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Synthesis, properties and microemulsion formulation of ibuprofen eugenol ester

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Ibuprofen-eugenol ester (IEE), a highly lipophilic compound, was synthesized from ibuprofen in order to reduce the common side effects induced by this classic anti-inflammatory drug. IEE was isolated as an amorphous whitish solid with a melting point at 40.2 ± 0.1 °C, whose structure was confirmed by IR, ^{1}H NMR and MS spectra. The hydrolysis results showed that the ester was stable over a wide pH range from 1.1–9.96. However, it could be hydrolyzed easily by enzymes from rat plasma and rat liver homogenate. A pharmaceutically acceptable microemulsion system was presented and characterized in terms of stability, droplet size distribution (DSD) and their solubilization capacity for IEE. The solubility of IEE in the optimized microemulsion formulation was about 21,000 times higher than that in water. The AUC of ibuprofen from the prodrug showed a remarkable increase compared to oral ibuprofen suspension. These results suggest that synthesizing the ibuprofen prodrug was justified and the presented microemulsion system might be a promising oral dosage form for poorly water-soluble drugs.

1. Introduction

Ibuprofen has been widely used to treat inflammatory diseases. However, side effects, such as ulcerogenic action, have been reported both in experimental animals and in clinical use (Laporte et al. 1991; Wynne et al. 1998). Since the use of prodrugs to temporarily mask the acidic group of nonsteroidal anti-inflammatory drugs (NSAIDs) has been postulated as an approach to decrease their gastrointestinal toxicity, conjugating ibuprofen with another entity to make a highly lipophilic prodrug that could transform back into the two entities *in vivo* was considered. Eugenol, a volatile composition extracted from a Chinese traditional herb, *Eugenia caryophyllata* Thunb., which has good anti-oxidative, analgesic, antipyretic and anti-inflammatory activity (Ji 1999), was chosen on the basis of conjugating two drugs having different pharmacological activities to make a mutual prodrug with synergistic and anti-inflammatory effects and reduced GI irritation (Otagiri et al. 1999; Mahmoud et al. 2002; Sharma et al. 2003; Tantishaiyakul et al. 2002). The resulting product of conjugation, ibuprofen eugenol ester (IEE) – a virtually non-soluble, highly lipophilic drug – was successfully entrapped in a microemulsion (ME), which provided a foundation for future research on the design of oral formulations of poorly water-soluble lipophilic drugs.

In oral drug delivery programs, ester prodrugs are commonly used to enhance membrane permeation and transepithelial transport of hydrophilic drugs by increasing the lipophilicity of the parent compound, resulting in enhanced transmembrane transport by passive diffusion (Balant et al. 1990; Tylor et al. 1996). Ideally, a prodrug aimed at oral administration should be chemically stable in the gastrointestinal tract and should be rapidly activated after traversing the gastrointestinal barrier (Bundgaard 1987). Given the known ability of rat plasma and liver homogenates to cleave esters and amides (Yoshikawa et al. 1995) the present study was undertaken to assess the hydrolysis of IEE by these systems and thereby to predict the liability to prodrug cleavage in vivo.

Microemulsions (ME) have been attracting considerable interest as drug delivery systems for drugs with poor solubility. ME are clear, isotropic, thermodynamically stable dispersions with low viscosity in the presence of a suitable surfactant or mixed surfactants, usually in conjunction with cosurfactants, which could be sterilized by filtration and produced on a large scale without subjecting them to high-energy homogenization. A microemulsion system is a good candidate for oral delivery of poorly water-soluble drugs because of its ability to improve drug solubilization and its potential for enhancing absorption in the gastrointestinal tract (GI), caused by surfactant-induced permeability changes. In the present study, to enhance the solubility and bioavilability of IEE after oral administration, a microemulsion system composed of oil, surfactant, and cosurfactant, was prepared, and its physicochemical properties and pharmacokinetic parameters were evaluated in comparision to a 0.5% CMC suspension of ibuprofen.

