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International Journal of Pharmaceutics 288 (2005) 315–323
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

A novel formulation design about water-insoluble oily drug: preparation of zedoary turmeric oil microspheres with self-emulsifying ability and evaluation in rabbits

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Received 8 August 2004; received in revised form 13 October 2004; accepted 16 October 2004

Abstract

To enhance in vivo absorption of zedoary turmeric oil (ZTO) and develop new formulations of a water-insoluble oily drug, novel ZTO microspheres with self-emulsifying ability, called self-emulsifying microspheres here, were prepared in a liquid system by the quasi-emulsion solvent diffusion method. The microspheres containing hydroxypropyl methylcellulose acetate succinate (HPMCAS-LG), Talc and Aerosil 200 formed the stable surfactant-free emulsion when exposed to the pH 6.8 phosphate buffer, and were significantly different from the conventional self-emulsifying systems (SES), defined as isotropic mixtures of oil, surfactant and drug. Micromeritic properties, the efficiency of emulsification and the drug-release rate of the resultant microspheres were investigated. The bioavailability of the microspheres to the conventional self-emulsifying formulation for oral administration was evaluated in 12 healthy rabbits. A HPLC method was employed to determine the plasma concentration of Germacrone, an indexical component found in ZTO. The release rates of ZTO and Germacrone from the microspheres were enhanced significantly with increasing amounts of dispersing agents, and the efficiency of self-emulsification greatly depended on the HPMCAS-LG/Aerosil 200 ratio. The emulsion droplets released from the microspheres were much smaller than that of the conventional SES. The microsphere bioavailability (*F*) to the conventional SES for oral administration was 157.7%. Our method greatly improved the bioavailability of the water-insoluble oily drug from the self-emulsifying microspheres over the conventional SES and it is useful for the oily drug to form solid preparations.

Keywords: Self-emulsifying; Microspheres; Zedoary turmeric oil; Germacrone; Bioavailability

1. Introduction

Some oily drugs have wide clinical application for their strong therapeutic activity. The conventional

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doi:10.1016/j.ijpharm.2004.10.005

strategies of their preparations were to form emulsions containing the oily drugs. Due to the convenience, compliance and safety to patients, the oral administration is preferential. However, for some oily drugs, emulsions for oral administration are hardly acceptable due to the disagreeable taste even if the drugs can be well absorbed in this formulation. Self-emulsifying formulations are a satisfying alternative for the well compliance to patients. In the absence of water, a mixture of an oil and a non-ionic surfactant forms clear and transparent isotropic solution that is known as a self-emulsifying system (SES). SES are used as a vehicle for drug delivery to improve in vitro dissolution, and therefore, to enhance in vivo absorption of lipophilic drugs (Charman and Rogge, 1992; Shah et al., 1994). This mixture is known to form a fine oil-in-water emulsion with gentle agitation, when exposed to aqueous media. For drugs having characteristics of dissolution rate-limited absorption, the SES could significantly enhance the rate and extent of absorption (Ja and Yong, 2000; Myles et al., 1990).

Curcuma zedoaria (Berg.) Rose. (Zingiberaceae), also called 'er-zhu' in Chinese, has long been used as a folk medicine. The essential oil, zedoary turmeric oil (ZTO), was extracted from the dry rhizome of *C. zedoaria*. From ZTO, some hepatoprotective sesquiterpene compounds were isolated (Mau et al., 2003; Hikino et al., 1968), including furanodiene, Germacrone, curdione, neocurdione, curcumenol, isocurcumenol, aerugidiol and zedoarondiol, which were found to present potent protective effect on D-galactosamine/lipopolysaccharide-induced liver injury in mice (Matsuda et al., 1998). A series of studies on ZTO indicated that it had strong pharmacological actions including suppression of tumors, anti-bacterial, increasing white blood cells, anti-thrombosis, and increasing motility of the stomach (Li et al., 2002a, b; Wei et al., 2003). However, the feasibility of developing new formulations was limited because of its irritant properties, instability, and volatility. In addition, poor water solubility results in low oral bioavailability of ZTO formulations.

