Triptolide-loaded microemulsion-based hydrogels: physical properties and percutaneous permeability

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KEY WORDS
Microemulsion-based hydrogel; Rheological properties; Environmental scanning electron microscopy; Triptolide; \textit{In vitro} permeation

Abstract Triptolide is a diterpenoid compound that inhibits the inflammation of rheumatoid arthritis (RA). However, the use of triptolide is limited due to its strong gastrointestinal toxicity. The purpose of this work was to develop a transdermal delivery system for triptolide to reduce this toxicity. A microemulsion-based hydrogel (MBH) was prepared from the combination of Gemseal 40-oleic acid as oil phase, Tween 80-labrasol as surfactant, anhydrous ethanol as co-surfactant, water as aqueous phase and Poloxamer 407 as hydrogel matrix. Rheological measurements, environmental scanning electron microscopy (ESEM) and transdermal experiments \textit{in vitro} were used to characterize triptolide-loaded and blank MBH preparations. The effects of Poloxamer 407 and triptolide on the rheological properties and microstructures of the MBH were determined. Transparent and homogeneous MBH could only be formed when the concentration of Poloxamer 407 in the selected O/W microemulsion was in the range of 14.0 – 16.0% (w/w). When the concentration of Poloxamer 407 increased, the rheological properties such as the yield stresses ($\sigma_y$), storage and loss moduli ($G'$, $G''$) of the formulations increased, and the network structures became more compact. The addition of triptolide did not change the interconnected network structures of the MBH preparations. MBH preparations afford a better sustained release profile when compared to microemulsions, a finding confirmed by an \textit{in vitro} permeation test in mice. MBH appears to be a promising vehicle for transdermal delivery of triptolide.

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1. Introduction

Triptolide, which possesses potent anti-inflammatory and immunosuppressive properties, is one of the major active components in the Chinese herbal remedy Tripterygium wilfordii Hook F (TWHF)1–4. Triptolide has been reported to be therapeutically efficacious in the treatment of rheumatoid arthritis (RA)5–7, but the strong toxicity of triptolide towards the gastrointestinal, hepatic, renal and reproductive systems has restricted its oral application to a great extent8–10. Triptolide also has a narrow therapeutic window, with an effective dose nearly equal to its toxic dose10. To overcome these disadvantages, chemical modification and biotransformation of triptolide has been investigated11–13. In addition, transformation of the dosage form has also been explored14,15, and a transdermal drug delivery preparation of triptolide, using a triptolide-loaded microemulsion has been developed by our laboratory16.

A microemulsion is defined as an oil/water (O/W) or water/oil (W/O) emulsion producing a transparent product which has a droplet size from 10 to 100 nm and does not coalesce17,18. Microemulsions are composed of oil phase, surfactant, cosurfactant and an aqueous phase at appropriate ratios19. Microemulsions have several specific physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability20–23, and are a promising route of drug administration in transdermal applications. However, the application of most microemulsions, especially in the pharmaceutical industry may be limited due to their inconvenient application, which is caused by their extraordinary low viscosity. In order to improve the viscosity of a preparation, hydrogel matrices such as Carbomer 940, Poloxamer 188, Poloxamer 407, xanthan gum and carrageenan have been utilized to form MBH, which are more suitable for transdermal applications as compared to microemulsions.

In the past few years a number of studies have been conducted on the microstructure of MBH24–27. Usually, small-angle neutron scattering28–30, electro-paramagnetic resonance31 and rheological measurements32–34 were applied to characterize MBH microstructure. A rheological method has been employed in this study along with ESEM to determine the dynamic and static macroscopic properties and microstructure of our preparations.

In this study, MBH was formed by an O/W microemulsion, which was composed of Tween 80-labrasol/Gemseal 40-oleic acid/anhydrous ethanol/water, and Poloxamer 407. The rheometer and ESEM were applied to study the effect of Poloxamer’s concentration on the properties of the MBH and find the best concentration for preparation of a MBH with good viscoelasticity and compact microstructure. We further investigated the effect of triptolide’s addition on the properties of the MBH. Based on these results, the percutaneous penetration rates of microemulsion and MBH were evaluated.

2. Materials and methods

2.1. Materials

Triptolide was supplied by Fujian Hantang Group Co., Ltd. (Fuzhou, China). Oleic acid (OA) was purchased from Xilong Chemical Factory (Shantou, China). Gemseal 40 was purchased from Shanghai Zhirou Chemical Co., Ltd. (Shanghai, China). Polysorbate 80 (TWEEN 80) was procured from Zhejiang Clear Pure Chemicals Co., Ltd. (Wenzhou, China). Caprylocaproyl macrogolglycerides (Labrasol) was a kind gift from Gattefossé (France). Ethanol was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals and solvents were of analytical reagent grade or chromatography reagent grades.

