Uniform Ethyl Cellulose Microspheres of Controlled Sizes and Polymer Viscosities and Their Drug-Release Profiles

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ABSTRACT: Monodisperse ethyl cellulose (EC) microspheres (MSs) of three size groups (20–35, 55–60, and 80–105 μm in diameter) were fabricated to study the effect of the MS size on the drug-release profiles with a novel scheme combining mechanical and hydrodynamic forces. More than 90% of the MSs were within ±3 μm of the average diameter, regardless of the EC viscosities used in the study. The effect of the polymer viscosity was also examined with ECs with two distinct viscosities (4 and 45 cp). The encapsulation efficiencies (EEs) of piroxicam and rhodamine were 6.4–51 and 63–80%, respectively. The drug distribution in the MSs showed a higher concentration near the particle surface, and this was more distinct with rhodamine. An approximately zero-order release was observed with the small MSs of 4-cp EC during 24 h without evident initial bursts. The MS size affected the surface-area-to-volume ratio, EE, and intraparticle drug distribution, affecting the drug-release profiles. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 850–857, 2009

Key words: biopolymers; drug delivery systems; microencapsulation; processing

INTRODUCTION

Controlled drug-delivery systems are of great interest because of their ability to regulate the release kinetics and spatial localization of therapeutic agents.[1] Drugs with a short biological half-life may require frequent administration or large doses to maintain their therapeutically efficacious levels, and this may cause undesirable toxicity or local side effects. By choosing an appropriate delivery system, therefore, one can protect drugs from the biological environment, thus prolonging their release, or target them to a site of interest more efficiently.

Biocompatible polymer microspheres (MSs) are good candidates for controlled drug-delivery systems.[2–4] Ethyl cellulose (EC), a water-insoluble and pH-independent polymer, has been widely used as a material for controlled drug release for various administration routes.[5–11] For example, indomethacin-loaded EC was studied as a rectal delivery vehicle and provided more than 5 h of prolonged release.[5,10] EC MSs loaded with potassium chloride, aspirin, fenoterol HBr, and so forth were studied as oral delivery vehicles, achieving continuous release for about 24 h.[6,7,12–14]

Several methods of fabricating drug-loaded EC MSs, including coacervation,[9,15–17] spray drying,[18,19] and emulsion techniques,[12,13,17,20–24] have been investigated, and the factors affecting the drug-release profiles have been studied. The nonsolvents used,[12,17,21] the amount of the emulsion stabilizer,[23] the rate of agitation,[10,14,23] and the molecular weight of EC[18,20] have been varied to examine their respective effects on drug release. However, possibly because of the difficulty in fabricating monodisperse EC MSs, none of these release studies were carried out without the uncertainty stemming from the polydispersity of MSs. It is well known that the shape, size, and size distribution of drug-loaded MSs are some of the critical determinants of drug-release profiles because the surface-area-to-volume ratio of the MSs strongly influences the rate of drug release and/or polymer degradation. For example, MSs with distorted spherical shapes should show different drug release than those with smooth spherical shapes even with the same volume. EC MSs obtained from coacervation and spray drying have often exhibited irregular shapes with wide size distributions.[16,18] Most of the MSs obtained from the emulsion method have exhibited smooth spherical shapes but still wide size distributions.[12,13,17,20–24] The reported standard deviations of the size distributions are 20–50% of the average diameters.

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We recently fabricated monodisperse MSs of poly (D,L-lactide-*co*-glycolide) (PLG), various hydrogel materials, and a few other polymers for use as drug-delivery systems with a precision particle fabrication (PPF) technique.[25–29] The PPF technique, which uses both acoustic and hydrodynamic forces for particle fabrication, evolved from the work of Kim and co-workers,[30–38] who demonstrated the ability to generate uniform solid and hollow MSs of frozen hydrogen and silica aerogels with precisely controlled sizes and thicknesses. Following the basic PPF scheme, we fabricated drug-loaded EC MSs with a uniform size and a precisely controlled size distribution in this work. Piroxicam, a nonsteroidal anti-inflammatory drug, and rhodamine B were encapsulated as model hydrophobic (solubility in water = 53.3 μg/mL at pH ~ 7) and hydrophilic drugs (7.8 mg/mL), respectively, having similar molecular weights.[27] By fabricating monodisperse EC MSs of different sizes and two distinct viscosities (4 and 45 cp), we investigated the effects of the particle size and polymer viscosity on the drug release without the uncertainties associated with the particle size variation. The *in vitro* drug-release studies were performed in a simulated biological fluid (i.e., pH 7.4 phosphate-buffered saline) for no longer than 24 h mainly to assess monodisperse EC MSs as controlled drug-delivery devices, possibly for oral or rectal administration. Although the release profile at low pHs, mimicking the stomach fluid for oral drug delivery, was not examined in this study, the results obtained herein could shed some light on their possible applications because disintegration, degradation, or swelling of the EC matrix at a low pH is thought to be unlikely.[5,6] The effects of the particle surface morphology and drug distribution on drug release were also examined.

