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Preparation of high solubilizable microemulsion of naproxen and its solubilization mechanism

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ABSTRACT

To improve the skin permeation of naproxen with larger dosage, microemulsion with high content of naproxen was investigated for transdermal delivery and its solubilization mechanism was studied. Naproxen micoremulsions composed of 4% isopropyl myristate, 18% Tween 80, 18% ethanol and water were prepared and phase inversion temperature (PIT) method was used to increase drug content. The using of PIT method resulted in the maximum content of naproxen in microemulsion increased from $1.98 \pm 0.13\%$ to $4.12 \pm 0.07\%$, accordingly the permeation rate of naproxen from microemulsion through excised mice skin increased from 135.13 ± 5.50 to $214.46 \pm 7.53\ \mu g\ cm^{-2}\ h^{-1}$. The analyses of Natural Bond Orbital and interaction energy using the B3LYP and MP2 (fc) methods suggested that the solubilization mechanism of microemulsion for naproxen mainly might be the formation of complex between the hydrogen atom of hydroxyl in Tween 80 and the oxygen atom of carbonyl group in naproxen, as is in accordance with the result from ^1H NMR experiments. The change of thermodynamic function with temperature confirmed that, because the complex was easy to be formed in high temperature and that formed at PIT became more stable when the temperature decreased to below PIT, the solubilization ability of microemulsion for naproxen could be improved by the PIT method. The powerful permeation enhancing ability of microemulsion induced by the solubilization of PIT method makes it a promising vehicle for the transdermal delivery of naproxen.

1. Introduction

Naproxen, 6-methoxy-α-methyl-2-naphthaleneacetic acid (Fig. 1A), is a non-steroidal anti-inflammatory drug (NSAID) that is used with increasing frequency in the treatment of rheumatic diseases and related painful conditions (Todd and Clissold, 1990). Naproxen exhibits high and variable binding to plasma proteins and is eliminated from the body primarily via intrahepatic processes (Held, 1980). As reported, the usual oral dosage of naproxen in clinical treatment was 0.75–2.25 g/day, and the elimination half life period was approximately 12–14 h. As other NSAIDs, by oral administration, naproxen might cause gastritis and peptic ulceration (Brogden et al., 1979), and the local drug level in the adjacent tissues obtainable was relatively low (Weber et al., 2001). In order to avoid the irritation of gastrointestinal tract, minimize systemic toxicity and achieve a better therapeutic effect, one promising method is to administer drug via skin. Unfortunately, the bioavailability of naproxen via percutaneous absorption is rather poor (Singh and Roberts, 1994; Suh et al., 1997). To improve skin permeation of naproxen, pro-drug approach (Weber et al., 2001; Bonina et al., 1993; Rautio et al., 2000) and use of penetration enhancers in appropriate vehicle (Degim et al., 1999) have been adopted. However, there are less of the relative literatures about microemulsion for local application of naproxen, although it has been widely used in pharmaceutics as an effective transdermal delivery vehicle (Yuan et al., 2006; Lee et al., 2005; Djordjevic et al., 2004; Chen et al., 2004).

Microemulsion is transparent, thermodynamically stable and has a droplet size <150 nm and does not have the tendency to coalesce (Kreilgaard, 2002). All the typical components composed of microemulsion such as oil, surfactant, cosurfactant and water are usually good permeation enhancers. In particular, microemulsion has powerful solubilization ability for poorly water-soluble drugs. All these merits of microemulsion make it shown significant enhancement effect on transdermal delivery over conventional formulation (Lawrence and Rees, 2000; Gasco, 1997).

Owing to the large dosage of naproxen, it is necessary to formulate a transdermal delivery microemulsion with high drug content. As reported (Wadle et al., 1993), phase transfer could be utilized for the production of finely dispersed emulsions with long-term stability. In particular, recently it has been found that heating microemulsion over phase inversion temperature (PIT)

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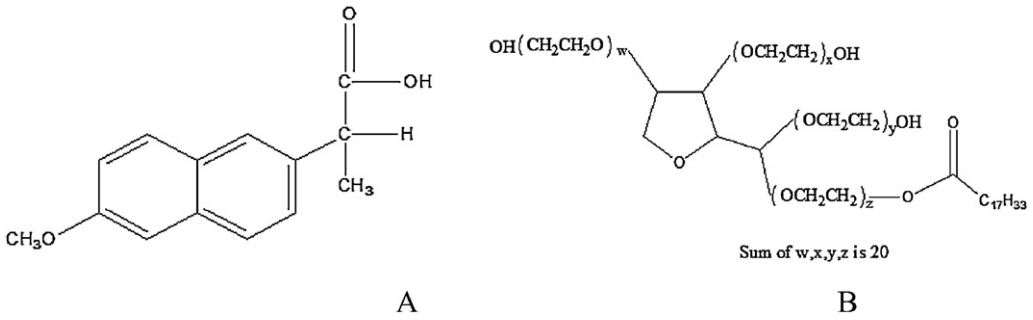

Fig. 1. Structure of Tween 80 and naproxen (A: naproxen, B: Tween 80).

of the emulsifiers can significantly improve the solubilization of microemulsion for drugs (Tian et al., 2009). The solubilization effect of microemulsion should be attributed to the using of large surfactant, therefore, intermolecular interactions between surfactants and drugs have been studied to explain solubilization mechanisms through physical measurement, e.g. UV–vis absorption spectra (Moreno et al., 2001), and NMR (Lv and Zheng, 2005). Although the results of experiments may partially show the structures of intermolecular interaction complexes, but the electronic configuration of the complexes cannot be indicated and it is usually difficult to investigate systematically the change of thermodynamic function with temperature for them. Obviously, there is a limitation to explain the nature of the interaction with experiment. Luckily, quantum chemistry approach can be used to reveal the nature of intermolecular interaction to explain further solubilization mechanisms because it is able to discover the electronic structures of complexes. In this paper, to improve the skin permeation of naproxen with larger dosage, microemulsion with high content of naproxen is prepared using PIT method and its solubilization mechanisms are revealed by experiment and quantum chemistry theoretical method.

2. Materials and methods

2.1. Materials

Naproxen (99.6% purity) was purchased from Zhejiang Charioteer Pharmaceutical Co., Ltd. (Zhejiang, China). Isopropyl myristate (IPM) and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). Water was purified by double distillation. Other chemicals were of HPLC or analytical grade.

2.2. Determination of the solubility of naproxen in some media

The solubility of naproxen was investigated in some media such as IPM, Tween 80, water, etc. An excess amount of naproxen was added to 5 mL of each selected solvent and shaken at 20 °C for 72 h. After the suspension was filtered through a membrane filter (0.45 μm), the concentration of naproxen (C, μg/mL) was determined by HPLC. The density of filtrate (ρ, mg/mL) was determined by measuring the mass in certain volume. The mass concentration (C, %) was calculated according to Eq. (1), and the solubility of naproxen (Sol., μg/mg) in medium was calculated by Eq. (2).

$$C(\%) = \frac{C(\mu g/mL)}{1000 \times \rho(mg/mL)} \times 100\% \quad (1)$$

$$\text{Sol.}(\mu g/mg) = \frac{C(\%)}{1 - C(\%)} \times 1000 \quad (2)$$

where Sol. (μg/mg) means the solubility that is the dissoluble mass of naproxen in unit mass of solvent.

