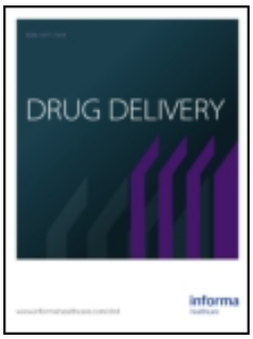

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Preparation of Various Solid-Lipid Beads for Drug Delivery of Enrofloxacin

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Solid-lipid beads were prepared to retard the release rate of enrofloxacin and to mask its bitter taste using carrageenan or sodium alginate as a shell material and either cacao butter or Witepsol W-35 as a solid lipid core. Sodium alginate was a better shell material than carrageenan and the highest loading efficiency was obtained using 2% sodium alginate. The alginate beads had a spherical morphology and a sturdy shell structure. The enrofloxacin release rate at room temperature was greatly reduced. Solid-lipid beads have the potential to mask the bitter taste of enrofloxacin and extend its release rate.

Keywords Carrageenan, Enrofloxacin, Sodium Alginate, Solid-Lipid Bead, Taste Masking

Fluoroquinolones, a group of drugs derived from nalidixic acid, have been developed as high potency antibacterial agents. These drugs are very useful in treating diseases caused by intracellular bacteria, such as *Mycobacterium, Mycoplasma, Chlamydia, Legionella*, and *Brucella*. Enrofloxacin is one of the fluoroquinolones that has been shown to be effective in treating the main bacterial processes affecting farm animals (Pons et al. 1995). Despite the excellent antibacterial properties of enrofloxacin, its bitter taste critically limits its use in animals.

Masking the unpleasant taste of a drug can improve patient compliance and the product value. Various masking techniques have been attempted. These include capsules, coating with water-insoluble polymers (Nakagami et al. 1991), adsorption to an ion-exchange resin (Fu Lu et al. 1991), microencapsulation with various polymers (Friend 1991), complexing with cyclodextrins (Takahashi et al. 1988; Uekama et al. 1983), and chemical modifications such as the use of insoluble prodrugs (Alexander et al. 1991). However, only a limited number of studies have reported the masking of unpleasant taste using microspheres with an edible lipid or polysaccharide such as alginate (Robson, Craig, and Deutsch 1999). Alginate as well as carrageenan has been used as an encapsulating material by many researchers (Park and Jung 2002: Suzuki and Lim 1994), and they are natural polysaccharides, which are suitable for oral consumption. The success of the alginate gel entrapment technique can be attributed to the gentle environment it provides for the entrapped material (Park and Jung 2002).

Recently, calcium-induced alginate gel beads were used as a unique vehicle for drug delivery. They are rapidly formed by the gelation of alginic acid in the presence of calcium ions and are able to incorporate some compounds such as drugs or polysaccharides in the gel matrix (Gombotz and Wee 1998). The beads have been used in various ways in the gastrointestinal tract, for example, for the sustained release of drugs or to absorb bile acids. Other studies used the gastroretention system to improve control over the drug release or to achieve a site-specific delivery (Murata et al. 2000).

Our study attempted to develop taste-masked formulations for enrofloxacin using solid-lipid beads composed of polysaccharides as a shell material and solid lipids with melting point near the body temperature as a core material. The encapsulated solid lipids may retard the drug diffusion rate from the solid-lipid beads because it is in the solid state at ambient temperature and inhibits drug migration toward the surface of the beads. Consequently, the bitter taste of enrofloxacin was greatly reduced and the drug release time can be extended.

MATERIALS AND METHODS

Materials

Sodium alginate was purchased from Yakuri Pure Chemicals (Tokyo, Japan). The κ-carrageenan and Witepsol W-35 was obtained from a local distributor. Cacao butter was purchased from OCG Cacao (MA, USA). The soybean oil, calcium chloride dihydrate, and potassium chloride were purchased from Junsei

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Chemical Co. (Tokyo, Japan). Enrofloxacin was provided by LG Chemical (Seoul, Korea).

