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Comparison of the degradation and release behaviors of poly(lactide-*co*-glycolide)–methoxypoly(ethylene glycol) microspheres prepared with single- and double-emulsion evaporation methods

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ABSTRACT: The purpose of this study was to compare the degradation and release behaviors of poly(lactide-*co*-glycolide) (PLGA)–methoxypoly(ethylene glycol) microspheres fabricated by the single-emulsion evaporation method (DEEM) and double-emulsion evaporation methods (DEEM). Vancomycin and mizolastine were used as the hydrophilic and hydrophobic model drugs, and they were encapsulated into microspheres through DEEM and SEEM, respectively. The two types of microspheres were similar in size distribution, but the mizolastine-loaded microspheres showed a much higher encapsulation efficiency than those loaded with vancomycin. Scanning electron microscopy, size, and molecular weight (Mw) analyses during the degradation revealed that the microspheres fabricated by DEEM underwent a bulk degradation process and showed a faster MW reduction rate during the early degradation period than the microspheres fabricated by SEEM, which exhibited a surface-to-bulk degradation process according to the Mw and morphological changes. The mass loss rates of the two types of microspheres were similar, but the mean size decrease rates showed a little difference. The mizolastine-loaded microspheres exhibited an approximately linear release profile after the initial burst release, whereas the vancomycin-loaded microspheres showed a more severe burst release, a faster release rate, and thus, a shorter time to full release. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41943.

KEYWORDS: biodegradable; biomaterials; degradation; drug delivery systems

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INTRODUCTION

In recent years, special attention has been paid to sustained-release microspheres based on biodegradable polymers,[1–4] such as poly(lactide-*co*-glycolide) (PLGA), poly(lactic acid), and poly(ε-caprolactone). Patient compliance, drug protection, and sustained release are part of the many benefits to encapsulating and releasing a therapeutic agent from polymer microspheres.[5] Polymeric microspheres have been prepared by various methods, such as phase separation,[6] solvent evaporation,[7] spray drying,[8] and supercritical fluid precipitation.[9,10] Among these methods, the single-emulsion evaporation method (SEEM) and double-emulsion evaporation method (DEEM) are the most widely used laboratory techniques for preparing microspheres encapsulating hydrophobic and hydrophilic drugs, respectively. Many studies have been conducted to optimize microsphere fabrication conditions with the solvent evaporation method[11,12] and to explore the drug-release mechanism. It has been reported that microspheres fabricated by SEEM and DEEM showed totally different interior structures;[13,14] thus, they may have a different in drug-release manner. Macromolecular drugs, such as proteins or peptides, have been preferred as hydrophilic model drugs in the studies, and they have always shown a typical triphasic release profile,[15,16] whereas hydrophobic model drugs have mostly been small in molecular weights (MWs) and have shown various release profiles according to the reports.[3,17] The release behaviors of small hydrophobic and hydrophilic drugs from microspheres of similar size have been scarcely studied and compared. Li *et al.*[17] studied methotrexate-loaded PLGA–methoxypoly(ethylene glycol) (mPEG) microspheres (50 μm) prepared by SEEM, and Avgoustakis *et al.*[18] investigated cisplatin-loaded PLGA–mPEG nanoparticles (150 nm) fabricated by DEEM. The two types of particles exhibited totally

