 and 300°C under nitrogen flow (sweep gas: 30 mL/min, protecting gas: 50 mL/min). Empty aluminum pan was used as reference.

### *In Vitro* Drug Cumulative Release Studies

To study the drug release behaviors, the GA-loaded PDLLA microspheres or beads (100 mg) were enclosed in dialysis bags and then were immersed in 40 mL of Tris-HCl buffer (pH = 7.4) with shaking speed of 100 rpm at 37°C. At predetermined time intervals, 5.0 mL of the release medium were removed and replaced by 5.0 mL fresh medium. The concentration of GA in the release buffer was then determined at 255 nm by HPLC, while the amount of BSA released was assayed by BCA assay kit at 570 nm using an Enzyme-labeled instrument (ELX800, Bio-Tek, USA). All samples were analyzed in triplicate. Morphology change

of the beads after the *in vitro* release experiment was observed by SEM.

### HPLC Measurement

An Agilent (model 1200) HPLC system, equipped with an Eclipse XDB-C18 (4.6 × 150 mm<sup>2</sup>, 5 μm) columns, was used. The mobile phase was methanol and 0.1% of phosphoric acid aqueous solution in the ratio of 87:13 (v/v). The mobile phase was filtered through 0.45 μm membrane filter. The flow rate of the mobile phase was maintained at 1.0 mL/min. The column temperature was set at 25°C and the detection was carried out with a UV-detector at wavelength 255 nm. The run time was set at 20 min and the volume of the injection loop was 20 μL. The column was equilibrated for at least 60 min with the mobile phase flowing through the system.

## RESULTS AND DISCUSSION

### Morphology Observation

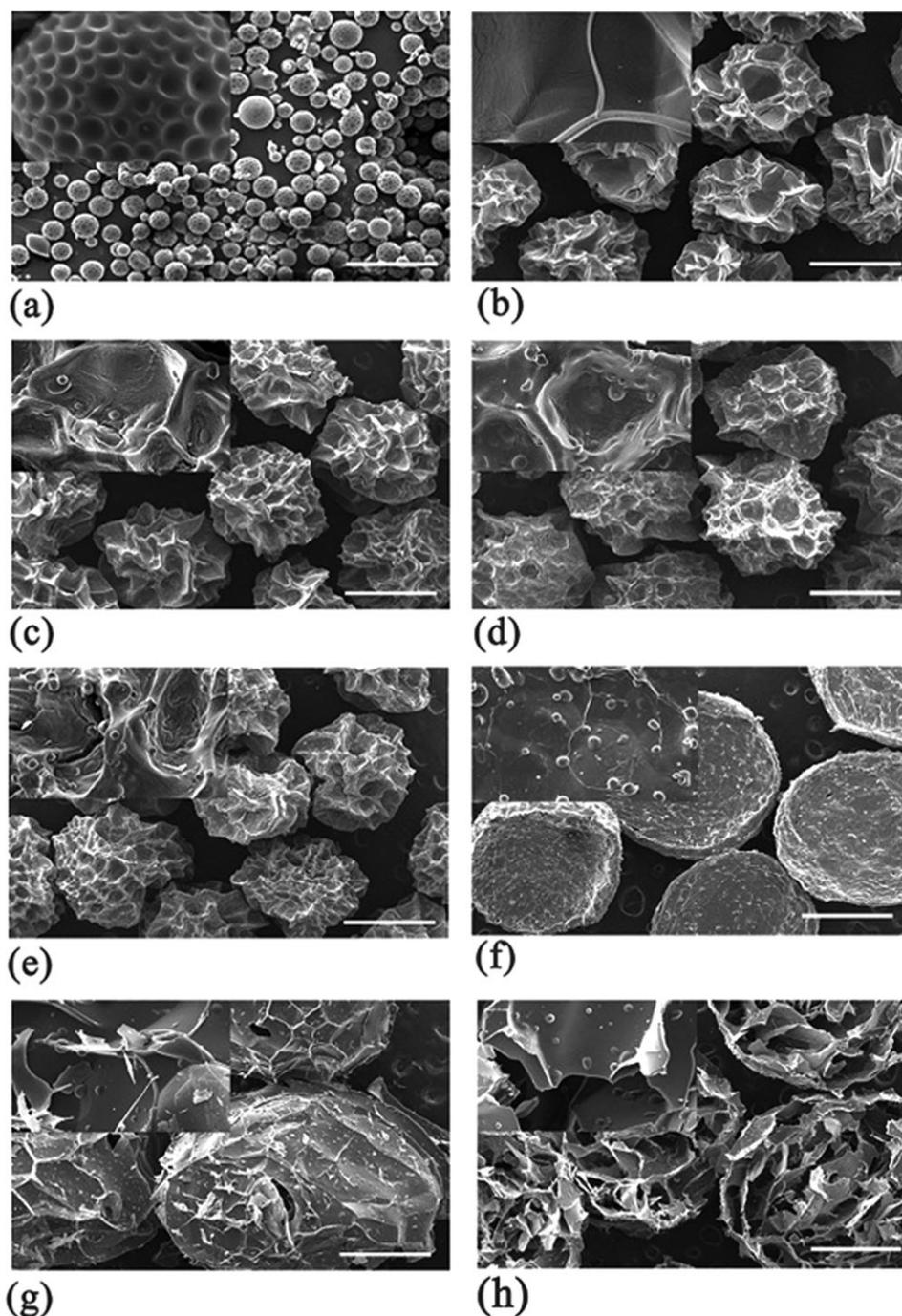
It is well-known that the morphology of polymeric microspheres could be a critical factor to affect the drug release kinetics.<sup>24</sup> As shown in Figure 3(a), the morphology of the PDLLA microspheres have spherical shape and relatively uniform size distribution (about 15–25 μm). Interestingly, the surface of the microspheres has many concave dents. Compared with smooth PDLLA microspheres, the PDLLA microspheres obtained in this study have larger specific surface area. The formation of the concave dents could be attributed to the evaporation rate of methylene chloride during preparation.