2.3. Preparation

According to previous screening result of blank microemulsion (Tian et al., 2008), naproxen microemulsions were prepared as follows. Approximate 5 mg naproxen was added into 18 mg Tween 80, and then mixed thoroughly with 4 mg IPM and 18 mg ethanol. Subsequently, 60 mg water was added to the mixture drop by drop under a magnetic stirring at ambient temperature. Dealing the preparations with the following three different methods, microemulsion A, B and C were obtained, respectively.

After suspended naproxen in the preparation was eliminated by passing through 0.45 μm polyvinyl difluoride filters, microemulsion A was obtained.

To increase the content of naproxen, PIT method (Brime et al., 2002) was adopted to deal with above preparation. According to the sharply change point of conductivity, the PIT of Tween 80 in the emulsion system of water with IPM (1:1, mass ratio) was 70 °C. Therefore, the preparation was heated at 80 °C (over PIT of the emulsifiers) for about 5 min until turbid emulsion showed, and then standing at room temperature till clear. Filtering through 0.45 μm polyvinyl difluoride filters to eliminate suspended naproxen, microemulsion B was obtained.

Microemulsion A was heated at 80 °C for about 5 min until turbid emulsion showed, and then standing at room temperature till clear, microemulsion C was obtained.

From formulations production, they were protected from the light by storing in dark-brown bottles.

2.4. Solubilization mechanisms

2.4.1. ^{1}H NMR spectroscopy

To ascertain solubilization mechanisms of microemulsion for naproxen, ^{1}H NMR measurements were performed at 289.7 K on a DRX 300 system to investigate the intermolecular interactions between Tween 80 and naproxen. Solutions of naproxen, Tween 80, and their mixture with DMSO as solvent were prepared and their chemical shifts were determined relative to internal tetramethylsilane.

2.4.2. Intermolecular interaction

In order to explain further the origin of the solubilization mechanisms of microemulsion for naproxen, the investigation on the intermolecular interactions between Tween 80 (Fig. 1B) and naproxen were carried out by the quantum chemistry theoretical calculation method.

Our preliminary calculations using semi-empirical AM1 method showed that the intermolecular interactions formed mainly between the hydroxyl in Tween 80 and the carboxyl group of naproxen, while other interactions, such as those between the $-OCH_2CH_2$ group, five-member-ring, $-OCOC_{17}H_{33}$ group in Tween 80 and naproxen, etc., were poor and slight. This result showed that

the origin of the solubilization mechanism might be the formation of the intermolecular interaction between the hydroxyl in Tween 80 and naproxen. Therefore, in this paper, only CH_3–O–C_2H_4–OH group with the hydroxyl was selected as the model of Tween 80 at high-level calculations.

All calculations were performed using GAUSSIAN 03 programs (Frisch et al., 2003). The structures of complexes were calculated at B3LYP/6-311G** and B3LYP/6-311++G** levels, and vibrational frequency calculations were also been performed by the same methods. Single point energy calculations were carried out using B3LYP and MP2 (fc) methods with 6-311G** and 6-311++G** basis sets. The binding energy (D_e) was calculated as the difference between the total energy of the complex and the sum of the total energies of the monomers. The D_e corrected for the basis set superposition error (BSSE) (Boys and Bernardi, 1970) was evaluated. Natural Bond Orbital (NBO) analysis (Reed et al., 1988) was carried out at the B3LYP/6-311++G** level.

2.4.3. Effect of temperature on intermolecular interaction

On the basis of the principle of statistical thermodynamics (Hill, 1960) standard molar heat capacity ($C^{\theta}_{p,m}$), standard molar entropy (S^{θ}_{m}) from 263 to 368 K were obtained. The thermodynamic parameters of the intermolecular interactions (ΔS_T, ΔH_T and ΔG_T) were calculated from standard expressions using rigid rotor-harmonic oscillatorideal gas approximations. ΔG_T was calculated from the expression:

$$\Delta G_T = \Delta H_T - T\Delta S_T$$

where ΔG_T, ΔH_T, T and ΔS_T are the change of the Gibbs free energy, the change of the enthalpy, temperature and the change of the entropy, respectively.

2.5. Characterization of microemulsion

Microemulsion systems were characterized in terms of viscosity, mean droplet size and image of transmission electron microscope (TEM).

The mean droplet sizes of the microemulsion samples were measured via dynamic light scattering (DLS) on a Zetasizer 3000 (Malvern Instruments, Ltd.). The backscatter measurement was performed at a fixed angle of 90° at 20 °C.

Dynamic viscosity (η) of each microemulsion sample was evaluated via an NDF-8S circumrotate viscometer (Shanghai Precision Instrument CO., Ltd., China), at a rate of rotation of 30 r/min and 20 ± 2 °C.

TEM was employed to characterize the microstructure of microemulsion. Samples were placed on a carbon-coated copper grid and then a drop of 1% phosphotungstic acid covered on it. The superfluous phosphotungstic acid on sample was wiped off by filter paper. The TEM images were obtained using a Tecnai G220 TEM (FEI Corp., German).

2.6. HPLC analysis

A Kromasil 100A 5U C_{18} column (4.6 mm × 250 mm, 5 μm, Dikma, USA) and a LC-10AT vp series HPLC equipment (Shimadzu, Japan) with an UV–vis detector set at a wave length of 275 nm were employed. The mobile phase consisted of 0.01 mol/L potassium dihydrogen phosphate (pH 3.0) – methanol (20/80) (v/v) was delivered at a rate of 1.0 mL/min. The retention time of naproxen was found to be 5.3 ± 4% min, and the method was successfully validated with RSD value of 0.71% and a determination coefficient (r) of 0.9998. All operations were carried out in triplicate for each sample at ambient temperature.

2.7. Skin permeation study

The abdominal skins were obtained from male mice (Provided from the Central of Test Animals of Shanxi Medical University, Production Approve Number: SYXK (Jin) 2009-0004) weighing 25 ± 2 g. After hair was shaved carefully with an electric clipper (Oster, USA), the skins were excised. The subcutaneous fat was removed, and then the skins were washed and examined for integrity. The permeation experiments were performed using a drug diffusion apparatus (RYJ-6A, Shanghai, China) with a recirculation water bath and 6 diffusion cells at 32 ± 1 °C. The skins were clamped between the donor and the receptor chamber of vertical diffusion cells with an effective diffusion area of 2.54 cm^2, and a 6.5 mL cell volume. The receptor chamber was filled with pH 7.4 phosphate buffer (the solubility of naproxen in buffer of pH 7.4 was 4187.7 μg/mL at 32 °C), and was constantly stirred at 600 rpm throughout the experiment. After the formulations (1.0 mL) were gently placed in the donor chamber, the receptor medium 2.0 mL was withdrawn every hour for up to 10 h, and an equal volume receiver solution was immediately replenished after each sampling. Collected samples were filtered through 0.45 μm polyvinyl difluoride filters, and naproxen was quantified by HPLC analysis as described above. The cumulative drug permeation per unit of skin surface area (Q_t) was calculated. The permeation rate of naproxen at a steady-state (J_s, μg cm^{-2} h^{-1}) through mice skin was calculated from the slope of linear portion of the plots of Q_t versus time (Li et al., 2005).