2. Investigations, results and discussion

2.1. Synthesis of the prodrug

The rationale of this work was to couple ibuprofen with eugenol to achieve many advantages related to synergistic analgesic and anti-inflammatory effects and reduced GI irritation without adversely affecting their bioactivity. Eugenol is a volatile oil with good anti-oxidative, analgesic, antipyretic and anti-inflammatory activity that has been widely used as a topical agent in surgery, but its utility is also limited to this due to its unstable and volatile nature. The conjugation of eugenol with ibuprofen as a reversible prodrug offers many advantages including stabilization, reduction of irritation and unpleasant smell, and versatility of use. This might provide a new approach to dealing with other physically or chemically unstable drugs, like many volatile oils or active entities extracted from traditional Chinese medicine (TCM). In addition, biotransformation of the prodrugs into the parent compounds at the target site or sites of activity might be used to achieve rate and time controlled drug delivery of the active entities (Bonian et al. 2001; Rautio et al. 2000; Thorsteinsson et al. 1999).

2.2. Characterization of IEE

The proposed structure of the product from the preceding synthesis was confirmed by the following data as IEE: it was an amorphous whitish solid; mp was 40.2 ± 0.1 °C; IR (KBr) 1758.2 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), 0.90 (d, 6 H), 1.6 (d, 3 H), 1.86 (m, 1 H), 2.46 (d, 2 H), 3.34 (d, 2 H), 3.68 (s, 3 H), 3.95 (m, 1 H), 5.08 (m, 2 H), 5.94 (m, 1 H), 6.71 (d, 2 H), 6.83 (d, 1 H), 7.13 (d, 2 H), 7.30 (d, 2 H); MS: (IE, 70 eV) m/z 352 [M]. The purity of the product was found to be $\geq$98% by HPLC. This is the first time that the synthesis of IEE has been reproted.

2.2.1. Solubility and partition coefficient

IEE was poorly soluble in water ($\approx 3\ \mu g \cdot mL^{-1}$). However, it was readily soluble in various organic solvents. There was no significant difference in IEE solubility in the various oils tested. However, in Miglyol 812, a medium-chain triglyceride, the solubility of IEE was slightly higher compared with other oils, although the difference was not statistically significant (Table 1). Therefore, Miglyol 812 was chosen as the candidate oil phase in this study.

The solubility of IEE was 15-fold higher as the ratio of PEG 400/ethanol increased from 0 : 1 to 7 : 3 in aqueous solutions with 10% cosurfactants (data not shown). As far as patient compliance was concerned, a PEG 400/ethanol ratio of 7 : 3 was chosen for further study.

The octanol-water partition (log P) of the ester could not be determined since the value was to high to obtain a reliable result by experimentation (Bruggeman 1982). Therefore it was estimated using the ClogP program (SYBYL, Tripos). The calculated log P value for the ester was 6.45, which indicated that its entrapment efficiency in microemulsion might be much higher than that of ibuprofen (log P = 3.6). The complete incorporation of IEE into the microemulsion was also validated in the following experiments.

Table 1: Solubility of IEE and ibuprofen in different vehicles (n = 3)

Vehicle	IEE ($mg \cdot g^{-1}$)
Water	0.003
Miglyol 812	587.65 ± 28.96
IPM	565.39 ± 21.67
Labrafac cc	530.45 ± 18.98

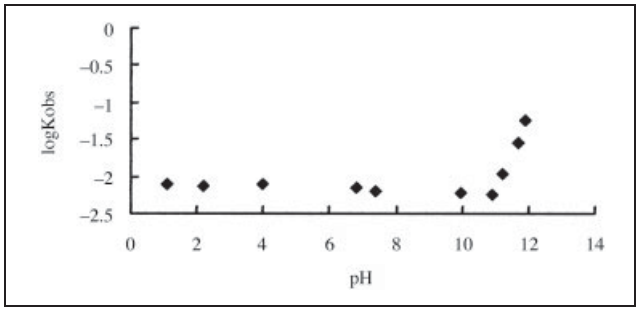

Fig. 1: pH-rate profile for chemical degradation of IEE at 37 °C

Table 2: Hydrolysis rate constants and corresponding half-lives of IEE in buffer solution at 37 °C ($\mu = 0.1$)

pH	K_{obs} (h^{-1})	Half-life (h)
1.1–9.96	$7.15 \times 10^{-3} \pm 7.66 \times 10^{-4}$	97.88 ± 10.80
10.91	$5.60 \times 10^{-3} \pm 1.21 \times 10^{-4}$	123.75
11.24	$1.09 \times 10^{-3} \pm 1.89 \times 10^{-4}$	63.58
11.68	$2.91 \times 10^{-3} \pm 2.01 \times 10^{-4}$	23.81
11.91	$5.85 \times 10^{-3} \pm 3.23 \times 10^{-4}$	11.85

