Synthesis of Ibuprofen Eugenol Ester and Its Microemulsion Formulation for Parenteral Delivery

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The purpose of this study was to investigate the possibility of parenteral delivery of poorly water-soluble lipophilic drugs using a phospholipid-based microemulsion system. Ibuprofen eugenol ester (IEE), a highly lipophilic compound, was synthesized from ibuprofen and eugenol, and isolated as an amorphous whitish solid with a melting point at 40.2±0.1 °C, which structure was confirmed by IR, ^{1}H-NMR and MS spectra. A pharmaceutically acceptable microemulsion system using Miglyol 812, soybean phosphatidylcholine (SbPC) and poly (ethylene glycol) (660)-12-hydroxystearate (Solutol® HS-15), and PEG400 and ethanol as oil phase, surfactants and cosurfactants, respectively, was presented and characterized in terms of stability, droplet size distribution (DSD) and their solubilization capacity of IEE. The solubility of IEE in the optimized microemulsion formulation consisting of 6.4% ibuprofen eugenol, 9.6% Miglyol 812, 6% SbPC, 6% HS-15, 8.4% PEG400, 3.6% ethanol and 60% distilled water (w/w) was about 21000 times higher than that in water. The ibuprofen blood concentration after intravenous administration of microemulsions was determined and compared with that of ibuprofen solution. It was concluded that the presented microemulsion system might be a promising intravenous dosage form of poorly water-soluble lipophilic drugs.

Key words synthesis; ibuprofen eugenol ester; prodrug; microemulsion; pharmacokinetic

Microemulsion (ME) has been attracting considerable concerns as delivery systems of drugs with poor solubility. ME is a clear, isotropic, and thermodynamically stable dispersion 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 large scale without exerting high-energy homogenization. o/w ME exhibits the benefits in the formulation of sparingly soluble drugs intended for parenteral delivery, where the administration of suspension is undesirable. It provides a considerable promise as means to increase aqueous solubility of poorly water-soluble lipophilic drugs to the extent (10^3- to 10^5-fold) necessary for the relatively high concentrations (1—100 mg/ml), which were frequently required in parenteral use. Several sparingly soluble lipophilic drugs have been formulated into o/w ME for parenteral delivery[1—3] which exhibiting a higher physical stability in plasma than lipsomes or other vesicles[4] and the internal oil phases were resistant against drug leaching due to the formation of a distinct core in the interior of the surfactant aggregate.

In this presented work, ibuprofen, an effective non-steroidal anti-inflammatory drug (NSAID) was chosen as a model drug for the following two points. The common side effects of ibuprofen were upper gastrointestinal (GI) irritation and bleeding, which might be avoided by making into intravenous formulations.[5] On the other hand, so far, there was still no intravenous formulation of ibuprofen in the market. However, it might not easy to make an o/w ME directly from ibuprofen due to its relatively good aqueous solubility (46 μg/ml). Thus, we took the conjugation of ibuprofen with another entity into consideration to make a highly lipophilic prodrug that could transform back into the two entities *in vivo* was taken. Eugenol, a volatile drug extracted from a Chinese traditional herb *Eugenia Caryophyllata Thunb.*, which is of good anti-oxidative, analgesic, antipyretic and anti-inflammatory activities,[6] was chosen according to the suggestion of conjugating two drugs having different pharmacological activities to expect synergistic analgesic, anti-inflammatory effects and reduced GI irritation.[7—9] The resulting product of conjugation, ibuprofen eugenol ester (IEE)—a virtually non-soluble, highly lipophilic drug was successfully entrapped in ME, which might provide a basis of foundation for future research on the designing of intravenous formulation of poorly water-soluble lipophilic drugs.

Experimental

Materials Eugenol and ibuprofen were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. 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® HS-15), Labrafac cc (Medium chain triglycerides EP), Isopropyl myristate (IPM) and Miglyol 812 were kindly donated by BASF (Germany), Gattefosse Francem, Croda (U.K.) and CONDEA Chemie GmbH, respectively. Ethanol and PEG400 were supplied by Shandou Yuwang Tech. Co., China and other reagents were all of analytical grade.