Preparation of Solid-Lipid Beads

To obtain drug-dispersed lipid suspension, the solid lipid (cacao butter or Witepsol W-35) was melted by heating and mixed with enrofloxacin using a homogenizer (Ultra-Turrax T25, IKA Labortechnik, Germany). A polysaccharide (sodium alginate or carrageenan) aqueous solution was added to the drug-dispersed lipid suspension at 40°C. Using a 1 ml syringe with a 26-gauge needle, the mixture was slowly dropped into an ice cooled aqueous solution containing either 2% $CaCl_2$ or 4% KCl as a cross-linker. The solid-lipid beads were obtained within 10 min. The beads were collected by filtration and washed with distilled water to remove the unreacted $CaCl_2$ or KCl. The washed beads were air-dried at ambient temperature for 12 hr.

Loading Efficiency of Enrofloxacin

The enrofloxacin-loaded solid-lipid beads were placed in a pH 7.4 phosphate buffer solution at 40°C and stirred vigorously. After stirring for 24 hr, the solution was filtered by a 0.45 μm syringe filter. The quantity of enrofloxacin was measured with an ultraviolet spectrophotometer (UV-1601, Shimadzu, Japan). The loading efficiency was calculated by comparing the amount of the drug initially used to prepare the beads with that encapsulated in the beads.

Particle Size Distribution

The particle size distribution of the beads was determined by sieving analysis using standard sieves (Chung-gye Industrial MFG., Korea).

In Vitro Release Studies

A drug release test was carried out using a dissolution tester (DST 600A, Fine Science Institute, Korea). The solid-lipid beads loaded with enrofloxacin were placed in 500 ml of a pH 7.4 phosphate buffer solution and stirred at 100 rpm at either 25°C or 37°C. An aliquot of the dissolution medium was withdrawn at predetermined time intervals and an equivalent amount of fresh medium was added to the dissolution medium. The collected samples were analyzed by a UV spectrophotometer to determine the amount of the enrofloxacin released from the beads. The bead morphology was examined by scanning electron microscopy (S-4700, Hitachi, Japan). The sample was mounted onto an aluminum stub and sputter-coated for 120 sec with platinum particles in an argon atmosphere.

RESULTS AND DISCUSSION

Effect of Shell Material and Core Lipid

Solid-lipid beads were prepared to retard the release rate and mask the bitter taste of enrofloxacin using either carrageenan or sodium alginate as the shell material and either cacao butter or Witepsol W-35 as the lipid core. To mask the taste, the drug should not be released during the administration process but should be released within the gastrointestinal tract. Lipids with the appropriate melting point can entrap the drug within the matrix at room temperature and prevent the drug from being released during administration. The entrapped drug can be released at body temperature. The drug was first dispersed in a melted lipid solution and then dispersed in an aqueous sodium alginate or carrageenan solution. At this point most of the drug was entrapped within melted lipid and the dispersion was dropped into an ice cooled 4% KCl or 2% $CaCl_2$ aqueous solution to rapidly solidify both the core lipid and the shell material.

When 1% carrageenan was used as the shell material, the transient droplets were easily broken and dispersed in a plate form before the solidification was complete. However, the carrageenan solution formed a fiber-like structure and aggregated when 2% carrageenan was used. It appears that the viscosity of the 2% carrageenan solution was too high to form small spherical droplets before solidification was complete. Although beads were formed when 1.5% carrageenan was used, its morphology was flatter and somewhat distorted. The beads collected after filtration had a soft and fragile shell, although the formed beads were sufficiently hard after complete drying. In contrast, sturdy and spherical beads were prepared when either 1%, 1.5%, or 2% of sodium alginate was used as a shell material.

Table 1 compares the loading efficiency of enrofloxacin in various solid-lipid beads. The loading efficiency was over 80% regardless of the core lipid used—cacao butter or Witepsol W-35. The enrofloxacin loading efficiency was the highest when 2% sodium alginate was used as a shell material regardless of the solid lipid used. The shorter congealing time of sodium alginate when compared with that of carrageenan appeared to reduce the time for the drug to diffuse from the lipid core, resulting in a higher loading efficiency. As the sodium alginate content was increased, the loading efficiency tended to increased and the shell became sturdier and more resilient.

