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Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration

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Abstract

In this work, a hydrogel-thickened microemulsion (HTM) was investigated for delivering an extremely low concentration of drug molecule. The pseudo-ternary phase diagrams were constructed using isopropyl myristate (IPM), Tween 80, propylene glycol and water. The various HTM were prepared and characterized. We described that HTM has the combination of o/w microstructure of microemulsion and the three-dimensional gel network of hydrogel in continuous phase using transmission electron microscope. The stability tests showed that HTM had good stability. The influence of the addition of hydrogel into microemulsions on the viscosity and permeation ability is investigated. The abilities of HTM to deliver an extremely low concentration of triptolide as a model drug were evaluated using the in vitro permeation studies. The permeation rates of triptolide from various HTM were 2.2–3.6 times over that from the control hydrogel. The addition of 2% menthol into HTM consisting of 3% IPM, 30% Tween 80, 15% propylene glycol, 0.75% carbomer 940 resulted in the highest permeation rate of $0.105 \pm 0.006\ \mu g\ cm^{-2}\ h^{-1}$, which was 5.8 times over control gel. The powerful permeation enhancing ability of HTM with a suitable viscosity makes it promising alternative carrier for transdermal administration of drug molecule at an extremely low concentration.

Keywords: Microemulsion; Hydrogel; Transdermal delivery; Triptolide

1. Introduction

Microemulsion is a clear, thermodynamically stable, isotropic mixture of oil, water, surfactant and cosurfactant, which has typically a droplet diameter of approximately 100 nm or less (Hoar and Schulman, 1943; Danielsson and Lindman, 1981; Schulman et al., 1959). It has found widespread uses in pharmaceutics (Lawrence and Rees, 2000), food (Garti, 2003), and many other industries (Campbell and Rusling, 1999; Zarur and Ying, 2000; Lee et al., 2003; Wu et al., 1999). The properties of microemulsion, e.g. enhanced drug solubility, good thermodynamic stability, ease of manufacturing and permeation enhancement ability over conventional formulations have been exploited in pharmaceutics and especially plays important role in drug delivery systems (Kreilgaard, 2002). Microemulsion offers a significant advantage of increasing solubility of active agents for delivering active agents through skin or mucous membrane and also can reduce their barrier due to its powerful permeation enhancing effect (Delgado-Charro et al., 1997; Baroli et al., 2000). However, many drug molecules with high bioactivity, e.g. estradiol, calcitonin are required to be delivered through mucous membrane or skin at extremely low dose of drug for reducing adverse side effect, increasing compliance or avoiding the waste of drug. However, there is lack of the relative literatures about microemulsions for delivering drugs at the low concentration of drug.

In addition, the low viscosity of microemulsion also restrains its application in pharmaceutical industry due to inconvenient use (Lawrence and Rees, 2000). Biocompatible hydrogels with weak interaction with surfactants have recently been found to change the rheology properties of microemulsion. The addition of hydrogels, e.g. carrageenan and carbomer 940 into microemulsion resulted in the formation of hydrogel-thickened

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microemulsion with a weak gel behavior and the change of viscosity (Lapasin et al., 2001; Valenta and Schultz, 2004; Gulsen and Chauhan, 2005). However, there is lack of the direct observation of the microstructure of microemulsions combined with hydrogels, even though the properties of microemulsions combined with hydrogels implied that the oily phase might be hosted within the three-dimensional gel network or microemulsions transformed to lamellar structure or a highly ordered microstructure (Lapasin et al., 2001; Peltola et al., 2003; Valenta and Schultz, 2004; Gulsen and Chauhan, 2005). Additionally, the influence of the incorporation of hydrogels into microemulsion on the permeation ability of microemulsion to deliver drug through skins is unexplored. The hydrogel-thickened microemulsion (HTM) system with a high viscosity and powerful permeation ability is expected to deliver an extremely low concentration of drug.