HPLC Analysis The chromatographic system consisted of a pump (Shimadzu LC-10AD), a UV detector (Shimadzu SPD-10A), 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, while using methanol–acetonitril–pH 4.0 phosphate buffer (65 : 5 : 30, v/v/v) 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.

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 crude brown oil that was then dissolved in acetone solution (200 ml) in presence of anhydrous potassium carbonate under stirring. Eugenol (1.5 ml, 0.009 mol) was then added in drop-wise and stirring was continued overnight at ambient temperature. The mixture was evaporated under reduced pressure after filtration to give 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.

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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 performed to validate the structure.

Solubility Determination The solubility of IEE in water, IPM, Miglyol 812, Labrafac cc and 10% PEG400/EtOH (different ratio) aqueous solutions was determined by adding an excess amount of IEE to each solvent or solution. Each mixture was sonicated for 15 min and then rotated for 48—72 h to ensure equilibrium. Aliquot of saturated solutions of IEE were analyzed by HPLC after adequate dilution. The experiments were performed in triplicate.

Construction of Phase Diagrams and Preparation of IEE Loaded Microemulsions The pseudo-ternary phase diagrams of oil, surfactant/cosurfactants and water were developed using water titration method: the mixture of oil and surfactant/cosurfactants at certain weight ratio were diluted with water in a drop-wise manner. Five phase diagrams were prepared with SbPC/HS-15/cosurfactant weight ratios defined at 1 : 1 : 4, 1 : 1 : 2, 1 : 1 : 1, 1.2 : 0.8 : 2, 0.8 : 1.2 : 2. For each phase diagram at specific surfactant/cosurfactants ratio, ten transparent and homogenous mixture 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 formed under magnetic stirring. Then, each mixture was titrated with water and visually observed for phase clarity and flowability.

After the identification of ME region in the phase diagrams, an optimized blank ME formulation was selected at desired component ratio. The preparation of selected ME was simply performed by adding the weighed components together under gently stirring.

The IEE loaded MEs were prepared by using the drug oil solution as oil phase. The amount of IEE entrapped in ME was determined by HPLC after filtration through 0.45 μm membrane (to remove the unentrapped IEE) and appropriate dilution with methanol.

Characterization of IEE Microemulsions Stability: MEs were stored at 4 °C or under room temperature. Their physical stabilities were measured by periodic inspection over 3 months for the presence of macroscopic cloudiness or the formation of two distinct layers and droplet size distribution. ME stability was also evaluated by means of dilution with distilled water.

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

Pharmacokinetic Evaluation 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 proved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University). Before administration, the rats were fasted overnight but were allowed free access of water and libitum. The IEE ME or ibuprofen aqueous solution containing adequate amount of propylene glycol was administrated to rats (10 mg/kg) *via* the tail vein. Blood samples (approximately 0.4 ml) were drawn by puncture of the retroorbital sinus before dosing, at 1/20, 1/6, 5/12, 2/3, 1, 3/2, 2, 3, 4, 5, 7 and 9 h after administration. Blood samples were collected in heparinized tubes and stored at −20 °C as soon as possible until assay. Specimens were thawed and allowed to reach room temperature before analysis.

The non-compartmental pharmacokinetic parameters such as area under the drug concentration–time curve (*AUC*), biological half-time ($T_{1/2}$), mean resident time (*MRT*), total clearance (*CL*) and apparent volume of distribution at steady state (V_{ss}) were calculated based on the reported method.[10] Levels of statistical significance ($p<0.05$) were assessed using the Student *t*-test between the two means for unpaired data. All results were expressed as mean±standard deviation (S.D.)

