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*Research Article*

# Correlation Between Rheological Properties, *In Vitro* Release, and Percutaneous Permeation of Tetrahydropalmatine

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***Abstract.*** The aim of the present work was to investigate the influence of formulation factors including different grades of Carbopol® matrices and penetration enhancers on the percutaneous permeation of tetrahydropalmatine (THP), rheological properties, and *in vitro* release; and the correlation behind rheological properties, *in vitro* release, and percutaneous permeation. Transdermal penetration of THP through excised rabbit skin and *in vitro* release of THP across transparent Cellophane® were performed by vertical Franz diffusion cell. Rheological analyses were proceeded in terms of "steady flow tests", "oscillation stress sweep", and "creep recovery". The result of percutaneous penetration of THP indicated that, the emulgel prepared with Carbopol® 971P (Cp 971P) as the matrix and *N*-methyl-2-pyrrolidone (NMP) as the penetration enhancer had the highest cumulative permeation amount (118.19 μg/cm$^2$). All the experimental data showed a good fit to the Casson model in viscosimetric studies no matter what the types of matrices or the kinds of penetration enhancers were. The release profile fitted the zero-order release kinetics model with Cp 971P as the matrix without any penetration enhancers. However, when adding penetration enhancers, *in vitro* release of THP presented anomalous (non-Fickian) release kinetics. Clarifying the relationship behind percutaneous permeation of THP, rheological properties, and *in vitro* release will provide us with profound insights and facilitate the design of specific emulgel.

**KEY WORDS:** Carbopol®; emulgel; *in vitro* release; rheological properties; THP.

## INTRODUCTION

*Corydalis yanhusuo*, the dried tuber of *C. yanhusuo* W.T. Wang, is well known as a traditional Chinese medicine (1). It has received ever-increasing attention because of its therapeutic effects on promoting blood circulation, reinforcing vital energy, and alleviating pain including headache, chest pain, abdominal pain, backache, arthralgia, and trauma (2). Tetrahydropalmatine (THP), which belongs to the isoquinoline alkaloid family is one of the active ingredients isolated from *C. yanhusuo* (3–6). The chemical structure of THP was shown in Fig. 1. The physicochemical property of THP such as low molecular mass (355.43 g/mol), suitable *n*-octanol/water partition coefficient *log*P (1.42) and low oral bioavailability (17%) provide a rationale of developing a transdermal delivery system, and enhanced efficacy, safety, and patient compliance could be expected. Hydrogel polymers play an important role in designing such delivery systems (7–11) and among hydrogel polymers, Carbopol® family was preferred because of its high stability, compatibility, bioadhesive properties, excellent organoleptic characteristics, and low toxicity (12–14). Carbopol® is a hydrophilic polyacrylic acid polymer and its carboxyl groups become highly ionized after neutralization, forming a gel due to electrostatic repulsion among charged polymer chains (15, 16). Carbopol® is a cross-linked network of water-soluble polymer, whose porosity permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule through the gel network.

The emulgel has the characteristics of emulsion and gel. When adding the oil phase and surfactant into the gel, the emulsion will form between the oil phase and the water phase in the gel matrix under the influence of surfactant in the stirring condition. The requirement that emulgel is suitable for a specific use, acceptable to the patient, and permit sustained release of the active principle to application site prompted us to work hard aimed at developing an effective semisolid topical formulation containing THP for alleviating pain. The central point in the parallel study of the percutaneous permeation, rheological characterization, and *in vitro* release kinetics of THP is to understand the correlation between percutaneous permeation, viscoelastic properties, and *in vitro* release kinetics. The viscoelastic properties of pharmaceutical semisolids have been reported to affect primary physicochemical properties, *e.g.*, drug release and diffusion (17). The transdermal absorption of drugs applied

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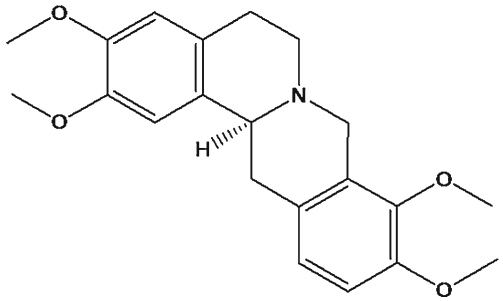

**Fig. 1.** The chemical structure of THP

topically depends on the rate of release and the permeability to skin (18–21) and satisfactory release of drug from dosage forms is a prerequisite for therapeutic efficacy. Penetration enhancers may be present in topical and transdermal systems in order to increase the skin permeability to drugs and can influence the physical properties of semisolid preparations (22). On the basis of these considerations, the effect of various grades of Carbopol® matrices and transdermal penetration enhancers such as *N*-methyl-2-pyrrolidone (NMP), Transcutol P, and Span 80 on percutaneous permeation, rheological characterization, and *in vitro* release were studied in this article and the optimized formulation was determined.