EXPERIMENTAL

Materials

ECs with two different viscosities (48% ethoxyl, 4 cp, and 49.3% ethoxyl, 45 cp) were obtained from Aldrich (St. Louis, MO). Poly(vinyl alcohol) (PVA; 88% hydrolyzed) was purchased from Polysciences (Warrington, PA). Rhodamine B was acquired from Sigma. Piroxicam freebase was a gift from Dongwha Pharmaceuticals (Seoul, Korea). High-performance-liquid-chromatography-grade dichloromethane (DCM) was purchased from Fisher Scientific (Pittsburg, PA).

MS preparation

As shown in Figure 1, EC MSs with two different viscosities (4 and 45 cp) were prepared with the PPF method.[26–38] Briefly, to fabricate the drug-loaded EC MSs, 1 g of EC was dissolved in 20 mL of DCM (5% w/v), which was followed by an initial loading of the drugs (piroxicam: 50 mg and 5% w/w; rhodamine: 100 mg and 10% w/w). A smooth jet of the resulting solution was generated with a dual nozzle carrying the solution of EC and drug into the inner nozzle and a carrier stream of an aqueous phase (1% w/v PVA in deionized water) into the outer nozzle. The jet was subsequently broken up into uniform drops by acoustic excitation, which were collected in a beaker containing an aqueous PVA solution (1% w/v) and subsequently hardened for 3 h. The resulting MSs were filtered, washed several times with deionized water, and lyophilized.

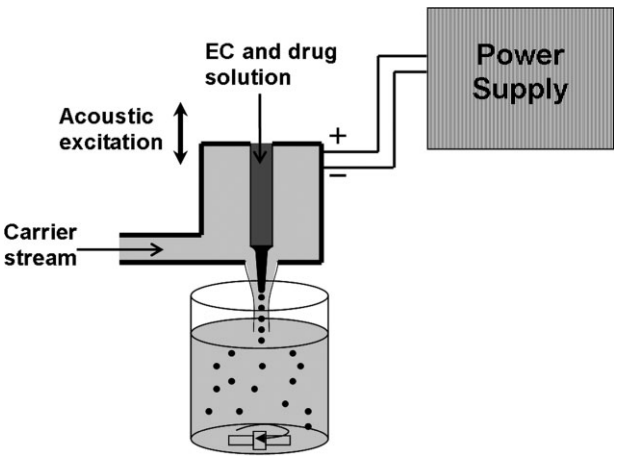

Figure 1 Schematic of the apparatus for the fabrication of the drug-loaded EC MSs.

Particle size distribution

A Coulter Multisizer 3 (Beckman Coulter, Fullerton, CA) equipped with a 200-μm aperture was used to determine the size distribution of the resulting EC MSs. The EC MSs were suspended in an Isoton electrolyte (Beckman Coulter) with a dispersant (Dispersant IA, Beckman Coulter) to prevent aggregation. More than 5000 MSs were counted for each sample.

Scanning electron microscopy (SEM)

The uniformity and surface morphology of the MSs were examined with a Hitachi (Schaumburg, IL) S-4700 scanning electron microscope. A droplet of an aqueous suspension of EC MSs was placed on a small piece of silicon wafer attached to a scanning electron microscope sample holder. The samples were dried overnight and sputter-coated with gold. The MSs were imaged at 2–10 kV.

Encapsulation efficiency (EE) study

Five-milligram samples of EC MSs, loaded with piroxicam and rhodamine, were completely dissolved

in 20 mL of DCM and methanol, respectively. The resulting solution was measured with a spectrophotometer (Cary 50, Varian, Palo Alto, CA) to determine the actual amount of the drug entrapped in the EC MSs. The initial loading amounts of piroxicam and rhodamine were 250 and 500 μg, that is, 5 and 10% w/w, respectively. The EE was obtained with the following equation:

$$\text{EE}\,(\%) = (\text{Actual loading amount}/\text{Initial loading amount}) \times 100 \quad (1)$$

All measurements were carried out in triplicate.

