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Physicochemical and pharmacokinetic characterization of a spray-dried malotilate emulsion

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ABSTRACT

Malotilate (MT) is a hepatoprotective drug administered orally. However, MT was found to be a poorly water-soluble drug with low oral bioavailability. In the present investigation, a novel spray-dried emulsion (SDE) loaded with MT was prepared, and its physicochemical properties were characterized by rheological evaluation, particle size measurement, *in vitro* release, and surface morphology. The pharmacokinetic study of SDE, in comparison to MT suspension with the pure MT powder homogeneously dispersed in 0.5% CMC–Na solution, was also performed in rats after a single oral dose. It was found that SDE exhibited a 2.9-fold higher peak plasma concentration (C_{max}) and 2.3-fold higher area under the curve (*AUC*) than MT suspension.

1. Introduction

As the most widely used lipid-based formulations, emulsion has its utility to improve the dissolution rate and increase the bioavailability of poorly water-soluble drugs by eliminating the dissolution step and amplifying the specific surface area. In addition, emulsion can extend the gastric emptying to provide longer dissolution time. However, creaming, flocculation, coalescence, and phase separation are often observed in emulsion, which gives physicochemical stability problems during storage (Welin-Berger and Bergenstahl, 2000). Besides, compliance problems of emulsion also limit its direct application as the delivery vehicles for oral drugs (Christensen et al., 2002). In order to overcome these drawbacks, self-emulsifying drug delivery system (SEDDS), an isotropic mixture of oils and surfactants and sometimes cosolvents, has been adopted for oral delivery of lipophilic drugs and several products have been commercially available, such as Neoral® (cyclosporine), Sandimmune® (cyclosporine), Norvir® (ritonavir), Fortavase® (saquinavir) and Aptivus® (tipranavir) (Morozowich and Gao, 2009). SEDDS forms fine oil-in-water (O/W) emulsions spontaneously under the digestive motility of the stomach. However, relatively high concentrations of surfactants in SEDDS (usually 30–60%) may cause obvious irritation to gastrointestinal mucosa (Gursoy and Benita, 2004). In addition, lack of general formulation guidance also limited SEDDS to be broadly applied (Buyukozturk et al., 2010).

Recently, dry emulsion (DE), a formulation containing none or small amounts of nonionic surfactants has been suggested as one way to circumvent such disadvantage of conventional emulsions and SEDDS (Shively and Thompson, 1995; Christensen et al., 2001; Jang et al., 2006; Yin et al., 2009). DE formulations are prepared by drying O/W emulsions containing soluble solid carriers (e.g. dextrin (Jang et al., 2006; Yin et al., 2009), lactose monohydrate (Yin et al., 2009), hydroxypropyl methylcellulose (Hansen et al., 2005; Christensen et al., 2001)) and/or insoluble solid carriers (e.g. magnesium aluminometasilate (Hansen et al., 2004)) in aqueous phase. After drying, the solid carriers encapsulate the dispersed lipid phase and form a matrix. The process of solidification of emulsion into DE can be performed by spray drying (Jang et al., 2006; Hansen et al., 2005), lyophilization (Corveleyn and Remon, 1998a,b) or vacuum distillation (Shively, 1993).

DE has been successfully applied as a potential oral drug delivery system for poorly soluble drugs to improve bioavailability (Dollo et al., 2003; Jang et al., 2006; Ge et al., 2008) as well as to improve photostability (Takeuchi et al., 1992; Jang et al., 2006), oxidation stability (Heinzelmann and Franke, 1999) and enzymatic stability (Ge et al., 2008).

Malotilate (MT), diisopropyl 1,3-dithiol-2-ylidenemalonate (Fig. 1), is a liver protein metabolism improved drug used in the treatment of chronic hepatitis and cirrhosis (Bührer et al., 1986;

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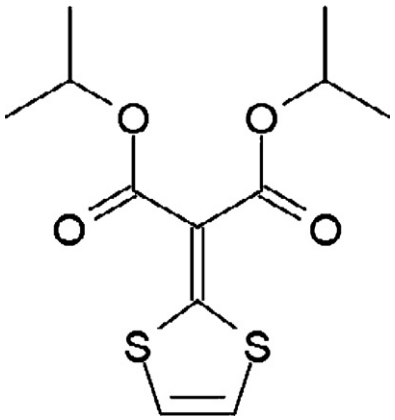

Fig. 1. Chemical structure of malotilate (molecular weight: 288.4).