In this study, to enhance in vivo absorption of ZTO as a water-insoluble oily model drug and develop new formulations, novel ZTO microspheres with self-emulsifying ability (called self-emulsifying microspheres here) were prepared using the quasi-emulsion solvent diffusion method, which is employed to produce a solid dispersion system with water-insoluble drugs to improve bioavailability (Kawashima et al., 1989b; Yang et al., 2003; Yang et al., 2004). The microspheres containing hydroxypropyl methylcellulose acetate succinate (HPMCAS-LG), Talc and Aerosil 200 were significantly different from the conventional SES defined as isotropic mixtures of oil, surfactant and drug. The conventional SES as a control preparation was prepared using ethyl oleate (EO) and a surfactant (Tween 85), which is expected to improve the dissolution characteristics for ZTO and results in higher oral bioavailability. Here we employed a HPLC method to detect the rabbit plasma concentration of Germacrone (a indexical component found in ZTO, 7.93%, w/w) and evaluated the microsphere bioavailability to the conventional SES.

2. Materials and methods

2.1. Chemicals and reagents

Zedoary turmeric oil was provided by Jiangxi Tongren Natural Perfume Co. (Jiangxi, China). The standards (Germacrone and Tanshinone IIA) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); hydroxypropyl methylcellulose acetate succinate (HPMCAS-LG) and hydroxypropyl methylcellulose (HPMC-60SH4000) were kindly supplied by Shin-Etsu Chemical Ind. Co. Ltd. (Japan); Aerosil 200 from RÖhm Pharma. (Germany); methanol and tetrahydrofuran of HPLC-grade were obtained from Concord Tech.Co. (Tianjin, China). All other chemicals, such as Talc, sodium dodecyl sulfate (SDS), dichloromethane, acetone, ethyl oleate (EO), Tween 85, Zine sulfate heptahydratate, anhydrous sodium sulfate were of analytical grade and provided by Shandong Yuwang Chemical Plant (Shandong, China).

2.2. Preparation of self-emulsifying microspheres of ZTO

The microspheres were prepared using a quasi-emulsion solvent diffusion method (Akbuga, 1989; Kawashima et al., 1989a). HPMCAS-LG (0.8 g) was dissolved in a mixed organic solvent containing ace-

tone (3.0 ml, as good solvent) and dichloromethane (7.0 ml, as bridging liquid). Then ZTO (1.0–2.0 ml) and Aerosil 200 (0.3–0.5 g) were added uniformly. Talc (0.4–0.8 g) was suspended under vigorous agitation as drug-dispersing agent in the drug-polymer solution. The resultant drug-polymer–Aerosil–Talc suspension was slowly introduced into 150 ml of distilled water (as poor solvent) containing 0.1% of SDS and 0.05% HPMC-60SH4000 while being moderately stirred at 500 rpm using a mechanical stirrer (Mode D-8401-WZ, Tianjin, China) at room temperature. The suspension was finely dispersed into quasi-emulsion droplets immediately under agitation, and the drug–Aerosil and polymers were coprecipitated in the emulsion droplets. After stirring the system for 10 min, 150 ml of poor solvent was added to the system at a rate of 2 ml/min using a constant pump (Mode DHL-A, Shanghai, China) to promote the diffusion of the good solvent from emulsion droplets into poor solvent resulting in enhancement of the solidification of quasi-emulsion droplets. The solidified microspheres were collected by filtration and washed with water, and the collected microspheres were dried at $-50\,^{\circ}$Cand $10-50$ Pa for 12 h using a freeze drier (Mode FD-1, Beijing, China). The content of ZTO in microspheres was measured by the method described in the Chinese Pharmacopoeia (Ch. P.) (N.P.C., 2000) using a spectrophotometer (Mode 752, The Third Analytical Instrument Plant, Shanghai, China). The drug loading and incorporation efficiency (%) were calculated by using Eqs. (1) and (2), respectively. The shape, surface topography and cross-section of microspheres were observed by a scanning electron microscope (SEM, Jsm-T20, Jeol Co. Ltd., Japan).

$$\text{Drug loading}(\%) = \frac{M_{\text{actual}}}{\text{weighed quantity of powder of microspheres}} \times 100 \quad (1)$$

$$\text{Incorporation efficiency}\,(\%) = \frac{M_{\text{actual}}}{M_{\text{theoretical}}} \times 100 \quad (2)$$

where M_{actual} is the actual ZTO content in weighed quantity of powder of microspheres and $M_{theoretical}$ the theoretical amount of ZTO in microspheres calculated from the quantity added in the fabrication process. The means of three assays were reported.