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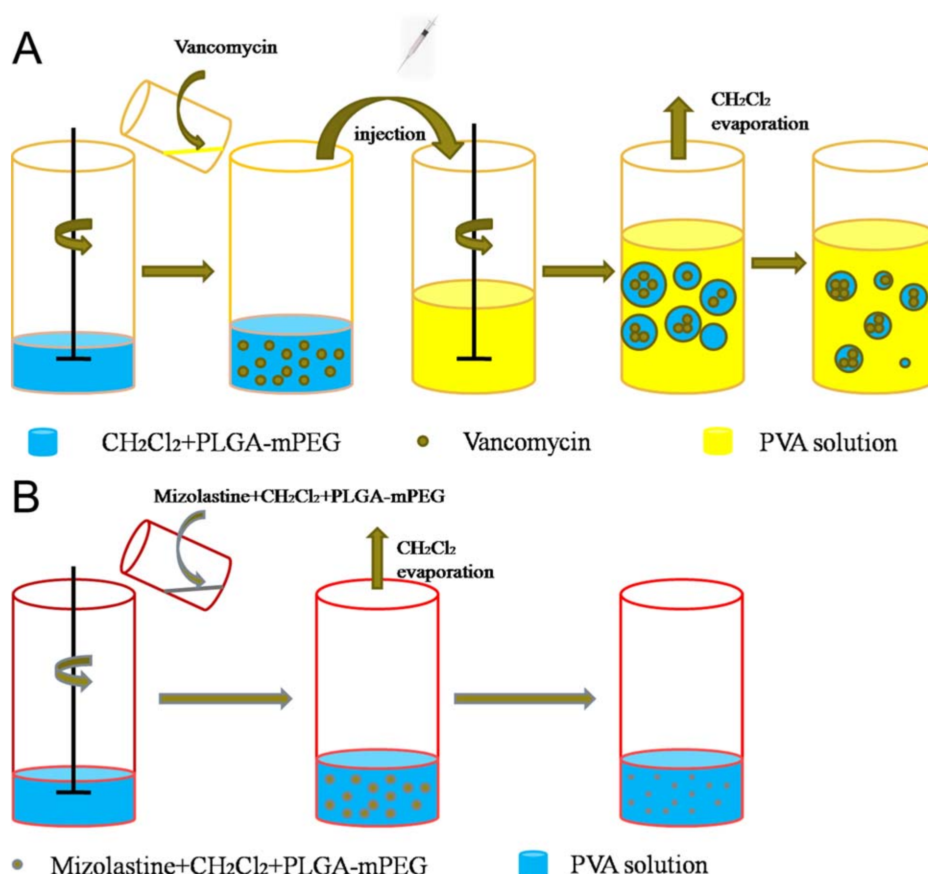

Figure 1. Schematic of the preparation procedures for (A) vancomycin-loaded and (B) mizolastine-loaded microspheres. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

different degradation and release behaviors, but we could not confirm that it was caused by the particle structure difference, as the particle size and matrix MW between the two studies were quite different.

In this study, PLGA–mPEG microspheres were used to encapsulate hydrophobic and hydrophilic drugs with small MWs through SEEM and DEEM. The microspheres degradation behaviors were compared through MW, polydispersity, and mass changes. The microsphere morphology alternation was characterized by scanning electron microscopy (SEM) to observe the microsphere erosion process. Nano Measurer 1.2 software was used to analyze the particle size changes. *In vitro* release profiles of the two drugs were also adequately studied and associated with their degradation behaviors.

EXPERIMENTAL

Materials

PLGA–mPEG [$PLGA_{9573}$–$mPEG_{5000}$ (where 9573 is the Mw of PLGA, and 5000 is the Mw of mPEG), MW 14,573, lactide/glycolide (LA/GA) = 3:1' was synthesized in our laboratory and characterized by gel permeation chromatography, IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy.[19] Poly(vinyl alcohol) (PVA; polymerization degree ≈ 1700 and hydrolysis degree ≈ 99%) from China National Medicines Corp., Ltd., was used as a stabilizer in the emulsion. Methylene chloride, as solvents, was analytical grade and was purchased from China National Medicines Corp., Ltd., and was used without purification. Vancomycin was obtained from Sigma, and mizolastine was purchased from Kang Bao Tai Fine Chemical Co., Ltd. (Wu Han, China).

Microsphere Preparation

Vancomycin-loaded microspheres were prepared with DEEM,[20] shown in Figure 1(A). First, 0.1 mL of vancomycin solution (100 mg/mL, in distilled water) was mixed in 4 mL of dichloromethane containing 250 mg of PLGA–mPEG, and the mixture was emulsified at 14,500 rpm for 10 s to obtain the water-in-oil emulsion. The primary emulsion was then injected slowly into 10 mL of a 0.25% w/v PVA solution and emulsified at 8500 rpm for 10 s to produce the water-in-oil-in-water emulsion. Next, the second emulsion was introduced into 120 mL of a 0.25% w/v PVA solution and continuously stirred at 600 rpm for 3 h to evaporate the dichloromethane. The solidified microspheres were then centrifuged for 2 min at 3000 rpm, washed three times with distilled water, lyophilized, and stored at 4°C.