Particle Size Distribution

Figure 1 shows the particle size distribution of the enrofloxacin-loaded solid lipid beads. The particle size of almost all the solid-lipid beads prepared ranged from 0.85 to 1.18 mm. Vegetable oils have been used to prepare microspheres to incorporate lipid soluble drugs and/or to improve the floatability of the microspheres

TABLE 1
Comparison of the enrofloxacin loading efficiencies in various solid-lipid beads

Polysaccharide		Loading efficiency (%)	
	Percent	Cacao butter	Witepsol W-35
Sodium alginate	1.0	91.4 ± 2.6	85.9 ± 2.8
	1.5	91.5 ± 1.1	89.4 ± 2.4
	2.0	94.4 ± 1.7	93.6 ± 1.4
κ-Carrageenan	1.5	80.7 ± 2.9	86.1 ± 4.4

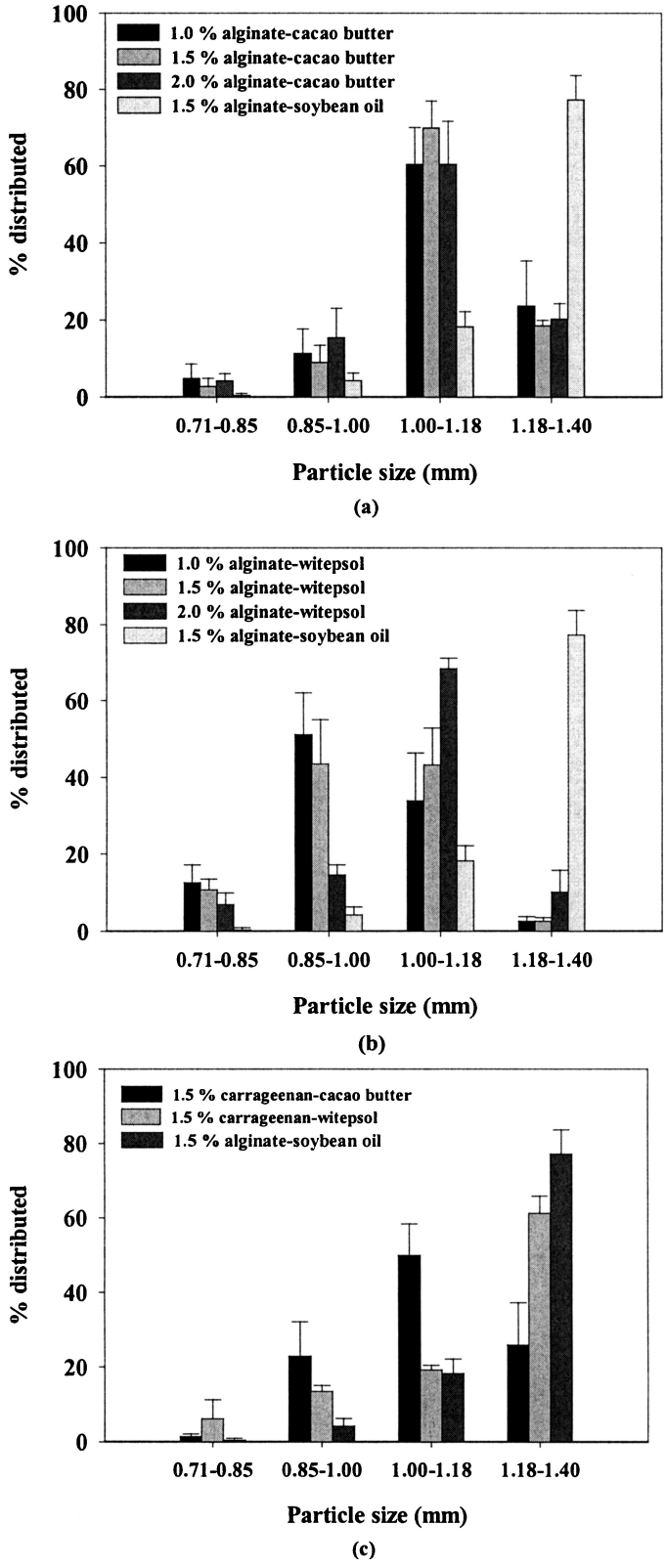

FIG. 1. Particle size distribution of the various enrofloxacin-loaded beads. Each bar represents an average of three measurements and the error bars represent the standard deviations.