2.8. Stability

The stability of microemulsions was evaluated via clarity and phase separation observation, droplet size determination and HPLC analysis of naproxen at 32 ± 2 °C, 60% ± 5% relative humidity for up to 6 months by a WD-A drug stability test chamber (Pharmacopeia standard equipment mill, Tianjin, China). The centrifugation was also carried out to assess the physical stability and microemulsions were centrifuged for 30 min at 13,000 rpm in the centrifuge tests.

2.9. Statistical analysis

The viscosity and skin permeation studies of microemulsion A, B and C were performed three times, respectively. The data were expressed as the mean value ± SD, and single factor one-way analysis of the variance (ANOVA) was performed with a 95% confidence interval on the viscosity and drug permeability data. This was done to determine if the differences among the data at different experimental conditions are greater than the errors due to random effects. A multiple comparison test was used to compare different between each group (A, B) and control (C). A p-value less than 0.05 indicated significant difference between the two groups of data.

3. Results and discussion

3.1. Preparation analysis

In this experiment, Tween 80 which had good biocompatibility and low skin irritant was selected as surfactant (Rhee et al., 2001). IPM with excellent fluidity and enhancement permeation ability (Lee et al., 2003) was selected as oil. Ethanol, an efficient permeation enhancer (Gao and Singh, 1998), was selected as cosurfactant. According to previous studies (Tian et al., 2008), the formulation of microemulsion was selected as 4% IPM, 18% Tween 80, 18% ethanol and water, owing to its excellent permeation ability for naproxen. Because of the much higher solubility of naproxen in Tween 80 than in IPM or ethanol (as listed in

Table 1
Solubility of naproxen in various mediums at 20 °C (mean ± SD, $n = 3$).

Media	Solubility (μg/mg)
Tween 80	91.42 ± 0.14
IPM	10.73 ± 0.03
Ethanol	19.36 ± 0.07

Table 1), naproxen was firstly dissolved into Tween 80 to prepare drug-loaded microemulsion. To increase the drug content of microemulsion, PIT method was used. By HPLC analysis, the maximum content of naproxen loaded in microemulsions prepared by ordinary method (referred as A) and PIT method (referred as B) was 1.98% and 4.12%, respectively. Obviously, the solubility of naproxen in O/W microemulsion was significantly increased in comparison with that in water (15.96 ± 0.12 μg/mL), which can be attributed to the solubilization of microemulsion.

The sum of cross product of the solubility of naproxen in every component composed of microemulsion and the mass fraction of their corresponding component was calculated according to following equation, which denoted the calculation value of the solubility of naproxen in microemulsion (labeled as $Sol._C$, %). Setting the $Sol._C$ as control, the effect of the preparation method of microemulsion on its solubilization for naproxen was evaluated. According to the solubility of naproxen (presented in Table 1), the $Sol._C$ was calculated to be 2.04%, where the content of naproxen dissolved in water was so small that it can be ignored in comparison with that in the other components. From the result of calculation, the maximum content of naproxen loaded in microemulsion A had a decrease of 2.9% in comparison with the $Sol._C$, however it increased 102% in microemulsion B. Obviously, using PIT method, the solubilization of microemulsion for naproxen was improved significantly. To reveal the nature of strong solubilization ability by PIT method, the solubilization mechanisms of microemulsion were investigated firstly.

$$Sol._C(\%) = \left(\sum \frac{Sol._i(\mu g/mg)}{1000} \times F_i \right) \times 100\%$$

where i denoted the component (Tween 80, ethanol, IPM and water) composed of microemulsion, F_i denoted the mass fraction of component i.

3.2. Solubilization mechanisms of microemulsion for naproxen

The solubilization of microemulsion for poorly water-soluble drugs can be contributed to the effect of large surfactant used in microemulsion, therefore the intermolecular interactions between Tween 80 and naproxen was investigated through ^{1}H NMR analysis and quantum chemistry theoretical calculation.

Carefully comparing the ^{1}H NMR spectra of naproxen and Tween 80 as well as their mixture, it is found that the chemical shift of "H" in naphthalene ring have no obvious change when naproxen is dissolved in Tween 80, while the proton of "α-methyl" changes from 1.444 ppm to 1.502 ppm. Meanwhile, the peak of Tween 80 at 2.483 ppm disappears, and the peak at 1.227 ppm is strengthened obviously. This result demonstrates new hydrogen bonds might be formed between hydroxyl groups in Tween 80 as proton donor and carbonyl groups in naproxen molecules as proton receptor, which results in "H" in α-methyl of naproxen lacking electron and moving to low field, and thus the increased chemical shift. Therefore, solubilization mechanism of microemulsion for naproxen might be due to the formation of hydrogen bond between naproxen and Tween 80.

The calculated results of intermolecular interactions between model of Tween 80 and naproxen have demonstrated there are three complexes ((a), (b) and (c)) found at the B3LYP/6-311G** and B3LYP/6-311++G** levels (see Fig. 2). However, only two complexes ((a) and (b)) corresponding to the minimum energy points (NImag = 0) at the molecular energy hypersurface have been obtained.

Table 2
Binding energies of complexes ($-D_e$ (kJ/mol)).

Parameters	MP2 (fc)/6-311G**	MP2 (fc)/6-311++G**	B3LYP/6-311G**	B3LYP/6-311++G**
(a)	79.33 (55.80)[a]	82.36 (43.06)	78.28 (58.81)	70.99 (56.79)
(b)	61.20 (47.61)	54.15 (46.22)	5169(46.37)	60.00 (46.71)
(c)	44.02 (38.31)	40.55 (39.01)	48.28 (40.64)	46.02 (39.39)

[a] The value in the parentheses is BSSE-corrected ($-D_{e(BSSE)}$).