Ryle and Dumont, 1987). MT is a symmetrical isopropyl ester of a sulphur-containing compound with very poor aqueous solubility. It was easily absorbed from the gastrointestinal (GI) tract after oral administration and undergoes extensive first-pass metabolism (Ryle and Dumont, 1987). Only 2–3% of MT was not eliminated by liver in health human body when administered orally (Bührer et al., 1986). The poor aqueous solubility and extensive first-pass effect resulted in low oral bioavailability of MT. However, very limited studies were reported to enhance the *in vivo* bioavailability of MT. Wu et al. (1999) prepared the amorphous matter of MT using colloidal silica as the carrier to improve the *in vitro* dissolution extent from 16.34 to 44.20% in 60 min. However, no attempt till now was conducted to improve the *in vivo* bioavailability of MT.

The main objective of the present study is to employ DE as the carrier for improving the dissolution rate and increasing the bioavailability of MT. The physicochemical properties of the liquid emulsion prepared by high pressure homogenization and DE obtained by spray-drying were characterized. In addition, *in vivo* pharmacokinetic study of the spray-dried emulsions (SDE) loaded with MT was performed, in comparison with MT suspension, to demonstrate the oral bioavailability enhancement of SDE.

2. Materials and methods

2.1. Materials

Malotilate (mean particle size about 150–300 μm) was purchased from Yabang Pharmaceuticals Co., Ltd. (Changzhou, China). Malotilate reference standard and indomethacin reference standard were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Labrafac® CC, Peceol® and Labrafil® M 1944CS were obtained from Gattefosse Corp. (Lyon, France). Glucidex® 12 was obtained from Roquette Freres (Lestrem, France). Crodamol® EO and Crodamol® IPM were obtained from Croda (Yorkshire, England). Neobee M-5 was obtained from Stepan Company (Illinois, USA). Peanut oil was purchased from Yihai Kerry Foodstuffs Marketing Co., Ltd. (Shenzhen, China). Soybean oil was purchased from Huanye Pharmaceuticals Co., Ltd. (Guangzhou, China). PVA-0486 was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). Methanol of HPLC grade was from Merck (Darmstadt, Germany). All other chemical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Solubility studies

The equilibrated solubilities of MT were determined in various oils (e.g. Crodamol EO, Crodamol IPM, Labrafac CC, peanut oil, soybean oil, Labrafil M 1944CS, Neobee M-5 and Peceol). Excess amount of MT was shaken with each oil by ZD-85 shaker (Guohua Instrument Co., Ltd., China) at 37 °C. Following equilibration (2 days), samples were centrifuged at 14,000 rpm for 10 min in a Sigma centrifuge (Model 1-15 K, Germany). Aliquot portions of the supernatant liquid were collected and filtered through a 0.45 μm filter, and then the concentrations of MT in various oils were analyzed by HPLC method. All samples were prepared in triplicates.

The HPLC system consisted of a Shimadzu LC-10AD pump and a SPD-10A UV detector (Kyoto, Japan). The analysis was carried out on a Waters ODS column (5 μm, 4.6 mm × 150 mm) which was held at 30 °C. The isocratic mobile phase was a mixture of methanol, water and 40% acetic acid (77:22.5:0.5, v/v/v) and was pumped at a flow rate of 1.0 ml/min with UV monitoring at 362 nm.

Table 1
Compositions of the homogenized emulsion.

Formulation	g/100 ml
Malotilate	4.11
Labrafac CC	6.32
PVA	6.80
Glucidex	10.33
Water	To 100 ml

2.3. Stabilizer selection by surface tension determination

The surface tension analyses were carried out with the Wilhelmy Plate method using a DCAT 21 Tensiometer (DataPhysics Instrument GmbH, Germany). This method utilized the interaction of a platinum plate (9.95 mm × 9.95 mm × 0.20 mm) with the surface being tested. In the plate method the liquid was raised until the contact between the surface and the plate was registered. All the experiments were performed at 25 °C, using the following instrumental parameters: small vessel 30 ml, motor speed 1 mm/s, and immersion depth 3 mm. Samples were prepared by dispersing selected oil phase into the aqueous solutions containing different concentrations of the stabilizer candidate (e.g. HPMC, PVA, PVP), using an Ultra-Turrax T18 stirrer (Jahnke & Kunkel, Staufen, Germany) at 14,000 rpm for 3 min. All samples were determined in triplicates.

2.4. Preparation of the emulsions

Labrafac CC with MT dissolving in it (650 mg/g) was dispersed in an aqueous solution containing stabilizer and Glucidex using an Ultra-Turrax T18 stirrer at 14,000 rpm for 3 min. Compositions of the liquid emulsion formulation were provided in Table 1. The obtained coarse emulsion (CE) was then homogenized *via* a high pressure homogenizer (Avestin Emulsiflex-05, Avestin Inc., Ottawa, Canada) at 30 °C. 10 cycles at 1600 bar were applied to obtain the homogenized emulsion (HE).