Mizolastine microspheres were prepared with SEEM,[3,13,21] as shown in Figure 1(B). A quantity of 10 mg of mizolastine was added to 3 mL of dichloromethane containing 440 mg of PLGA–mPEG. The solution was then mixed with 10 mL of a 1% w/v PVA solution under homogenization at 6700 rpm for 30 s to obtain the oil-in-water emulsion. This emulsion was then added to 100 mL of a 0.1% w/v PVA solution and stirred

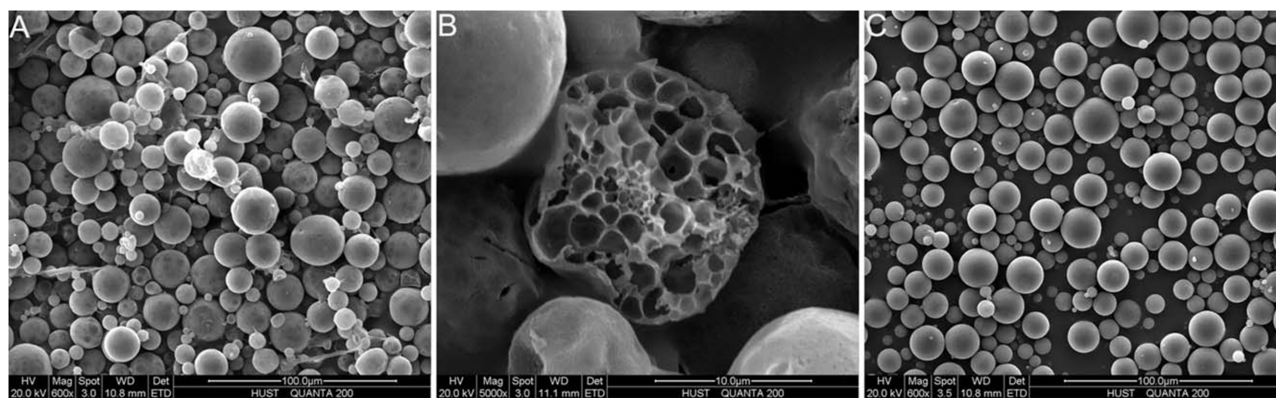

Figure 2. Scanning electron micrographs of the drug-loaded microspheres: (A) vancomycin-loaded PLGA–mPEG microspheres, (B) interior structure of the vancomycin-loaded PLGA–mPEG microspheres, and (C) mizolastine-loaded PLGA–mPEG microspheres.

continuously for 3 h at 600 rpm to evaporate the dichloromethane completely. The resulting mizolastine microspheres were then centrifuged for 2 min at 3000 rpm, washed three times with distilled water, freeze-dried overnight, and stored at 4°C.

In addition, the unloaded PLGA–mPEG microspheres were also prepared under the same conditions for degradation analysis.

Characteristics of the Microspheres

The surface morphology of the microspheres was observed with SEM (Quanta 200, Holland, FEI). The microspheres were mounted onto metal stubs with double-sided adhesive tape. After they were vacuum-coated with a thin layer of gold, the microspheres were examined by SEM. For the mean size and size distribution analysis, 300 microspheres in each group were randomly chosen from the SEM micrograph, and the software (Nano Measurer 1. 2) was applied to counting the mean size and size distribution.

The vancomycin and mizolastine encapsulation efficiencies of the microspheres were measured by ultraviolet–visible (UV–vis) spectrophotometry and high-performance liquid chromatography (HPLC), respectively. Briefly, a quantity of 10 mg of dried vancomycin-loaded microspheres was dissolved in 1 mL of methylene chloride under stirring, and 3 mL of phosphate buffer saline (PBS; pH 7.4, 0.01% sodium azide, 0.02% Tween 80) was then added. The mixture was vigorously agitated for 5 min to extract vancomycin into PBS from the methylene chloride. After centrifugation, the aqueous phase was withdrawn, and the amount of drug was analyzed with UV–vis spectrophotometry. To determine the mizolastine encapsulation efficiency, 10 mg of mizolastine-loaded microspheres was dissolved in 1 mL of methylene chloride, and then, we added 50 mL of the HPLC mobile phase (acetonitrile to 0.02 mol/L ammonium acetate = 2:3) and stirred the mixture for 1 h. The resulting mixtures were filtered through 0.45-μm nylon filters and then analyzed by HPLC. The drug encapsulation efficiency was expressed as follows:

$$\text{EE}(\%) = (\text{Actual drug in PLGA–mPEG}) / (\text{Initial drug in PLGA–mPEG}) \times 100$$

All of the experiments were run in triplicate, and the data are shown as the mean plus or minus the standard deviation.