2.2.2. Chemical stability

The observed hydrolysis of IEE followed pseudo-first order kinetics and the degradation products of the ester were ibuprofen and eugenol. Pseudo-first oder plots for the decomposition of IEE were constructed from the logarithm of remaining ester versus time. The pseudo-first-order rate constants (k_{obs}) are shown in Table 1 and the pH-rate profile is presented in Fig. 1. The total rate equation for hydrolysis can be written as: $-dc/dt = k_{obs}C$, $k_{obs} = k_0 + k_{OH^-}[OH^-]$, where k_{obs} is the apparent first-order rate constant, $[OH^-]$ is the hydroxide ion concentration, k_{OH^-} is the rate constant for specific base-catalyzed hydrolysis, and k_0 is the apparent first-order rate constant for spontaneous or water-catalyzed hydrolysis. As seen in Fig. 1, the minimum rate of hydrolysis occurs at pH values below 9.96, where the rate of reaction is apparently pH-indepentend. The average of k_{obs} (or k_0) in this ph range is $7.15 \times 10^{-3} h^{-1}$ (Table 2). With increasing pH above 9.96, the ester degrades progressively faster to yield ibuprofen and eugenol. The plot of log k_{obs} vs. pH 10.91–11.91 gave a straight line with s slope of 1.0 (r = 0.997), indicating that the hydroxide ion concentration is the major factor that influences the hydrolysis rate of the ester. Ibuprofen eugenol ester exhibited high aqueous stability at pH 1.1–9.96. This indicates that the ester might be sufficiently stable to pass through the gastrointestinal tract intact.

2.2.3. Enzymatic stability

The observed hydrolysis half-lives of IEE in rat plasma and liver homogenate were 11.7 min and 4.8 min (Ta-

Table 3: Hydrolysis rates and half-life of prodrug in different incubation mixtures at 37 °C

Incubation mixtures	k_{obs}	Half-life
80% rat plasma	59.4×10^{-3} min^{-1}	11.67 min
20% rat liver homogenate	0.143 min^{-1}	4.85 min

ble 3), respectively. The results suggested that the ester was readily hydrolyzed into its parent drugs in biological media. The data indicated that enzymes from rat liver homogenate increased the rate of hydrolysis are compared to those from rat plasma. Since the protein content in plasma is higher, it is possible that the observed slow rate of hydrolysis in plasma may be due to binding of the ester to plasma proteins, protecting some of the substrate molecules from hydrolysis. This results is consistent with previous findings (Brunner et al. 1995; Tantishaiyakul et al. 2002).

2.3. Phase behavior

Since phosphatidylcholines (SbPC) are naturally occurring non-toxic, biocompatible surfactants, the preparation of lecithin-based ME is of considerable pharmaceutical interest. However, since SbPC has a strong tendency to form liquid crystalline structures, particularly in the aqueous phase (Bergenstaho and Fontell 1983), mixed surfactants were taken into consideration and SbPC/HS-15 was chosen to form a larger ME region in the phase diagrams and give a more stable ME. The added HS-15 formed a mixed monolayer with SbPC between the water and oil domains, the flexibility of this mixed film being increased compared with that of the rigid film formed by using SbPC as a single surfactant because the different molecular structures of HS-15 and SbPC prevent close packing of the molecules at the interface (Von Corswant et al. 1998).

Pseudo-ternary phase diagrams with various weight ratios of SbPC/HS-15/cosurfactant are shown in Fig. 2 and Fig. 3. The translucent and low viscosity microemulsion region and the relatively high viscosity region are presented in the phase diagrams as the black and gray areas. From these phase diagrams, the influence of relative surfactant/cosurfactant concentration on the ME area can be clearly seen.

Fig. 2 shows the effect of surfactant/cosurfactant ratio on the phase behavior of the pseudo-ternary systems. While keeping SbPC/HC-15 (surfactant) constant at 1 : 1, and changing the surfactant (S): cosurfactant (Cos) ratio, such as SbPC/HS-15/Cos = 1 : 1 : 4, 1 : 1 : 2, 1 : 1 : 1, the area of the ME region increased with increasing ratio of S/Cos from 1 : 1 to 2 : 1, indicating that a higher proportion of oil could be incorporated in microemulsions. However, as shown in Fig. 2. There is a grey area exhibiting relatively high viscosity and low stability.