In order to enhance the physical stability of liquid emulsion, the prepared HE was subsequently spray dried using a mini spray-dryer equipped with a high performance cyclone (Büchi B-290: Büchi Labortechnik AG, Switzerland) and a 0.7-mm nozzle. The following standard operating conditions were used: inlet temperature, 155 °C; aspirator setting, 100% (40 m^3/h); spray flow rate, 600 l/h; pump setting, 5 ml/min; these conditions resulted in an outlet temperature around 100 °C. The content of MT in dry emulsion was 149.1 mg/g. Stability data by HPLC determination showed that no significant change in drug content was observed, which confirmed that the homogenization and spray drying process did not cause any significant chemical degradation of MT in lab-scale batch process.

2.5. Reconstitution of SDE

0.67 g of spray-dried powder was dispersed with 200 ml distilled water in a 500 ml beaker by glass stirring rod until the homogenous reconstituted emulsion was obtained. Samples were withdrawn for further characterization.

2.6. Droplet size and zeta potential analysis

The droplet size and zeta potential were determined using a Zetasizer 3000 (Malvern Instruments, U.K.) by photon correlation spectroscopy and laser Doppler electrophoresis at 25 °C, respectively. The measurements yield the volume weighted mean droplet size, the polydispersity index (PI) and zeta potential of HE and reconstituted SDE. All samples were analyzed in triplicates.

2.7. Rheological properties

Samples were transferred into the small sample adapter of the Brookfield LVDV-III Ultra rheometer (Brookfield Engineering Laboratories Inc., MA, USA), which was pre-heated to the assay temperature (25 ± 0.3 °C). The ratio of inner radius to outer radius was 0.92 in the concentric cylinder system. The measuring element, spindle number 18 (SC4-18), was then introduced and sheared at 6.60 s^{-1} rate for 15 min in the rheometer before the measurement cycles started. The rotating speed of the spindle was programmed for control. Shear stress (τ) was measured as a function of increasing shear rate (γ). The rheograms obtained during the procedure were fitted to the first-order model. The model was verified by statistical analysis using origin 7.5 (Originlab Corp., Northampton, USA) and the rheological parameters were calculated.

2.8. Differential scanning calorimetry (DSC)

DCS profiles were generated using a differential scanning calorimeter (NETZSCH DSC 204, Germany) to characterize the solid form of MT in SDE. The physical mixture (PM) of all the ingredients with the same ratio in SDE, drug-free SDE, SDE containing MT and the pure MT were studied. The DSC runs were performed over a temperature range of 40–250 °C at a heating rate of 10 °C/min in an open pan using alumina as a reference material.

2.9. Scanning electron microscopy (SEM)

The surface morphology of SDE was examined by scanning electron microscope (Hitachi S3400, Tokyo, Japan). Powder samples were glued and mounted on metal sample plates. The samples were gold coated (thickness ≈ 15–20 nm) with a sputter coater using an electrical potential of 2.0 kV at 25 mA for 10 min.

2.10. In vitro release study

In vitro release profiles of MT from various formulations were generated using the USP 2 dissolution apparatus (Sotax A7 Dissolution Apparatus, Sotax Ltd., London, U.K.) with the paddle rotating at 100 rpm. MT suspension (obtained by dispersing the pure MT powders homogeneously in 0.5% of CMC–Na aqueous solution), CE, HE and reconstituted SDE were placed in dialysis bag located in 900 ml of release media (the mixture of diluted hydrochloric acid (1%, v/v) and isopropanol (7:3, v/v; 37 °C), respectively. Samples (4 ml) were collected and filtered through a membrane filter (0.22 μm) for analysis at predetermined time points. The MT concentrations were measured at 362 nm using UV spectrophotometer (Purkinje General TU1900, Beijing, China).

2.11. In vivo absorption study

2.11.1. Animals

The study was approved by the Ethical Committee of China Pharmaceutical University. Male Sprague-Dawley rats weighting between 230 and 270 g were obtained from the Laboratory Animal Center, China Pharmaceutical University (Nanjing, China). Animals were housed in standard cages on 12 h light-dark cycles, fed with standard animal chow and tap water daily. Prior to the experiment, the animals were subjected to fasting for 12 h but allowed free access to water. All animals used in this study were handled in accordance with the guidelines of the Principles of Laboratory Animal Care (State Council, revised 1988).

2.11.2. Experimental protocol

Bioavailability of SDE was compared with that of the MT suspension. Before gavage administration to the rats, solid powders of SDE were reconstituted with distilled water.