Triptolide is reported to have immunosuppressive, anticancer and anti-fertility activity and has been used for the treatment of autoimmune diseases such as psoriasis and rheumatoid arthritis clinically (Tengchaisri et al., 1998; Lu et al., 1999). In our previous studies, triptolide was found to be effective by topical administration at a relatively low therapeutic dose of 50–200 μg/day (Xia and Zheng, 1992; Li et al., 2005). We validated the transdermal delivery of microemulsion systems containing 0.025% triptolide (Mei et al., 2003; Chen et al., 2004). Due to the low viscosity of microemulsion, waste of scarce, potential skin irritation and side effects at high concentration or dose of triptolide, it is necessary to explore a microemulsion-based dosage form with a powerful permeation enhancing ability and a suitable viscosity for transdermal delivery to deliver triptolide at an extremely low concentration of 0.003%. Then, triptolide was selected as the model active molecule in this work.

In this work, we study HTM with a high viscosity and powerful permeation ability to deliver triptolide at an extremely low dose of 0.003%. We report the observation of HTM using transmission electron microscopy (TEM) and demonstrate that HTM shows the combination of the of o/w spherical droplet microstructure and gel network of hydrogel. We describe the influence of hydrogel on the viscosity of microemulsion and penetration of triptolide. This thickened microemulsion may be readily administered by transdermal route. Permeation experiments carried out on mice show that HTM induced significant transdermal uptakes of triptolide when compared with the control gel. The mechanisms of HTM for delivering triptolide at an extremely low concentration are also elucidated.

2. Materials and methods

2.1. Materials

Isopropyl myristate (IPM) was purchased from Haiyan chemical Factory (Zhejiang, China). Tween 80, propylene glycol (PG) and triethanolamine (TEA) was obtained from Shanghai Chemical Reagent Corporation (Shanghai, China). Triptolide was obtained from Fujian Medical Sciences Institute (Fuzhou, China). Menthol was purchased from Shangai Xinhua Perfumery Factory (Shanghai, China). Other chemicals are of HPLC or analytical grade.

2.2. Construction of pseudo-ternary phase diagrams

In order to find out the existence range of microemulsions, pseudo-ternary phase diagrams were constructed using H_2O titration method at ambient temperature (25 °C). Three phase diagrams were prepared with the 1:1, 2:1 and 3:1 weight ratios of Tween 80 to PG, respectively. For each phase diagram at specific surfactant/cosurfactant weight ratio, the ratios of IPM to the mixture of surfactant and cosurfactant were varied as 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 4.5: 5.5, 5:5, 5.5:4.5, 6:4, 6.5:3.5, 7:3, 7.5:2.5, 8:2, 8.5:1.5, 9:1, 9.5:0.5. The mixtures of oil, surfactant and cosurfactant at certain weight ratios were diluted with H_2O dropwise, under moderate magnetic stirring. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions, crude emulsions or gels. No attempt was made to distinguish between o/w, w/o or bicontinuous type microemulsions.

2.3. Preparation

2.3.1. Preparation of triptolide-loaded microemulsion

According to the microemulsion regions (MR) in the phase diagrams, the microemulsion formulations were selected as described in Table 1. In order to prepare the drug-loaded microemulsions, a stock solution containing triptolide was prepared with the mixture of IPM and PG (Chen et al., 2004). The clear oily phase containing triptolide was obtained by diluting the weighed amounts of stock solution with IPM, PG and Tween 80. Then water was added to the clear oily phase drop by drop. The o/w microemulsions containing triptolide were obtained under a magnetic stirring at ambient temperature.