Fig. 3 shows the influence of SbPC/HS-15 ratios on the microemulsion region. The phase diagrams with SbPC/HS-15: cosurfactant ratios of 1.2 : 0.2 : 2, 1 : 1 : 2 and 0.8 : 1.2 : 2 indicate that the ME region is markedly affected by the SbPC/HS-15 ratio and the largest ME region is given at ratio of 1 : 1. As seen from Fig. 2 and Fig. 3, the optimized weight ratio of SbPC/HS-15/Cosurfactant was 1 : 1 : 2.

2.4. Solubilization of IEE in the microemulsion system

The optimized formulation of blank microemulsion with a mean droplet size (MDS) of 30 nm, narrow droplet size distribution (DSD) and good stability comprised 16% oil, 6% SbPC, 6% HS-15, 12% cosurfactant and 60% H_2O. The IEE-loaded microemulsions were evaluated in terms of solubilization capacity, droplet size and stability of IEE ME.

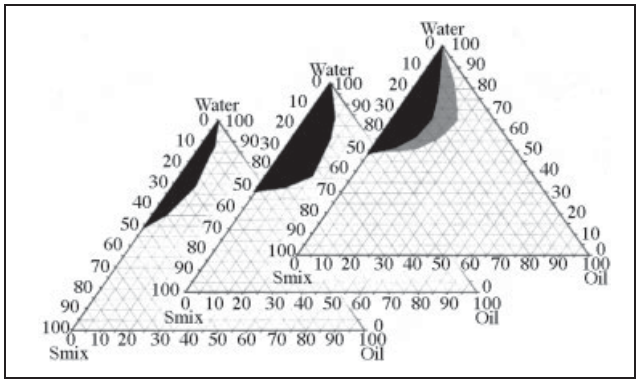

Fig. 2: Phase diagrams of oil/surfactant/cosurfactant/H_2O system at different SbPC/HS-15/Cosurfactant ratios of 1 : 1 : 4, 1 : 1 : 2 and 1 : 1 : 1: Influence of ratio of surfactant to cosurfactant

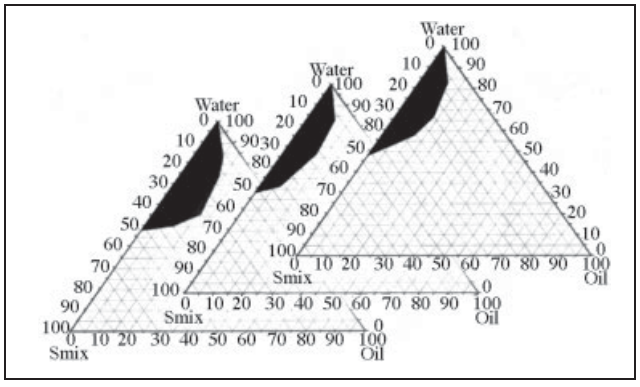

Fig. 3: Phase diagrams of oil/surfactant/cosurfactant/H_2O systems at different SbPC: HS-15: Cosurfactant of 1 : 1 : 2, 1.2 : 0.8 : 1 and 0.8 : 1.2 : 1 Influence of ratio of SbPC to HS-15