The surface morphologies of the freeze-dried beads with different amount of the PDLLA microspheres are shown in Figure 3(b–e). From these SEM micrographs, the freeze-dried beads have uniform size and wrinkles on their surfaces regardless of the addition of the microspheres into the beads. Some PDLLA microspheres are attached on the surface of the beads. To achieve a controlled drug release, the drug-loaded microspheres must be homogeneously incorporated into the matrix of the hydrogel beads. To further reveal the distribution of the PDLLA microspheres in the interior of the beads, the wet beads were cross-sectioned and then freeze-dried. As shown in Figure 3(f), the PDLLA microspheres are homogeneously distributed in the entire cross-section. Therefore, it can be inferred that the microspheres were uniformly embedded in the beads.

The morphologies of the beads after 15 days of *in vitro* release are shown in Figure 3(g,h). The beads were still spherical, while the surfaces had small cracks and fluffy structure and the beads size increased. The interior structures of the beads disintegrated, which should be attributed to the degradation of calcium alginate crosslinking network. In addition, a few PDLLA microspheres were still visible in the beads, demonstrating that the PDLLA microspheres did not completely degrade.

### Drug Content and Loading Efficiency

The feeding weight ratio of GA to PDLLA was taken as 0.6:2 (theoretical drug content of 23.1%) during preparation of drug-loaded microspheres. The drug content and loading efficiency were listed in TABLE II. It can be seen that, the GA drug content in PDLLA microspheres was 7.7% and the GA loading efficiency was 33.6%. Similar loading efficiency of lidocaine in



**Figure 3.** SEM images of the PDLLA microspheres and the hydrogel beads: (a), the PDLLA microspheres (Sample A), scale bar 100  $\mu\text{m}$ ; (b), the calcium alginate beads (Sample B); (c), the beads loaded with the PDLLA microspheres (Sample C); (d), the beads loaded with the PDLLA microspheres (Sample D); (e), the beads loaded with the PDLLA microspheres (Sample E); (f), the cross-section of the beads loaded with the PDLLA microspheres; (g), the beads loaded with the PDLLA microspheres after 15 days of *in vitro* release; (h) the cross-section of the beads loaded with the PDLLA microspheres after 15 days of *in vitro* release. (b)–(h) scale bar 1 mm.

