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RESEARCH PAPER

# Preparation, Evaluation, and NMR Characterization of Vinpocetine Microemulsion for Transdermal Delivery

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## ABSTRACT

A novel microemulsion was prepared to increase the solubility and the in vitro transdermal delivery of poorly water-soluble vinpocetine. The correlation between the transdermal permeation rate and structural characteristics of vinpocetine microemulsion was investigated by pulsed field gradient nuclear magnetic resonance (PFG-NMR). For the microemulsions, oleic acid was chosen as oil phase, PEG-8 glyceryl caprylate/caprate (Labrasol®) as surfactant (S), purified diethylene glycol monoethyl ether (Transcutol P®) as cosurfactant (CoS), and the double-distilled water as water phase. Pseudo-ternary phase diagrams were constructed to obtain the concentration range of each component for the microemulsion formation. The effects of various oils and different weight ratios of surfactant to cosurfactant (S/CoS) on the solubility and permeation rate of vinpocetine were investigated. Self-diffusion coefficients were determined by PFG-NMR in order to investigate the influence of microemulsion composition with the equal drug concentration on their transdermal delivery. Finally, the microemulsion containing 1% vinpocetine was optimized with 4% oleic acid, 20.5% Labrasol, 20.5% Transcutol P, and 55% double-distilled water (w/w), in which drug solubility was about 3160-fold higher compared to that in water and the apparent permeation rate across the excised rat skin was $36.4 \pm 2.1$ μg/cm$^2$/h. The physicochemical properties of the optimized microemulsion were examined for the pH, viscosity, refractive index, conductivity, and particle size distribution. The microemulsion was stable after storing more than 12 months at 25°C. The irritation study showed that the optimized microemulsion was a nonirritant transdermal delivery system.

*Key Words:* Vinpocetine; Microemulsion; Transdermal drug delivery; Self-diffusion coefficients.

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## INTRODUCTION

Vinpocetine, an alkaloid of the common periwinkle plant (*Vinca minor*), is widely used in the treatment of cerebral disease originating from a vascular or cerebral metabolic disturbance.[1–4] However, it undergoes a marked first-pass effect after oral administration. Approximately 75% of the substance is metabolized, leading to an absolute bioavailability of 7% in man.[5]

Because of this poor bioavailability, transdermal drug delivery has received increasing attention as an efficient route of the drug administration. For example, it can avoid the hepatic metabolism and provide steady-state plasma drug levels and long-term therapy from a single dose.[6] It has been reported that vinpocetine is a good candidate for transdermal delivery due to its appropriate partition coefficient (log $K_{o/w} = 3.56$). Therefore, its various transdermal formulations, including patch, gel, and cream, have been reported.[7–9] However, improvement of drug permeability through the skin is always a difficult problem of its clinical use. One way is to use skin penetration enhancers reported by Kobayashi et al.[7] and the other way is to develop appropriate vehicles to increase the solubility and thermodynamic activity of the drug and then to increase the permeation.

Microemulsion is a clear, isotropic, and thermodynamically stable dispersion with low viscosity in the presence of a suitable surfactant, usually in conjunction with a cosurfactant. It has several interesting characteristics, such as enhanced drug solubility, good thermodynamic stability, simplicity of preparation, and increased local or systemic delivery.[10] Previous reports have confirmed that the incorporation of a lipophilic drug into the internal phase of an oil-in-water microemulsion has become an attractive technique for percutaneous administration of drug due to the high solubilizing capacity.[11] This approach is to favor high concentration gradient across the diffusion membrane, leading to an increase in the activity of the drug in the vehicle and thereby improving the drug diffusion rates. High dose of drug can be incorporated into this system as a consequence of the supersolvent properties of microemulsion and the dispersed phase can also act as a reservoir, making it possible to maintain an almost constant concentration gradient over the skin for a long time.[12] Furthermore, the surfactant and cosurfactant may reduce the diffusional barrier of stratum corneum by acting as permeation enhancers. The percutaneous absorption of drug will also increase due to the hydration effect of the stratum corneum if the water content in microemulsion is high enough.[13]