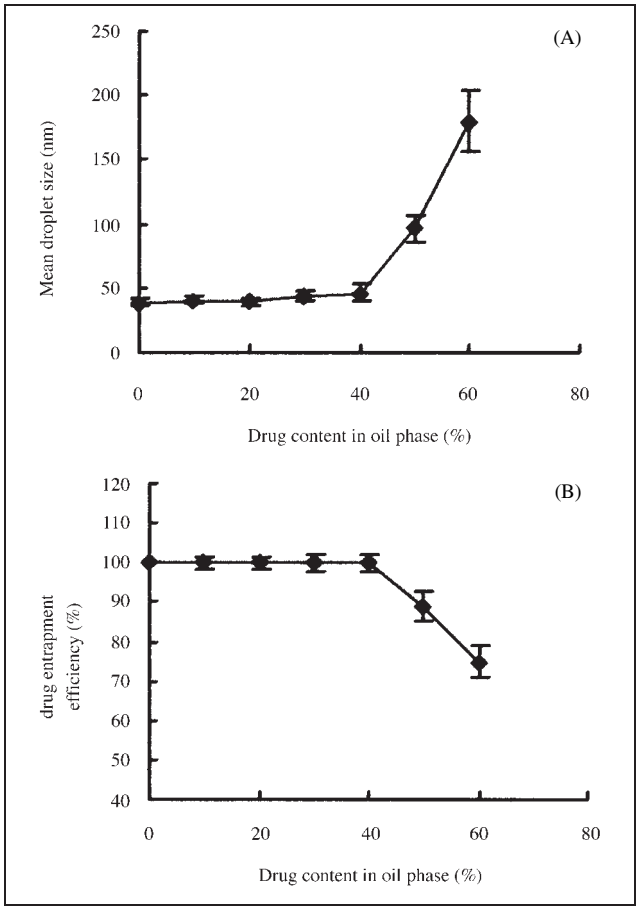

Fig. 4: Particle size distribution of microemulsions (A) and percentage IEE dissolved in microemulsions (B) as a function of IEE content (%, w/w) of the oily phase

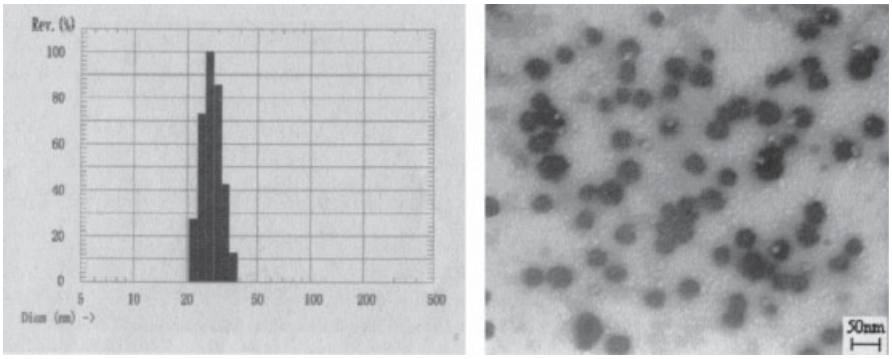

Fig. 5:
DSD and TEM photomicrograph of IEE-ME

Due to its poor water solubility and high lipophilicity, IEE could be readily solubilized in the oil, forming a distinct core in the interior of the surfactant aggregate increasing the apparent aqueous solubility of IEE to 64 mg · mL^{-1}, which was about 21,000-fold greater than the solubility of IEE in pure water (3 μg · mL^{-1}). The drug loading of IEE in ME could be as high as 40% so that it was easy to meet the requirements of high concentration for oral use and reduced volume. The effect of drug loading on MDS is shown in Fig. 4A, in that below a loading of 40% in the oil phase, the MDS of IEE-ME exhibited no significant difference to that of blank ME while the MDS increased dramatically with increasing drug loading above 40%. Moreover, the DSD and stability of IEE-ME also became undesirable at a loading above 40%. The relationship between drug percentage in the oil phase and drug entrapment efficiency is shown in Fig. 4B where the entrapment of IEE was complete when the drug percentage in the oil phase was below 40%.

2.5. Characterization of IEE microemulsions

The DSD and a TEM photomicrograph of the optimized IEE-ME are shown in Fig. 5. No significant changes of PSD or drug leakage were found with the optimized ME over 3-months storage at 4 °C and room temperature.

2.6. Pharmacokinetic evaluation

IEE could only be detected in plasma at very early times after administration, which might be due to fast enzyme hydrolysis as stated above, so the *in vivo* plasma concentration curves are given for ibuprofen. The plasma concentration curve and the main pharmacokinetic parameters are given in Fig. 6 and Table 4, respectively. Administered in equimolar doses, the ibuprofen plasma concentrations with

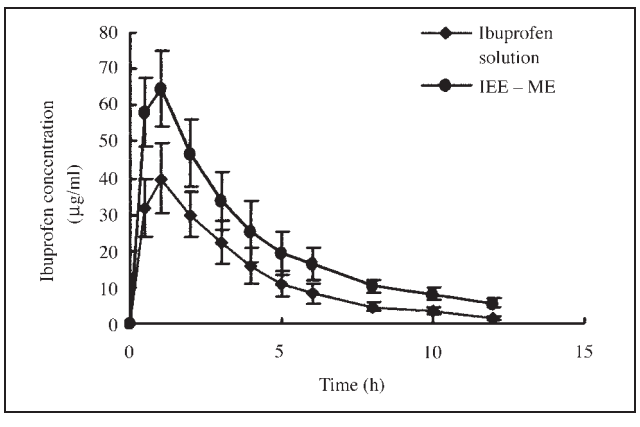

Fig. 6: Ibuprofen plasma concentration vs. time after administration of ...