The rats were randomly allocated into three groups (5 rats in each group) and administered orally with reconstituted SDE, CE and MT suspension at dose of 36 mg/kg as MT. 300 μl of blood samples were collected through orbital sinus into heparinized tubes at 0, 5, 10, 20, 30, 40, 50, 60, 90, 120, 180, 240 and 360 min. Plasma was separated by centrifugation (10 °C, 10,000 rpm, 5 min) using the refrigerated table top centrifuge and kept frozen at −20 °C until analysis.

2.11.3. Analysis of MT in rat plasma

In this study, a modified HPLC/UV method was employed to determine the plasma concentration of MT using a Shimadzu HPLC system. MT was separated by a C18 column (Shimadzu VP-ODS column, 250 mm × 4.6 mm, 5 μm) guarded with a C18 precolumn (Shimadzu) and detected at 362 nm. Isocratic mobile phase consisted of methanol and 0.01 mol/l acetic buffer (pH 4.7) in a volume ratio of 76/24. The mobile phase was pumped at a flow rate of 1.0 ml/min. The column temperature was set at 30 °C.

Frozen plasma samples were thawed at room temperature just before sample preparation. 100 μl of rat plasma was mixed with 200 μl of indomethacin solution (internal standard, 1.0 mg/ml in methanol) and vortex-mixed for 5 min. The mixture was then centrifuged for 5 min (10 °C, 14,000 rpm) and a 20 μl volume of supernatant was injected into the HPLC system for analysis as described above.

2.11.4. Data analysis

Pharmacokinetic analysis was performed by means of a compartmental method using the PKSolver computer program (issued by China Pharmaceutical University) (Zhang et al., 2010). The area under the plasma concentration versus time curve from zero to 6 h (AUC_{0-6h}) was calculated using the trapezoidal rule. The peak plasma drug concentration (C_{max}) and the time to reach C_{max} (t_{max}) were directly obtained from plasma data.

All results were expressed as mean ± SD. The data from different formulations were compared for statistical significance by ANOVA. Results were considered statistically significant with p-values < 0.05.

3. Results and discussion

3.1. Solubility studies

The oily phase in emulsions should have good solvent properties to allow more amount of drug to be dissolved in it. The solubility of MT in various oils is displayed in Table 2. Among the vehicles tested, Labrafac CC gave the highest drug solubility and was chosen as the

Table 2
Solubility of malotilate in various oily phases at 37 °C ($n = 3$).

Oily phases	Solubility (mg/g)	Oily phases	Solubility (mg/g)
Soybean oil	410.8 ± 19.7	Labrafac CC	797.4 ± 58.6
Peanut oil	666.2 ± 29.5	Labrafil M 1944CS	540.2 ± 28.7
Crodamol EO	405.0 ± 13.7	Neobee M-5	736.9 ± 32.3
Crodamol IPM	458.7 ± 1.3	Peceol	464.4 ± 14.9

oily phase. In addition, Labrafac CC also had a moderate viscosity and showed a good emulsion forming ability.

3.2. Surface tension studies and selection of stabilizer

Stabilizers with better emulsifiability can reduce the surface tension significantly between aqueous and oily phases and can establish a higher barrier to prevent coalescence. The surface tensions of Labrafac CC-water systems containing different concentrations of HPMC, PVA or PVP as the stabilizer are presented in Fig. 2. It is depicted that the addition of all three stabilizers reduced the surface tension dramatically, and the surface tension achieved the platform when the concentration of stabilizer was above 2%. Among them, the systems containing PVA gave the lowest values, thus the amphiphilic PVA was chosen as the stabilizer.

3.3. Homogenization parameters

The mean droplet sizes of HE depended mainly on the power density of the homogenizer, the number of homogenization cycles, temperature and also the size of the coarse emulsion. The mean droplet size became constant when certain cycles were carried out, that means the given power density was enough. The droplet sizes of HE are presented in Fig. 3 after different cycles at 1600 bar at 30 °C. It was found that high pressure homogenization had strong ability to break CE with a particle size of 976.6 nm and broad polydispersity (PI = 0.902). After just two cycles, the resultant HE had a sharply reduced particle size (194.9 nm) and uniform distribution (PI = 0.484). The extremely rapid reduction in size demonstrated the ability of PVA in emulsifying and stabilizing the resultant emulsion. After then, the droplet size was in a relatively constant state. The particle size was about 180 nm and the PI value decreased to less than 0.2, which indicated the obtained HE had a small variation in droplet sizes. The optimal number of cycles was found to be between 4 and 8 with the minimum droplet size and small PI value. Finally, 6 cycles were used to prepare the emulsions for the following study.