2.2. Preparation of microemulsion

Formation of a triptolide-loaded microemulsion was based on our previous study and was composed of OA-Gemseal 40 (4:6, 7.8%, w/w), Labrasol-TWEEN 80 (4:1, 16.3%, w/w), ethanol (8.1%, w/w) and water (67.8%, w/w)16. The method of preparation is as follows: OA, Gemseal 40, Labrasol, TWEEN 80, ethanol and triptolide were first mixed together, and then water was added into the above mixture drop by drop with magnetic stirring at ambient temperature. After the resulting system was equilibrated with gentle magnetic stirring for 30 min, a microemulsion containing triptolide was obtained.

2.3. Preparation of MBH

Poloxamer 407 was chosen as hydrogel matrix based on previous experiments. Triptolide-loaded MBH was prepared according to a method described by Chen et al.35. Poloxamer 407 was combined with differing volumes of a triptolide-loaded microemulsion for 24 h to yield highly viscous solutions (MBH). The concentration of Poloxamer 407 in these MBH preparations was 12%, 13%, 14%, 15%, 16%, 17% and 18% (w/w).

2.4. Rheological measurements

The rheological properties of the MBH samples were measured with Anton Paar MCR101 Rheometer (Anton Paar Company, Australia) at 30 ±0.1 ºC. The cone angle was 0.5 º and the cone diameter was 20 mm. The sample thickness in the middle of the sensor was 1.0 mm. In oscillation experiments, a small amplitude oscillation mode of frequency sweep was used, and a 1-Hz fixed frequency was adopted when the measurements of storage modulus G’ and loss modulus G” was determined over a stress sweep between 3 and 500 Pa. In addition, the area of the thixotropic loop was measured while the shear velocity varied from 2 to 50/s and then back to 2/s. Moreover, the apparent viscosity of samples was investigated while the shearing rate varied from 1 to 200/s. Finally, we determined the linear viscoelastic area and the best concentration of Poloxamer 407. The effects of triptolide addition on the rheological properties of MBH were also determined.

2.5. ESEM observations

Samples were imaged using an ESEM (Quanta 200F, FEI, USA). The beam energy was 30 kV and signals were collected using a gaseous secondary electron detector (GSED). Water vapor was used as the imaging gas with constant pressure of approximately 1000 Pa. The temperature was maintained at 25 ºC during the experiment. All samples were enlarged 5000 × by the ESEM for visualization before and after the addition of triptolide.

2.6. Skin irritation study

The skin irritation potential of triptolide-loaded MBH was conducted in rabbits. The backs of rabbits (2.0–2.5 kg, Experimental
2.8. **HPLC analysis of triptolide**

The amount of permeated triptolide was determined by HPLC using Agilent 1200 series consisting of a quaternary pump, autosampler, diode-array detector and workstation. The column was an ODS-2 HYPERSIL column (250 mm × 4.6 mm, 5 μm). The elution was performed at 35 °C and the mobile phase was a methanol-water (45:55, v/v) mixture with a flow rate of 1.0 mL/min. The detection wavelength was monitored at 218 nm and the retention time was 7.4 min. The assay was linear (r²=0.9999) over a concentration range of 0.3–30 μg/mL. The detection limit of this method was 0.1 μg/mL. The RSD value for precision was below 0.5% and the percentage recoveries ranged 99.0–101.0%. No interference of the other formulation components was observed.

3. **Results and discussion**

3.1. **Preparation of MBH**

In order to obtain microemulsions with high drug-loading rate and high permeability, a pseudo-ternary phase diagram and conductivity determination was utilized from our previous study. The optimized formulation consisted of oil (7.8%, w/w), surfactant (16.3%, w/w), co-surfactant (8.1%, w/w) and water (67.8%, w/w). After addition of this microemulsion to Poloxamer 188, Poloxamer 407, sodium carboxyl methyl cellulose (CMC-Na), HPMC K4m, HPMC E4m, Carbomer 934, Carbomer 940 or Carbomer 941 matrices for 24 h, only the Poloxamer viscous solutions remained clear. One possible reason is that the Poloxamer 188 and Poloxamer 407 matrices were not dissociated from the hydrated state by surfactant and co-surfactant in the microemulsion, and had no significant influence on the formation of MBH. However, the viscosity of microemulsion-Poloxamer 188 mix was too low and so the Poloxamer 407 was chosen for further research.