2.3.2. Construction of HTM

Carbomer 940 was selected as the gel matrix to prepare HTM. Carbomer 940 was slowly mixed with water. The oily phase was

Table 1
The composition of the different microemulsions

Microemulsions	IPM (g)	Tween 80 (g)	PG (g)	Triptolide (g)	H_2O (g)	Average size (nm)
A	3	30	15	0.003	51.997	43.0 ± 0.3
B	3	40	20	0.003	36.997	29.9 ± 0.4
C	3	45	15	0.003	36.997	22.2 ± 0.3
D	3	30	30	0.003	36.997	41.6 ± 0.4

obtained by mixing IPM, Tween 80, PG with the stock solution. After carbomer 940 was entirely swelled in the water and pH was adjusted by adding TEA, HTM was obtained by mixing the swelled gel in water with the oily phase. Menthol as a permeation enhancer was dissolved in oily phase.

In order to obtain an aqueous control gel, triptolide was solubilized in water solution containing 20% PG, followed by adding carbomer 940 and adjusting pH.

2.4. Characterization

2.4.1. Measurement of pH and viscosity

The pH values were measured at 25 °C using a pHS-3C digital acidimeter (Shanghai Rex Instruments Factory, Shanghai, China). The viscosity was measured at 25 °C using a NDJ-8S digital viscometer (Shanghai Precision & Scientific Instrument, Shanghai, China) with a rotor (No. 4) at 3 rpm.

2.4.2. Photon correlation spectroscopy

The average droplet size and polydispersity index of samples were measured by photon correlation spectroscopy (Nano ZS90, Malvern Instruments, U.K.) at 633 nm. The measurements were performed at 25 °C using a He–Ne laser.

2.4.3. Transmission electron microscopy

TEM was employed to characterize the microstructure of HTM. Samples were placed on a carbon-coated copper grid and then a drop of 1% phosphotungstic acid covered on HTM. The superfluous phosphotungstic acid on sample was wiped off by filter paper. The TEM images were obtained using a Tecnai G2 20 TEM (FEI Corp., German).

2.5. Stability

The stability of HTM was studied via clarity and phase separation observation, droplet size determination and HPLC analysis of triptolide at 30 °C for up to 6 months. The centrifuge test was also carried out to assess the physical stability and HTM were centrifuged for 30 min at 13,000 rpm in the centrifuge tests.

2.6. HPLC analysis

Triptolide was analyzed by reversed phase HPLC using Agilent 1100 series. The HPLC system consisted of quaternary pump, autosampler, diode array detector and workstation. The column was a Lichrospher C18 column (5 μ, 4.6 mm i.d. × 25 cm). The mobile phase was a methanol–0.05 mol/l potassium dihydrogen phosphate (65:35, v/v) mixture with a flow rate of 0.50 ml/min. The detection wavelength was set at 218 nm and the retention time was 9.5 min. The assay was linear ($r^2 = 0.9999$) in the concentration range of 0.05–100.0 μg/ml with a lowest detection limit at 0.015 μg/ml. The R.S.D. value for precision is below 0.4%. The percentage recoveries ranged from 99.0 to 101.0%. No interference of the other formulation components was observed. All samples filtered through an aqueous 0.45 μm pore size membrane filter before injection.

2.7. In vitro permeation studies

The abdominal skins were obtained from male mice weighing 25 ± 2 g. After hair was removed with a depilatory, the skins were excised. The subcutaneous fat was removed, and then the skins washed and examined for integrity. The permeation experiments were performed using a diffusion instrument (TK-12A, Shanghai, China) with a re-circulating water bath and 12 diffusion cells. The skins were clamped between the donor and the receptor chamber of vertical diffusion cells with an effective diffusion area of 2.8 cm^2 and a 7 ml cell volume. The receptor chamber was filled with freshly prepared physiological saline. The receptor chambers were set at 37 °C and the solution in the receptor chambers was stirred continuously at 300 rpm. The formulations (1.5 g) were gently placed in the donor chamber. At 2, 4, 6, 8 h, 0.5 ml of the solution in the acceptor chamber was removed for HPLC determination and replaced immediately with an equal volume of fresh physiological saline. Each sample was performed three times. Cumulative corrections were made to obtain the total amount of triptolide permeated at each time interval.