(Murata et al. 2000; Ribeiro et al. 1999). To investigate the effect of liquid oil use as a core material, soybean oil was used instead of the solid lipid to prepare the beads. When soybean oil replaced either cacao butter or Witepsol W-35, the particle size range increased to 1.18 to 1.40 mm. One of the reasons why smaller beads were obtained when the solid lipid was used as a core material is that when the solid-lipid in the liquid state was dropped into a cold calcium or potassium aqueous solution, it solidified and its volume correspondingly reduced. In contrast, the volume of soybean oil did not change because it is still in the liquid state at the experimental condition.

The particle size of the beads also was different depending on the solid lipid used as a core material. As shown in Figure 1(a) and 1(b), the particle size distribution of the beads was smaller using Witepsol W-35 than that using cacao butter. When dropped into a cold aqueous solution from a needle by gravitation, the droplet size of the Witepsol W-35 solution was smaller than that of cacao butter solution. The polysaccharide concentration did not have a significant effect on the size distribution of the alginate-cacao butter beads. However, smaller beads were obtained as the sodium alginate concentration was increased in case of the alginate-Witepsol W-35 beads.

In Vitro Drug Release

Figure 2 shows the effect of the temperature and the sodium alginate concentration on the release rate of enrofloxacin from the solid-lipid beads using cacao butter as the core material in the pH 7.4 phosphate buffer solution. The enrofloxacin release rate from the solid-lipid beads were greatly reduced when compared with that from the powder, and the release rate at 25°C was

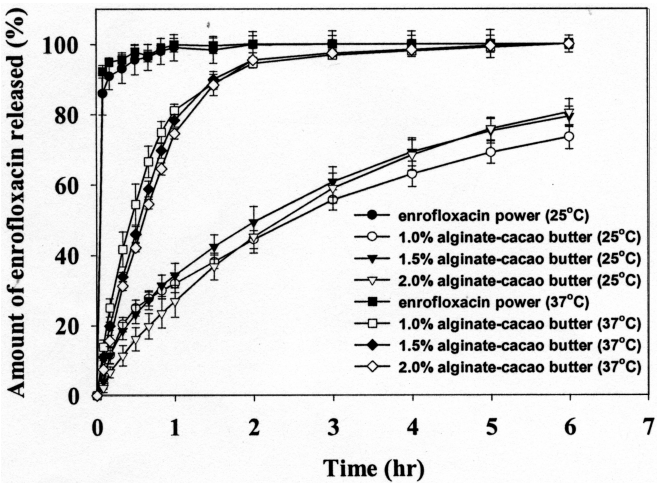

FIG. 2. Effect of temperature and concentration of sodium alginate on the enrofloxacin release rate from the solid-lipid beads using cacao butter as a core material in the pH 7.4 phosphate buffer solution. Enrofloxacin powder was used as a control.

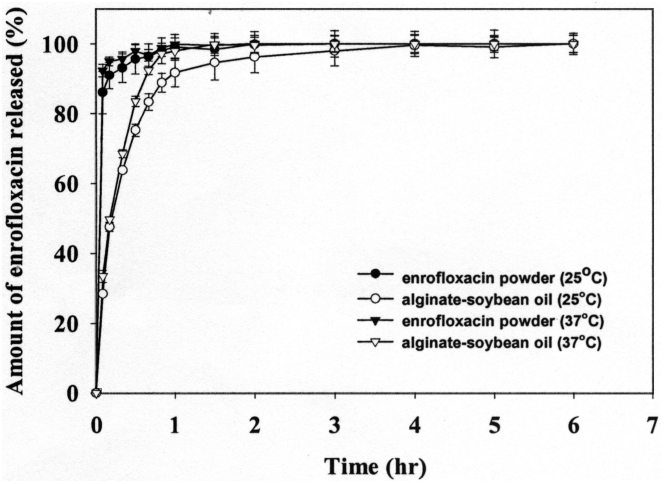

FIG. 3. Effect of temperature on the enrofloxacin release rate from 1.5% alginate-soybean oil beads in the pH 7.4 phosphate buffer solution.