3.2. **Rheological measurements**

In the oscillation experiment, if the modulus of energy ∗∗ was a function of ∗∗ while shear stress was above critical stress. The sample could bear stronger elastic behavior and external stress below or equal to critical stress value and the values of ∗∗ were higher than that of ∗∗ as a function of ∗∗ and ∗∗ were higher than ∗∗ while shear stress was below or equal to critical stress value and the values of ∗∗ were lower than ∗∗ while shear stress was above critical stress. An evident linear viscoelastic region could be found in this

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Animal Center of Jiangxi University of Traditional Chinese Medicine, Jiangxi, China), were clipped free of fur with scissors 24 h prior to application of a patch. Just prior to the test, the rabbits were randomly divided into two groups, one intact-skin group and one skin-injury group, obtained by scarifying intact skin until capillary hemorrhage. The two groups were subdivided into a single-application subgroup and a multiple-applications subgroup. The skin surface was further divided into four regions for triptolide-loaded microemulsion, triptolide-loaded MBH, blank microemulsion and blank MBH applications. For the single application, 0.8 g of both preparations described above was used to cover the applications and gauze tape was used to fasten the polyethylene for 24 h until the drug was removed with warm water. Inspection on the administration site for the presence of erythema and edema began 1 h after removal of the drug. For multiple applications, two preparations were applied on the same skin regions for 6 h and 1 h after removal of the drug, then the sites were assessed for signs of skin irritation. The test procedure was repeated for 6 consecutive days. The irritation scores of the test area were determined by judging the extent of erythema and edema according to the criteria proposed by Chen et al.38. Erythema and edema were graded as follows: zero for no visible reaction, one for slight reaction, two for moderate reaction, three for severe reaction, and four for extremely serious reaction. The total scores for the irritation test in each condition were calculated using the following equation.

Average irritation score = (Erythema reaction score + Edema reaction score) / Amount of animal

2.7. **In vitro permeation studies**

2.7.1. **Preparation of skins**

Male Kunming mice weighing 20 ± 2 g were purchased from the Experimental Animal Center of Jiangxi University of TCM (Jiang Xi, China) for the permeation studies. The protocol of the study was approved by the Ethical Committee of Jiangxi University of TCM. Skin was obtained from the abdominal region of mice after removing hair carefully with scissors, and the subcutaneous fat and connective tissue were trimmed after that. Then the excised skin was washed with physiological saline solution and examined for integrity. Afterwards, the skin was kept at −20 °C for a maximum of 7 days before use.

2.7.2. **In vitro permeation studies**

This experiment was performed by using Franz diffusion cells at 37 ± 0.5 °C. The Franz diffusion cells have an effective diffusion area of 0.64 cm² and a receiving pool capacity of 5 mL. The excised skin was mounted on the receptor chamber with the subcutaneous side facing upwards into the donor chamber and the dermal side facing downwards into the receptor chamber, which was filled with 5 mL of 20% (v/v) alcohol in physiological saline solution. One gram of microemulsion or MBH (containing 0.03% triptolide) was added to the donor chamber. The receptor medium was stirred at 300 rpm throughout the experiment. 1 mL of receptor medium was extracted at 2, 4, 6, 8, 10, 12, 24 h and then the same volume of 20% (v/v) alcohol in physiological saline solution was immediately added into the receptor chamber. All samples were filtered through a 0.22 μm pore size cellulose membrane filter and analyzed by HPLC. All experiments were performed in triplicate.
concentration range. This indicated that the structure of gel network had formed. In order to characterize the LVE-R of blank preparations directly, the terminal shear stress data for LVE-R were determined and are shown in Table 1. It can be clearly seen that the system in which the concentration of Poloxamer 407 was 16% (w/w) was already at saturation point. Since all the water and Poloxamer 407 had taken part in the formation of this saturated sample, the viscoelastic properties were strongest and critical stresses were maximum, which means that the system would not be easily destroyed by external force. When the concentration of Poloxamer 407 was higher than 16.0% (w/w), the values of $G'$ and $G''$ were respectively about one to two orders of magnitude higher than those of other systems in the investigated stress range, indicating that the system became more solid-like at higher concentration of Poloxamer 407.

Fig. 2A shows the apparent viscosity of the two MBH preparations containing 15% Poloxamer 407. It was found that the apparent viscosity of triptolide-loaded MBH was markedly lower than that of blank MBH preparation. Hence, the addition of triptolide decreased the viscosity of MBH. Further information gained on the thixotropy of the two MBH preparations is shown in Fig. 2B. The results of blank and triptolide-loaded MBH preparations were 3245.37 Pa/s and 3763.47 Pa/s, respectively. The addition of triptolide affected the interconnected network to a noticeable extent, but this did not damage the stability of this structure.