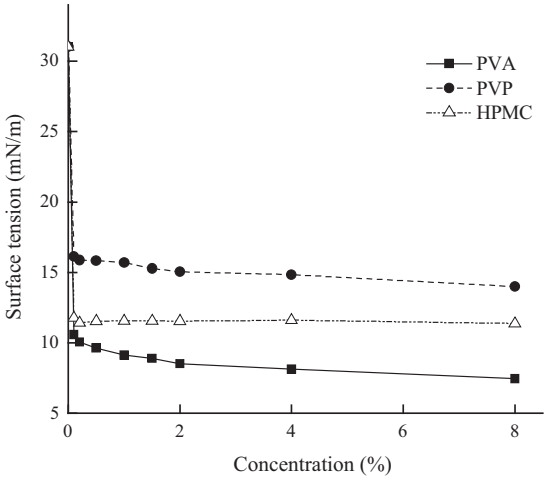

Fig. 2. Surface tensions of Labrafac CC-water systems containing different stabilizers.

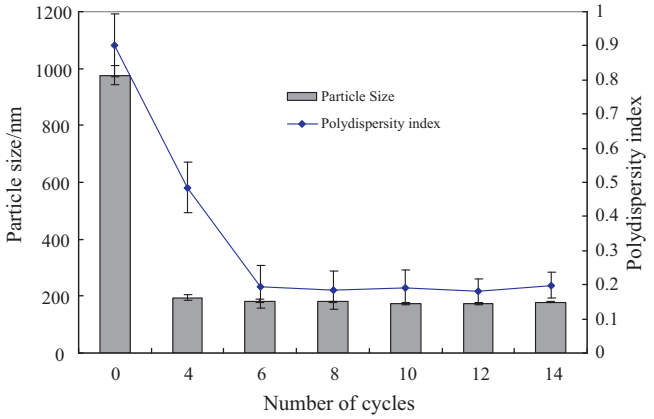

Fig. 3. Droplet size and PI of emulsions as a function of the number of homogenization cycles (pressure = 1600 bar, $n = 3$).

The effect of preparation temperature on droplet size was also considered. The droplet sizes of HE processed at 20, 35 and 50 °C were 175.8 ± 5.30, 177.7 ± 2.28 and 205.2 ± 4.76 nm, respectively. It was found that the droplet size values were of no significant difference at 20 and 35 °C, but increased significantly at 50 °C ($p < 0.05$). When the preparation temperature increased, the viscosity of oil phase decreased and the Brownian motion of droplets increased, which led the droplets of water phase would acquire more kinetic energy in which the collisions between droplets of water phase would be accelerated. Therefore, the interaction between these collisions improved the separation between oil and water phase, which resulted in the coalescence of emulsion (Shinoda and Saito, 1969; Ghannam, 2005; McClements, 1999). Finally, 30 °C was selected for the preparation temperature.

3.4. Rheological properties

The relationships of shear rate (γ) and the shear stress (τ) of the drug-free HE and HE containing MT are shown in Fig. 4 and described by first-order model:

Drug-free HE: $\gamma = -1.02 + 72.33 \times \tau$, $r = 1.000$.

HE containing MT: $\gamma = -1.98 + 48.11 \times \tau$, $r = 0.9999$.

It was demonstrated that both HEs were Bingham plastic fluids whose shear stresses more than yield values were linearly increased with shear rates (McClements, 1999). The viscosity values of drug-free HE and HE containing MT were the reciprocals

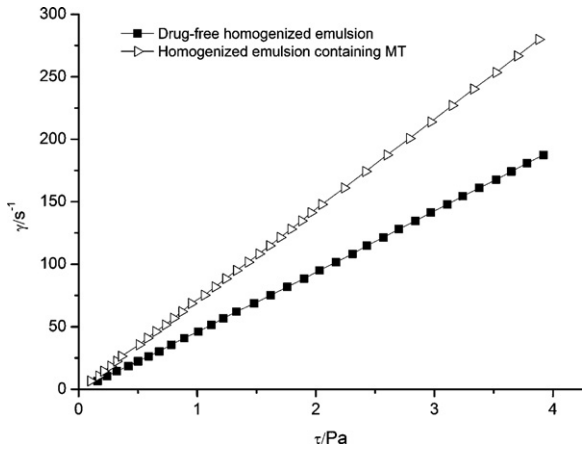

Fig. 4. Rheology curves of the drug-free HE and HE containing MT at 25 °C.

Table 3
Droplet size and zeta potential studies of CE, HE and reconstituted SDE ($n = 3$).