## MATERIALS AND METHODS

### Materials

THP (purity 99.5%) was purchased from Xi'an Xiao Cao Botanical development Co., Ltd. (Shanxi, China); Carbopol® (934NF, 974P, 971P, 980NF) were purchased from Lubrizol Co. Ltd. (USA) and the viscosity (cP) at 0.5% (*w*/*w*), pH=7.5 are 30,500~39,400, 29,400~39,400, 4,000~11,000, 40,000~60,000 separately; transparent Cellophane® membrane, which is natural, and is made of natural raw material cotton, was supplied by ShangYu Cellophane Co., Ltd. (Zhejiang, China); isopropyl palmitate (IPP) was supplied by China National Medicines Co., Ltd. (Shanghai, China); 1,2-propylene glycoland (PG), *N*-methyl-2-pyrrolidone (NMP), and Transcutol P were purchased from Beijing Chemical Co. Ltd. (Beijing, China); oleic acid and Span 80 were purchased from Tianjin Bio Chemical Co., Ltd. (Tianjin, China); triethanolamine was obtained from Tianjin BoDi Chemical Hoding Co., Ltd. (Tianjin, China); methanol that was of high-performance liquid chromatography (HPLC) grade was supplied by Hanbon Science and Technology (Jiangsu, China). Other chemicals were of the highest reagent grade available.

### Methods

#### *HPLC Determination of THP*

The determination of THP was performed by HPLC, which was equipped with a Hitachi instrument (Pump L-7100, UV-VIS Detector L-7420, T2000L workstation) and Diamonsil $C_{18}$ reversed-phase column (200×4.6 mm i.d., 5 μm; Dikma Technologies, Beijing China). The mobile phase was a mixture of methanol and distilled water (with 1% triethanolamine) at a ratio of 68:32 (*v*/*v*), and the pH was adjusted to 6.30 with glacial acetic acid. Aliquots of 20 μl from each sample were injected and eluted at a flow rate of 1.0 ml/min. Measurements were taken at a wavelength of 230 nm and the column temperature was maintained at 40°C.

### Preparation Emulgels

Emulgel formulations were prepared by a three-step method: (1) polymer dispersion in water, (2) neutralization of the polymeric aqueous dispersion, and (3) emulsification of the oil phase. Four different types of Carbopol®, namely Cp 934NF, Cp 971P, Cp 974P, and Cp 980NF were suspended in deionized water; then a period of 48-h settle was allowed to ensure the formation of a stable reticular structure, followed by adding triethanolamine for neutralizing under 200-rpm agitation to a final pH value of 6.0. In order to obtain a complete polymer hydration, the gels were reserved at 4°C for 24 h before the addition of the oil phase; successively, the oil phase IPP was slowly added to the gels with a stirring of 800 rpm and the temperature was maintained at 80°C. Once the mixture was homogeneous, the heat process was stopped and the agitating process continued until the material got cooled to room temperature, then THP was added to emulgel system under gentle manual agitating to verify homogeneity of the formula. The composition of emulgel formulas for the following experiments was shown in Table I.

### Skin Preparation

Male rabbits weighing 2.0~2.2 kg were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The Care and use of laboratory animals was carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (23). The hair of the abdominal skin was carefully clipped under anesthesia with urethane (20% *w*/*v*, i.p.) and the abdomen regions were carefully shaved with a razor after removal of hair by electric clippers (model 900, TGC, Japan). Full-thickness skin (epidermis with SC and dermis) was excised from the shaved abdominal site. The integrity of the skin was carefully checked by microscopic observation, and any skin which was not uniform was rejected. The skin was washed immediately with phosphate-buffered saline, wrapped in aluminum foil, and stored at −70°C till further use (used within 1 week of preparation). Before the experiment, skin was allowed to reach room temperature for at least 10 h.