In Vitro Microsphere Release Behavior Analysis

The *in vitro* microspheres release experiment was conducted by the suspension of microspheres in PBS (pH 7.4, 0.01% sodium azide, 0.02% Tween 80) and maintained at 37°C under continuous shaking (50 strokes/min). In consideration of the different solubility of the two drugs in PBS, we designed different release conditions. In triplicate, a certain amount of microspheres (30 mg of vancomycin-loaded microspheres and 15 mg of mizolastine-loaded microspheres) were suspended in PBS (5 mL for vancomycin-loaded microspheres and 30 mL for mizolastine-loaded microspheres) and installed in centrifuge tubes. The tubes were then sealed and placed in a shaking water bath. At scheduled time intervals, the tubes were taken out and centrifuged; then, a certain amount of supernatant (3 mL for vancomycin-loaded microspheres and 28 mL for mizolastine-loaded microspheres) was withdrawn to determine the amount of drug released and was replenished by the same volume of fresh medium. The concentration of vancomycin was measured by UV–vis spectrophotometry, and that of mizolastine was measured by HPLC.

In Vitro Microsphere Degradation Analysis

Unloaded microspheres prepared under the same conditions were applied in a degradation study as it was reported[5] that unloaded microspheres degraded in a manner that was very similar to those loaded with the drugs. The degradation behavior of the microspheres was evaluated by the effects of MW reduction, total mass loss, morphology, and size changes with time on their incubation in PBS at 37°C, as described in our previous work.[19] Unloaded microspheres which were applied for degradation analysis were incubated under the same conditions as those used for the drug-release experiment. As in the *in vitro* release experiments, the samples were centrifuged, and the supernatants were removed at fixed time points for drug-release analysis. Thus, in the degradation experiment, all of the samples were centrifuged at the same time point, and the same amount of supernatant was discarded to remove any acidic degradation products; this contributed to the autocatalytic degradation of PLGA–mPEG. If the samples were degraded further, the same amount of PBS was added back to the tube; if not, the samples were lyophilized and weighed. The dried PLGA–mPEG microspheres were then used to measure the MW and MW

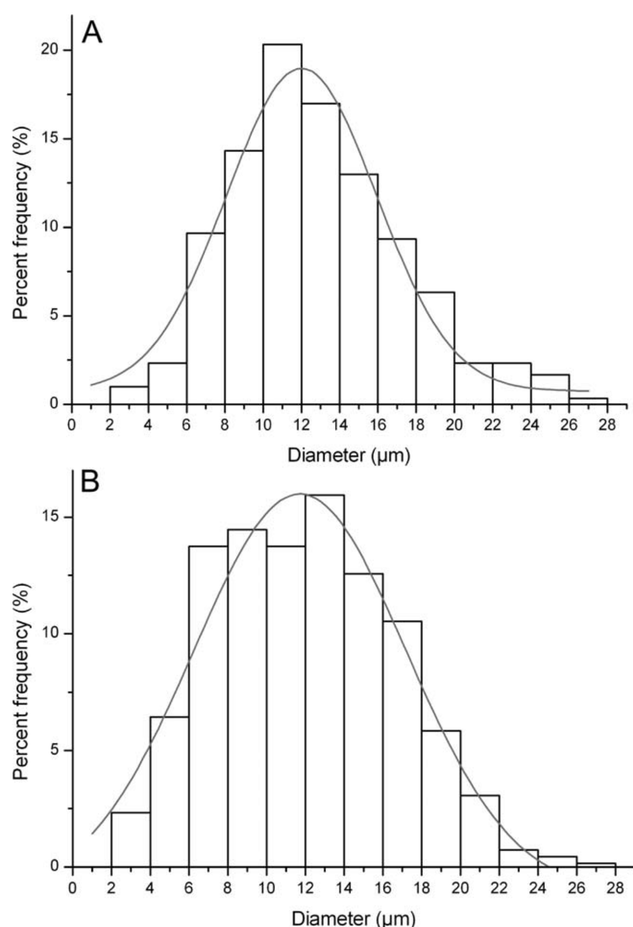

Figure 3. Size distribution of the drug-loaded microspheres: (A) vancomycin-loaded PLGA–mPEG microspheres and (B) mizolastine-loaded PLGA–mPEG microspheres.

distribution by gel permeation chromatography. The microspheres mean size changes during degradation were determined by the software of Nano Measurer 1. 2.