Confocal microscopy

The drug-loaded EC MSs were imaged with a laser scanning confocal microscope (Fluoview FV 300 laser scanning biologic microscope, Olympus, Center Valley, PA). The images from the MS midsections were analyzed to determine the drug distribution at the center.

In vitro drug release

The drug-loaded EC MSs (1–2 mg) were incubated in 1.5 mL of a phosphate-buffered saline solution (pH = 7.4; Mediatech, Manassas, VA) containing 1% Tween 20 (Sigma, St. Louis, MO) at 37°C for 24 h with continuous agitation. At scheduled intervals, 1 mL of the supernatant was collected, and the medium was refilled with a fresh phosphate-buffered saline solution. The collected supernatant was measured spectrophotometrically (Varian 50) to determine the released amount. The drug-release data were calculated as the cumulative percentage release based on the actual amount of drug encapsulated in the EC MSs. The addition of 1% Tween 20 increased piroxicam solubility to more than 1 mg/mL and helped to maintain adequate sink conditions.[26] The experiments were carried out in triplicate for each sample; the standard deviations were also obtained and incorporated into the release data.

RESULTS AND DISCUSSION

Characterization of drug-loaded EC MSs

The factors affecting *in vitro* drug-release profiles include the size and size distribution of EC MSs and the EC viscosity, that is, the degree of polymerization. For a given drug and carrier polymer, the particle size is considered to be an important factor influencing the EE, intraparticle drug distribution, and diffusive drug release, which dictate the drug-release kinetics.[26,27,29] However, because of the unavailability of monodisperse EC MSs as drug-delivery vehicles, the effect of the MS size on the drug release was not elucidated. Therefore, the EC MSs fabricated by the PPF method herein enabled us to elucidate the parameters affecting the drug release without the uncertainties resulting from the nonuniformity of the particle size.

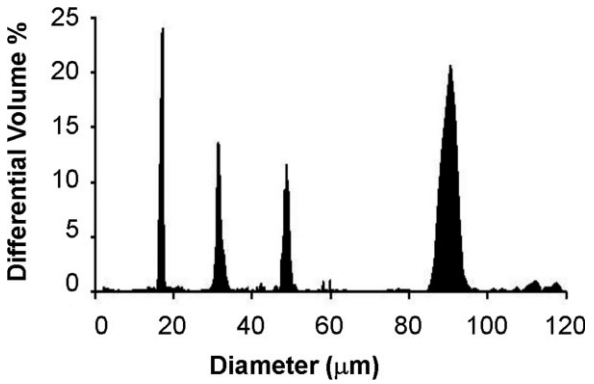

Figure 2 Size distributions of four different batches of EC MSs fabricated with the PPF method.

As shown by the size distributions in Figure 2, uniform EC MSs of various sizes were prepared with more than 90% of the MSs within ±3 μm of the average diameter. The initial loadings of piroxicam and rhodamine were 5 and 10% w/w, respectively. Figure 3 shows the SEM images of 4- and 45-cp EC MSs, illustrating the excellent uniformity of the MSs fabricated by the PPF method. Three different MS sizes were fabricated for each viscosity. For the encapsulation of piroxicam, MSs with diameters of 35, 55, and 85 μm and 30, 55, and 90 μm were fabricated with 4-cp EC and 45-cp EC, respectively (Table I). For rhodamine, MSs with diameters of 30, 60, and 105 μm and 20, 60, and 90 μm were fabricated with 4-cp EC and 45-cp EC, respectively (Table I). All EC MSs exhibited porous surface morphologies, as shown in Figure 4.

EE

EE was analyzed in light of drug diffusion, which was intricately dictated by the size, polymer viscosity, hardening time of the MSs, and drug/polymer interactions. It should also be noted that unlike the hardened EC MSs, the EC solution droplets were under more dynamic conditions, such as solvent evaporation, size reduction, and highly mobile drug molecules in solution.