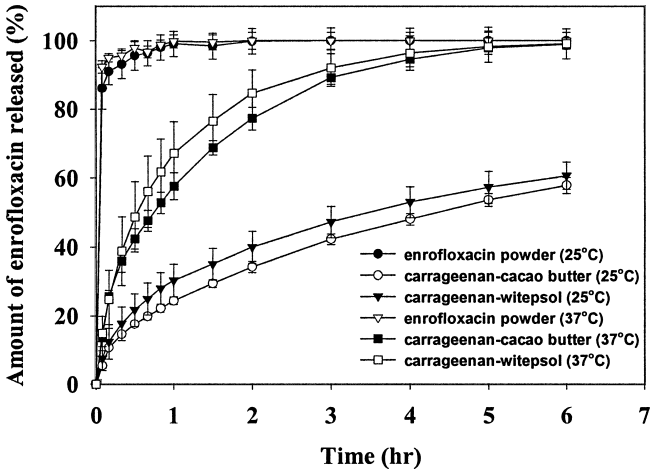

FIG. 5. Effect of temperature and solid-lipids on the enrofloxacin release rate from the solid-lipid beads using carrageenan as a shell material in the pH 7.4 phosphate buffer solution.

slower than that at 37°C. The release rate at temperatures below the cacao butter melting point (solid lipid) was lower than that at the temperature above the melting point because diffusion rate in the solid state is lower than that in the liquid state. To confirm this, the effect of temperature on the enrofloxacin release rate from the alginate beads containing soybean oil was investigated. As can be seen in Figure 3, the release rate of enrofloxacin from the alginate-soybean oil beads at 25°C was similar with that at 37°C. Moreover, the amount of enrofloxacin released in 1 hr was approximately 94% at 25°C and 97% at 37°C. The release rates from the alginate-soybean oil beads were only slightly slower than that of the powder and much faster than that from the solid lipid beads. These results clearly indicate that soybean oil cannot be used as a core material to entrap the enrofloxacin and mask the taste.

Figure 4 shows the effect of the temperature and the sodium alginate concentration on the enrofloxacin release rate from the solid-lipid beads containing Witepsol W-35 as a core material in a pH 7.4 phosphate buffer solution. The effect of temperature on the release rate of the alginate-Witepsol W-35 beads was similar to that of the alginate-cacao butter beads. Regardless of the core material used, either cacao butter or Witepsol W-35, the effect of the alginate concentration on the release rate was minimal, which indicates that the retarded release rate was mainly due to the core lipids.

Figure 5 shows the effect of the temperature and the solid lipids on the enrofloxacin release rate from the solid-lipid beads using carrageenan as a shell material in the pH 7.4 phosphate buffer solution. The enrofloxacin release rate from the beads containing cacao butter was slightly lower than that from the

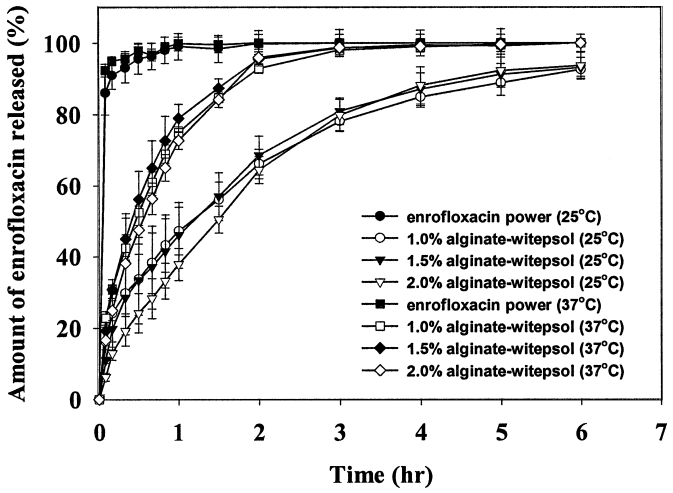

FIG. 4. Effect of temperature and sodium alginate concentration on the enrofloxacin release rate from the solid-lipid beads using Witepsol W-35 as a core material in the pH 7.4 phosphate buffer solution. Enrofloxacin powder was used as the control.