RESULTS AND DISCUSSION

Characteristics of the Microspheres

The drug-loaded microspheres fabricated by DEEM and SEEM exhibited a spherical and smooth morphology, as shown in Figure 2. Few vancomycin-loaded microspheres showed broken morphologies, but all of the mizolastine-loaded microspheres revealed a sphere shape and smooth surface; this could be ascribed to their different fabrication methods. The vancomycin-loaded microspheres exhibited a porous, honeycomb interior structure; this could also be speculated through the preparation procedures shown in Figure 1(A). The size distribution analysis showed a similar result (Figure 3) between the vancomycin- and mizolastine-loaded microspheres, with average particle sizes both around 12 μm; this prevented interference in the degradation and drug release analysis, which may have been caused by the particle size difference. The encapsulation efficiency was found to be $55.26 \pm 3.68\%$ for vancomycin in the PLGA–mPEG microspheres, whereas mizolastine showed a much higher encapsulation efficiency of $93.3 \pm 2.15\%$; this was attributed to the lipophilic nature of mizolastine. Thus, the drug tended to remain entrapped in the microspheres instead of being partitioned into the external aqueous phase during the preparation process.[3,22] Vancomycin-loaded microspheres revealed a lower encapsulation efficiency, as part of the hydrophilic drug was expulsed to the outer water phase during the preparation process;[13] this caused a loss of drug.

In Vitro Microsphere Degradation Analysis

The microsphere degradation experiment was conducted under the same conditions with the *in vitro* drug-release

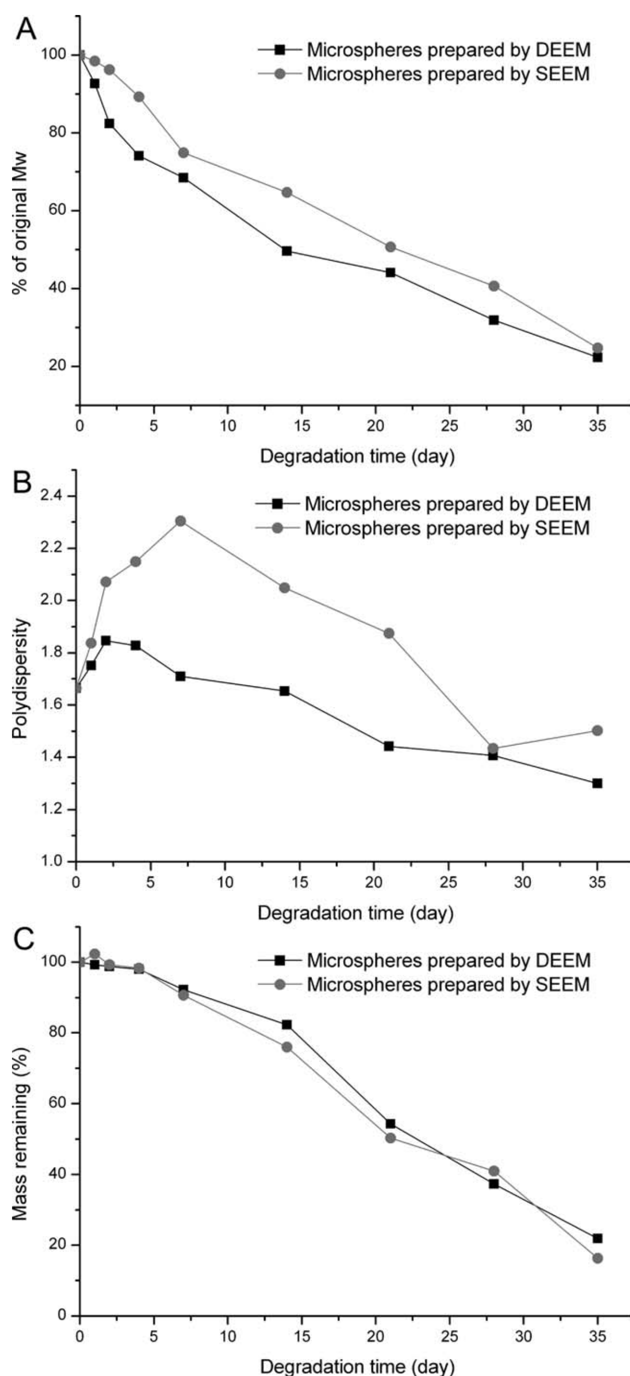

Figure 4. (A) Polymer MW, (B) polydispersity, and (C) total mass changes of the unloaded PLGA–mPEG microspheres during degradation in PBS.