Samples	Mean droplet size (nm)	Zeta potential (mV)
CE	976.6 ± 32.76	−10.3 ± 1.2
HE	170.9 ± 1.8	−13.5 ± 0.7
Reconstituted SDE	175.3 ± 5.3	−12.6 ± 0.9

CE: coarse emulsion, HE: homogenized emulsion, SDE: spray-dried emulsion.

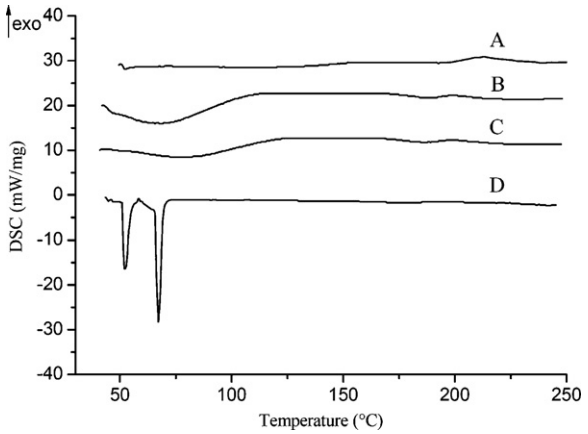

Fig. 5. DSC thermograms of PM (A), drug-free SDE (B), SDE containing MT (C) and the pure MT (D).

of slopes, 13.82 and 20.78 mPa s, respectively, much higher than the corresponding drug-free CE (8.23 mPa s) and CE containing MT (14.78 mPa s). It is implied that the addition of MT thickened the oil phase, leading to increased viscosity of emulsions. The fitted yield values of drug-free HE and HE containing MT were 0.014 and 0.041 Pa, respectively, indicating emulsions can flow under subtle shearing force, a favorable property for spray drying process. After reconstitution, the SDE had the similar viscosity value of 19.28 ± 1.21 mPa s, demonstrating that the spray drying and reconstitution process had no significant effect on the viscosity.

3.5. Droplet size and zeta potential determination

The mean droplet size and zeta potential of HE and reconstituted SDE were demonstrated in Table 3. No significant change in mean droplet size and zeta potential was observed ($p > 0.05$) after solidification of HE, suggesting that the combination of Labrafac CC and PVA was sufficient to stabilize the emulsions during the spray drying process.

3.6. DSC studies

The thermograms of the physical mixture (PM) containing MT and all the ingredients with the same ratio as in SDE (as shown in Table 1 except the water), drug-free SDE, SDE containing MT and the pure MT powder were studied to examine the solid form of MT in SDE (Fig. 5). The pure MT showed two sharp endothermic peaks with onset 50 °C and 59 °C which attributed to crystal form transition and melting point (Uchida et al., 1987), respectively. PM sample only showed a very slight endothermic transition at 52 °C attributed to the dissolving of solid MT into the existing oil. Similar to drug-free SDE, the DSC thermogram of SDE containing MT did not show any melting endothermic peak. These results demonstrated that MT in SDE was at an amorphous state, probably contributing its higher *in vitro* dissolution rate.

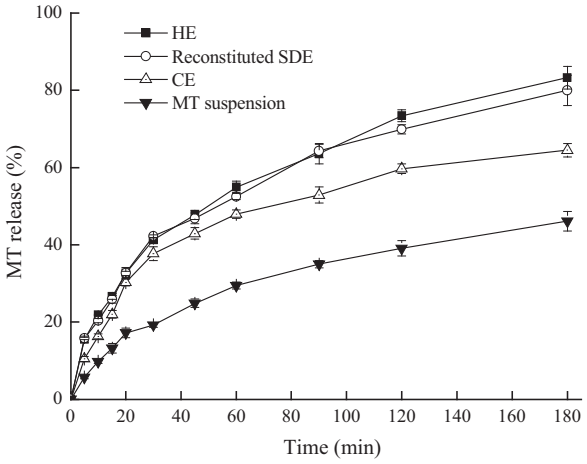

Fig. 7. *In vitro* release profiles of MT formulations using dialysis bag ($n = 6$).

3.7. Morphology

SEM was employed to visualize and compare the structural and surface morphology of SDE stored for 6 months under room temperature compared with the fresh prepared. The micrographs of the SDE powders appeared to be the separated, uniform and spherical particles with relatively deep dents (Fig. 6A), which had been inferred by Pedersen (Pedersen et al., 1998) that the deep surface dents were rendered by maltodextrin (Glucidex) to diminish the agglomeration among granules, even under high humidity. No considerable change of surface morphology appeared after 6 months' storage (Fig. 6B).