Results and Discussions

Synthesis of IEE The rationale of this work was to couple ibuprofen with eugenol to achieve many advantages related to synergistic analgesic, anti-inflammatory effects and reduced GI irritation without adverse affection on their bioactivity. Eugenol was a volatile oil with good anti-oxidative, analgesic, antipyretic and anti-inflammatory activities, and was widely used as a topical reagent in surgery, but its utility was restricted due to its nature of unstability and volatilization. The conjugation of eugenol with ibuprofen as a reversible prodrug lends it many advantages including stabilization, reduction of irritation and unpleasant smell, and versatility of usage. This approach might provide a new idea when dealing with other physically or chemically unstable drugs, like many volatile oils or active entities extracted from traditional Chinese medicines (TCM). In addition, the biotransformation of the prodrugs into the parent compounds at its target site or sites of activity might be used to achieve rate and time controlled drug delivery of the active entities.[11—13]

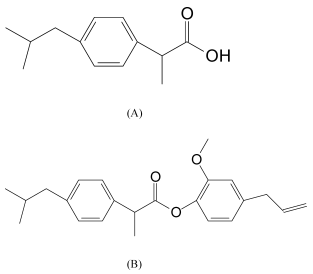


Fig. 1. Molecular Structure of Ibuprofen (A) and IEE (B)

Table 1. Solubility of IEE and Ibuprofen in Different Vehicles ($n=3$)

Vehicle	Solubility (mg/g)	
	IEE	Ibuprofen
Water	0.003	0.046
Miglyol 812	587.65±28.96	43.4±0.67
IPM	565.39±21.67	49.2±1.24
Labrafac cc	530.45±18.98	44.6±1.62

Characterization of IEE The proposed structure of IEE 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}; ^{1}H-NMR (300 MHz, $CDCl_3$), 0.90 (d, 6H), 1.6 (d, 3H), 1.86 (m, 1H), 2.46 (d, 2H), 3.34 (d, 2H), 3.68 (s, 3H), 3.95 (m, 1H), 5.08 (m, 2H), 5.94 (m, 1H), 6.71 (d, 2H), 6.83 (d, 1H), 7.13 (d, 2H), 7.30 (d, 2H); MS: (IE, 70 eV) *m*/*z* 352 [M]. The molecular structure of ibuprofen and IEE were shown in Fig. 1. The purity of the product was found to be ≥98% by HPLC.

It was the first time for IEE to be synthesized and reported.

Solubility and Partition Coefficient IEE was poorly soluble in water (*ca.* 3 μg/ml). However, it was readily soluble in various oils. There was no significant difference of the IEE solubility in the 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). It was found that the solubility of IEE in Miglyol 812 was about 13.5-time greater than that of ibuprofen. Therefore, Miglyol 812 was chosen as a candidate oil phase in this study.

The solubility of IEE was 15-fold higher as the ratio of PEG400/ethanol increased from 0 : 1 to 7 : 3 in 10% cosurfactants aqueous solution (data was not shown). As far as patients' compliance was concerned, the ratio of PEG400/ethanol at 7 : 3 was chosen for further studies.

The octanol–water partition (logP) of the ester could not be determined since the value was too high to obtain a reliable result by experimentation.[14] Therefore it was estimated using the ClogP program (SYBYL, Tripos). The calculated

logP value for the ester was 6.45, which indicated that its entrapment efficiency in ME might be much higher than that of ibuprofen (logP=3.6). The complete incorporation of IEE into the ME was also validated in the following experiments.

Construction of Phase Diagrams Surfactants suitable for IV injection purpose were limited to SbPC, Cremophor EL® and Poloxamer188 for many years. Now, according to the European Pharmacopoeia, HS-15 (polyethylene glycol 660 ester of 12-hydroxy stearic acid) is also acceptable for this purpose. Surfactants used in this work were chosen from three ones other than Cremophor EL® due to its histamine release and anaphylactic reactions which have been extensively reported recently. The single use of the three surfactants was not able to give stable MEs, because this might due to the lipophilic or hydrophilic property of single surfactant according to the results reported by Schurtenberger *et al.*[15] Therefore, mixed surfactants were taken into consideration and SbPC/HS-15 were chosen for forming larger ME region in the phase diagrams and giving more stable ME. The addition of HS-15 formed a mixed monolayer with SbPC between the water and oil domains, and then the flexibility of this mixed film was increased compared with that of the rigid film formed by using SbPC as single surfactant, because the different molecular structures of HS-15 and SbPC prevents a close packing of the molecules at the interface.[2]

The pseudo-ternary phase diagrams with various SbPC/HS-15/cosurfactants weight ratios are displayed in Figs. 2 and 3. The translucent and low viscosity ME region and relatively high viscosity region were presented in the phase diagrams as blackened and gray area, respectively.