Besides the individual characteristics of the applied constituents for pharmaceutical microemulsion formulations, the transdermal drug delivery potential of the microemulsion system has been reported to be highly dependent on the composition and internal structure of the phases.[14] It has also been reported that the determination of self-diffusion coefficients of the components by pulsed field gradient nuclear magnetic resonance (PFG-NMR) is necessary to characterize the microemulsion structure. A correlation exists between the self-diffusion coefficient and transdermal potential, which could be used to predict the optimized formulation.[15,16]

To our knowledge, no report has dealt with the transdermal delivery of vinpocetine from microemulsion. The aim of this study, therefore, was to develop a novel microemulsion containing vinpocetine by screening oils and optimizing the formulation to achieve high solubility and skin permeation rate. The influence of the self-diffusion rate of the drug in the vehicles was also investigated on its transdermal delivery rate. In addition, the physicochemical stability and skin irritation of the optimized microemulsion containing vinpocetine were evaluated.

## MATERIALS AND METHODS

### Materials

Vinpocetine was purchased from Dongbei Pharmaceutical Corporation (Shenyang, China). Isopropyl isostearate (ISIP) was purchased from Yangjia Chemical Ltd. (Shanghai, China). Oleic acid was purchased from Shenyang Chemical Plant (Shenyang, China). Isopropyl myristate (IPM) and caprylic/capric triglyceride (GTCC) were kindly donated by Croda (UK), and glyceryl monolinoleate (Maisine 35-1), lauroyl macrogolglycerides (Glucire 44/14), PEG-8 glyceryl caprylate/caprate (Labrasol®), and diethyleneglycol monoethyl ether (Transcutol P®) by Gattefosse (France). All other chemicals were of analytical grade.

### Construction of Phase Diagrams and Preparation of the Microemulsions

Pseudo-ternary phase diagrams were constructed to obtain the appropriate components and their concentration ranges that can result in large existence area of microemulsion.[17,18] Surfactant (Labrasol, S) was gently mixed with cosurfactant (Transcutol P, CoS) in fixed ratios (0.5:1, 1:1, 2:1, and 3:1). Aliquots of

each surfactant-cosurfactant mixture ($S_{mix}$) were then mixed with oil. Double-distilled water was added to the mixture drop by drop under gentle agitation until a clear, isotropic and thermodynamically stable dispersion with low viscosity was obtained.

The mixture was kept at ambient temperature (25°C) to reach equilibrium. The staining method was used to further identify the formation of O/W microemulsion or not.[19] Then the physical states were represented on a pseudo-ternary phase diagram with one axis representing water, one representing oil, and the third representing the $S_{mix}$. The influence of the weight ratio of S/CoS on the area of O/W microemulsion region was investigated. Unless stated otherwise, the composition of microemulsion and drug concentration (%) was represented as w/w.

## Solubility of Vinpocetine in Various Mediums

The solubility of vinpocetine was determined in six oils and also in prepared microemulsions. Oils employed were oleic acid, isopropyl isostearate (ISIP), isopropyl myristate (IPM), caprylic/capric triglyceride (GTCC), glyceryl monolinoleate (Maisine 35-1), and lauroyl macrogolglycerides (Glucire 44/14). An excess amount of vinpocetine was added to each medium and the mixture was shaken reciprocally for 72 h at 37±1°C in oils and at 25±1°C in microemulsions, respectively. Triplicate samples were centrifuged at 10,000 rpm for 10 min. Then aliquots of supernatant were filtered through 0.45-μm membrane filters and the solubility of vinpocetine was determined by high performance liquid chromatography (HPLC) after dilution with methanol of HPLC grade. For each sample, three replicated assays were performed.