From Fig. 2, in the C1 symmetrical complex (a), the hydrogen atom of hydroxyl in Tween 80 points towards the oxygen atom of carbonyl group in naproxen. The distance between them is 2.441 Å, and a considerable increase of the O—H bond in Tween 80 (more than 0.88 Å) is observed upon complexation, suggesting that the hydrogen bond interaction between the hydrogen atom of hydroxyl in Tween 80 and the oxygen atom of carbonyl group in naproxen is formed. Allowing the hydroxyl of Tween 80 moving into the hydroxyl of the carboxyl in naproxen, the C1 symmetrical complex (b) is subsequently optimized at B3LYP/6-311++G** level. The greatest variation occurs in the hydroxyl of Tween 80, in which the O—H bond changes from 0.987 to 1.104 Å. The distance between the hydrogen atom of the hydroxyl in Tween 80 and the oxygen of the hydroxyl of the carboxyl in naproxen is found to be 2.327 Å at B3LYP/6-311++G** level, indicating that the hydrogen bond interaction between them is possible. The C1 symmetrical hydrogen-bonded complex (c), in which the hydrogen atom of the hydroxyl in Tween 80 points toward the oxygen atom of the ether group of naproxen, is also found. The O—H bond changes from 0.987 to 1.109 Å. The distance between the hydrogen atom of the hydroxyl in Tween 80 and the oxygen of the ether group of naproxen is found to be 2.416 Å at B3LYP/6-311++G** level. Unfortunately, the C1 complex (c) was not found to be the true minimum, and vibrational analysis gives two imaginary frequencies corresponding to the wagging of the O—H bonds.

Table 2 gives both uncorrected and corrected interaction energies after correction of the BSSE by means of the counterpoise method at four levels. From Table 2, the BSSEs are in the range of 5.71–23.53 kJ/mol at MP2 (fc)/6-311++G** level. Even if the BSSEs are included, they almost do not change the ordering of the interaction energies since the binding energies differ by at least 17.18 kJ/mol employing MP2 (fc)/6-311++G** method. The proportion of the BSSE correlated interaction energies for the complexes to their total binding energies, defined as $[(-D_e) - (-D_{e(BSSE)})]/(-D_e)$, is up to 22.15% at B3LYP/6-311++G** level. This result suggests that it might not be necessary to check the BSSE corrections at B3LYP/6-311++G** level, as is consistent with our previous investigations (Ren et al., 2009a,b, 2008; Wu et al., 2009; Yang and Ren, 2010). However, the proportion of the BSSE is up to 29.66 or 47.72% employing the MP2 (fc)/6-311G** or MP2 (fc)/6-311++G** method, indicating that it must be considered for the MP2 (fc)/6-311++G** method.

The deformation energy (ΔE_{def}) is defined as the energy difference between the monomer frameworks at the geometry of the complex. It could not be negligible for the interactions. On the other hand, ΔE_b is obtained by subtracting the energies of the monomer frameworks in complex from the energy of the complex. Thus, the binding energy (D_e) is divided into the deformation energy (ΔE_{def}) and ΔE_b. It can be expressed as follows:

$$D_e = \Delta E_{def} + \Delta E_b$$

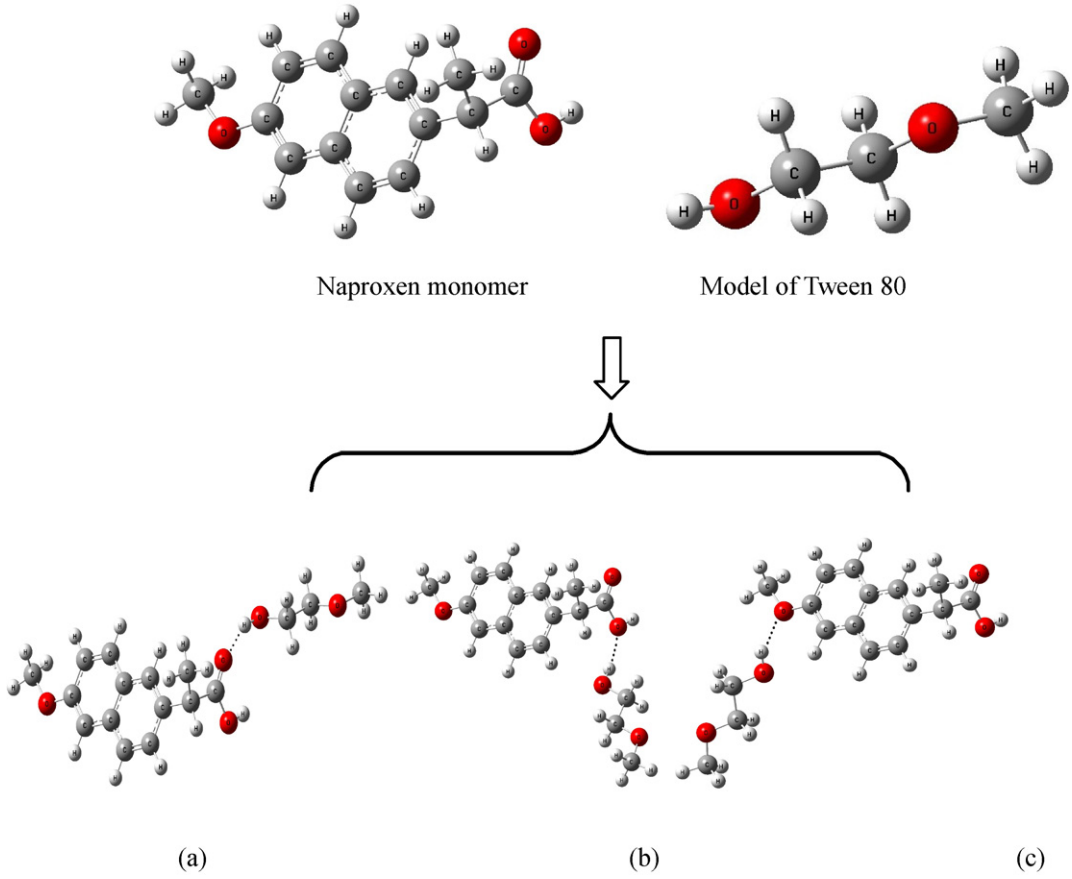

Fig. 2. The 3D structures of naproxen monomer, model of Tween 80 and their three possible complexes (in the complex (a), hydrogen bond interaction between the hydrogen atom of hydroxyl in the model of Tween 80 and the oxygen atom of carbonyl group in naproxen is formed; in (b), hydrogen bond interaction between the hydrogen atom of the hydroxyl in the model of Tween 80 and the oxygen of the hydroxyl of the carboxyl in naproxen is found; in (c), that between the hydrogen atom of the hydroxyl in the model of Tween 80 and the oxygen atom of the ether group of naproxen).

It is worth mentioning that ΔE_{def}, ΔE_b and D_e are overestimated at 6-311G** basis set compared to those from 6-311++G**. This shows that the complexation energy depends on the quality of the basis set. On the other hand, we have found that the ΔE_b values for the complexes show a maximum of 26.08 kJ/mol (19.25% of relative error) between the expensive MP2 (fc)/6-311G** and MP2 (fc)/6-311++G** method, indicating that ΔE_b might be estimated using the MP2 (fc)/6-311G** approach instead of the more expensive MP2 (fc)/6-311++G** method.

As can be seen from Table 2, at four levels, the orders of intermolecular interaction energies are all (a) > (b) > (c). This result shows that the complex (a) is the most stable. Thus, the origin of solubilization mechanism might be mainly the formation of the intermolecular interaction between the hydrogen atom of hydroxyl in Tween 80 and the oxygen atom of carbonyl group in naproxen, as is in accordance with above the result by ^{1}H NMR experiment.