### *In Vitro* Percutaneous Permeation

Skin permeation experiments were performed according to the method of Fang *et al.* (24). Modified Franz vertical diffusion cell systems containing eight cells, which were stirred magnetically in a constant speed at 600 rpm and thermoregulated with a water jacket at 32°C, were used as the experimental equipment. About 5 $cm^2$ of rabbit abdominal skin was mounted between the two half-cells, with the SC side of the skin facing the donor compartment and the dermis side facing the receiver compartment. Excess skin at the sides was trimmed off to minimize lateral diffusion. The donor cell was filled with 1.0 g of test emulgel and occluded with aluminum

**Table I.** Composition of THP Emulgel (100 g) Formulations with Different Grades of Carbopol® as Matrices With or Without Different Permeation Enhancers at the Concentration of 3% (*w*/*w*)

| | Formula | | | | | | |
|---|---|---|---|---|---|---|---|
| Formula composition | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Carbopol® 934 NF | 0.5 | – | – | – | – | – | – |
| Carbopol® 974 P | – | 0.5 | – | – | – | – | – |
| Carbopol® 980 NF | – | – | 0.5 | – | – | – | – |
| Carbopol® 971 P | – | – | – | 0.5 | 0.5 | 0.5 | 0.5 |
| THP | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| IPP | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| PG | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Tween 80 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| NMP | – | – | – | – | 3.0 | – | – |
| Transcutol P | – | – | – | – | – | 3.0 | – |
| Span 80 | – | – | – | – | – | – | 3.0 |
| Triethanolamine | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Distilled water | 88.75 | 88.75 | 88.75 | 88.75 | 85.75 | 85.75 | 85.75 |

foil. The receiver solution was 40% PEG/water. The effective surface area available for permeation was 0.95 $cm^2$. Samples of 5.0 ml were withdrawn at 4, 8, 12, 24, 28, 32, 36 h from the receiver side and an equal volume of receiver medium was immediately added. THP concentration was determined with HPLC method.

## Rheological Study

Rheological analyses were performed using a AR 2000ex rheometer (TA Instruments-waters LLC, UK) equipped with a cone-plate geometry (cone diameter 40 mm, angle 1°) and a gap of 63 μm. This configuration was chosen because of the high consistency of the samples. All the rheological measurements were carried out in triplicates.

### *Steady Flow (Viscometry)*

We researched the influence of different types of Carbopol® as matrices on THP emulgel viscometry, the initial stress ($\sigma$) was setted at 20 Pa, and the final stress ($\sigma$) was setted at 800 Pa for formula 1, 500 Pa for formulas 2 and 3, and 100 Pa for formula 4. The initial stress ($\sigma$) was setted at 20 Pa, and the final stress ($\sigma$) was setted at 100 Pa for formula 5~7. The rheograms "shear stress (pascal) *vs* shear rate (per second)" were recorded.

### *Oscillation Stress Sweep*

The viscoelasticity of the emulgels was tested applying an oscillating shear stress. When a sinusoidal stress was applied to the sample, modulus which induced response was measured. The linear viscoelastic region was identified in a frequency range of 0.1~40 Hz, at the temperature of 25°C.

Formula 5 was used as the sample for this experiment. To characterize the influence of temperature on viscoelastic properties, the sample was exposed to a frequency scan at a constant stress (6.4 Pa); 0.1~40-Hz frequency range, in the field of linear viscoelasticity, at 25°C (to simulate the storage condition) and 32°C (to simulate the application site). The temperature unit had a stability of ±0.1°C, and before each test prior to the measurement, the emulgel was equilibrated for 5 min at each temperature, in order to evenly adjust for the correct temperature of the sample. The storage ($G'$) and viscous ($G''$) modulus were recorded for emulgel as a function of frequentry of oscillatory stress applied.

### *Creep-Recovery Test*

Formula 5 was used as the sample for this experiment and was subjected to a constant shear stress (6.4 Pa) for a period of 60 s, and the compliance modulus ($J$) was measured, then the stress was removed and recovery was recorded for 60 s. The experiment temperature was setted at 25°C.

## *In Vitro* Release of THP

The emulgels (1.0 g) containing THP (0.1%) were used for release test with 40% PEG/water in the modified Franz vertical diffusion cell to simulate the topical release. The diameter of the cell was 1.1 cm, providing 0.95 $cm^2$ effective constant area, and the volume of receptor chamber was 7.4 ml. The Cellophane® membrane was mounted on the top of receptor compartment of diffusion cells. The prepared emulgels were placed in intimate contact with synthetic membrane, whose surface previously moistened with the receiving phase, the donor cap was covered with parafilm and clamped. The sampling port was sealed with a parafilm to prevent the evaporation of the receptor medium. The receptor compartment was filled with the receptor solution, which was stirred by a magnetic stirring bar and maintained at 32°C by a circulating water bath. Aliquots (5 ml) of the receiving phase were withdrawn at proper time intervals at 0.5, 2, 4, 6, 8, 12, 24, 28, 32, 36, 48, 52, 56, 60, 72 h and replaced with an equal volume of fresh buffer solution. Sink conditions were maintained during the experiments.