## In Vitro Skin Permeation Study

Skins were obtained from male Wistar rats weighting 250 ± 20 g. After hair was shaved carefully with an electric shaver one day before the penetration study, a patch of skin was excised from the dorsal portion from each sacrificed rat just before the experiment. Then, subcutaneous fat was trimmed. Rats were used in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University. The epithelium of fresh human cadaver skin excised from the chest and isolated by the trypsin digestion method was utilized as the barrier.[20]

The permeation studies were performed in Vertical Franz cells. The effective diffusion area of the cells surface was 1.77 $cm^2$.The skin samples were mounted on diffusion cells with the stratum corneum side up. Donor solutions consisted of 1 mL of test microemulsion containing vinpocetine. The receiving chamber was filled with 11.5 mL of 40% polyethylene glycol 400/water (40% PEG) and magnetically stirred at 600 rpm. It has been reported that 40% PEG has been used as the receiver solution for vinpocetine transdermal delivery to prevent dissolution limiting into the receiver medium and maintain ''sink condition.''[7] It was also reported that the skin barrier function was not influenced by 40% PEG.[21] The diffusion cells were thermostated at 32 0±5°C using a recirculating water bath (79HW-1, Zhejiang, China). At hourly intervals, the entire contents of the receptor cells were withdrawn and the cells were refilled with fresh receiving solution equilibrated at 32 ± 5°C. The samples were filtered (0.45 μm) and analyzed by HPLC. At least three samples of each formulation were evaluated.

The cumulative amount of vinpocetine permeated through the skin (Q, μg/$cm^2$)was plotted as a function of time (t, h). The drug flux at steady-state ($J_s$, μg/$cm^2$/h) was calculated as the slope from the linear part of the curve by linear regression analysis. The intercept on the X-axis was taken as the lag time ($T_{lag}$, h).

## NMR Spectroscopy

The NMR measurements were performed at 25°C on a Varian UNITY+500 MHz system, which delivered a maximum gradient strength of 30 Gauss/cm. Chemical shifts of all microemulsion components were determined relative to internal $D_2O$.

Self-diffusion coefficients were measured using pulsed field gradient nuclear magnetic resonance (PFG-NMR). The Gradient Compensated Stimulated Echo Spin Lock (GCSTESL) pulse sequence was shown in Fig. 1,[22] modulating the amplitude of fixed-length gradient pulses. Fifteen amplitude values were used for

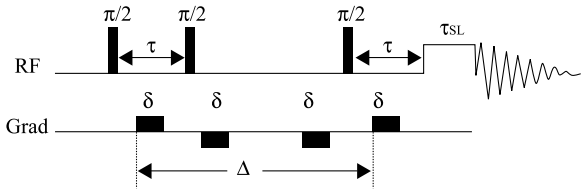


***Figure 1.*** The gradient compensated stimulated echo spin lock (GCSTESL) pulse sequence with time interval τ=2.3 ms, gradient duration δ=2 ms, and spin lock time τSL=1.5 ms.

each experiment. Self-diffusion coefficients (D) were derived from Eq. 1:

$$I = I_0 \exp[-(\gamma\delta G)^2(\Delta - \delta/3)D] \quad (1)$$

Where I and $I_0$=intensities of NMR signal in the presence and absence of field gradient pulses, $\gamma$=the gyromagnetic constant for $^1H$, $\delta$=the duration of the z gradient pulse, and $\Delta$=the time interval between the gradient pulses.[23] Microemulsion formulations containing $D_2O/H_2O$ in a ratio of 1:1 were used in the diffusion measurements.

### Physicochemical Characterization of Study Microemulsion

Physicochemical parameters were measured at 25±1°C. pH was determined using a pH meter (pH S-2C, Shanghai, China). Viscosity was measured using a circumrotate viscometer (NDJ-1, Shanghai, China). Conductivity was measured using a conductivity meter (DDS-11C, Tianjing, China). The refractivity index was measured using a refractometer (Shijiazhuang, China). A Malvern Photo Correlation Spectrometer (Zetasizer 3000, Malvern, UK), equipped with an argon laser model 2000, was employed to monitor the particle size of microemulsion. Light scattering was monitored at a 90° angle at 25±1°C.