2.3. Preparation of conventional SES

Conventional SES was prepared by methods reported in the literature (Li et al., 2002). The mixture of ZTO, EO and Tween 85 (40:70:30, w/w/w) was prepared by adding ZTO, EO and Tween 85 into a glass test tube followed by vortexing for 2 min.

2.4. Drug release rate of the microspheres

The drug release tests of the microspheres were carried out for 60 min under 100 rpm by the paddle method specified in Ch. P. (2000 ed.). The microspheres of ZTO (100 mg) were weighed and put into 1000 ml of the phosphate buffer (pH 6.8) kept at $37 \pm 0.5\,^{\circ}$C. Five milliliters of the dissolution medium was sampled at intervals, and fresh dissolution medium was simultaneously replaced in the apparatus to keep the volume constant. The withdrawn sample was filtered through a membrane filter (0.8 μm). The concentration of ZTO in filtrate was measured by above-described method. The concentration of Germacrone in filtrate was measured using a HPLC method at 245 nm reported in the literature with some modifications (Wang and Wang, 2001).

2.5. Measurement of particle size of resultant emulsion

Self-emulsifying microspheres and conventional SES, which had the same ZTO dose of 100 mg were separately dispersed to 500 ml of phosphate buffer (pH 6.8) controlled at $37 \pm 0.5\,^{\circ}$C. The resultant emulsions were prepared by stirring for 10 min at 100 rpm using a paddle method. Both emulsions were hermetically stored for a period of 48 h and phase separation was observed visually. The particle size and distribution of the resultant emulsions was determined by laser diffractometry using a particle size analyzer (LS230, Beckman Coulter^{TM}, USA).

2.6. Animal study

Male rabbits weighing 2.5 ± 0.3 kg were provided by the Animal Center of this University (Shenyang, China). The rabbits were fasted overnight but were allowed free access to water. The animals were divided into two groups (six animals each), and each animal received 80 mg/kg of ZTO dose in one of the following dosage forms: (1) self-emulsifying microspheres for oral administration, (2) conventional SES orally administered. The formulations were administered at the same time (09:30 h) to avoid chronopharmacokinetic effects (Malmary et al., 1995). The blood samples (about 1.0 ml) were withdrawn via the marginal ear vein. The samples were collected at predetermined time intervals up to 24 h after the drug administration. The plasma was separated by centrifuging at $10\,000 \times g$ for 5 min and stored at $-20\,^{\circ}$C until the assay.

2.7. Determination of Germacrone in plasma

The HPLC system consisted of a pump (Hitachi L-7110), a μBondapak C18 column, (Diamonsil^{TM}, 200 mm × 4.6 mm, 5 μm particle size), a guard column (10 mm × 4.6 mm) packed with C18 material, a column oven (Shimadzu CTO-10AS) and a UV–vis detector(Hitachi L-7420). The mobile phase was prepared by mixing 800 ml methanol with 200 ml water and 50 ml tetrahydrofuran. The solvent was filtered through a 0.45 μm filter and degassed.

To determine the amount of Germacrone and remove the disturbance of protein and endogenous compounds in plasma, a method of sample preparation (precipitating protein-solvent extracting method) was employed. Thawed specimens (0.5 ml) were mixed by vortexing with 10 μl internal standard (3.3 μg/ml Tanshinone IIA in methanol) for 30 s, and 100 μl Zine sulfate heptahydrate solution (0.5 g/ml) was added in order to precipitate protein. The solution was centrifuged at $12\,000 \times g$ for 5 min to obtain a clean supernatant. After the supernatant was separated, 80 μl of acetonitrile and 80 mg of anhydrous sodium sulfate (as the dehydrating agent) were sequentially added to the precipitate and vigorously agitated by vortexing for 10 min. The resultant solution was centrifuged at $12\,000 \times g$ for 10 min again. The obtained clear supernatant was transferred into a clean glass tube and the concentration of Germacrone in the supernatant was determined at 245 nm by injecting 20 μl volume into the column with a flow rate of 0.8 ml/min. All chromatography was performed at 30 °C.

2.8. Bioavailability evaluation

Area under the plasma concentration-curve (AUC) was calculated using the trapezoidal rule. The bioavailability of the self-emulsifying microspheres to the conventional SES was calculated through comparing those two AUCs using the Eq. (3).

$$F\ (\%) = \frac{(\mathrm{AUC}/D)_{\mathrm{A}}}{(\mathrm{AUC}/D)_{\mathrm{B}}} \times 100 \tag{3}$$

where F is the relative bioavailability, AUC the area under the plasma-concentration–time curve, D the dose administered, A the self-emulsifying microspheres and B the conventional SES.