## Data Analysis

The rheological data was analyzed by rheology advantage data analysis software. All the data presented in this article was

analyzed by one-way analysis of variance (ANOVA) and the significance level was set to 0.05.

## RESULTS AND DISCUSSIONS

### *In Vitro* Percutaneous Permeation

The percutaneous permeation curves of THP from the emulgels with different types of Carbopol® as matrices were presented in Fig. 2. Formula 4 showed the highest cumulative amount of permeation. Cp 971P (0.5%, *w/w*) as the matrix of formula 4 was found to possess optimum consistency and was easy to handle, therefore it was used during the following experiments. The percutaneous permeation profiles of THP from emulgels with different permeation enhancers at the concentration of 3% were shown in Fig. 3. The enhancement order (NMP>Transcutol P>control≥Span 80) was obtained. The most outstanding penetration enhancer was NMP, which had the $Q_{36h}$ up to 118.19 μg/cm$^2$, providing an almost 2.69-fold increase in permeation amount, followed by Transcutol P with a 1.68-fold increase. There were no distinguished difference of percutaneous permeation amounts between Span 80 and control, with $P>0.05$. Therefore, NMP was chosen as the penetration enhancer in the optimized formula. Although the mechanism of drug diffusion across the skin appears complex, it is controlled by fundamental physicochemical concepts, the most important of which are the partition coefficient, thermodynamic activity, and diffusion coefficient of the drug (25). In order to increase the permeation of drugs across the skin, penetration enhancers need to act on one or more of these parameters. NMP altered the solubilizing ability of the aqueous regions of stratum corneum between the polar lipid head groups of the bilayers, thereby promoting drug partition into skin, which subsequently results in increased drug permeation (26). Transcutol P had a slightly lower promoting effect compared with NMP. Transcutol P is a hygroscopic compound that can absorb water from the skin thereby improving the skin penetration of certain drugs by maximizing their thermodynamic activity due to a change in their solubility (27–29). Span 80 is a nonionic surfactant with a polar head combined with a long alkyl chain making the SC layer less rigid by inserting its C16 alkyl chain into the lipid domain. The effect of Span 80 as a penetration enhancer could be attributed to the improvement in drug diffusion through the SC barrier (30). In view of the *log*$P$ of THP, it is generally considered that the partitioning of THP into SC rather than the diffusivity process may be the rate-limited step.

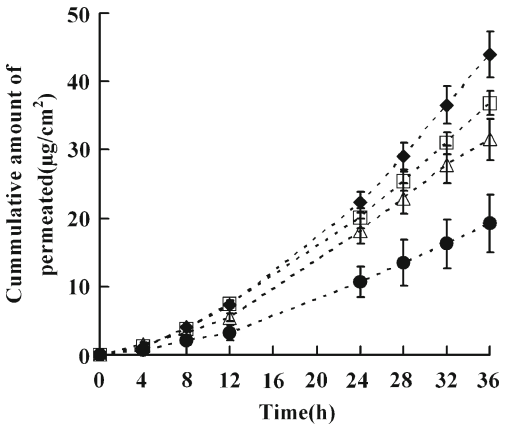


**Fig. 2.** The percutaneous permeated amount of THP emulgel with different grades of Carbopol® matrices: *diamond*, Cp 971P; *white square*, Cp 980NF; *white diamond*, Cp 934NF; *black circle*, Cp 974P in 36 h (mean±S.E., $n=4$)

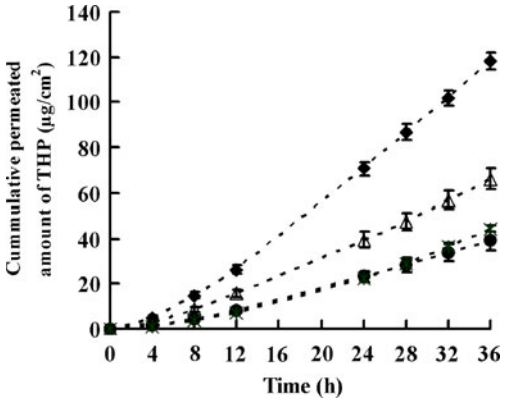


**Fig. 3.** The percutaneous permeated amount of THP emulgel with different permeation enhancers: *asterisk*, control (formula 4); *diamond*, NMP (formula 5); *triangle*, Transcutol P (formula 6); *circle*, Span 80 (formula 7), at the concentration of 3% (*w/w*) in 36 h across rabbit skin (mean±S.E., $n=4$)