### Irritancy Test

Concerning the transdermal delivery system, the skin irritancy test should be performed to confirm its safety. For the irritancy test, a single dose of 10 μL of the test microemulsion was applied to the left ear of the mouse, with the right ear as a control. The development of erythema was monitored daily for 6 days using the method of Utelly and Van Abbe.[24]

### HPLC Analysis of Vinpocetine

Vinpocetine was assayed by reversed HPLC (Shimadzu LC-10AT vp). The mobile phase consisted of methanol/0.1 $mol \cdot l^{-1}$ ammonium carbonate aqueous solution/glacial acetic acid at a ratio of 9:1:0.05 (v/v/v). The flow rate was fixed at 1 $mL \cdot min^{-1}$ and the UV detector (Shimadzu, SPD 10- AV) was set at $\lambda$=268 nm. The injection volume was 20 μL. The peak area correlated linearly with vinpocetine concentrations ($r^2$=0.9999) in the range 0.144∼11.520 $\mu g \cdot mL^{-1}$. Limit of quantitation was 0.1 $\mu g \cdot mL^{-1}$. Coefficient of variation and accuracy were 1.2%, 100.36% at 0.144 $\mu g \cdot mL^{-1}$ and 1.0%, 100.15% at 5.76 $\mu g \cdot mL^{-1}$ and 0.8%, and 100.01% at 11.520 $\mu g \cdot mL^{-1}$, respectively.

### Statistical Analysis

The data were expressed as mean ± SD (standard deviation) and were analyzed statistically by the one- and two-way not balanced analysis of variance (ANOVA) test and by the one-population and two-population Student's t-test (level of significance for $P < 0.05$).

## RESULTS AND DISCUSSION

### Solubility of Vinpocetine in Various Oils

The solubility of vinpocetine was determined in six oils, which were oleic acid, isopropyl isostearate (ISIP), isopropyl myristate (IPM), caprylic/capric triglyceride (GTCC), glyceryl monolinoleate (Maisine 35-1), and lauroyl macrogolglycerides (Glucire 44/14). Figure 2 shows the solubility of vinpocetine in various oils. The highest solubility was observed in Maisine 35-1 (72.3 ± 4.5 mg/mL), followed by Gelucire 44/14 (52.5 ± 3.8 mg/mL), and oleic acid (50.2 ± 3.1 mg/mL). The solubility in other oils was relatively low. Therefore, oleic acid, Maisine 35-1, and Glucire 44/14 were chosen for the preparation of microemulsion.

### Preparation of Pseudo-ternary Phase Diagram

Pseudo-ternary phase diagrams were constructed to obtain appropriate components and their concentration ranges for the microemulsion. Figure 3 shows the phase diagram of O/W microemulsion consisted of oleic acid, Labrasol (S), Transcutol P (CoS), and distilled water. The S/CoS ratio was varied as 0.5:1,

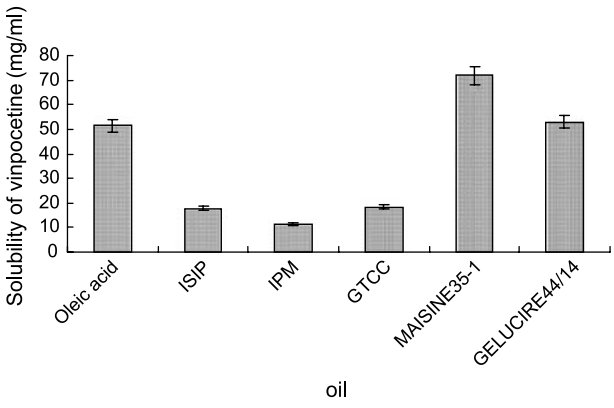


*Figure 2.* The solubility of vinpocetine in various oils at 37±1°C (mg/mL, mean ± SD, n=3).