Figure 2 shows the effect of surfactant : cosurfactants ratio on the phase behavior of the pesudo-ternary systems. While keeping SbPC/HS-15 (Surfactant) constant 1 : 1, and changing surfactant (S) : cosurfactants (Cos) ratio, such as SbPC/HS-15/Cos=1 : 1 : 4, 1 : 1 : 2, 1 : 1 : 1, the area of ME region increased with increasing ratio of S/Cos from 1 : 2 to 2 : 1. It indicated that a higher proportion of oil could be incorporated in MEs. Although MEs in the gray area were also translucent, the viscocity was relatively high so that the formulations in this area were not suitable for injection purpose.

Figure 3 showed the influence of SbPC/HS-15 ratios on the ME region. The phase diagrams with SbPC/HS-15/cosurfactants ratio at 1.2 : 0.8 : 2, 1 : 1 : 2 and 0.8 : 1.2 : 2 indicated that ME region were markedly affected by the SbPC/HS-15 ratio and the largest ME region was given at ratio of 1 : 1. As seen from Figs. 2 and 3, the optimized weight ratio of SbPC/HS-15/Cosurfactants was 1 : 1 : 2.

Characterization of IEE Microemulsions The optimized formulation of blank ME with a mean droplet size (MDS) of 40 nm, narrow droplet size distribution (DSD) and good stability was consisted of 16% oil, 6% SbPC, 6% HS-15, 12% cosurfactants and 60% water (Fig. 4). The IEE-loaded MEs were evaluated in terms of solubilization capacity, droplet size and stability of IEE-ME.

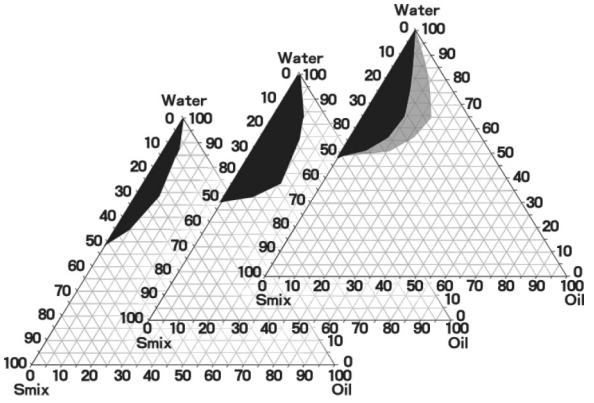


Fig. 2. Phase Diagrams of Oil/Surfactant/Cosurfactants/Water System at Different SbPC/HS-15/Cosurfactants of 1 : 1 : 4, 1 : 1 : 2 and 1 : 1 : 1 Influence of Ratios of Surfactant to Cosurfactants

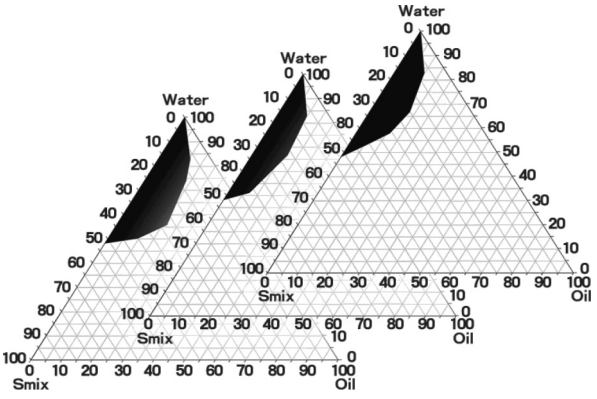
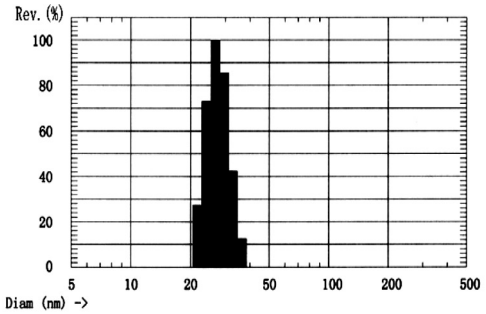
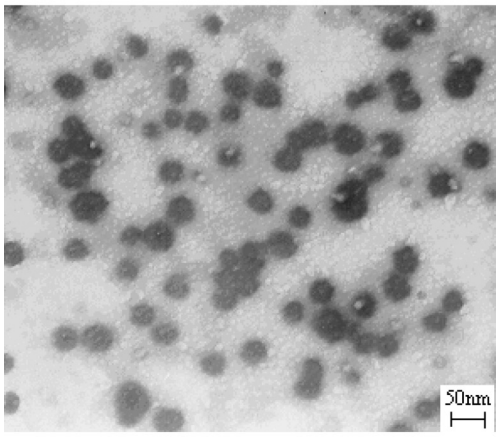