Table 4: Ibuprofen pharmacokinetic parameters after oral administration (mean ± SD, n = 5)

Parameter	Compound Ibuprofen	IEE-ME
C_{max} (μg · mL^{-1})	39.94 ± 9.72	64.6 ± 10.34*
T_{max} (h)	1	1
AUC_{0-12} (μg · h · ml^{-1})	155.07 ± 39.77	270.31 ± 58.22*
MRT_{0-12} (h)	3.62 ± 0.54	3.69 ± 0.06

*$P < 0.05$ compared to ibuprofen

IEE-ME were significantly higher than with ibuprofen suspension, the C_{max} with IEE-ME being 1.64-fold higher than that with of ibuprofen suspension. The AUC_{0-12} with IEE-ME was increased by 1.74-fold compared with that with ibuprofen suspension, which resulted in an obvious increase in bioavailability with IEE-ME. The enhanced bioavailability was probably due to the good solubilization and fine dispersion of the drug in the ME (Constantinides 1995; Kim 2001).

3. Experimental

3.1. Materials

Eugenol and ibuprofen were purchased from Sigma Chemical Co., St. Louis, MO. and Xinhua Pharmaceutical Co., Shandong, China, respectively. Dimethyl sulfoxide was obtained from Shenyang Chemical Plant. Anhydrous potassium carbonate and anhydrous magnesium sulfate were provided by Tianjin Fuchen Chemical Plant. Acetonitrile, tetrahydrofuran, methanol of HPLC grade and acetone were obtained from Tianjin Concord Tech. Co., China.

Soybean phosphatidylcholine (SbPC) was obtained from Shanghai Taiwei Pharmaceutical Co.; poly (ethylene glycol)(660)-12-hydroxystearate(12-HSA-EO15, Solutol® HS15), Labrafac cc, isopropyl myristate (IPM) and Miglyol 812 were kindly donated by BASF (Germany), Gattefosse France, Croda (UK) and CONDEA Chemie GmbH, respectively. Ethanol and PEG400 were supplied by Shandou Yuwang Tech. Co., China and other regeants were all of analytical grade.

3.2. HPLC analysis

The chromatographic system consisted of a pump (Shimadzu LC-10AD), a UV detector (Shimadzu SPD-10A) and a 20 μL loop (Rhenodyne model 7725i). A Diamonsil™ C18 column (200 mm × 4.6 mm, 5 μm, Dikma Technologies) and a Phenomenex C18 securityguard (4 mm × 3.0 mm, 5 μm, Torrance) were utilized for drug separation, using methanol-acetonitril-pH 4.0 phosphate buffer (65 : 5 : 30) as mobile phase A and acetonitrile-methanol-0.2%trifluoroacetic acid-tetrahydrofuran (500 : 100 : 150 : 20 v/v/v/v) as mobile phase B for determination of ibuprofen and IEE, respectively. The flow rate and UV wavelength were 1.0 ml/min and 230 nm, respectively.

3.3. Synthesis of IEE

A mixture of ibuprofen (1.92 g, 0.009 mol) and dimethyl sulfoxide (1 mL, 0.014 mol) was refluxed on an oil bath (80–90 °C) for 1 h. The mixture was concentrated under reduced pressure to give a crude brown oil that was then dissolved in acetone solution (200 mL) in the presence of anhydrous potassium carbonate under stirring. Eugenol (1.5 mL, 0.009 mol) was then added drop-wise and stirring was continued overnight at ambient temperature. The mixture was evaporated under reduced pressure after fil-

tration to give a yellowish oil. The oil was then dissolved in ethyl acetate (30 mL) and dried with anhydrous magnesium sulfate after washing with NaOH solution. Ethyl acetate was removed by distillation under reduced pressure.
The final product was recrystallized by petroleum ether and dried in a vacuum oven overnight. The product was assayed for purity on TLC, and IR, ^{1}H NMR and MS spectra were determined to validate the structure.

3.4. Characterization of IEE

3.4.1. Solubility determination

The solubility of IEE in water and other solvents was determined by adding an excess amount of IEE. The mixture was sonicated for 15 min and then rotated for 48–72 h to ensure equilibrium. Aliquots of saturated solutions of IEE were analyzed by HPLC after adequate dilution. The experiments were performed in triplicate.