### Rheological Characteristics

#### *Steady Flow (Viscometry)*

In viscosimetric studies, the applied shear stress was presented as a function of shear rate for different types of Carbopol® as matrices and different penetration enhancers (NMP, Transcutol P, and Span 80) with the concentration of 3% in THP emulgel in Figs. 4 and 5, respectively. In order to better understand the rheological properties of the tested emulgels, different parameters were calculated by fitting the experimental data with specific models. The rheological behavior were fitted with the Casson model in Eq. 1:

$$\tau^{1/2} = \tau_C{}^{1/2} + (\eta C\gamma)^{1/2} \quad (1)$$

where $\tau$ is the shear stress [pascal], $\gamma$ the shear rate [per second], $\tau_C$ and $\eta C$ are the Casson yield stress and viscosity

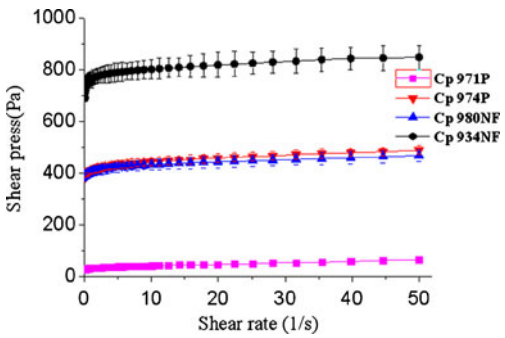


**Fig. 4.** Shear stress curves as a function of shear rate of THP emulgel with different grades of Carbopol® as matrices (mean±S.E., $n=3$)

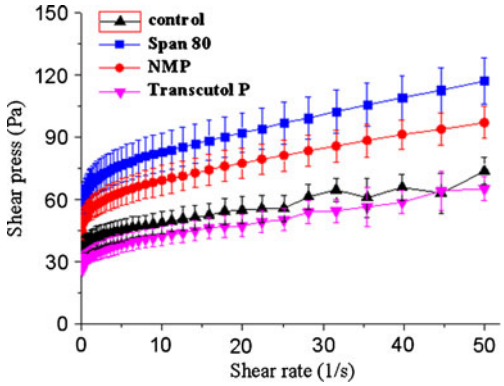


**Fig. 5.** Influence of different penetration enhancers (NMP, Transcutol P, and Span 80) at the concentration of 3% (*w*/*w*), in viscometry study of THP emulgel with Cp 971P as the matrix (mean±S.E., $n=3$)

coefficient, respectively. The two model constants are obtained from the "Casson plot" of shear stress, τ, and shea rate, γ, and the model constants $\tau_C$ and ηC are the intercept and slope, respectively. All the formulations demonstrated good fit to the Casson model and the parameters (yield stress and viscosity coefficient) was shown in Table II. According to the values of yield stress and viscosity in Table II (lines 1~4), the viscosity of emulgel with Cp 971P as the matrix undoubtedly was the lowest among these formulas with different types of Carbopol® as matrices. As can be seen from the data listed in Table II (lines 5~7), the viscosity of emulgel with penetration enhancer (Transcutol P) reduced, while the viscosity of emulgel with penetration enhancer (NMP or Span 80) increased in different degrees.

*Oscillation Stress Sweep*

The parameters obtained were the complex modulus $G^*$ consisting of the elastic modulus $G'$ and the viscous modulus $G''$. According to their correlations the parameters are calculated by Eqs. 2~4:

$$G' = G \times \mathrm{Cos}(\delta) \tag{2}$$

$$G'' = G \times \mathrm{Sin}(\delta) \tag{3}$$

$$\tan\delta = G''/G' \tag{4}$$

**Table II.** Yield Stress and Viscosity Coefficient of Casson Model for THP Emulgels

| Formula | Yield stress $\tau_C$ (Pa) | Viscosity coefficient $\eta C$ (Pa s) |
|---|---|---|
| 1 | 740.2 | 0.09971 |
| 2 | 395.6 | 0.1161 |
| 3 | 392.2 | 0.07888 |
| 4 | 35.64 | 0.1042 |
| 5 | 50.49 | 0.1583 |
| 6 | 28.58 | 0.1394 |
| 7 | 58.94 | 0.1962 |

The ratio of loss and storage moduli ($G''/G'$) is called loss tangent (tanδ), tanδ can be used to investigate the rheological changes due to ingredient interaction such as gel formation giving a lower tanδ because the material becomes more elastic (31). When the value of tanδ ($G''/G'$) is unity, the same degree of viscous and elastic can be expected (32).