PDLLA microspheres prepared with the same method was reported by Chung.<sup>25</sup> The low loading efficiency of GA in the microspheres could be due to GA entry into the aqueous phase during the evaporation process, where it likely formed microcrystalline deposits due to its low partition coefficient. These deposits were lost in the washing steps.<sup>26</sup>

The drug content and loading efficiency of BSA in the beads were shown in TABLE II. Obviously, the drug content and loading efficiency declined with the increase of the amount of GA-loaded microspheres added. This could be because the microstructure of the beads was destroyed by the addition of the microspheres, and the destroyed degree increased with increasing the amount of the

**Table II.** Drug Content and Loading Efficiency of GA and BSA

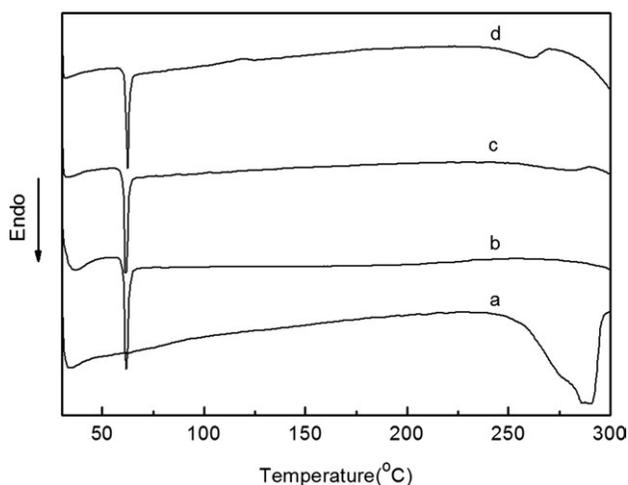
Samples	Drug content (%)	Loading efficiency (%)
GA-loaded PDLLA microspheres (sample A)	7.7 ± 0.1	33.6 ± 0.6
BSA-loaded beads (sample B)	0.89 ± 0.03	88.51 ± 2.95
BSA-loaded beads (sample C)	0.71 ± 0.04	71.44 ± 4.26
BSA-loaded beads (sample D)	0.65 ± 0.04	64.72 ± 4.32
BSA-loaded beads (sample E)	0.49 ± 0.03	49.29 ± 3.40

The values presented are the average of three experiments with standard deviation.

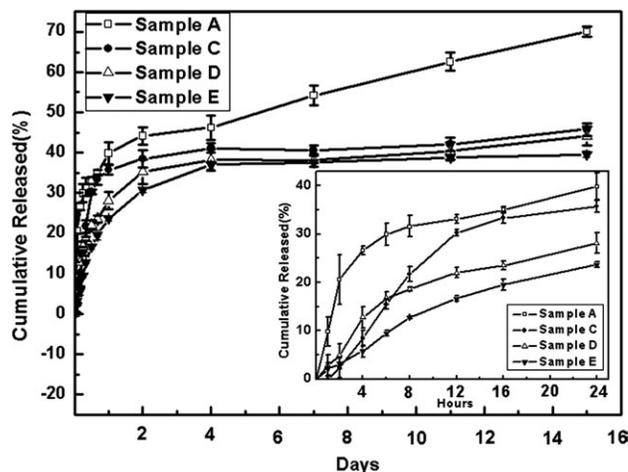
microspheres added. Further, the destroyed microstructure led to more BSA leakage from the beads.

### DSC Analysis

DSC has been shown to be a powerful analytical tool in the characterization of solid state interactions between drug and its carrier.<sup>27</sup> As shown in Figure 4, the DSC curve of GA exhibited a wide single endothermic peak at 285–294°C, which corresponded to its intrinsic melting points. DSC curve of the blank PDLLA microspheres exhibited a sharp endothermic peak at 61.7°C, corresponding to its glass transition temperatures ( $T_g$ ). Beyond that, the PDLLA had no other melt endothermic response as expected from an amorphous polymer. The DSC curve of the physical mixture of GA and blank PDLLA microspheres showed the peaks resulting from simple superposition of the DSC curves of their individual components. By contrast, the GA-loaded PDLLA microspheres showed a wide endothermic peak of GA melting, while it shifted to a lower endothermic



**Figure 4.** DSC thermograms of (a) GA, (b) blank PDLLA microspheres, (c) physical mixture of GA and blank PDLLA microspheres with an equivalent GA to PDLLA mass ratio as in the GA-loaded microspheres, (d) GA-loaded microspheres.