### 3.3. ESEM observations

In this study, ESEM was used to observe the formation and interconnected network structures of the MBH preparations. Fig. 3 shows ESEM images of the blank MBH preparation with different concentrations of Poloxamer 407 (Fig. 3A–C) and the triptolide-loaded MBH preparation with 16% (w/w) Poloxamer 407 (Fig. 3D).

Fig. 3A shows that the network structures of the O/W microemulsion containing 13% (w/w) Poloxamer 407 had not been formed completely, and that the system was still loose and colloidal. We found that as the concentration of Poloxamer 407 increased, the whole microemulsion would contribute to the formation of the hydrogel. When the concentration of Poloxamer 407 was 14% (w/w), the interconnected network structures had been formed. By comparison, the network structures of systems containing 16% (w/w) Poloxamer 407 (Fig. 3B) were more compact and the rheological measurement revealed that the dynamic viscoelasticity of this system was strongest. When the concentration of Poloxamer 407 increased to 17% (w/w) (Fig. 3C),

### Table 1 Terminal shear stress of LVE-R of blank MBH containing different concentrations of Poloxamer 407.

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<th>Concentration of Poloxamer 407 (%)</th>
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Figure 1  Rheological images of (A) Blank MBH with different concentrations (12–18%) of Poloxamer 407 and (B) triptolide-loaded MBH with different concentrations (14–16%) of Poloxamer 407.
the structures were relatively disorganized. This may be the result of incomplete swelling of the matrix at this concentration.

Fig. 3D shows the effect of triptolide on the microstructure of MBH. The concentration of triptolide was fixed at 0.03% (w/w) and the concentration of Poloxamer 407 was 13%, 14%, 15%, 16%, 17%, 18% (w/w). Compared to the blank MBH preparations, the interconnected network structures of triptolide-loaded MBH preparations were less well defined. However, when the concentration of Poloxamer 407 was 16% (w/w), the network structures of systems were most compact.
3.4 Skin irritation study

The results of skin irritation test are shown in Tables 2 and 3. On the basis of Chen et al.\(^{38}\) where scores of \(<0.49\) indicated no irritation, \(0.5–2.99\) indicated slight irritation, \(3–5.99\) indicated moderate irritation, and \(6–8\) indicated severe irritation. The tested microemulsions and MBH preparations had no irritation on intact skin after a single application or multiple applications. However, slight skin irritation could be observed on injured skins after single application or multiple applications of microemulsion. We attributed this result to a change in the microemulsion after adding Poloxamer 407. In addition, the formed network structures and increased viscosity may decrease the contact between skin and microemulsion. Therefore, irritation by the MBH preparations was much weaker, which was also shown by the blank microemulsion and MBH preparation.

3.5 In vitro permeation studies

Mouse skin has been used to study skin permeation of triptolide in microemulsions and MBH preparations\(^{24}\). To ensure stable collection conditions, 20% (v/v) alcohol in physiological saline solution was used as receptor fluid. The transdermal permeation profiles were typical steady-state profiles with a lag time (Fig. 4).

Table 4 shows that the cumulative amount of triptolide obtained from the microemulsion was 355.4 ± 9.9 μg/cm\(^2\), which was 3.4 times that of the MBH preparation at 24 h (104.4 ± 12.4 μg/cm\(^2\)) after application. The percutaneous absorption rate (\(J_a\), μg/cm\(^2\)·h) of microemulsion was 3.6 times that of MBH, which showed that addition of Poloxamer 407 to a microemulsion could decrease the permeability of triptolide markedly. This may be attributed to the formation of interconnected network structures and increased viscosity\(^{41,42}\). Therefore, it can be concluded that the addition of Poloxamer 407 into the microemulsion should delay drug release.

The MBH preparation containing 14–16% (w/w) Poloxamer 407 with suitable viscosity was used to deliver triptolide for transdermal administration. In this study, the optimal concentration of Poloxamer 407 was established. The addition of triptolide

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Figure 4 Percutaneous permeation profiles of triptolide from the microemulsion and MBH (Mean ± SD; \(n=5\)).
to MBH weakened the interconnected network structures of the system to a certain extent, but the networks were not destroyed. The results of the in vitro permeation test in mice showed that MBH and microemulsion both facilitate the permeation of triptolide. It can be concluded that MBH provides strong permeability with low irritation and should prove to be a promising transdermal drug delivery system.

Acknowledgments

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