The values of log $G'$ and log $G''$ *vs* the logarithm of frequency were shown in Fig. 6, in order to investigate the frequency dependence of modulus. The changes in rheological parameters can be divided into three phases. During the first phase, $G'$ was always higher than $G''$ over the frequency range. It was evident that the storage modulus $G'$ showed almost no dependence with frequency, and the value of tanδ was less than 1.0 over the frequency range, the sample was characterized by predominant elastic properties behavior ($G'>G''$), indicating that the sample had formed a continuous network structure and these features are characteristic of a "strong gel". In the second phase, when the frequency was increased above some critical level, values of both $G'$ and $G''$ suddenly changed, and the value of tanδ sharply increased and exceeded 1.0. The $G'$–$G''$ crossover (tanδ=1.0) time might be sol/gel transition time (33). Frequency-dependent property of $G'$ and $G''$ might be due to the sudden changes of modulus that can be connected with the structural breakdown by mechanical shear, a faster molecular mobility at higher frequencies. The last phase consisted of stabilization, values of both $G'$ and $G''$ reached plateau, the value of tanδ was more than 1.0 over the frequency range; furthermore, the sample was characterized by regnant viscous behavior ($G''>G'$), and the rheological behavior was typical of a "weak gel".

The objective of this work was to characterize the influence of temperature on viscoelastic properties of Carbopol®. The frequency range and the $G'$–$G''$ values were plotted in logarithmic scale. As illustrated in Fig. 7, an increase in temperature (from 25°C to 32°C) provoked decreased values of both $G'$ and $G''$ as we expected. The influence of temperature on the apparent viscosity at a specified shear rate can be calculated by the Arrhenius equation (34, 35). It means that when the THP emulgel was applied at skin surface (application site temperature, 32°C), fluidity and spreadability would increase comparing with the condition of 25°C. These rheological characteristics are important to ensure if the formulation is acceptable to the patient and it permits sustained release of the active principle to application site.

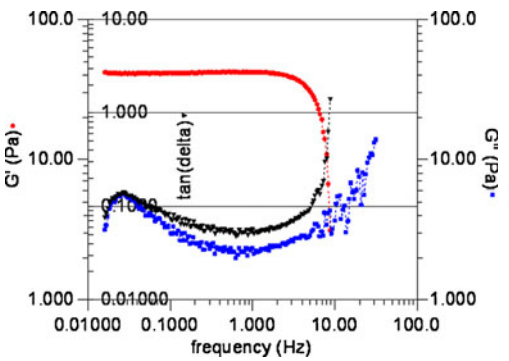


**Fig. 6.** Storage ($G'$) and viscous ($G''$) modulus plotted as a function of the frequency for THP emulgel with Cp 971P as the matrix

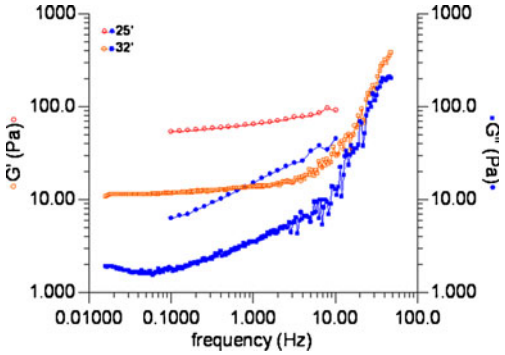


**Fig 7.** Influence of the temperatures (25°C and 32°C) in oscillometry study of THP emulgel with Cp 971P as the matrix. Storage ($G'$) and viscous ($G''$) modulus were recorded as a function of the frequency of the oscillatory stress applied

### *Creep Recovery*

The creep and recovery curves of viscoelastic THP emulgel at a constant temperature (25°C) were shown in Fig. 8, and was characterized by two phases, namely the creep phase and the recovery phase. The cut-off point from the upward-sloping part of the creep curve on the ordinate in Fig. 8 represents instantaneous unit deformation at $t=0$ ($J_0$) or the instantaneous elastic response of the final formulation. The creep-recovery percent $\delta J$ can be calculated from Eq. 5.

$$\delta J = \frac{J(60s) - J(120s)}{J(60s)} \times 100 \tag{5}$$

$\delta J$ was approximately equal to 1.0, which means that the final formulation presented irreversible shear breakdown, thixotropy, and the structure completely built-up. For linear viscoelastic materials, the instantaneous compliance ($J_0$) upon the loading is solely an elastic component, which can be entirely recovered on unloading. Creep phase is the time-dependent increase in compliance ($J$) under sustained stress ($\tau_0$); the time-dependent deformation is recoverable with time after the removal of stress. For topical transdermal permeation semisolid, the plasticity is a specially desirable characteristic.