3. Results and discussion

3.1. Preparation of microspheres by quasi-emulsion solvent diffusion method

When the resultant mixed solution of good solvent and bridging liquid-containing drug and additives was poured into the poor solvent under stirring, finely dispersed droplets were formed immediately and semi-transparent emulsions were observed visually. With the diffusion of the good solvent out of the droplets into the poor solvent, the drug and the polymers were coprecipitated in the droplets. The preparation of microspheres was controlled by three processes, such as forming emulsion droplets, consolidation, and solidification. The sizes of the resultant microspheres were dependent on the sizes of the emulsion droplets formed at the initial stage. Prolonging the induction period of the coacervation of the droplets was required for forming uniform microspheres. To decrease the diffusion rate of the good solvent and bridging liquid from the emulsion droplets into the poor solvent, a slow perfusion (2 ml/min) of the poor solvent was carried out after a part of the amount of the poor solvent (i.e., 150 ml) was used. It was found that HPMC (over 0.05% (w/v) in poor solvent) effectively prevented the cohesion of droplets during the consolidation period, while the excess concentration (i.e., 0.1%, w/v) of HPMC signifi-

cantly decreased the sizes of the emulsion droplets and resulted in a small size of the resultant microspheres After freeze-drying for 12 h, the residual amount of acetone in the resultant microspheres was less than 10 ppm, and residual amount of dichloromethane was not detected using GC (GC-17A, Shimadzu Co., Japan).

In this formulation, HPMCAS-LG was used as a bond agent to bind the Aerosil 200 and Talc into microspheres and formed a polymeric matrix. Aerosil 200 was used to adsorb ZTO, and Talc as the dispersing agents was included. It was found that the ZTO microspheres having a good spherical shape were easy to form under strong agitation. It was indicated that HPMCAS-LC was one of the suitable polymers for the preparation of the ZTO microspheres using this method due to its good plastic deformation property. In addition, HPMCAS-LG compared with other polymers, such as HPMCP and Eudragit, was found to be helpful for the stability of ZTO from accelerating condition test of its physical mixture with ZTO under 60 °C, in which the content of Germacrone and other components in ZTO had no significant change after 10 days, while the amount of Germacrone was less than 50% compared with that at 0 day after ZTO mixed with HPMCP or Eudragit was carried out the same test.

Table 1 indicates the content of ZTO in microspheres and also drug loss. Microspheres with high drug loading were obtained. It was found that the drug loss occurred after dryness for 12 h using the freeze drier, but the losing amount was little because the incorporation efficiencies was high, always exceeding 85%. It was mainly due to the strong affinity between ZTO and some excipients, as sorbents for their large specific surface area, such as silica and Talc. For example the Aerosil 200 has a mean typical diameter of 12 nm and its BET surface area was determined to be 224.38 m^2/g using a surface area analyzer (SA3100, Beckman Coulter^{TM}, USA). As increasing the ratio of drug to excipients, the drug loading of microspheres was increased. The high content of ZTO in microspheres was believed to be due to the poor solubility of drug in poor solvent. These suggested that the present method was suitable for the preparation of ZTO self-emulsifying microspheres. The surface morphology and the cross section of the microspheres were investigated using a SEM. As seen in Fig. 1, the microsphere was spherical and exhibited apertured and uneven surfaces. The micropores were formed on the surface due to the further diffusion of the organic solvents out of the microsphere. The Talc particles and polymers were commixed uniformly in dense texture.

3.2. Self-emulsifying process of microspheres

Emulsions can be stabilized by solid particles in the absence of surfactant. Aerosil was used to stabilize so-called three-phase-emulsions through a layer of solid particles which arrange between continuous and dispersed phase (Binks et al., 2003; Kruglyakov et al., 2004). Hydrophilic polymer is a known emulsifier as well as a common viscosity enhancer used in emulsion formations to improve the stability, such as HPMC as emulsifier for sub-micron emulsions (Michaela et al., 2000). It was found that Aerosil with a hydrophilic polymer-made emulsification easier, and the emulsions' stability was defined strongly by the type of Aerosil and hydrophilic polymer as well as the ratio of these excipients.