To clarify the nature of the complexation, the NBO analysis was carried out. The NBO approach mainly yields only one kind of hybridization which involves the formation of the hydrogen bond. According to the NBO analysis, all the complexes have two units, as is in agreement with the character of most intermolecular interaction systems. In this study, delocalization effects between these two units can be identified from the presence of off-diagonal elements of the Fock matrix in the NBO basis, and the strengths of these delocalization interactions, $E^{(2)}$, can be estimated by second-order perturbation theory. The results of $E^{(2)}$ indicate that in complex (a), the major interaction is that the oxygen atom of carbonyl group in naproxen offers the $sp^{2.08}$-hybridization electrons to the contacting $\sigma_{(O–H)*}$ antibonding orbital of the hydroxyl in Tween 80 and this interaction has stabilized the system by 50.66 kcal/mol. For complex (b), the major interaction is $LP_O \rightarrow \sigma_{(O–H)*}$, and it has stabilized the system by 22.38 kcal/mol. In complex (c), the oxygen atom of the ether group of naproxen offers the pure p electron to the $\sigma_{(O–H)*}$ antibonding orbital of the hydroxyl of Tween 80. Furthermore, the net charge transfer is evaluated to be from acceptor to proton donors by 87.6, 65.1 and 16.8 me, respectively. These results are in accordance with above the analyses of the structure and energy.

The calculated result under the B3LYP and MP2 (fc) methods as well as the analysis of Natural Bond Orbital have indicated that the solubilization mechanism might be mainly the formation of complex a. According to the analogic steric configuration of complex a, it can be presumed that naproxen is solubilized on the hydrophile surface of microdroplets.

3.3. Effect of PIT on solubilization

In fact, besides the hydrogen-bond interaction between the hydrogen atom of hydroxyl in Tween 80 and the two oxygen atoms of carboxyl group in naproxen, those between the hydroxyl of Tween 80 and water or ethanol in microemulsion could also be formed. However, it is well known that the density of charge in the oxygen of carboxyl is larger than that in the oxygen of hydroxyl. Thus, the hydrogen-bond interaction between the oxygen of carbonyl and the hydrogen of hydroxyl is greater than that between the oxygen of hydroxyl and the hydrogen of hydroxyl, as is well

Table 3
Thermodynamics parameters for the complex (a) at B3LYP/6-311++G** level.

	Temperature (K)	$C^{\theta}_{p,m}$ (J mol^{-1} K^{-1})	S^{θ}_{m} (J mol^{-1} K^{-1})	ΔS_T (J mol^{-1} K^{-1})	ΔH_T (kJ/mol)	ΔG_T (kJ/mol)
Model of Tween 80	263	84.34	311.09			
	273	88.43	318.28			
	283	93.27	327.20			
	298.15	99.92	336.18			
	308	106.01	349.21			
	318	113.29	366.47			
	328	120.54	392.78			
	338	128.93	431.37			
	348	136.05	476.39			
	358	145.34	507.88			
	368	155.66	549.01			
Naproxen	263	223.85	500.97			
	273	232.98	512.10			
	283	244.13	522.04			
	298.15	256.76	534.37			
	308	267.86	552.07			
	318	279.04	580.59			
	328	291.47	621.67			
	338	303.06	677.96			
	348	316.87	726.17			
	358	327.04	779.65			
	368	340.55	846.18			
Complex (a)	263	330.83	698.62	−113.44	−37.28	−7.45
	273	342.28	710.60	−119.78	−37.10	−4.40
	283	354.67	720.21	−129.03	−36.66	−0.14
	298.15	367.23	734.06	−136.49	−36.52	4.17
	308	380.55	763.57	−137.71	−36.01	6.40
	318	394.21	799.02	−148.04	−35.21	11.87
	328	409.67	846.51	−167.94	−34.08	21.00
	338	424.88	935.87	−173.46	−32.28	26.35
	348	439.64	1016.05	−186.51	−30.01	34.90
	358	455.29	1077.12	−210.41	−27.88	47.45
	368	462.04	1125.63	−269.56	−24.11	75.09

agree with the result from the quantum chemistry methods (see (a) and (b) in Table 2 and Fig. 2). Therefore, according to the result of hydrogen-bond in Tween 80 and naproxen (a) is the most stable, the interaction between Tween 80 and naproxen is larger than that between Tween 80 and water or ethanol. At PIT, the thermal motion of molecules in microemulsion is strengthened, and with the increasing of the probability of molecular collision, more stable Tween 80 complexes of naproxen than those of water or ethanol will be formed through molecular reorganization. This might be the solubilization mechanisms of PIT method, as is in accordance with the result of investigation on temperature-dependence of the solubilization of dipalmitoylphosphatidylcholine by the non-ionic surfactant Triton X-100 by Schnitzer (Schnitzer et al., 2003).

In order to explain further the origin of PIT method in improving the solubilization ability of microemulsion for naproxen, the effect of temperature on the intermolecular interaction of Tween 80 and naproxen for the complex (a) was carried out by the quantum chemistry theoretical calculation method.

As it can be seen from Table 3, the thermodynamic functions $C^{\theta}_{p,m}$, S^{θ}_{m} and ΔS_T increase with temperature evidently. This is because the main contributions to the thermodynamic functions are from the translations and rotations of molecules when temperature is low, however, at the higher temperature, the vibrational movement is intensified and therefore makes more contributions to the thermodynamic properties, which lead to the increase in the thermodynamic functions.

The changes of entropies and the changes of enthalpies (ΔS_T, ΔH_T) are both negative but the changes of the Gibbs free energies (ΔG_T) become from negative to positive when the temperature rises from 283 to 298.15 K. Therefore, the formation of the complex should be an exothermic process accompanied by the decrease of the systematic entropies. According to Wang et al. (Ju et al., 2003; Wang et al., 2007, 2009), the larger the ΔG_T, the stronger the intermolecular interaction becomes. The results from Table 3 suggest that the intermolecular interaction between Tween 80 and naproxen become stronger as the temperature decreases. Thus, when the temperature decreases from PIT to room temperature, even to 283 K, the intermolecular interaction between Tween 80 and naproxen becomes stronger enough to form the more stable complex than that formed at PIT via spontaneous process. That is to say, on one hand, the complex is easy to be formed in high temperature (i.e. in PIT) due to the great thermal motion of molecules; on the other hand, the complex which is formed at PIT becomes more stable when the temperature decreases to below PIT. Therefore, the solubilization ability of microemulsion for naproxen can be improved by PIT method.

3.4. Characterization

To investigate the effect of preparation method on the characterizations of microemulsion, microemulsion C with equal drug content to microemulsion A was prepared by PIT method and set as control microemulsion. According to the results in Table 4 and Fig. 3, all the microemulsions were transparent, their mean droplet sizes were no more than 100 nm, viscosities were lower than 200 mPa s, all these were accordance with the characterization of microemulsion (Yuan et al., 2006).