Table I summarizes the EEs (Std. < ±1.5%) and the actual loading amounts of the EC MSs fabricated in this work. For piroxicam, the EEs of both small (30 and 35 μm) and large (80 and 90 μm) MSs exhibited moderate values (>30%), regardless of the viscosity. Yang et al.[12,13] reported that fast hardening of EC MSs, resulting in rough surfaces and large pores, was responsible for the low EE, which increased with the EC viscosity because of the retarded

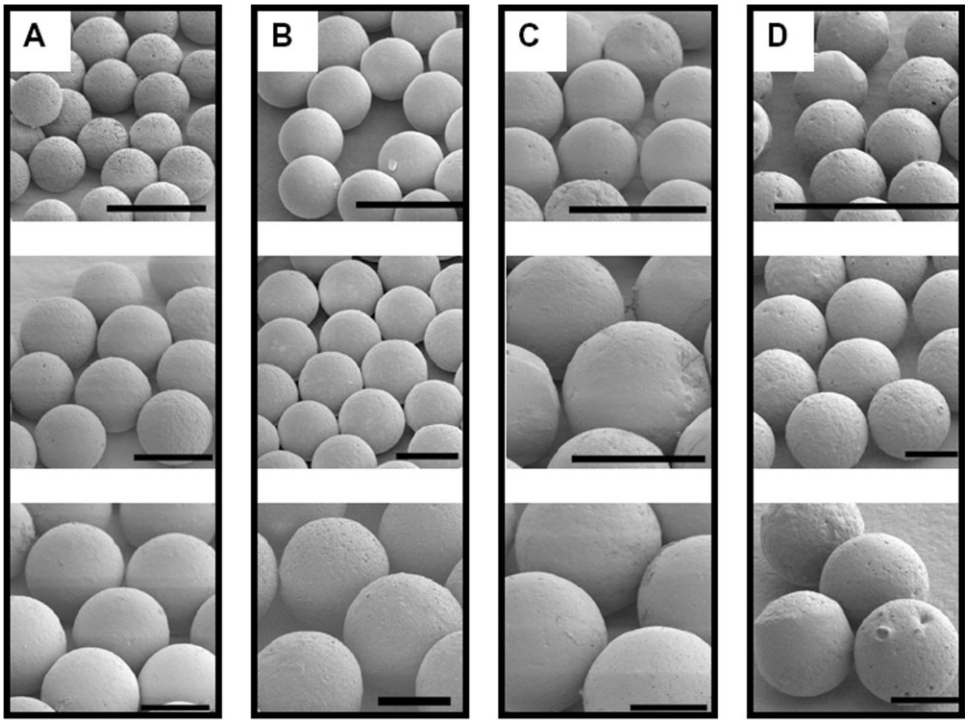

Figure 3 SEM images of drug-loaded uniform EC MSs with different sizes and EC viscosities. The loaded drugs and EC viscosities were (A) piroxicam and 4 cp, (B) piroxicam and 45 cp, (C) rhodamine and 4 cp, and (D) rhodamine and 45 cp. The diameters of the MSs were (A) 35, 55, and 80 μm; (B) 30, 55, and 90 μm; (C) 30, 60, and 105 μm; and (D) 20, 60, and 90 μm (from top to bottom). The scale bars are 50 μm.

diffusion. However, the monodisperse EC MSs used in this study revealed that the EE of piroxicam was affected by not only the EC viscosity but also other factors that originated from the MS size. For instance, the large surface-area-to-volume ratio of the small MSs facilitated the drug diffusion, lowering the EE, whereas it also facilitated the solvent removal to speed up the hardening of the MSs and to arrest piroxicam in the polymer matrix, improving the EE. The EE of the small MSs (30 and 35 μm) was measured to be 41.5% for 4-cp EC and 51.0% for 45-cp EC, that is, 20.8 and 25.5 μg of drug/mg of MSs, respectively. As the MS size increased, because of the decreased surface-area-to-volume ratio, the hardening of the MSs was retarded, and this facilitated the diffusion of the drug through the liquid droplet. On the other hand, the increased distance for the hydrophobic drug to diffuse out to the nascent droplet surface in conjunction with the decreased surface-area-to-volume ratio retarded the drug diffusion. As a result, the EEs of the large MSs (80 and 90 μm), which were 47.6% for 4 cp and 33.3% for 45-cp EC, that is, 23.8 and 16.7 μg of drug/mg of MSs, respectively, were not much different from those of the small MSs. Interestingly, the medium (55 μm) MSs exhibited much lower EEs, 13.7% for 4-cp EC and 6.4% for 45-cp EC, that is, 6.9 and 3.2 μg of drug/mg of MSs, respectively. The shorter diffusion distance compared to that of the large MSs and the slower hardening process compared to that of the small MSs could have contributed to such low EEs of the midsize MSs. A similar phenomenon has been observed for piroxicam-loaded PLG MSs.[27]