The cumulative amount of triptolide permeated through mouse skins was plotted as a function of time. The permeation rate of triptolide at a steady-state (J, μg cm^{-2} h^{-1}) through mouse skin was calculated from the slope of linear portion of the cumulative amount permeated through the mouse skins per unit area versus time plot (Li et al., 2005).

2.8. Skin irritation studies

The selected HTM and excipient were selected as the tested formulations for skin irritation studies. All samples were applied to the shaved skin on the back of six Japanese rabbits, and then the rabbits were secured. The animals were observed and evaluated for any sign of erythema, oedema or erosion for a period of 7 days.

2.9. Statistical analysis

All the skin permeation studies were repeated three times and data were expressed as the mean value ± S.D. Statistical data were analyzed by one-way analysis of variance. A multiple comparison test was used to compare different formulations and $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Phase studies

The studied systems were composed of IPM, PG, Tween 80 and water. The phase diagrams were used to find out the microemulsion regions. The pseudo-ternary phase diagrams with various weight ratios of Tween 80 to PG are described in Fig. 1. The translucent microemulsion region is presented in phase diagrams. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsions was observed. The rest of the region on the phase diagram represents the turbid and

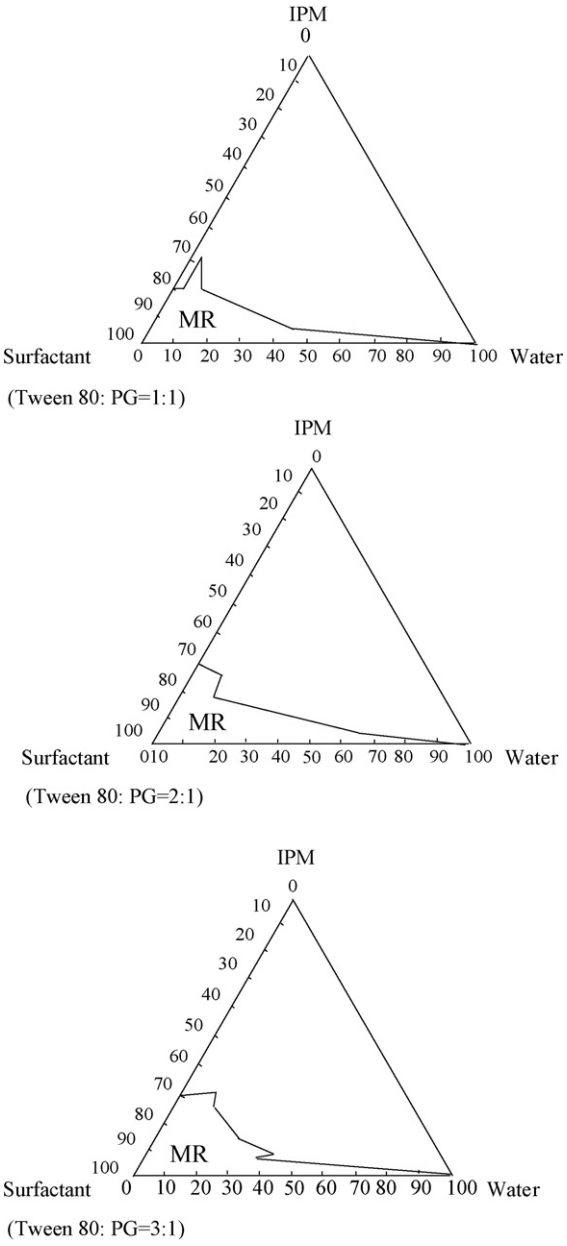

Fig. 1. The pseudo-ternary phase diagrams of the oil–surfactant–water system at the 1:1, 2:1, and 3:1 weight ratios of Tween 80 to PG at 25 °C. MR represents microemulsion region.

conventional emulsions based on visual observation. No liquid crystalline structure was observed using cross polarizer. MR regions were changed slightly in size with the increasing ratio of Tween 80 to PG. This phase diagrams showed significant difference when compared with that of the systems consisting of oleic acid, Tween 80 and PG in our previous work (Chen et al., 2004).