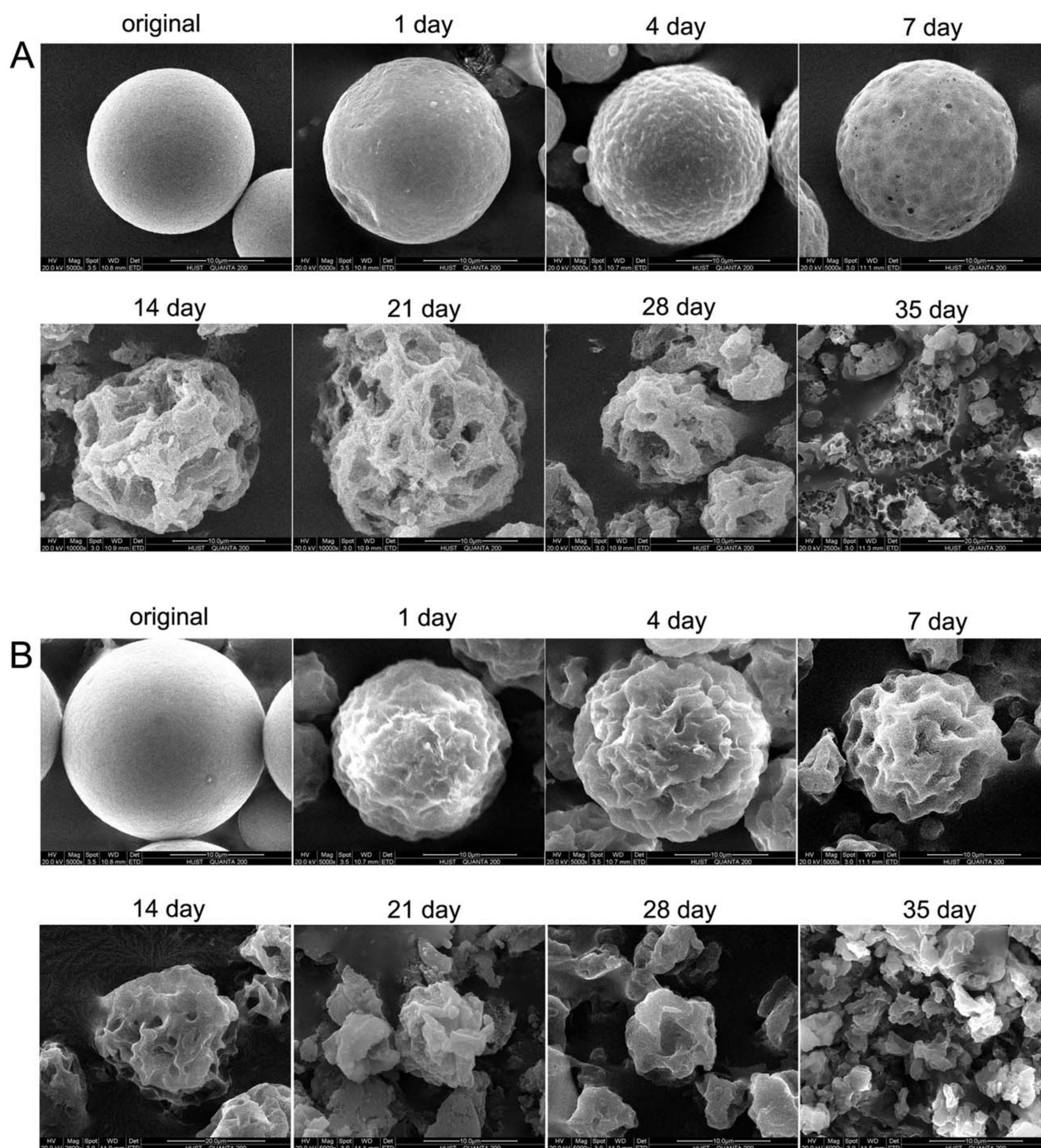

Figure 5. Scanning electron micrographs of the PLGA–mPEG microsphere morphological changes during degradation in PBS: (A) unloaded PLGA (9.5)–mPEG (5) microspheres prepared by the double-emulsion evaporation method and (B) unloaded PLGA (9.5)–mPEG (5) microspheres prepared by SEEM.

experiment. Blank microspheres were applied for degradation analysis, as it showed the same degradation behavior with the drug loaded. The MW reduction results revealed that the microspheres prepared by DEEM showed a faster reduction rate than the microspheres prepared by SEEM during the initial degradation period, and the reduction rates both slowed down in the latter degradation stages, as shown in Figure 4(A). PLGA–mPEG with a higher MW revealed faster a MW reduction rate for microspheres prepared by DEEM, especially in the early degradation period, as described in our previous work.[19] However, the PLGA–mPEG initial MW caused no significant MW reduction difference for microspheres prepared by SEEM in the early period, as shown in Figure S1 in the Supporting Information. Within an early period of 4 days, 74.07% of the original MW remained for the PLGA–mPEG microspheres prepared by DEEM, whereas, the percentage came to 89.33% for the PLGA–mPEG microspheres prepared by SEEM.