For rhodamine, high EEs (63.7–79.9%, i.e., 63.7–79.9 μg/mg of EC MSs) were obtained for MSs of all sizes, except for the 20-μm MSs of 45-cp EC (17.1%, i.e., 17.1 μg/mg of EC MSs). The low EE of the small MSs of 45 cp was rather unexpected. However, the

TABLE I
EEs of Piroxicam and Rhodamine in the EC MSs

Model drug	Polymer viscosity (cp)	Average diameter (μm)	EE (%)	Encapsulated drug in EC MSs (μg/mg)
Piroxicam	4	35 (±1.5)	41.5	20.8
		55 (±2.6)	13.7	6.9
		80 (±3.0)	47.6	23.8
	45	30 (±2.4)	51.0	25.5
		55 (±3.0)	6.4	3.2
		90 (±2.5)	33.3	16.7
Rhodamine B	4	30 (±1.5)	77.9	77.9
		60 (±1.5)	79.9	79.9
		105 (±2.3)	79.7	79.7
	45	20 (±1.0)	17.1	17.1
		60 (±2.0)	72.6	72.6
		90 (±2.5)	63.7	63.7

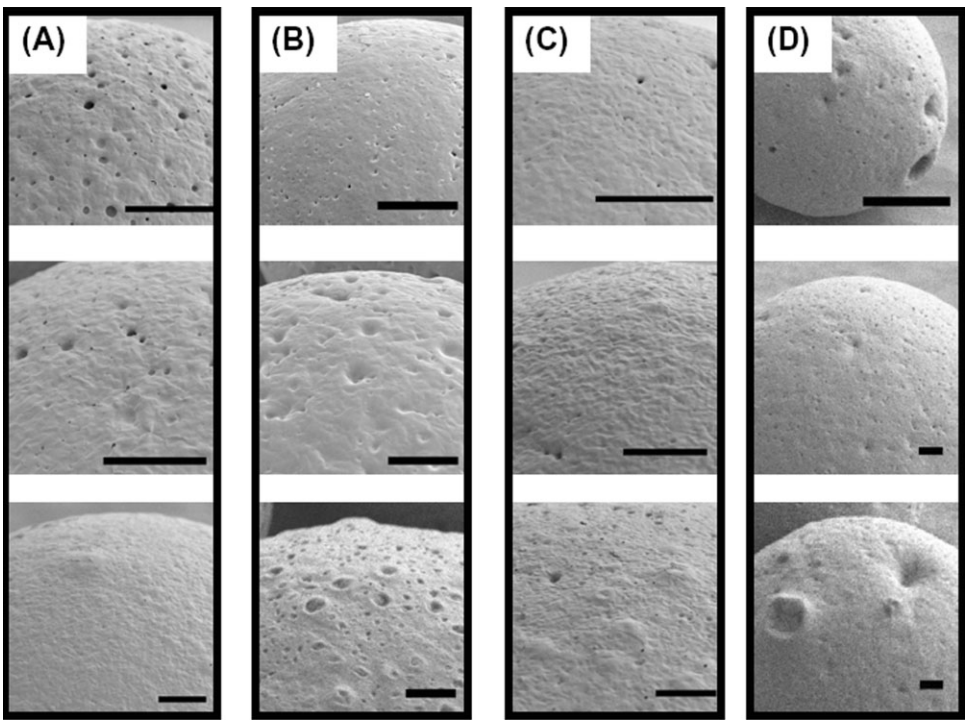

Figure 4 Surface morphologies of drug-loaded uniform EC MSs with different sizes and EC viscosities. The loaded drugs and EC viscosities were (A) piroxicam and 4 cp, (B) piroxicam and 45 cp, (C) rhodamine and 4 cp, and (D) rhodamine and 45 cp. The diameters of the MSs were (A) 35, 55, and 80 μm; (B) 30, 55, and 90 μm; (C) 30, 60, and 105 μm; and (D) 20, 60, and 90 μm (from top to bottom). The scale bars are 5 μm.

high surface-area-to-volume ratio, accelerating the drug diffusion, could have affected the low EE. In addition, the effect of the EC viscosity on the retardation of MS solidification seemed to be more significant than the effect on the retardation of drug diffusion. The higher overall EEs of rhodamine (17.1–79.9%, i.e., 17.1–79.9 μg/mg of EC MSs) versus those of piroxicam (6.4–47.6%, i.e., 3.2–25.5 μg/mg of EC MSs) could be attributed to the electrostatic attractive interactions between the oppositely charged EC and the basic dye rhodamine. EC is known to be negatively charged at neutral pH because of the presence of carboxyl groups.[39]