Statistical comparison of the viscosity showed that the viscosity of microemulsion B had only 14.8 mPa s higher than that of C ($p < 0.05$), while the viscosity of microemulsion A was much smaller ($p < 0.05$) than that of C. This result indicated that the increase of naproxen in microemulsion B had no significant influence on the viscosity, however, the use of PIT method resulted in significant increase of viscosity. As above analysis, PIT method could accelerate

Table 4
Characterizations of microemulsion.

Microemulsions	Preparation method	Drug content (%)	Appearance	η (mPa s)	Droplet size (nm) after 2 days	PDI
A	Ordinary	1.98	Transparent, pale yellow	38.4 ± 0.8	16.9 ± 0.7	0.246
B	PIT	4.12	Transparent, yellow	187.6 ± 2.1	91.2 ± 0.6	0.379
C	PIT	1.98	Transparent, yellow	172.8 ± 2.6	76.4 ± 1.2	0.312

molecular reorganization and strengthen molecular interactions, so the viscosity increased.

The mean droplet sizes of microemulsions prepared after 2 days were measured and also listed in Table 4. As can be seen from Table 4, the droplet size and its polydispersity index (PDI) of microemulsion B were larger than that of C while the droplet size of microemulsion A and its PDI were far smaller than that of C. This result showed that, although both the content of naproxen in microemulsion and the preparation method had influence on the distribution of micro-droplet, more notable influence was found by PIT method.

The microstructure of microemulsion and its micro-droplet size could be also represented by TEM images. In comparison with the image of blank microemulsion (Fig. 3(I)), there existed a lot of aggregates beside microdroplets in initially prepared microemulsion A (Fig. 3(II)). Because naproxen was initially dispersed in Tween 80 in the preparation of microemulsion, these aggregates might be the micelle formed by Tween 80 and naproxen. After a week, no obvious aggregates were observed in its TEM image, and spherical micro-droplets became irregular (Fig. 3(III)). After 3 months, micro-droplets regained spherical, and its sizes increased (Fig. 3(IV)). These results demonstrated that naproxen incorporated in microemulsion might experience a transformation from aqueous phase to the interface of micro-droplets, and further verified that naproxen was solubilized on the interface of micro-droplets. Therefore, the mean droplet size increased with the increasing of the content of naproxen. In addition, the micro-droplets of the prepared microemulsion B were constantly

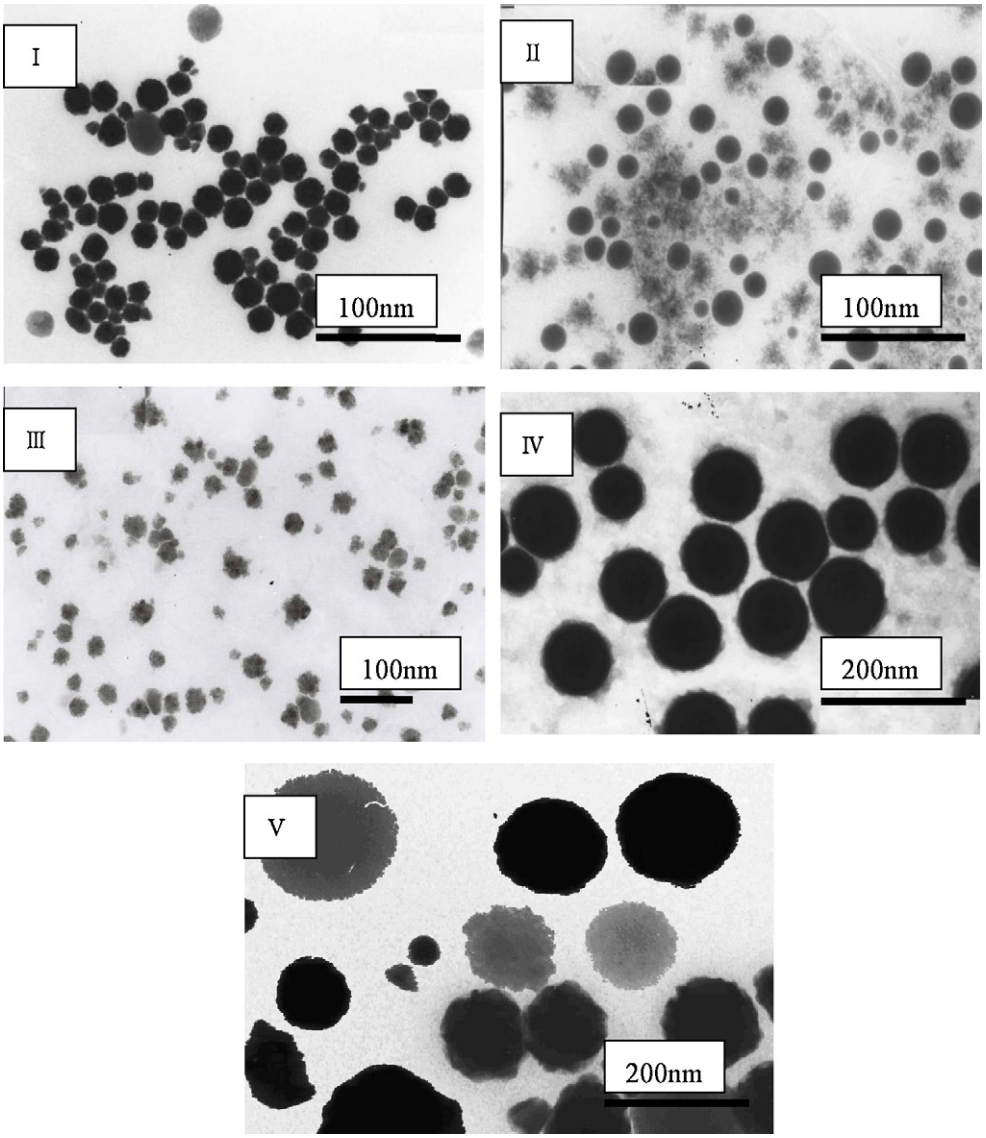

Fig. 3. The TEM images of microemulsion (I: blank microemulsion, II: initially prepared microemulsion A, III: microemulsion A after a week, IV: microemulsion A after 3 months, and V: microemulsion B).

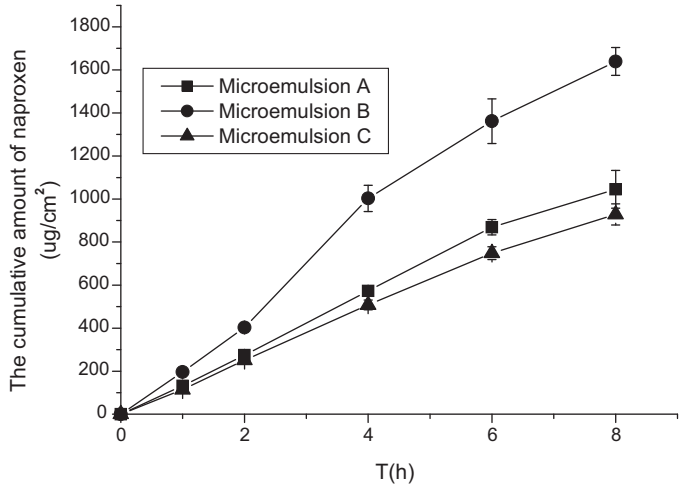

Fig. 4. Permeation profiles of naproxen through mouse skins from microemulsions (mean ± SD, $n = 3$).

spherical, and its size had no obvious change for at least 6 months (Fig. 3(V)). This result suggested that PIT method could accelerate the transformation of naproxen from aqueous phase to the interface of micro-droplets, which resulted in the mean droplet size of microemulsion increased. Therefore, PIT method had significant influence on the mean droplet size of microemulsion, which resulted in the increase of the mean droplet size of microemulsion.