When the resultant microspheres containing ZTO, Aerosil 200, HPMCAS-LG and Talc were immersed into aqueous media (pH 6.8 phosphate buffer) under gentle agitation, HPMCAS-LG was dissolved rapidly in a few minutes and ZTO with Aerosil 200 was released. The stable surfactant-free emulsion was obtained by virtue of HPMCAS-LG and Aerosil 200. Phase separation studies revealed a great dependency on the HPMCAS-LG/Aerosil 200 ratio with respect to the resultant emulsion stability: emulsions are unstable or do not even form at a surplus of HPMCAS-LG. Vice versa, a surplus of Aerosil 200 produces increasing par-

Table 1
Effect of feeding drug on drug loading and incorporation efficiency of microspheres ($n = 3$, $\bar{x} \pm$ S.D.)

ZTO:HPMCAS-LG:Aerosil 200:Talc	Drug loading (%)	Incorporation efficiency (%)
1.0:0.8:0.4:0.8	28.5 ± 0.36	86.9 ± 1.10
1.5:0.8:0.4:0.8	38.8 ± 0.40	91.7 ± 0.96
2.0:0.8:0.4:0.8	44.9 ± 0.65	90.8 ± 1.32

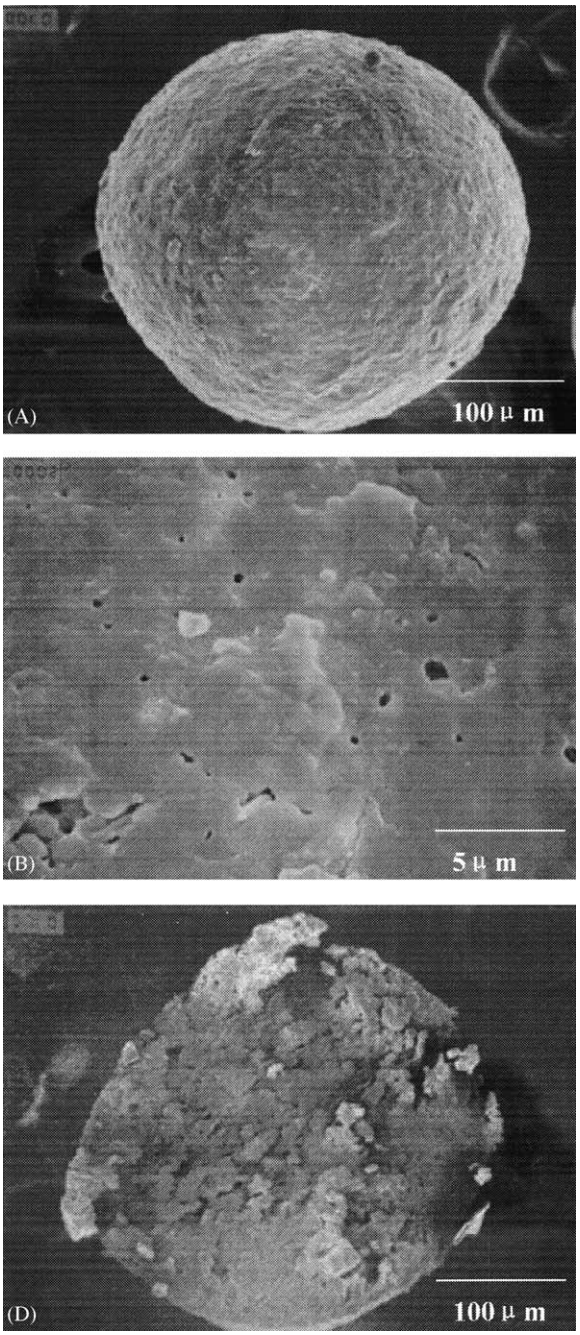

Fig. 1. Scanning electron microphotographs of microspheres. Key: (A) whole image (×100); (B) surface (×2000); (C) cross-section (×100).

ticle sizes (Fig. 2). It could be explained by the arrangement of Aerosil 200 particles on the ZTO droplet surface in the resultant emulsion and the adsorption of Aerosil 200 to HPMCAS-LG. The only presence of HPMCAS-LG would result in the resultant emulsion, which was not stable enough (Fig. 3A). Fig. 3B indicated droplets with a layer of Aerosil 200. Bonding with HPMCAS-LG Aerosil formed a layer (Fig. 3C) between continuous and dispersed phase, which lead to more stable resultant emulsion than that of Fig. 3B. Increase of particle sizes with surplus of Aerosil 200 was because of the congregation of Aerosil 200 particles. In this study, the best ratio (0.8:0.4) of HPMCAS-LG/Aerosil 200 was determined. The release rates of ZTO and Germacrone from the microspheres, which were equal to the rate of self-emulsification, were enhanced significantly by increasing the amount of Talc (as a dispersing agent) (Fig. 4), while the droplet sizes of resultant emulsion had no significant difference if the Talc in the microspheres formulation was included or not.