Intraparticle drug distribution

The midsection of the drug-loaded MSs was inspected with confocal microscopy to study the drug distribution across the MSs. The intensity of the emission light corresponded to the concentration of the drug. Grattard et al.[18] previously examined the distribution of fluorescein-labeled protein in spray-dried EC MSs and observed inconsistent distribution profiles possibly due to the polydispersity of EC MSs. The monodisperse EC MSs fabricated in this work, however, elucidated drug distributions specific to each precisely controlled size.

Figure 5 shows the distributions of piroxicam and rhodamine inside the EC MSs. Regardless of the MS sizes and EC viscosities, both drugs exhibited higher concentrations near the surface. In particular, the localized distribution of rhodamine was conspicuous. Interestingly, our previous study showed that the distribution of piroxicam in the PLG MSs was localized at the center and that of rhodamine was localized at the surface, and this was attributed to the different affinities of the drugs to the polymer and aqueous phase.[26] During the hardening process, piroxicam, which is more hydrophobic than PLG, would move toward the center, and the hydrophilic rhodamine would move toward the surface, that is, the oil–water interface. Therefore, the preferential distribution of piroxicam near the surface of the EC MSs could be ascribed to the hydrophobicity of EC, which is more hydrophobic than the drug.[5,6] Undoubtedly, the localized distribution became more prominent for rhodamine.

In vitro drug release

Figure 6 shows the *in vitro* drug-release profiles of piroxicam and rhodamine from the EC MSs. For piroxicam, the release rate decreased with the MS size, reflecting the surface-area-to-volume ratio of the MSs [Fig. 6(A,B)]. The larger the MS was, the smaller

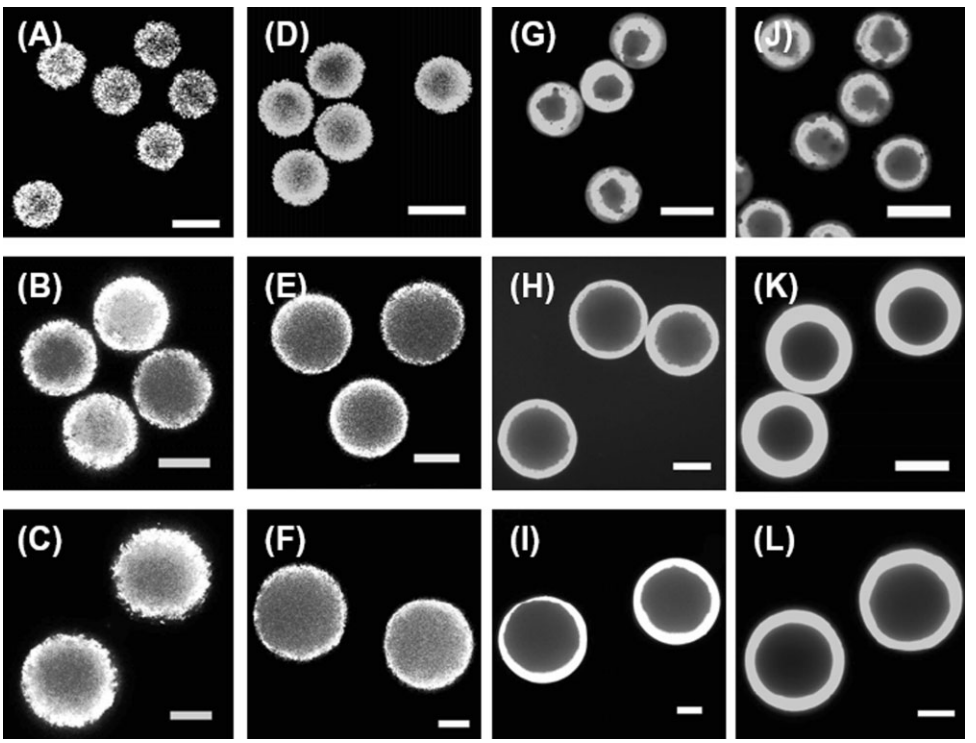

Figure 5 Intraparticle drug distribution profiles of the EC MSs as measured by the fluorescence output. The bright color indicates piroxicam and rhodamine distributions. The scale bars are 30 μm. The loaded drugs were (A–F) piroxicam and (G–L) rhodamine. The EC viscosities were (A–C) 4, (D–F) 45, (G–I) 4, and (J–L) 45 cp. The diameters were (A) 35, (B) 55, (C) 80, (D) 30, (E) 55, (F) 90, (G) 30, (H) 60, (I) 105, (J) 20, (K) 60, and (L) 90 μm.