3.2. Preparation analysis

The average particle size of microemulsions was also reported in Table 1. According to Table 1, microemulsion A and D had similar average diameters. The increase of PG in microemulsion D did not result in the significant increase of average size when compared with microemulsion A. However, the increase of Tween 80 in microemulsion C led to a decrease of 20.81 nm in average size in comparison with microemulsion A. Tween 80 as a surfactant might have more significant influence on the droplet size than PG due to the effective interfacial activity.

In this work, carbomer 940 and microemulsion A were chosen for constructing HTM. The influence of the order of the addition of carbomer 940 on the formation of HTM was investigated. Carbomer 940 was directly added into microemulsion A or the aqueous phase of microemulsion A, respectively. When carbomer 940 was directly added into microemulsion, it cost much more time to be swelled in microemulsion than in water (aqueous phase). It might attribute to the relatively high increase of the viscosity of microemulsion. After TEA was added to adjust the pH of microemulsion containing the swollen carbomer 940, HTM with a high viscosity was obtained. However, some tiny agglomerates of carbomer 940 could be observed, because carbomer 940 was not entirely swollen in the microemulsion with the relatively high viscosity. If carbomer 940 was swelled in the aqueous phase and the pH of swollen gel matrix was adjusted using TEA, the homogeneous HTM could also be obtained by mixing the swollen gel matrix with the oily phase of microemulsion A. According to the above results, it is possible that the gel network have no influence on the spontaneous dispersion of oily phase in aqueous phase or the spontaneous formation of microemulsion, even though the gel network arose the increase of the viscosity of the system. Carbomer 940 as an aqueous gel matrix in continuous phase, displayed non-covalent intermolecular associations deriving from disparate forces such as coulombic, van der waals and hydrogen-bond interaction and showed a weak gel behavior.[14] These physical interactions could lead to the formation of the three-dimensional gel network and the dispersed oil droplets were reasonably hosted within the meshes of the three-dimensional gel network. In conclusion, the order of the addition of carbomer 940 had no significant influence of the formation of HTM, but might influence the homogenization swelling of carbomer 940. In the subsequent study, HTM was prepared by mixing the swollen gel matrix with the aqueous phase.

3.3. Influence of carbomer 940 on viscosity

The influence of the different concentrations of carbomer 940 on the viscosity of microemulsion A was evaluated. 0.5%, 0.75% and 1.0% carbomer 940 were swollen in aqueous phase, respectively. After 0.675%, 1.0% and 1.35% of TEA were used to adjust pH of the swollen gel matrices, HTM were prepared by mixing the oily phase with various gel matrices. All the microemulsions in Table 1 had a low viscosity, which was similar to that of water. The incorporation of carbomer 940 into microemulsions resulted in the significant increase of viscosity of microemulsions. The viscosity of HTM containing 0.5%, 0.75% and 1.0% carbomer 940 were 60.92, 142.6, 164.5 Pa s, respectively. HTM containing 0.5% carbomer 940 had a relatively high fluidity. However 1% carbomer 940 resulted in a

Table 2
The composition of the different HTM and control gel

Vehicles	IPM (g)	Tween 80 (g)	PG (g)	Triptolide (g)	Carbomer (g)	TEA (g)	Menthol (g)	H_2O (g)	J ($\mu g\,cm^{-2}\,h^{-1}$)
HTM 1	3	30	15	0.003	0.75	1.0	0	50.247	0.064 ± 0.004
HTM 2	3	40	20	0.003	0.75	1.0	0	35.247	0.043 ± 0.001
HTM 3	3	45	15	0.003	0.75	1.0	0	35.247	0.040 ± 0.004
HTM 4	3	30	30	0.003	0.75	1.0	0	35.247	0.051 ± 0.004
HTM 5	3	30	15	0.003	0.75	1.0	1	48.247	0.105 ± 0.006
Control gel	0	0	20	0.003	0.75	1.0	0	78.247	0.018 ± 0.003

too high viscosity of HTM, even though Lapasin reported that 1% of carbomer 940 was also suitable (Lapasin et al., 2001). HTM containing 0.75% carbomer 940 had a most appropriate fluidity for topical administration. So 0.75% carbomer 940 as the optimum gel matrix was added to microemulsion A, B, C and D to prepare the various HTM systems in this work (Table 2).