3.4.2. Chemical stability

The hydrolysis of IEE was studied in the aqueous buffer solutions, with pH ranging from 1.10 to 11.91, at 37 °C. An ionic strength of 0.1 was maintained for each buffer by adding an appropriate amount of KCl. The reaction was initiated by adding 100 μL of a 0.01 M stock solution of ester in methanol to 10 ml of buffer solution, pre-equilibrated at 37 °C, in screw-capped test tubes. At appropriate times, reaction samples were withdrawn and analyzed by HPLC as described above. Pseudo-first order rate constants (k) were calculated from the slopes of linear plots of the logarithm of residual ester concentration against time, and the corresponding half-life obtained from the equation: $t_{1/2} = 0.693/k$. Triplicate samples were analyzed, and the mean value of the rate constant was calculated.

3.4.3. Enzymatic stability

The hydrolysis of IEE was studied in rat plasma or liver homogenate in phosphate buffer (pH 7.4). The reaction was initiated by adding 100 μL of IEE methanol solution (0.01 mol · L^{-1}) to 5.0 ml of prewarmed (37 °C) rat plasma (80%) or liver homogenate (20%). Keeping the mixture in a 37 °C water bath, samples (200 μL) were withdrawn at fixed time intervals and analyzed by HPLC after appropriate pretreatment before injection. Triplicate samples were analyzed and the mean value of the rate constant was calculated.

3.5. Construction of phase diagrams and preparation of IEE-ME

The pseudo-ternary phase diagrams of oil, surfactant/cosurfactant and H_2O were developed using the H_2O titration method: mixtures of oil and surfactant/cosurfactants of given weight ratios were diluted with water in a dropwise manner. Five phase diagrams were prepared with SbPC/HS-15/cosurfactant weight ratios defined as 1 : 1 : 4, 1 : 1 : 2, 1 : 1 : 1, 1.2 : 0.8 : 2, 0.8 : 1.2 : 2. For each phase diagram at a specific surfactant/cosurfactant ratio, ten transparent and homogenous mixtures of oil/(SbPC/HS-15/cosurfactants) at 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8 and 1 : 9 (w/w) were prepared under magnetic stirring. Then each mixtures was titrated with H_2O and visually observed for phase clarity and flowability.
After identification of the microemulsion region in the phase diagrams, an optimized blank microemulsion formulation was selected at the desired component ratio. The preparation of the selected microemulsion was performed by simple adding the weighed components together under gentle stirring.
The IEE loaded microemulsions were prepared using the drug oil solution as the oil phase. The amount of IEE entrapped in the ME was determined by HPLC after filtration through a 0.45 μm membrane (to remove unentrapped IEE) and appropriate dilution with methanol.

3.6. Characterization of IEE microemulsions

3.6.1. Stability

Microemulsions were stored at 4 °C or at room temperature. Their physical stability was measured by periodic inspection over 3 months for the presence of macroscopic cloudiness or the formation of two distinct layers.

3.6.2. Mean droplet size (MDS) and droplet size distribution (DSD)

MDS and DSD of the IEE microemulsions were measured using a Nicomp 380-Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA) at a fixed angle of 90 °C at 25 °C. Microemulsions were diluted with aqueous phase before analysis. Transmission Electron Microscope (TEM) (CM10, Phillips, Japan) photographs were also taken.

3.7. Pharmacokinetic evalution

Wistar rats (male and female, 12 weeks old, 200 ± 30 g) were provided by the Animal Center of Shenyang Pharmaceutical University (the experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University). Before administration, the rats were fasted orvernight but were allowed free access to water *ad libitum*. The IEE microemulsion or ibuprofen aqueous suspension in 0.5% carboxymethylcellulose was administrated by gavage to the rats (40 mg · kg^{-1}, calculated as ibuprofen). Blood samples (approximately 0.5 mL) were drawn by puncture of the retroorbital sinus before dosing, and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h after administration. Blood samples were collected in heparinized tubes and as soon as possible stored at −20 °C until assay. Specimens were thawed and allowed to reach room temperature before analysis.
The area under the drug concentration-time curve from 0 to 12 h (AUC_{0-12}) was calculated using the trapezoidal rule. The maximum plasma concentration of the drug (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were obtained directly from plasma data. The data from different formulations were compared for statistical significance by one-way analysis of variance (ANOVA). All results are expressed as mean ± S.D.

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