The degradation diversity between the two types of microspheres were ascribed to their different interior structures. According to the reports,[13,14] microspheres prepared by DEEM

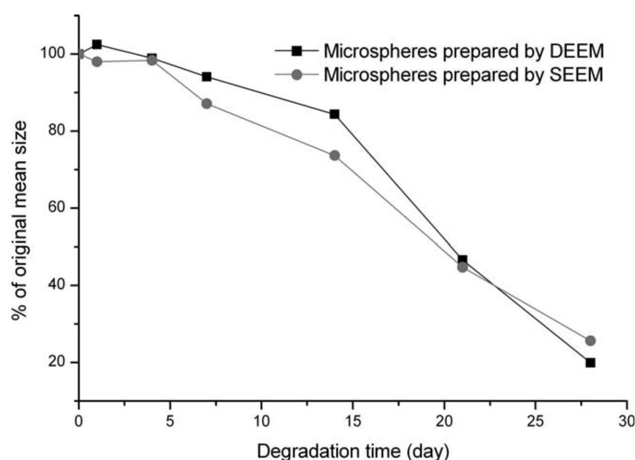

Figure 6. Mean size change of the unloaded PLGA–mPEG microspheres during degradation in PBS.

exhibited a capsule or honeycomb interior structure, and microspheres prepared by SEEM were solid spheres. Through the ultrasonic pulverization of microspheres, we confirmed that the microspheres prepared by DEEM in this study possessed a honeycomb interior structure [Figure 2(B)], but the microspheres prepared by SEEM was difficult to break; this, in turn, suggested that it was a solid sphere as reported.

On the basis of the MW reduction results, we assumed that the microspheres prepared by DEEM underwent a bulk degradation process, as water could infiltrate into the particle easily because of its numerous connecting holes inside. Therefore, the degradation process occurred in the bulk of the microspheres because of the complete wetting of the system; this resulted in a fast MW reduction rate in the early period. On the basis of this assumption, PLGA–mPEG with a higher MW would exhibited a faster MW reduction rate because of its more hydrolytic cleavable ester groups in the polymer chain; this was confirmed in our previous study.[19] In the latter stage of degradation, the MW reduction rate slowed down because of the decrease of cleavable ester bonds, and the MW decreased to a low value, as shown in Figure 4(A). For the solid microspheres prepared by SEEM, water was relatively difficult to infiltrate into the particle, the particle may have undergone a surface-to-bulk degradation in the early period, and the external matrix eroded first. This resulted in a slower MW reduction rate of the whole microsphere as compared to that prepared by DEEM. The surface-to-bulk degradation process also explained the similar MW reduction rates in the early stage among the PLGA–mPEG microspheres of different MWs (Figure S1). Furthermore, the surface-to-bulk degradation process caused a wider PLGA–mPEG MW distribution than in the microspheres prepared by DEEM, as shown in Figure 4(B). With sustained water infiltration, the microspheres were gradually filled with water, and the degradation proceeded in the bulk of the particles in the latter stage.

The assumption of the two types of degradation process was also supported by the microsphere morphology and size alterations, as shown in Figures 5 and 6. The degradation step directly controlled the erosion process. Both types of microspheres were subjected to surface modifications (increases in the roughness) once they were incubated in PBS; this should have been due to the rearrangement of the polymer chain in the surface as water infiltration. The microspheres prepared by DEEM exhibited bulk corrosion behavior along with the process of degradation [Figure 5(A)], whereas microspheres prepared by SEEM revealed an obvious surface-to-bulk erosion process [Figure 5(B)], which in turn, confirmed that the degradation behaviors were probably the same as we assumed. Microspheres prepared by DEEM showed a slight swelling once they were incubated into PBS (Figure 6); this was ascribed to the influx of water. However, no obvious swelling was observed for the microspheres prepared by SEEM; this indicated a slow water infiltration into the particles. Both types of microspheres lost their structure after 7 weeks of degradation, and thus, we only analyzed the size alternation among 6 weeks.