**Figure 5.** *In vitro* release profiles of GA from Samples A, C, D, and E for 15 days.

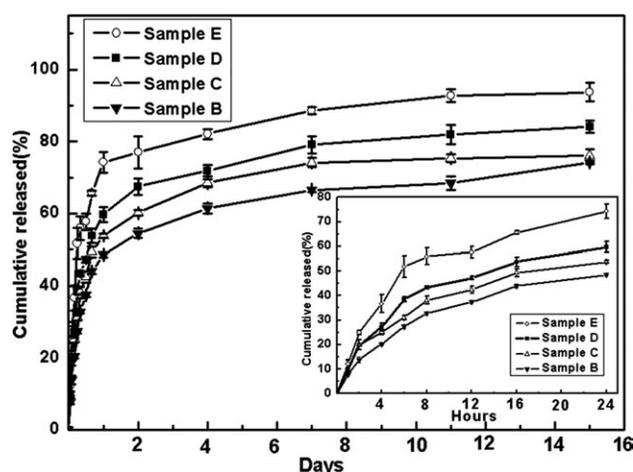
peak of at about 256–269°C. These results indicated that the GA entrapped in the microspheres remained the crystalline or microcrystalline form. In addition, the  $T_g$  of the polymer observed at the almost same endothermic peak was not influenced obviously by GA. From these results, there was certain interaction between GA and PDLLA, and the crystal structure of the encapsulated GA was influenced by the polymer.

### *In Vitro* Release Profiles

Figure 5 shows the cumulative release profiles of GA from the PDLLA microspheres (Sample A) and the beads (Samples C, D, and E), and the enlarged release curves of GA are given in the inset of Figure 5. There was an obvious burst release of GA at the very beginning of the release profile of Sample A. This might be a little GA adsorbed physically on the surface of naked PDLLA microspheres. Such rapid drug release is not desirable for controlled release, because the drug could diffuse into the blood or tissue fluid too quickly, leading to uncontrollable drug release rate.<sup>3</sup> By contrast, the beads (Samples C, D, and E) showed a lower cumulative release percentage of GA than the naked PDLLA microspheres. This may be due to the fact that the calcium alginate outer-layer acts as a diffusion barrier.

As reported, drug loading amount may affect the drug release rate.<sup>24</sup> Samples C, D, and E had different GA loading amounts and their release profiles are shown in Figure 3. It can be seen that, higher drug loading resulted in slower release, which could be attributed to the change of drug diffusivity caused by the different drug loading levels. Different amounts of GA, in other words, the GA-loaded PDLLA microspheres may affect the microstructure in the beads, leading to different drug diffusion rates. Obviously, the GA release was not complete, and lower GA percentage was released from the beads (Samples C, D and E) than from naked PDLLA microspheres (Sample A) after 15 days. Specifically, Sample C released about 45% of the loaded GA at 15 days, Sample D about 44%, and Sample E about 39%, while Sample A released about 70%. The results demonstrate that increasing PDLLA/alginate ratio can decrease the GA release.

The release profiles of BSA from the beads (Samples B–E) are shown in Figure 6, and the enlarged release curves of BSA are



**Figure 6.** *In vitro* release profiles of BSA from Samples B, C, D, and E for 15 days.

given in the inset. Obviously, there was an initial quick release followed by a slow release. BSA was released from the beads from 49 to 74% in 24 h, and about from 74 to 94% in 15 days. The initially released BSA should be the BSA close to the surface of the beads. Compared with the single drug-loaded bead (Sample B), the composite beads loaded with the PDLLA microspheres had a lower BSA cumulative release. Moreover, the release profiles of BSA from Samples C, D, and E were reversed to the GA release profiles. In addition, it can be seen that higher PDLLA/alginate ratio resulted in faster BSA release, which may be due to the interference of the beads microstructure by the introduction of the microspheres.

## CONCLUSIONS

In this study, a dual-drug delivery system was fabricated by embedding PDLLA microspheres within calcium alginate beads matrices. In this system, GA was encapsulated in the PDLLA microspheres, while BSA was loaded in calcium alginate matrices. SEM observation confirmed that the PDLLA microspheres were homogeneously distributed in the calcium alginate beads. DSC measurement suggested certain interaction between GA and PDLLA, and the crystal structure of GA was influenced by the polymer. The dual release *in vitro* showed rapider BSA release but sustained GA release in all the dual-drug delivery systems. Furthermore, the release rate of BSA was significantly accelerated by increasing PDLLA/alginate ratio, while the release rate of GA was decreased.

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