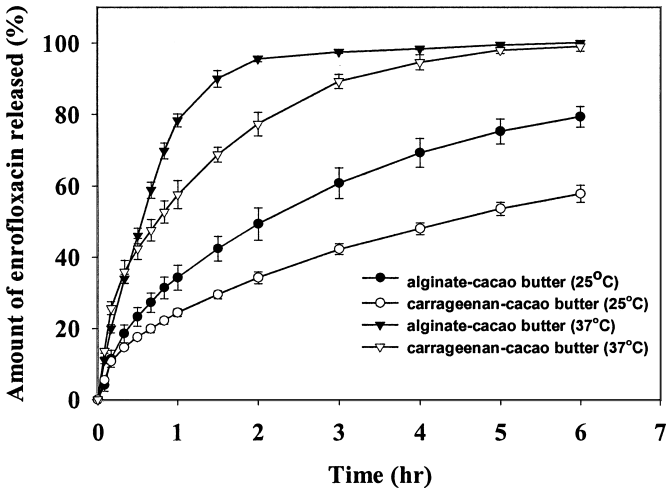

FIG. 6. Effect of temperature and polysaccharide (alginate or carrageenan) on the enrofloxacin release rate from the solid-lipid beads using cacao butter as a solid lipid in the pH 7.4 phosphate buffer solution.