3.4. TEM imaging

In order to observe the oily droplets, phosphotungstic acid was used to visualize the microstructure of HTM. Fig. 2 showed the TEM images of the HTM 1. Additionally, In order to verify the oily droplets in the HTM, a hydrogel consisting of Tween 80, PG, and the swollen carbomer gel matrix was prepared by removing IPM from HTM 1. According to the images, the spherical droplets and the gel network in HTM were observed. The diameters of all the droplets in HTM 1 were smaller than 100 nm. HTM still maintained the microstructure of microemulsion, even though Peltola et al. thought that the addition of carbomer 940 resulted in the transform from microemulsion to lamellar structure or a highly ordered microstructure and the transform from microemulsion to a highly structured cubic microgel by adding carrageenan into microemulsion was also reported (Valenta and Schultz, 2004; Peltola et al., 2003). No droplet was observed in the hydrogel in absence of IPM and only the gel network was observed in Fig. 2C. The hydrogel showed a porous structure of gel network. So it is concluded that the spherical droplets in HTM 1 were the dispersed IPM and the dispersed oily droplets were distributed in the three-dimensional gel network of continuous phase. HTM remained the o/w microstructure of microemulsion within three-dimensional gel network.

3.5. Stability

All microemulsion and HTM systems were stable at 30 °C in the presence or absence of triptolide. No change of particle size, phase separation and degradation of triptolide were observed during 6 months. The centrifuge tests showed that all microemulsions and HTM had good physical stability. In addition, the degradation of triptolide in HTM was not detected during 6 months. HTM systems still maintain a good thermodynamic stability similar to microemulsion. The o/w structure of HTM and suitable pH of HTM ranging from 6.0 to 7.0 provided a suitable circumstance for avoiding the hydrolysis of drug (Chen et al., 2004).

3.6. In vitro permeation studies

In our previous studies, oleic acid showed an important effect on the transdermal delivery of triptolide from microemulsion. So in this work we compared the permeation ability of Vehicle 1 with that of Vehicle 2 to evaluate the influence of oleic acid and IPM on the pemeation rates of triptolide from microemulsions (Table 3). In addition, the addition of carbomer 940 had significant influence on the viscosity of microemulsions. So in order to evaluate the influence of carbomer 940 on the permeation ability of microemulsion, we compared the permeation rates of Vehicle 2 with that of Vehicle 3 using the in vitro permeation studies (Table 3). The permeation rates of Vehicles 1, 2 and 3 were 0.028 ± 0.008, 0.067 ± 0.011 and $0.060 \pm 0.009\ \mu g\,cm^{-2}\,h^{-1}$, respectively. Vehicle 2 containing IPM showed a higher permeation rate than vehicle 1. This is because IPM might show much more powerful permeation ability than oleic acid for transdermal delivery of triptolide. The oil with permeation enhancing ability had significant influence on the penetration of triptolide.