3.8. In vitro release study

HE underwent rapid release with 50% of MT released within 1 h and approximately 80% of MT released within 3 h (Fig. 7). The reconstituted SDE had a very similar release profile with that of HE, which indicated that both spray drying and reconstituting processes did not affect the drug release.

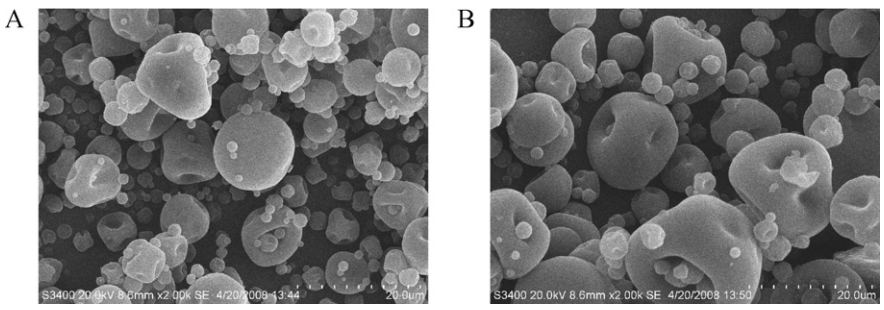

Fig. 6. Scanning electron micrographs of SDE: (A) fresh prepared before storage and (B) after storage for 6 months at room temperature.

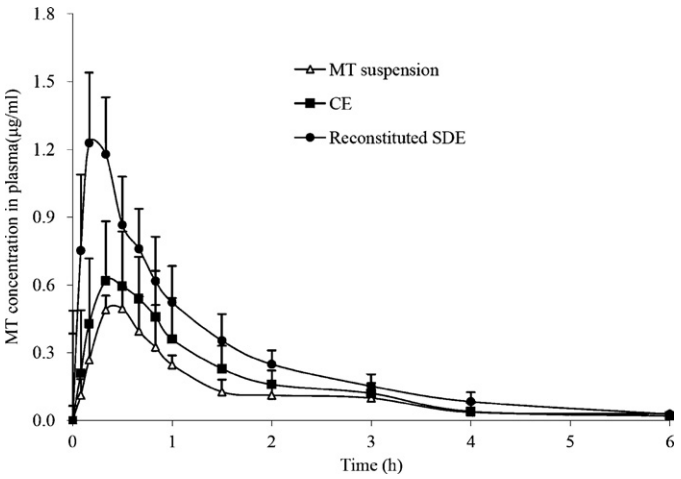

Fig. 8. Plasma concentration profiles of MT formulations ($n = 5$).

Compared to the HE and reconstituted SDE, the MT suspension and CE exhibited much slower and incomplete release profiles, with only 46.11% and 64.47% of MT released, respectively. It was observed that the release extent of MT was enhanced by decreasing droplet size. In other words, samples with increased surface area could release more completely.

However, the release profile of CE was comparable with that of the HE and reconstituted SDE at the first 20 min, which may be dominated by the dispersion of dissolved MT from oil in all three samples to media, whereas the MT suspension dissolved more slowly due to its poor wettability.

3.9. In vivo absorption study

For assay validation, quantification was based on the peak area ratio of MT and indomethacin, with retention time of 10.5 min and 8.0 min, respectively. Good linearity was obtained over the MT concentration range of 0.04–2 μg/ml ($r = 0.9999$, $n = 6$). At concentrations of 0.04, 0.2 and 2 μg/ml, spiked recoveries of MT from rat plasma were (91.7 ± 1.8)%, (99.2 ± 3.0)%, and (95.0 ± 2.8)% ($n = 3$); the intra-day coefficients of variation were 3.3%, 2.9%, and 4.0%; and the inter-day coefficients of variation were 8.5%, 3.0%, and 5.3%, respectively. The limit of quantification for 100 μl of rat plasma was 0.01 μg/ml. After storage for 14 d at −20 °C and freeze-thawing for three cycles, MT was found to be stable in rat plasma.

To estimate the SDE as a potential oral dosage form in improving the bioavailability of MT, an *in vivo* absorption study was carried out in rats using CE as a comparison and the MT suspension as the control. The mean plasma concentrations versus time profiles of the two formulations are shown in Fig. 8. The MT plasma concentrations in rats after oral administration of reconstituted SDE were significantly higher than that of CE and the MT suspension from 5 min to 2 h. Mean pharmacokinetic parameters for SDE, CE and the MT suspension are listed in Table 4. The C_{max} and AUC_{0-6h} of MT after the oral administration of reconstituted SDE were 2.9-fold and 2.3-fold higher than those of the MT suspension formulation. Although significantly weaker than SDE and no significant effect on accelerating the absorption, the C_{max} and AUC_{0-6h} of CE were 1.38-fold and 1.33-fold higher than those of the MT suspension. It can be obviously found that both *in vivo* absorption in Fig. 8 and the *in vitro* release shown in Fig. 7 demonstrated the effect of the droplet size of emulsion. Finer the particles, more absorption can be achieved.