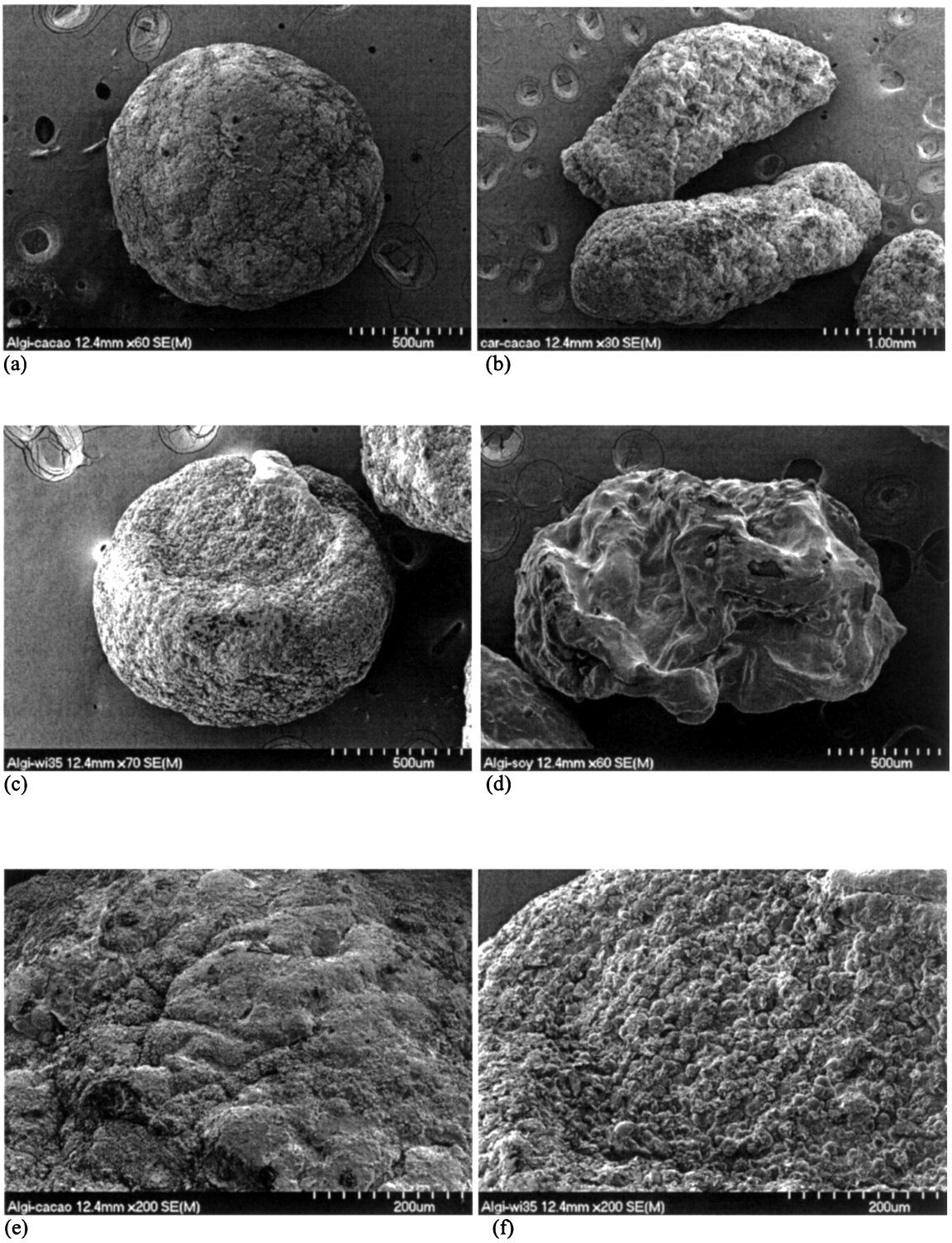

FIG. 7. Morphology of solid-lipid beads. (a) sodium alginate as a shell material with a cacao butter core; (b) carrageenan as a shell material with a cacao butter core; (c) sodium alginate as a shell material with Witepsol W-35; (d) sodium alginate as a shell material with soybean oil as a core; (e) surface morphology of the bead (a); and (f) surface morphology of the bead (c).

beads containing Witepsol W-35 at both temperatures tested. Once the carrageenan beads were swollen after being immersed in the dissolution medium, Witepsol W-35 came out of the beads much faster than the cacao butter in the pH 7.4 phosphate buffer solution. Witepsol W-35 disintegrated more easily than cacao butter. As a result, a faster release rate was obtained from the beads using Witepsol W-35 because some of the drug loaded in the Witepsol W-35 would be released more easily as it diffuses out of the carrageenan shell. A similar trend was observed in case of the beads using sodium alginate as a shell material at 37°C.

Figure 6 shows the effect of temperature and the shell material on the enrofloxacin release rate from the solid-lipid beads in the pH 7.4 phosphate buffer solution. The effect of temperature on the release rate from the solid-lipid beads using carrageenan as a shell material was similar to that of the solid-lipid beads using sodium alginate as a shell material, i.e., slower at 25°C and faster at 37°C. However, the enrofloxacin release rate from the beads using carrageenan was slower than that using sodium alginate in both temperatures tested. This might be because the swelling rate of the beads using sodium alginate was much faster than that using carrageenan in the pH 7.4 phosphate buffer solution. Moreover, the beads using sodium alginate were gradually eroded with time, while the beads using carrageenan maintained their shape after 4 hr of the release test.

Morphology

The morphology of solid-lipid beads using sodium alginate as a shell material was spherical and sturdy, whereas those using carrageenan was flatter and slightly distorted (Figure 7a and 7b). When Witepsol W-35 was used as the core material in the alginate bead, the surface morphology (Figure 7f) was quite different from that using cacao butter (Figure 7e). In the case of Witepsol W-35, small solid lipid particles were observed on the surface, which is one of the reasons why the beads with Witepsol W-35 showed a faster release rate in the drug release test. As can be seen in Figure 7d, soybean oil was observed on the surface of the alginate-soybean oil beads. This is another disadvantage of beads or microspheres containing vegetable oils in the core, which will cause long-term storage problems. It also explains why the release rate from the alginate-soybean oil beads was only slightly slower than that of the powder and why it was much faster than that from the solid lipid beads, as shown in Figure 3.

CONCLUSION

Sturdy and spherical solid-lipid beads were prepared using sodium alginate as a shell material and either cacao butter or Witepsol W-35 as a core material. The enrofloxacin release rate at room temperature was greatly reduced due to the solid-lipid in the core. Solid-lipid beads showed the possibility of effectively masking bitter taste of enrofloxacin and retarding its release rate.

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