Fig. 3. Phase Diagrams of Oil/Surfactant/Cosurfactant/Water System at Different SbPC : HS-15 : Cosurfactant of 1 : 1 : 2, 1.2 : 0.8 : 1 and 0.8 : 1.2 : 1 Influence of Ratios of SbPC to HS-15

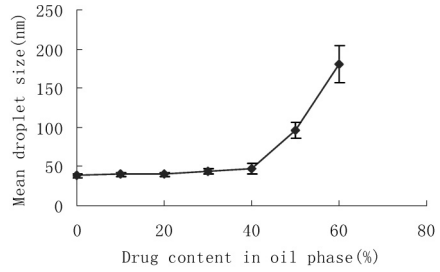


(A)

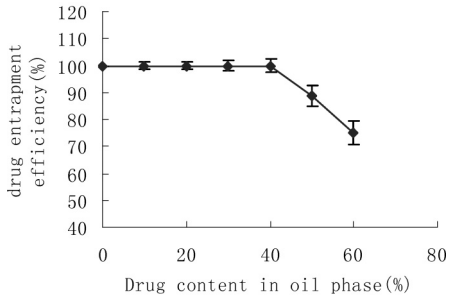


(B)

Fig. 4. The Droplet Size Distribution and TEM Photomicrograph (Magnification ×180000) of Blank ME



(A)



(B)

Fig. 5. Droplet Size of MEs (A) and Drug Entrapment Efficiency in MEs (B) as a Function of the IEE Content (%, w/w) in the Oily Phase

Solubilization of IEE in the Microemulsion System Due to its poor water solubility and high lipophilicity, IEE could be readily solubilized in the oil, to increase the apparent aqueous solubility of IEE up to 64 mg/ml, which was about 21000-fold greater than the solubility of IEE in pure water (3 μg/ml). As described below, the drug loading of IEE in ME could be as high as 40% in the oil, so that it was easy to meet the requirement of high concentration for parenteral use and reduced the volume of injection.

The effect of drug loading on MDS was shown in Fig. 5A that below a loading of 40% in oil phase, the MDS of IEE-ME exhibited no significant difference from that of blank ME, while the MDS increased dramatically with the increasing in drug loading above 40%. Moreover, the DSD and stability of IEE-ME were also become undesirable with a loading above 40%.

The relationship between drug content in oil phase and drug entrapment efficiency was shown in Fig. 5B where the entrapment of IEE was complete when the drug content in oil phase was below 40%. The optimized microemulsion formulation was consisted of 6.4% ibuprofen eugenol, 9.6% Miglyol 812, 6% SbPC, 6% HS-15, 12% cosurfactant (PEG400 : ethanol, 7 : 3) and 60% distilled water (w/w).

No significant changes of DSD or drug leakage was found with the optimized MEs over 3-month-storage at 4 °C and room temperature. Once given by intravenous route, the stability of ME was challenged by dilution of great amount of blood. However, there were no standard methods to test it *in vitro*. The most popular method at present was to dilute the ME with distilled water or saline. In this work, the author diluted the ME with distilled water and it was shown in Fig. 6. No obvious changes in droplet size were observed up to a 100-time volume dilution. The result was in consistent with other author,[16] indicating that the ME system might be relatively stable in blood circulation.