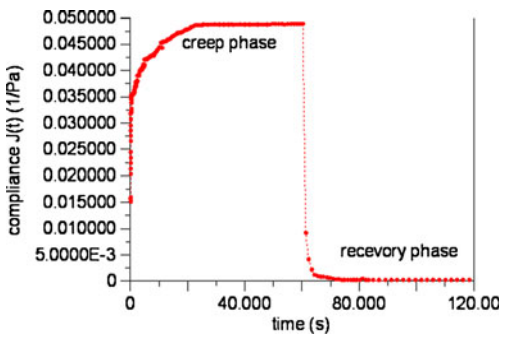


**Fig. 8.** Creep and recovery behavior to an applied constant stress for linear viscoelastic THP emulgel with Cp 971P as the matrix

### *In Vitro* Release of THP

The release of a drug from emulgel applied to the skin surface and its transport to the systemic circulation is a multistep process. It has become a popular method to assess *in vitro* release of drug from semisolid dosage forms using the Franz diffusion cell (36–38).

In order to better characterize the drug release behavior, the release kinetic parameters was calculated in Table III, data were fitted to the Korsmeyer–Peppas equation (39–42):

$$\frac{M_t}{M_\infty} = kt^n \tag{6}$$

where $M_t$ is the amount of released drug at time $t$, $M_\infty$ is the amount of loaded drug in the gel, $k$ is the rate constant, and $n$ is the release exponent characterizing the release mechanism. The calculated exponent, $n$, gives an indication of the release mechanism. When $n=1$, the release is zero-order kinetic, which controlled is by time dependence (case II). When $0.5<n<1$, the release is called "anomalous" and both swelling and diffusion play an important role (43, 44).

When the drug diffusion rate is slower than the relaxation rate of the polymeric chains, the diffusion is Fickian; whereas when the relaxation process is slow compared to diffusion, zero-order release kinetics occurs. When the drug diffusion rate and the polymeric relaxation rate are of the same order of magnitude, anomalous diffusion is observed and the value of $n$ falls between 0.5 and 1.0 (40, 45, 46). As can be seen from the data listed in Table II (line 1), the THP release presented zero-order release kinetics. This means that the Carbopol® relaxation process is slow compared to THP diffusion; the THP releases from emulgel principally through a diffusion-controlled mechanism. In this condition, the amount of released drug corresponds to the concentration of THP in the emulgel.

**Table III.** Release Kinetic Parameters

| Formula | $n$[a] | Mechanism of release | Correlation coefficient, $r$[b] |
|---|---|---|---|
| 4 | 1 | Case II | 0.9986 |
| | 0.9 | Anomalous | 0.9949 |
| | 0.8 | Anomalous | 0.9885 |
| | 0.7 | Anomalous | 0.9633 |
| 5 | 1 | Case II | 0.9841 |
| | 0.9 | Anomalous | 0.9918 |
| | 0.8 | Anomalous | 0.9974 |
| | 0.7 | Anomalous | 0.9854 |
| 6 | 1 | Case II | 0.9863 |
| | 0.9 | Anomalous | 0.9918 |
| | 0.8 | Anomalous | 0.9977 |
| | 0.7 | Anomalous | 0.9894 |
| 7 | 1 | Case II | 0.9947 |
| | 0.9 | Anomalous | 0.9977 |
| | 0.8 | Anomalous | 0.9994 |
| | 0.7 | Anomalous | 0.9847 |

[a] $X=t^n$
[b] $r$=correlation coefficient

However, it was notable that all emulgels (with different penetration enhancers) showed a rather similar release behavior, and the release fitted the anomalous model, with the release exponent $n=0.8$. This means that the release mechanism was controlled by the THP diffusion rate and the relaxation rate of Carbopol®. On the basis of these considerations, both the drug diffusion and the polymeric chain relaxation could control drug release kinetics. In order to indentify the proportional contribution controlling drug release kinetics, a nonlinear regression of the release date fitted Eq. 7 (47, 48).

$$\frac{M_t}{M_\infty} = k_1 t^{1/2} + k_2 t \tag{7}$$

Where $\frac{M_t}{M_\infty}$ is the fraction of released drug in time $t$; $k_1$ and $k_2$ are kinetic constants describing diffusion and relaxation contribution, respectively.