3.5. In vitro permeation studies

The permeation rates of naproxen from microemulsion A and B were compared with that of microemulsion C to evaluate the influence of preparation method and drug content on the permeation ability of naproxen. The permeation rates of microemulsion A, B and C through excised mouse skins were 135.13 ± 5.50, 214.46 ± 7.53, $118.79 \pm 4.91\ \mu g\,cm^{-2}\,h^{-1}$, respectively. The permeation profiles of naproxen from various microemulsions were shown in Fig. 4. The permeation rates of naproxen from microemulsion B were significantly larger than that from C ($p < 0.05$), which indicated that the increase of drug resulted in a significant increase of permeation rate. The thermodynamic activity of drug in the formulation is a significant driving force for the release and penetration of drug into skin (Walters et al., 1998). The high saturation of drug in vehicle can result in high thermodynamic activity, which can induce a high permeation rate (Higuchi, 1960; Chen et al., 2007). Owing to the stronger molecular interaction in the naproxen complex with Tween 80 obtained from PIT method than that by ordinary method, the thermodynamic activity of naproxen in microemulsion C was lower than that of in A. Thus, the permeation rates of naproxen from microemulsion A was $14\ \mu g\,cm^{-2}\,h^{-1}$ larger than that from C. However, statistical comparison of permeation rate showed there had no obvious difference ($p > 0.05$) between microemulsion A and C. The results demonstrated that the effect of drug content on the thermodynamic activity of naproxen was stronger than that of molecular interaction. The powerful solubilization of PIT method still played an important role in improving the permeation ability of microemulsion for naproxen.

3.6. Stability

The stability of microemulsion A and B was studied by a WD-A drug stability test chamber at $32 \pm 2\,^{\circ}C$, $60\% \pm 5\%$ relative humidity. All the preparations were protected from the light by storing in dark-brown bottles from formulations production.

After centrifugation, microemulsion A and B were isotropic transparent dispersions and no phase separation was observed. After 6 months, the mean droplet sizes of the tested formulations were no more than 100 nm, their viscosities had no obvious change, and no naproxen crystal was observed. HPLC analysis of naproxen demonstrated that drug degradation of the tested microemulsions was no more than 1%. This fact indicated that microemulsion A and B were stable for a long time.

3.7. Conclusion

In this study, naproxen micoremulsions were prepared using PIT method and characterized according to viscosity and mean droplet size as well as the image of TEM. The solubilization mechanisms were elucidated by the ^1H NMR analysis and quantum chemistry theoretical calculation. Intermolecular interaction between Tween 80 and naproxen were also investigated using the B3LYP and MP2 (fc) methods. The using of PIT method resulted in the maximum content of naproxen in microemulsion increased from 1.98% to 4.12%, and the permeation rate of naproxen through excised mice skin increased from 135.13 ± 5.50 to $214.46 \pm 7.53\ \mu g\,cm^{-2}\,h^{-1}$. The analyses of ^1H NMR, NBO and the change of thermodynamic function with temperature indicated that the origin of solubilization mechanism might be mainly the formation of the intermolecular interaction between the hydrogen atom of hydroxyl in Tween 80 and the oxygen atom of carbonyl group in naproxen. PIT method can improve the solubilization of microemulsion for naproxen, leading to the significantly improved skin penetration of naproxen in microemulsion.

References

Bonina, F.P., Montenegro, L., Guerrera, F., Bonina, F.P., Montenegro, L., Guerrera, F., 1993. Naproxen 1-alkylazacycloalkan-2-one esters as dermal prodrugs: in vitro evaluation. Int. J. Pharm. 100, 99–105.

Boys, S.F., Bernardi, F., 1970. The calculation of small molecular interactions by the differences of separate total energies. Some procedures with reduced errors. Mol. Phys. 19, 553–566.

Brime, B., Moreno, M.A., Frutos, G., Ballesteros, M.P., Frutos, P., 2002. Amphotericin B in oil–water lecithin-based microemulsions: formulation and toxicity evaluation. Pharm. Sci. 91, 1178–1185.

Brogden, R.N., Heel, R.C., Speight, T.M., 1979. Naproxen up to date: a review of its pharmacological properties and therapeutic efficacy and use in rheumatic disease and pain states. Drugs 18, 241–277.

Chen, H.B., Chang, X.L., Weng, T., Zhao, X.Z., Gao, Z.H., Yang, Y.J., Xu, H.B., Yang, X.L., 2004. A study of microemulsion systems for transdermal delivery of triptolide. J. Control. Release 98, 427–436.

Chen, H.B., Mou, D.S., Du, D.R., Chang, X.L., Zhu, D.D., Liu, J., Xu, H.B., Yang, X.L., 2007. Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. Int. J. Pharm. 341, 78–84.

Degim, I.T., Uslu, A., Hadgraft, J., Atay, T., Akay, C., Cevheroglu, S., 1999. The effects of Azone and capsaicin on the permeation of naproxen through human skin. Int. J. Pharm. 179, 21–25.

Djordjevic, L., Primorac, M., Stupar, M., Krajisnik, D., 2004. Characterization of caprylocaproyl macrogolglycerides based microemulsion drug delivery vehicles for an amphiphilic drug. Int. J. Pharm. 271, 11–19.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., et al., 2003. Gaussian 03, Revision B.03. Gaussian, Inc., Pittsburgh, PA.

Gao, S., Singh, J., 1998. Effect of oleic acid/ethanol and oleic acid/propylene glycol on the in vitro percutaneous absorption of 5-fluorouracil and tamoxifen and the macroscopic barrier property of porcine epidermis. Int. J. Pharm. 165, 45–55.

Gasco, M.R., 1997. Microemulsions in the pharmaceutical field. In: Perspectives and Applications, Industrial Applications of Microemulsions. Marce Dekker Inc., New York, pp. 97–122.

Held, H., 1980. Serumproteinbindung und eliminations-halbwertszeiten yon Naproxen bei Patienten mit hepatozellu-larem bzw. obstruktivem Ikterus. Arzneimittelforsch 18, 843–846.

Higuchi, T., 1960. Physical chemical analysis of percutaneous absorption process from creams and ointments. J. Soc. Cosmet. Mater. 11, 85–97.

Hill, T.L., 1960. Introduction to Statistic Thermodynamics. Addison-Wesley, New York.