The particle sizes of ZTO emulsion droplets released from the self-emulsifying microspheres (ZTO:HPMCAS-LG:Aerosil 200:Talc = 2:0.8:0.4:0.8) and the conventional SES were 182.0 ± 15.3 nm and 437.3 ± 20.1 nm, respectively. These results indicated that the self-emulsifying microspheres produced the resultant emulsion with a smaller mean size and a narrower particle size distribution with respect to the conventional SES (Fig. 5).

3.3. Bioavailability evaluation

Blank plasma samples spiked with seven different concentrations of Germacrone were processed as described above and all chromatograms obtained were estimated by peak-area measurement. The linear calibration curves obtained with peak-area ratio (y) of Germacrone to internal standard versus drug concentration (x) were found to be linear between 8.08 and 808 ng/ml (correlation coefficient $r = 0.9994$). The precision and recovery of the assay were estimated by analyzing the quality control (QC) samples with low, middle and high concentration. The concentrations of QC samples were calculated from the calibration curve performed on the same day. The within-day and between-day precisions (R.S.D.) ranged from 2.79 to 5.21% and from 3.48 to 5.47%, respectively. The mean method

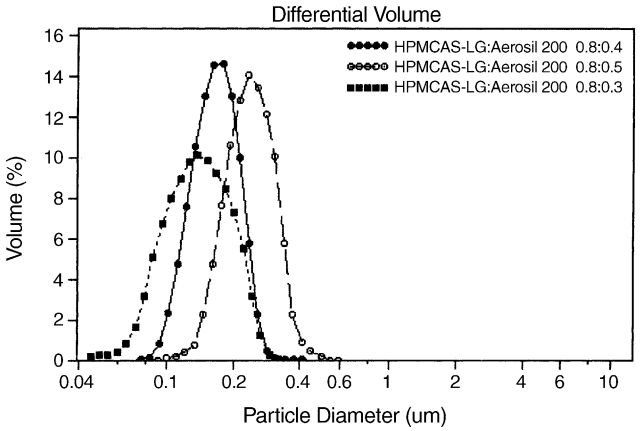

Fig. 2. Dependence of HPMCAS-LG/Aerosil 200 ratios on particle size. HPMCAS-LG:Aerosil 200 = 0.8:0.3 (■), 0.8:0.4 (●) and 0.8:0.5 (○).

recovery (accuracy) was 99.53% and the extract recovery of Germacrone was within the range of 81–91%. The internal standard showed mean extract recovery of 67.5% with R.S.D. < 5%. The limit of quantitation (LOQ) was 8.08 ng/ml Germacrone in plasma. Fig. 6 shows the mean plasma concentration–time profiles of Germacrone after oral administration of the self-emulsifying microspheres (ZTO:HPMCAS-LG:Aerosil 200:Talc = 2:0.8:0.4:0.8) and the conventional SES. The microsphere bioavailability (*F*) to the conventional SES was 157.7%. This data indicated that self-emulsifying microspheres significantly improved the bioavailability of ZTO over the conventional SES. It could be explained that smaller emulsion droplet re-

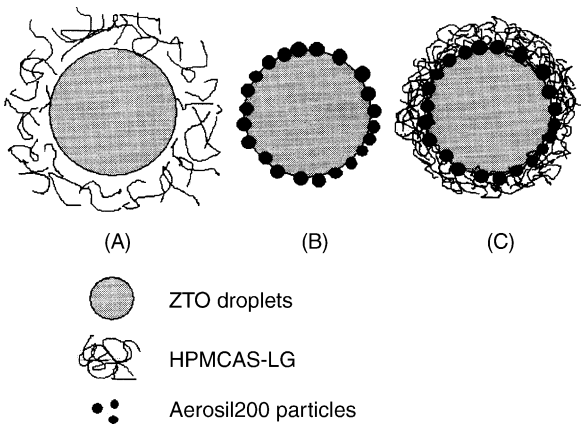

Fig. 3. Models for stabilizing ZTO droplets in resultant emulsion through HPMCAS-LG (A), Areosil 200 particles (B) or HPMCAS-LG/Aerosil 200 (C).