the surface-area-to-volume ratio was, and this reduced the flux of piroxicam out of the particles. Thus, the total release from 4-cp EC MSs during the first 24 h increased from 6 to 23% as the MS size decreased from 80 to 35 μm. For 45-cp EC, the total release also increased from 11 to 23% with the decrease in the MS size from 90 to 30 μm. The release from the medium MSs (50- and 55-μm MSs) was not compared with the others because of their much lower EEs (Table I). The effect of the EC viscosity on piroxicam release appeared to be insignificant. The total release was in the range of 6–23% for both 4- and 45-cp EC MSs.

Meanwhile, the effect of the polymer viscosity on rhodamine release was quite dramatic.[40] The total release from the 45-cp EC MSs during the first 24 h was significantly suppressed versus that from the 4-cp EC MSs, that is, 1–8% for the former and 20–60% for the latter [Fig. 6(C,D)]. For the 60-μm MSs of 45-cp EC, the total release increased from 8 to 40% as the EC viscosity decreased to 4 cp, and it decreased to 2% as the MS size increased to 90 μm. The release from the 20-μm MSs was not compared because of their much lower EE (Table I). The increase in the total release exhibited by the 4-cp EC MSs could be attributed to the high solubility of the drug in water in combination with the lower EC viscosity, that is, less hindrance for the diffusion of the drug. The former could be supported by the lower release percentage demonstrated by the less soluble piroxicam. The size effect was manifested for all three sizes of the 4-cp EC MSs, reflecting their surface-area-to-volume ratios. Figure 6(C) shows that the total release during the first 24 h for the 105-μm MSs was 20%, and this increased to 40 and 60% as the MS size decreased to 60 and 30 μm, respectively.

Reduction of the initial burst has been widely pursued for MS-based drug delivery because a significant initial burst can not only cause local or systemic toxicity but also be undesirable for long-term release.[41] It was reported that the initial burst of ketoprofen, a nonsteroidal anti-inflammatory drug, was reduced by the coating of Eudragit particles with a mixture of carboxymethylethylcellulose and EC.[42] To reduce the initial burst release of fenoterol HBr, a β-agonist, from EC MSs, a nonsolvent such as petroleum benzin was added during the MS hardening process.[14] However, these methods would require additional processes, toxic chemicals, or unnecessary additive materials in the drug-delivery carriers.

In this work, we demonstrated that the uniform MSs of 4-cp EC reduced the initial burst often observed with MS-based drug-delivery systems. The small EC MSs exhibited an appreciable reduction in initial bursts, leading to an approximately linear release of both lipophilic and hydrophilic