Ju, X.H., Xiao, J.J., Xiao, H.M., 2003. Theoretical study on intermolecular interactions and thermodynamic properties of water–hydrogen peroxide clusters. J. Mol. Struct. (THEOCHEM) 626, 231–238.

Kreilgaard, M., 2002. Influence of microemulsions on cutaneous drug delivery. Adv. Drug Deliv. Rev. 54, 77–98.

Lawrence, M.J., Rees, G.D., 2000. Microemulsion-based media as novel drug delivery systems. Adv. Drug Deliv. Rev. 45, 89–121.
Lee Jr., C.T., Ryoo, W., Smith Jr., P.G., Arellano, J., Mitchell, D.R., Lagow, R.J., Webber, S.E., Johnston, K.P., 2003. Carbon dioxide-in-water microemulsions. J. Am. Chem. Soc. 125, 15268–115268.
Lee, J., Lee, Y., Kim, J., Yoon, M., Choi, Y.W., 2005. Formulation of microemulsion systems for transdermal delivery of aceclofenac. Arch. Pharm. Res. 28, 1097–1102.
Li, Z., Hui, H., Ji, F., Peng, Z., 2005. The triptolide-loaded ointment clinically treated 248 patients with psoriasis. Chin. J. Dematol. 38, 182–183.
Lv, F.F., Zheng, L.Q., 2005. Phase behavior of the microemulsions and the stability of the chloramphenicol in the microemulsion-based ocular drug delivery system. Int. J. Pharm. 301, 237–246.
Moreno, M.A., Frutos, P., Ballesteros, M.P., 2001. Lyophilized lecithin based oil–water microemulsions as a new and low toxic delivery system for amphotericin B. Pharm. Res. 18, 344–351.
Rautio, J., Nevalainen, T., Taipale, H., Vepsäläinen, J., Gynther, J., Laine, K., Järvinen, T., 2000. Piperazinylalkyl prodrugs of naproxen improve in vitro skin permeation. Eur. J. Pharm. Sci. 11, 157–163.
Reed, A.E., Curtis, L.A., Weinhold, F.A., 1988. Intermolecular interactions from a natural bond orbital, donor–acceptor viewpoint. Chem. Rev. 88, 899–926.
Ren, F., Cao, D., Wang, W., Hou, S., Chen, S., 2008. Can B═B double bond be as potential proton acceptor: a UB3LYP and UMP2 theoretical study on unusual intermolecular T-shaped X—H···π interactions between the triplet state HB═BH (3Σ-g) and HF, HCl, HCN or H_2C_2. J. Mol. Struct. (THEOCHEM) 870, 43–48.
Ren, F., Cao, D., Wang, W., Ren, J., Hou, S., Chen, S., 2009a. A theoretical study on unusual intermolecular T-shaped X—H···π interactions between the singlet state HB═BH and HF, HCl, HCN or H_2C_2. J. Mol. Model. 15, 515–523.
Ren, F., Cao, D., Wang, W., Ren, J., Chen, S., 2009b. Can the BB multiple bonds be as the stronger hydrogen-bond proton acceptors than the CC multiple bonds: a comparative theoretical investigation on unusual intermolecular T-shaped X—H···π interaction between HF and HB═BH (3Σ-g), HB═BH (1Δg), OCB≡BCO, H_2C═CH_2 or HC≡CH. J. Mol. Struct. (THEOCHEM) 896, 38–43.
Rhee, Y.S., Choi, J.G., Park, E.S., Chi, S.C., 2001. Transdermal delivery of keto-profen using microemulsions. Int. J. Pharm. 228, 161–170.
Schnitzer, E., Lichtenberg, D., Koazlov, M.M., 2003. Temperature-dependence of the solubilization of dipalmitoylphosphatidylcholine (DPPC) by the non-ionic surfactant Triton X-100, kinetic and structural aspects. Chem. Phys. Lipids 126, 55–76.
Singh, P., Roberts, M.S., 1994. Skin permeability and local tissue concentrations of nonsteroidal anti-inflammatory drugs after topical application. J. Pharmacol. Exp. Ther. 268, 144–151.
Suh, H., Jun, H.W., Dzimianski, M.T., Lu, G.W., 1997. Pharmacokinetic and local tissue disposition studies of naproxen following topical and systemic administration in dogs and rats. Biopharm. Drug. Disp. 18, 623–633.
Tian, Q.P., Li, P., Qiu, L.X., et al., 2008. Application of genetic algorithm in formulation optimization of microemulsion for transdermal delivery. Acta Pharm. Sin. 43, 1228–1232.
Tian, Q.P., Li, P., Xie, K.C., 2009. Investigation of microemulsion system for transdermal drug delivery of Amphotericin B. Chem. Res. Chin. Univ. 25, 86–94.
Todd, P.A., Clissold, S.P., 1990. Naproxen. A reappraisal of its pharmacology, and therapeutic use in rheumatic diseases and pain states. Drugs 40, 91–137.
Wadle, A., Förster, T.h., Rybinski, W.V., 1993. Influence of the microemulsion phase structure on the phase inversion temperature emulsification of polar oils. Colloids Surf. A: Physicochem. Eng. 76, 51–57.
Walters, K.A., Brain, K.R., Green, D.M., James, V.G., Watkinson, A.C., Sands, R.H., 1998. Comparison of the transdermal delivery of estradiol from two gel formulations. Maturitas 29, 189–195.
Wang, Z.X., Zhang, J.C., Wu, J.Y., Cao, W.L., 2007. Theoretical investigation on intermolecular interactions between HCN and HNC: the nature and thermodynamic properties. J. Mol. Struct. (THEOCHEM) 806, 239–246.
Wang, G.X., Gong, X.D., Liu, Y., Xiao, H.M., 2009. A theoretical study on the vibrational spectra and thermodynamic properties for the nitro derivatives of phenols. Spectrochim. Acta Part A 74, 569–574.
Weber, H., Steimer, U., Mannhold, R., Cruciani, G., 2001. Synthesis, in vitro skin permeation studies, and PLS-analysis of new naproxen derivatives. Pharm. Res. 18, 600–607.
Wu, Y., Ren, F., Li, B., 2009. A B3LYP and MP2 theoretical investigation on unusual cation–π interaction between the singlet state HB═BH (1Δg) and H^+, Li^+, Na^+, Be^{2+} or Mg^{2+}. J. Mol. Struct. (THEOCHEM) 909, 79–85.
Yang, J., Ren, F., 2010. (U) MP2 (full) and (U) CCSD (T) theoretical investigation on the substituent effect on the cation–π interactions between Na^+ and LCCL (L = –H, –CH3, –OH, –F, –Cl, –CO, –NN, CN–, NC– and OH–). J. Mol. Struct. (THEOCHEM). 956, 1–9.
Yuan, Y., Li, S.M., Mo, F.K., Zhong, D.F., 2006. Investigation of microemulsion system for transdermal delivery of meloxicam. Int. J. Pharm. 321, 117–123.