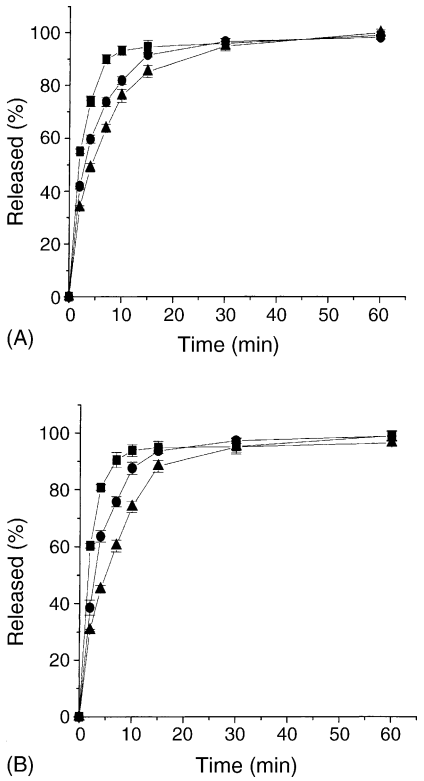

Fig. 4. Release behaviors of ZTO (A) and Germacrone (B) from self-emulsifying microspheres prepared at different amount of Talc. ZTO:HPMCAS-LG:Aerosil 200:Talc = 2:0.8:0.4:0.8 (■), 2:0.8:0.4:0.6 (●) and 2:0.8:0.4:0.4 (▲).

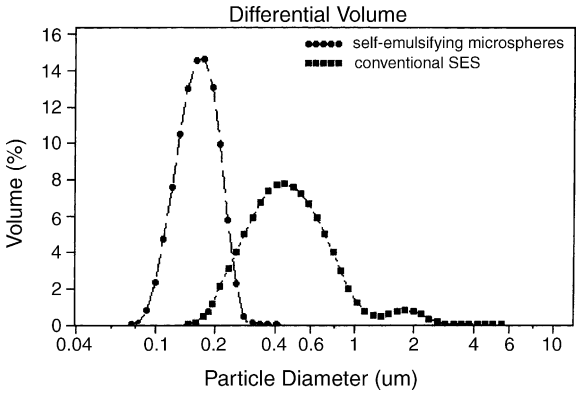

Fig. 5. Particle size distribution of emulsion droplets released from self-emulsifying microspheres (●), conventional SES (■).

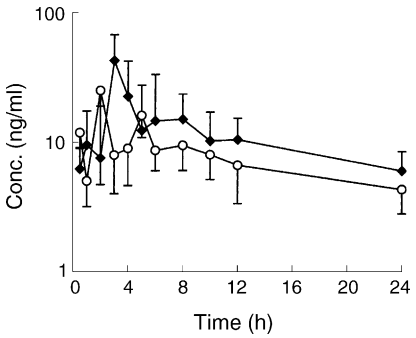

Fig. 6. The mean plasma concentration–time profiles of Germacrone after oral administration (80 mg/kg ZTO dose) of the conventional SES (◯) and the self-emulsifying microspheres (♦), each point represents the mean (S.D.) ($n = 6$).

leased from self-emulsifying microspheres compared with conventional SES (Fig. 5) much improved in vitro dissolution and the drug-disperse degree in the media, and therefore, enhanced in vivo absorption of ZTO.

4. Conclusion

In this study, novel self-emulsifying microspheres containing ZTO, Aerosil 200, HPMCAS-LG and Talc were prepared, which were significantly different from the conventional SES. The surfactant-free emulsion was produced by the virtue of Aerosil 200 and HPMCAS-LG when the microspheres were immersed into the phosphate buffer (pH 6.8) under gentle agitation. The evaluation of the microsphere bioavailability to the conventional SES for oral administration suggested that the self-emulsifying microspheres much improved the bioavailability of the water-insoluble oily drug (ZTO) over the conventional SES. In addition, the preparation method of self-emulsifying microspheres is a useful method for forming solid preparations of oily drugs.

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