The values of $k_1$, $k_2$ and ratio of $k_1/k_2$ obtained from the curve fitting were listed in Table IV. It is possible to hypothesize that certain interactions such as hydrogen bonding, might take place between Carbopol® matrix and enhancers (NMP, Transcutol P, and Span 80). Considering the bulk properties of the Carbopol® gel, it can be concluded that polymer-enhancers interactions alter the microenvironment in which drug release occurs. The ratio of $k_1/k_2$ was 5.20 when adding Transcutol P, indicating that the THP release in this condition was predominantly controlled by the diffusion mechanism. According to the Stokes–Einstein equation, the diffusion coefficient of the drug is inversely proportional the viscosity of the matrix. Since the THP release was predominantly controlled by the diffusion mechanism when adding Transcutol P, the low viscosity (shown in Fig. 5) facilitated the THP release from the emulgel. As expected, the highest release percentage (91.88%) was shown in the THP emulgel with Transcutol P as the penetration enhancer. The ratio of $k_1/k_2$ was 0.54 when adding Span 80, specifying that the THP release was principally relaxation controlled. Span 80 is a non-ionic surfactant with a polar head combined with a long alkyl chain. Aggregation processes occur between the tail long alkyl chain of Span 80 and the backbone of Carbopol® and electrostatic interactions appear between the polar heads of Span 80 and the charged groups of Carbopol®. When very small amount of Span 80 is added to the Carbopol® matrix, the inter-polymeric micelles between Span 80 and Carbopol® matrix form and create a three-dimensional network. However, more Span 80 above the critical micellar concentration (the cmc of Span 80 is 0.007 mg/ml) is added, the formation of intra-polymeric micelles becomes possible and the inter-polymeric micelles diminished, and the three-dimensional network disintegrates (49). The macroscopic effect is, however, not reflected in a decrease but in an increase in microviscosity(estimated in dynamic light scattering) owing to the formation of larger Carbopol®/Span 80 aggregates or the free micelles that contribute significantly to the obstruction of diffusional path (50). As can be seen in Fig. 9, the release percentage was the lowest with Span 80 as the penetration enhancer. The ratio of $k_1/k_2$ was 0.91 when adding NMP, demonstrating that diffusion rate of THP and the relaxation rate of Carbopol® chains were of the same order of magnitude. Since interactions between Carbopol® and enhancers could change the mobility of the chains, and Carbopol®/enhancer interaction are mainly through hydrogen bonding, it can be concluded that once adding NMP, carboxylic groulps of Carbopol® would be ionized, then the electrostatic repulsion between charged polymer chains would rise, followed the mobility of the chains would decrease and the viscosity would increase. As a result, the release percentage with NMP as the enhancer (57.32%) was less than the control (65.41%).

**Table IV.** The Kinetic Constants of Diffusion and Relaxation, $k_1$ and $k_2$, as well as the Ratio of Kinetic Constant $k_1/k_2$

| Formula | $k_1$ ($h^{-1/2}$) | $k_2$ ($h^{-1}$) | $k_1/k_2$ ($h^{1/2}$) |
|---|---|---|---|
| 5 | 0.78 | 0.86 | 0.91 |
| 6 | 4.21 | 0.81 | 5.20 |
| 7 | 0.52 | 0.96 | 0.54 |

## CONCLUSIONS

Investigation on the *in vitro* percutaneous penetration, rheological properties, and *in vitro* release of THP emulgel provided us with profound insights to design the reasonable formula. Not only the high cumulative permeation amount is a prerequisite for the optimized formula, but satisfactory rheological properties and suitable *in vitro* release kinetics are also important. The optimized emulgel formulation was determined from the *in vitro* percutaneous penetration test, then the emulgel was demonstrated reasonable from rheological properties and *in vitro* release viewpoints. The penetration enhancers in emulgel affected the rheological properties, the rheological behaviors impacted the THP release, and the release influenced the percutaneous penetration. Therefore, we highlight that the clarification of the relationship between rheological properties, *in vitro* release, and percutaneous permeation of THP

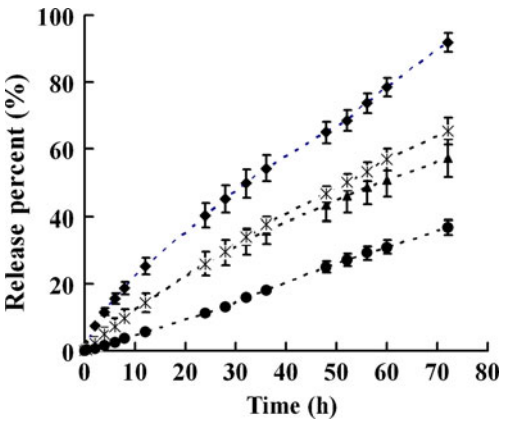


**Fig. 9.** The accumulated release percent of THP emulgels through Cellophane® membrane with or without different penetration enhancers: *diamond*, Transcutol P; *triangle*, NMP; *circle*, Span 80; *asterisk*, control (mean±S.E., $n=4$)

will provide us with profound insights and facilitate the design of emulgel.

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