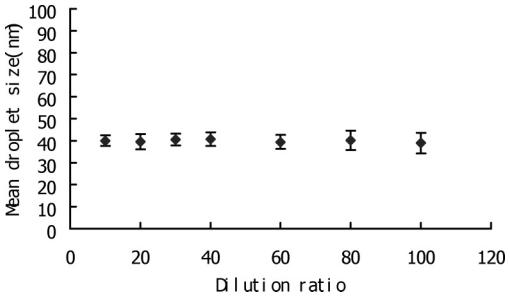


Fig. 6. Effect of the Aqueous Phase for ME

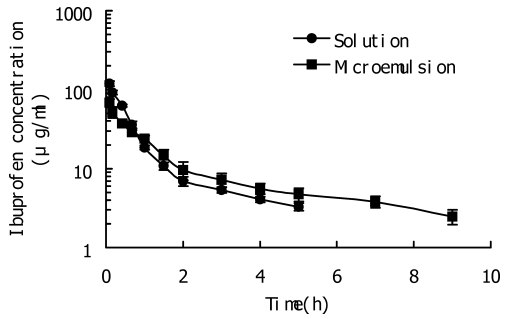


Fig. 7. Mean Plasma Concentration of Ibuprofen after Intravenous Administration of IEE-ME and Ibuprofen Solution of 10 mg/kg to Five Rats (n=5)

Table 2. Comparison of Pharmacokinetic Parameters between IEE Solution and ME

Parameter	Unit	Ibuprofen solution	Microemulsion
$T_{1/2}$	h	2.79±0.29	6.30±0.56[a)]
AUC	μg h ml^{-1}	94.52±8.84	106.42±12.77
MRT	h	2.28±0.18	5.74±0.41[a)]
V_{ss}	ml kg^{-1}	243.12±6.87	543.62±64.68[a)]
CL	ml h^{-1} kg^{-1}	112.17±7.00	94.93±12.09[a)]

a) Significantly different from the solution ($p \leq 0.05$).

Pharmacokinetic Evaluation Figure 7 shows the plasma concentration–time profiles of ibuprofen after administration of IEE-ME and ibuprofen aqueous solution to rats at a dose of 10 mg/kg (calculated by ibuprofen), respectively. The non-compartmental pharmacokinetic parameters in Table 2 were calculated based on the observed plasma levels of ibuprofen. In the case of microemulsion, the plasma concentration–time profile was determined by ibuprofen due to the fast hydrolysis of IEE into the parent drugs in plasma (the plasma level of IEE could only be detected within 1 h after administration). The plasma ibuprofen levels of IEE-ME were much lower than that of ibuprofen solution during the first 1 h while higher after that ($p<0.05$). It can be seen from the calculated pharmacokinetic parameters in Table 2, there was no obvious difference between the *AUC* of the two drug formulations. However, the $T_{1/2}$, *MRT* and V_{ss} were significantly longer or larger and the *CL* was lower comparing IEE-ME to the ibuprofen solution ($p<0.05$), which indicated that with the equimolar dosage, the IEE-ME exhibited an obvious prolonged acting time and the IEE-ME might have a much widely drug distribution *in vivo*.

The prolonged acting time of IEE-ME was largely due to

HS-15 protection of the ME droplet from recognition and uptake by RES that prolonged the circulation time significantly.[17] More studies are necessary to verify the above speculation and to elucidate the real mechanism of the prolonged acting time of IEE-ME. There will also be further research to validate the drug distribution of IEE-ME *in vivo*.

Conclusion

IEE, a purified whitish prodrug with high lipophilic property was synthesized from ibuprofen and eugenol, which could be hydrolyzed into parent drugs easily *in vivo*. Oil-in-water ME prepared with Miglyol 812 and SbPC/HS-15/co-surfactants could solubilize IEE up to 64 mg/ml, which was 21000-fold increase compared with the IEE solubility in water. Increased solubility in this ME implies the possibility of intravenous administration of IEE. The pharmacokinetic study in rats shows that esterification of ibuprofen and entrapment of the prodrug in ME lends ibuprofen the merit of prolonged acting time. Therefore, it was suggested that this ME system could be used as a parenteral drug carrier for poorly water-soluble lipophilic drugs.

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