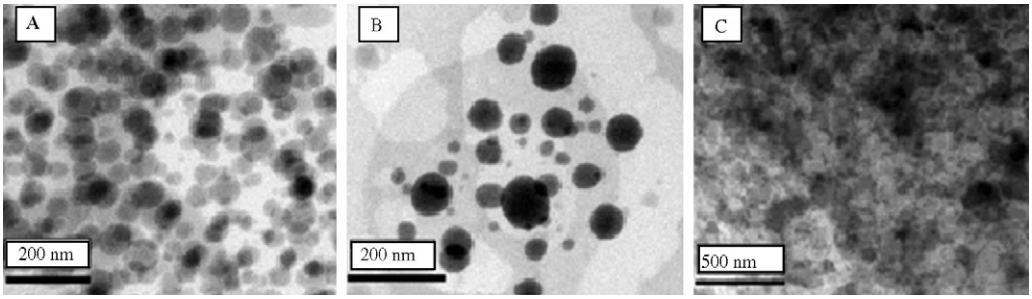

Fig. 2. The TEM images of HTM 1 and the hydrogel in absence of IPM. Images A and B describe the microstructure of HTM 1, which is the combination of o/w spherical droplets and gel network of carbomer 940. Image C shows the gel network of the hydrogel, which consists of Tween 80, PG and the swollen carbomer gel matrix.

Table 3
The compositions of the various vehicles

Vehicle	Triptolide (g)	IPM (g)	Oleic acid (g)	Tween 80 (g)	PG (g)	H_2O (g)	Carbomer (g)	TEA (g)	J ($\mu g\,cm^{-2}\,h^{-1}$)
1	0.003	0	3	25	35	36.997	0	0	0.028 ± 0.008
2	0.003	3	–	25	35	36.997	0	0	0.067 ± 0.011
3	0.003	3	–	25	35	35.247	0.75	1.0	0.060 ± 0.009

There is no significant difference of the permeation rate of triptolide between Vehicles 2 and 3 ($P > 0.05$). Peltola studied the influence of carbomer 940 on the permeability of estradiol. The addition of carbomer 940 into microemulsion decreased the permeability of estradiol and it might attribute to the increased viscosity and transform from microemulsion to lamellar structure or a highly ordered microstructure (Trotta, 1999; Peltola et al., 2003). Valenta found that carrageenan could facilitate the skin permeation of a model compound due to good adhesiveness, even though the microstructure is a highly structured cubic microgel. It might be due to the good adhesiveness of carrageenan on skin (Valenta and Schultz, 2004). In this work, the addition of 0.75% carbomer 940 had no significant influence on the permeation rates of triptolide.

The permeation parameters of various HTM were presented in Table 2. The permeation profiles of triptolide through mouse skins from various HTM and the control gel were shown in Fig. 3. Statistical comparison of the flux throughout 8 h showed that HTM provided fluxes (J) ($P < 0.01$) higher than the control gel, which had only a low permeation rate of 0.018 $\mu g\,cm^{-2}\,h^{-1}$. The permeation rates of triptolide from HTM 1, HTM 2, HTM 3 and HTM 4 were 2.2–3.6 times higher when compared with the control gel. HTM 1 had the highest permeation rate of $0.064 \pm 0.004\,\mu g\,cm^{-2}\,h^{-1}$. HTM 2, HTM 3 and HTM 4 showed a significant decrease of the permeation rates of triptolide when compared with HTM 1. It might be due to the increase of Tween 80 and PG, which might reduce the thermodynamic activity of triptolide (Tenjarla, 1999; Rhee et al., 2001). The equation of $J = PC/h = DKC/h$ can be expressed as an alternative form, $J = (D \times C_s/h) \times (C/C_m)$, where K can be defined as the ratio of saturated concentration in the skin (C_s) to the saturated concentration in microemulsion (C_m) and C/C_m can be defined as the extent of saturation, which directly indicates the thermodynamic activity of drug in vehicle. Then 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., 2006). 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 thermodynamic activity could still affect the penetration of drug, even though triptolide was extremely unsaturated in HTM. In addition, the increase of Tween 80 in HTM had more significant influence on the permeation rates of triptolide than that of PG. It might be relative to the more powerful solubilization of Tween 80.