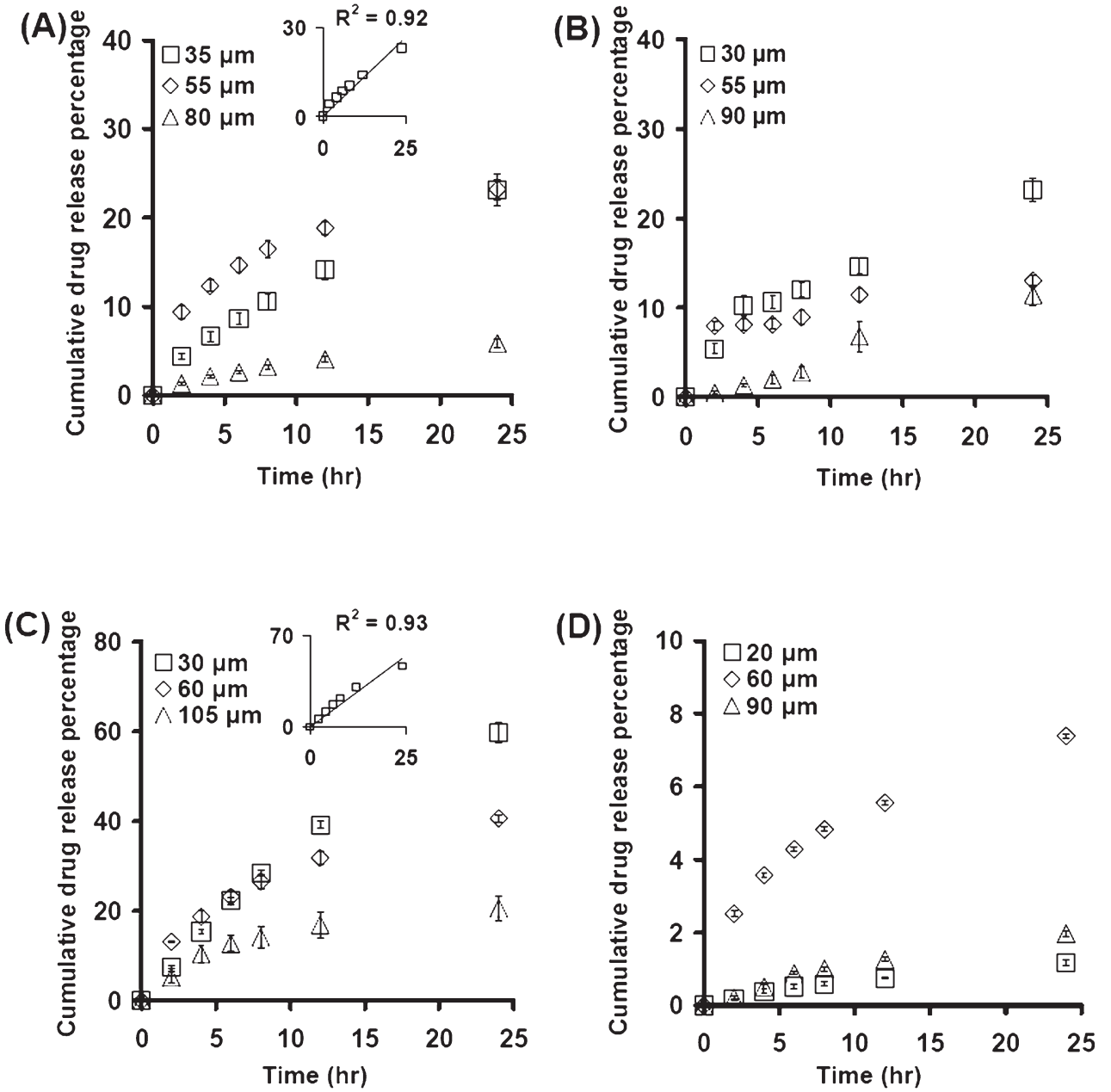

Figure 6 *In vitro* drug-release profiles of the EC MSs of different sizes and EC viscosities. The drugs and EC viscosities were (A) piroxicam and 4 cp, (B) piroxicam and 45 cp, (C) rhodamine and 4 cp, and (D) rhodamine and 45 cp.

compounds. The insets in Figure 6(A,C) show the linear least square fits to the *in vitro* release data from the 35- and 30-μm MSs of 4-cp EC for piroxicam and rhodamine, respectively, suggesting fairly good correlations with zero-order release ($R^2 > 0.92$). Although the release patterns in general followed Higuchi kinetics,[43] this result suggested that the monodisperse EC MSs could be a viable approach for potential oral/rectal drug delivery. It would require further study to understand the factors; however, we speculate that the short diffusion distance in conjunction with its less localized distribution profile in the small MSs facilitated the piroxicam release at the later stage to achieve the apparent zero-order release profile. On the other hand, the attractive interaction of rhodamine with EC could have affected the release profile, suppressing the diffusional release to some extent. Because of the localization of rhodamine at the surface, such an effect would be more significant at the early stage.

CONCLUSIONS

Uniform EC MSs with precisely controlled sizes and size distributions were prepared by the PPF method with both acoustic and hydrodynamic forces so that

the effects of the MS size and EC viscosity on the drug-release kinetics could be examined without possible errors caused by the size variation. The polymer viscosity showed no significant effect on the release of piroxicam but did show an effect on the release of rhodamine. On the other hand, the MS size affected the release of both piroxicam and rhodamine for 4-cp EC MSs. Rhodamine was released faster than piroxicam because of its higher water solubility. The small MSs of 4-cp EC showed approximately zero-order release without explicit initial bursts for both lipophilic and hydrophilic model drugs. With the capability of controlling the MS size, this study revealed that the MS size indeed affected the drug-release kinetics, influencing the drug diffusion and distribution in MSs, and it suggested that the release profile could be tailored through the control of the MS size in conjunction with other processing parameters to realize the optimal release kinetics, that is, zero-order release.

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