Although degradation steps were different between the two types of microspheres prepared by SEEM and DEEM, they revealed a similar mass loss process. Both the two types of microspheres showed a slight mass loss during the first week [Figure 4(C)]; this could probably have been due to the few soluble degradation products generated in this period. At the latter stage, the mass loss rate sped up as abundant soluble degradation products generated and dissolved in the PBS; this was also confirmed by the obvious morphology corrosion and mean size decrease of the microspheres, as shown in Figures 5 and 6.

In Vitro Microsphere Release Behavior Analysis

The hydrophilic vancomycin and hydrophobic mizolastine were used as small model drugs and were encapsulated into microspheres through DEEM and SEEM, respectively. Vancomycin-loaded microspheres exhibited a honeycomb interior structure, and the drug was distributed in the numerous holes inside the microspheres, whereas mizolastine was dispersed throughout the solid microspheres, mixing evenly with the matrix.

The *in vitro* vancomycin and mizolastine release behaviors from the PLGA–mPEG microspheres are given in Figure 7. Both of the microspheres exhibited an obvious initial burst release during the first day, and this was mostly due to the poorly entrapped or surface-associated drugs. Compared with the mizolastine-loaded microspheres, vancomycin showed a more severe burst release as part of the inner small drug distributed in the interior pores, which may have transported through the polymer phase because of microsphere swelling and polymer rearrangement caused by the fast water infiltration. Mizolastine exhibited a linear-like release rate after the initial burst release, and the release lasted for 5 weeks, whereas vancomycin showed a faster release rate, and it took only 3 weeks for the drug to be released in full. The different release profiles between vancomycin and mizolastine may have been ascribed to their different microsphere interior structures, drug distributions and microsphere degradation behaviors. Bulk degradation happened in vancomycin microspheres may have caused the interior holes to interlinked; thus, the drug could diffuse out quickly from the polymer phase, and the pores formed in the microsphere surface. This was caused by the sustained degradation (Figure 5). However, according to our previous study,[19] a macromolecular hydrophilic drug such as BSA shows a much slower release rate (lag period) and a longer time for drug to be released in full, as the macromolecular drug

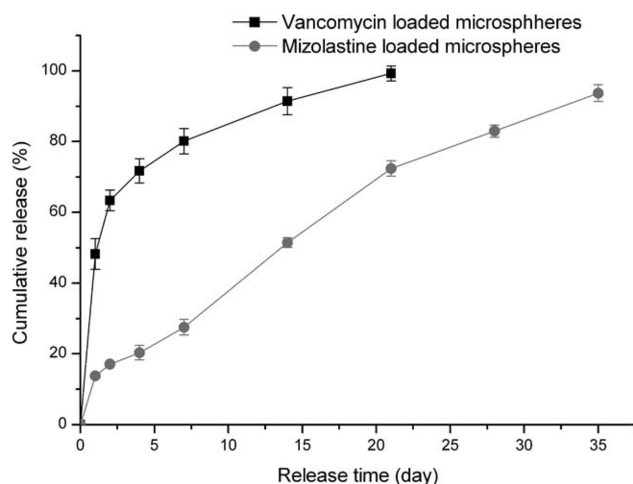

Figure 7. Release behaviors of (A) vancomycin and (B) mizolastine from the PLGA–mPEG microspheres.

could not diffuse out easily until the formation of pores big enough connecting to the out water.

Mizolastine dispersed throughout the solid microspheres and released out mostly depended on the microsphere degradation. Water infiltration and polymer chain arrangement also supplied a route for the small hydrophobic drug to diffuse out.[23] Microsphere degradation happened immediately once they were incubated into PBS; this was accompanied by mizolastine release. Therefore, the drug-in-matrix blending structure and the surface-to-bulk erosion behavior may have resulted in the linear-like release curve. This was also reflected by the microsphere morphology and mean size changes, as shown in Figures 5 and 6. About 6.3% of mizolastine was retained in the matrix when degradation eventually proceeded to the point of eradication of any existing microsphere structure within approximately 5 weeks; this could be explained by its drug-in-matrix blending structure.

CONCLUSIONS

In this research, vancomycin- and mizolastine-loaded PLGA–mPEG microspheres were successfully prepared by DEEM and SEEM, respectively. The two types of microspheres possessed different interior structures and drug distributions. Through degradation analysis and SEM observation, we found that the two types of microspheres exhibited totally different degradation behaviors: a bulk degradation (corrosion) process for the microspheres prepared by DEEM and a surface-to-bulk degradation (erosion) process for microspheres prepared by SEEM; this was mostly ascribed to their different interior structures. The difference in the interior structures and drug distributions also resulted in different drug-release behaviors, and the erosion process also contributed to the drug-release profiles.

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