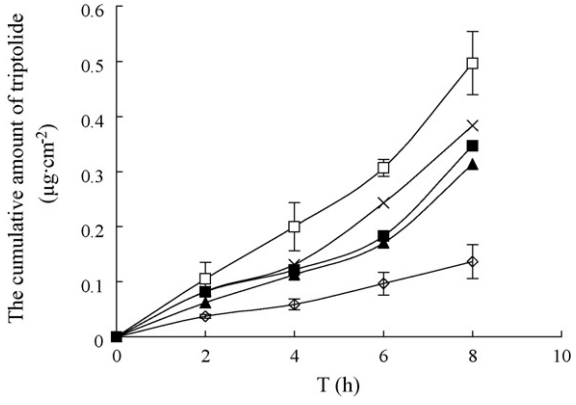

Fig. 3. Permeation profiles of triptolide through mouse skins from HTM systems and control gel (mean ± S.D., $n = 3$). (□) HTM 1, (■) HTM 2, (▲) HTM 3, (×) HTM 4, (◊) control gel.

In previous studies, oleic acid as the oily phase of microemulsion acted as the drug reservoir where drug is released from the inner phase to the outer phase and then further onto the skin. However, the semisolid HTM might restrain the movement of the droplets. Then, it might theoretically slow the transfer and penetration of triptolide. In HTM system, the high permeation rate showed that the oily phase as the drug reservoir should not be the key factor affecting the permeation of drug due to the extremely low concentration and unsaturation state of triptolide in HTM. Even though the permeation mechanism is not still elucidated, we can infer that the static droplets in HTM could closely contact with the skin due to adhesiveness of carbomer 940 and a large amount of inner IPM in HTM might penetrate into stratum corneum due to the small diameters of droplets (Peltola et al., 2003). IPM as a permeation enhancer had a strong permeation enhancing effect and could increase the diffusion coefficient (D) in skin, which could result in the increase of permeation coefficient. Moreover, the close contact with skin might contribute to the direct release of triptolide from oily droplets into skin without the transfer of drug in continuous phase. IPM acted as a more important role in penetration when compared with oleic acid (Paolino et al., 2002).

Because microemulsion has powerful solubilization ability, permeation enhancer with a high concentration can still be incorporated into HTM. So it is still necessary to improve the permeation rate of triptolide by the greatest extent using permeation enhancer (Naik et al., 2000). HTM 5 was prepared by incorporating 2% menthol into HTM 1. HTM 5 showed the highest permeation rate of $0.105 \pm 0.006\,\mu g\,cm^{-2}\,h^{-1}$, which was 5.8 times and 1.6 times higher than those from the control gel and HTM 1, respectively. The addition of menthol might increase the diffusion coefficient of drug in skin and reduce the barrier of stratum corneum. So, the solubilizing ability of HTM for permeation enhancer also comes into being an additive route for facilitating penetration of drug with a low dose.

3.7. Skin irritation studies

No obvious erythema, oedema or erosion was observed on the skins of rabbits after application of HTM 5 in the irritation studies. The encapsulation of drug in HTM and the extremely concentration of drug contributed to the avoidance of the skin irritation.

4. Conclusions

In this work, HTM system with suitable viscosity was constructed to deliver an extremely low concentration of triptolide for topical administration. We first reported that HTM remained the o/w microstructure of microemulsion and the spherical droplets were found to distribute in the three-dimensional gel network of continuous phase. The addition of carbomer 940 into microemulsions resulted in the increase of the viscosity and had no significant influence on the penetration of triptolide. The permeation rates of triptolide from HTM were 2.2–5.8 times higher than that from the control hydrogel. The close contact of the static droplets in semisolid HTM with skin and IPM with powerful permeation enhancing ability acted as the key role for the transdermal delivery of triptolide from HTM systems. Two percent menthol had a significant permeation enhancing effect on the penetration of triptolide through skin. The solubilizing ability of HTM for permeation enhancer also comes into being an additive route for facilitating penetration of drug at an extremely low dose. The HTM system is one of promising alternative carriers for transdermal delivery of drug molecule at an extremely low concentration.

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