Several factors could be involved in the improvement of MT bioavailability. Due to the small molecular weight and the relatively high hydrophobicity (Log $P = 3.58$, calculated by ALOGPS 2.1 program, http://www.vcclab.org), MT could have a good permeability through epithelia cells of gastrointestinal (GI) tract (Moss and Croninm, 2002; Camenisch et al., 1998). Actually, MT was rapidly absorbed in rat after oral administration (Ryle and Dumont, 1987). However, the poor aqueous solubility (45.30 μg/ml, calculated by ALOGPS 2.1 program) of MT limited its dissolution from marketed tablet and resulted in a low bioavailability. After oral administration of reconstituted SDE, the emulsion readily dispersed, and no dissolution of poor soluble drug MT was required since the drug was dissolved in the oil phase of the emulsion. Usually, drugs dissolved in oil phase were absorbed mainly *via* the aqueous phase, and the amount of the drugs in the aqueous phase was a critical factor for absorption from the O/W emulsion (Kakemi et al., 1972). The larger amount of drugs in aqueous phase, the more drugs was absorbed. In the present investigation, the *in vitro* release study demonstrated that MT could release much faster and more completed from the reconstituted SDE than that of the MT suspension, which finally contributed in the higher C_{max} and *AUC* obtained after oral administration of reconstituted SDE.

Except for liver, part of drugs can also undergo biotransformation in GI tract where numerous of enzymes existed (Hartiala, 1973). Ge et al. (2008) have reported that the SDE drug delivery system could protect lovastatin against intestinal metabolism by microsomal metabolism study and intestinal metabolism study. Since MT was a drug easy to be hydrolyzed by esterase and S-oxidation by oxygenase (Ryle and Dumont, 1987), protection against intestinal metabolism by SDE may also be a factor contributed in absorption enhancement of MT, which will be investigated in our further study. In addition, decreased droplet size and increased surface area of emulsions may lead to increased hydrolysis and absorption of Labrafac CC which was used as oil phase in MT SDE formulation. Such enhancement may accelerate the drug release from emulsion in GI tract. In addition, Labrafac CC (Caprylic/Capric triglyceride), a median chain triglyceride, could induce the activation of the so-called "ideal brake mechanism" which slowed down GI transit time (Maljaars et al., 2008); moreover, it was reported that median chain triglyceride like Labrafac CC had an enhanced effect on the intestinal cells to allow the lipid particles through the cell layer (Xiong et al., 2008; Constantinides et al., 1994, 1996). Both effects of median chain triglyceride may also contribute in the absorption enhancement of MT loaded in present SDE formulation.

Table 4
Pharmacokinetic parameters in rats after oral administration of MT formulations ($n = 5$, mean ± SD).

Parameters	MT suspension	CE	Reconstituted SDE
t_{max} (h)	0.43 ± 0.12	0.36 ± 0.09	0.25 ± 0.12[a]
C_{max} (μg/ml)	0.45 ± 0.08	0.62 ± 0.11[a]	1.31 ± 0.29[b,c]
$AUC_{0\to 6h}$ (μg ml/h)	0.65 ± 0.25	0.87 ± 0.28[a]	1.50 ± 0.41[b,c]
$AUC_{0\to\infty}$ (μg ml/h)	0.66 ± 0.26	0.88 ± 0.28[a]	1.50 ± 0.41[b,c]

MT: malotilate, CE: coarse emulsion, SDE: spray-dried emulsion.
[a] $p < 0.05$.
[b] $p < 0.01$ versus the MT suspension.
[c] $p < 0.05$ versus CE.

4. Conclusions

In the present investigation, the potential of SDE as a novel drug delivery system for MT has been evaluated based on its physicochemical and pharmacokinetic characterization. This novel system appeared to be the separated, uniform and spherical particles in morphology, and exhibited a much more rapid release profile than the MT suspension. High pressure homogenization process maintained the emulsion at reduced droplet sizes, which apparently increased the dissolution rate and therefore improved the bioavail-

ability. The liquid emulsion prior to spray drying was demonstrated to be a Bingham plastic fluid behavior. Considering the significant increment of pharmacokinetic parameters (*AUC* and C_{max}) and the thermodynamically and chemically stable drug delivery system, the spray dried emulsion may be an effective formulation strategy for the other oil-soluble drugs with low oral absorption.

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