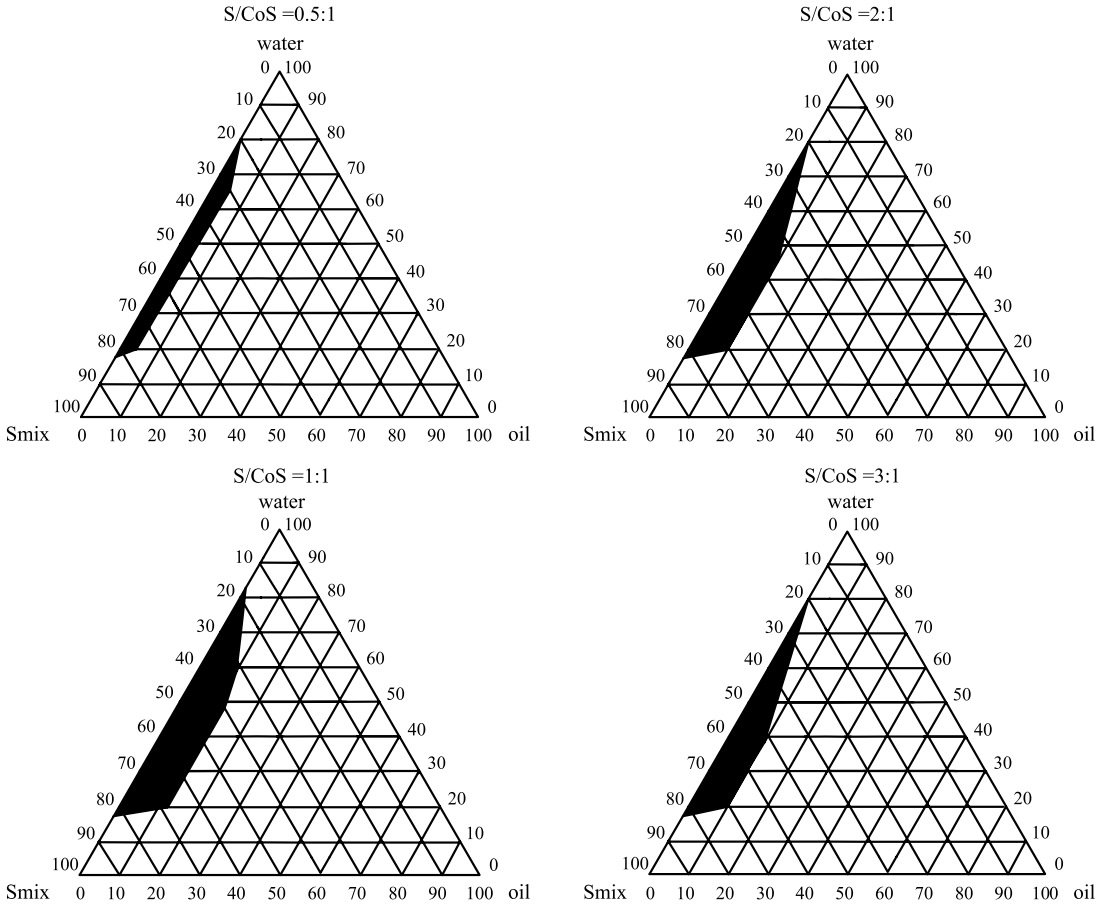


***Figure 3.*** Pseudo-ternary phase diagram composed of oleic acid, Labrasol-Transcutol P mixture ($S_{mix}$) (S/CoS = 0.5:1, 1:1, 2:1, 3:1) and distilled water. One axis represents water, one represents oil, and the third represents the $S_{mix}$. The concentration of each component is in weight percent (w/w). The shaded region represents the area of o/w microemulsion existence.

1:1, 2:1, and 3:1. Similar results were obtained from phase diagrams composed of Maisine 35-1 and Glucire 44/14 as oils (not shown). From the diagrams, we can see that the area region of microemulsion increased with the decreasing ratio of S/CoS from 3:1 to 1:1, reaching a maximum at S/CoS of 1:1. When S/CoS was 0.5:1, the region of microemulsion was too narrow to form stable formulation. Based on these results, different microemulsions within the shaded region were prepared to evaluate the effects of microemulsion composition with various oils on the drug solubility.

## Screening of Oils for Microemulsion

The solubility of vinpocetine in various microemulsions is shown in Fig. 4. The test microemulsions consisted of 4% of oil (oleic acid, Maisine35-1, or Gelucire 44/14), 41% of Labrasol-Transcutol P mixture with different S/CoS ratio (1:1, 2:1, and 3:1), and 55% of distilled water. Although the drug solubility in oleic acid was lower than that in Maisine 35-1 and Gelucire 44/14, the drug solubility in the microemulsion containing oleic acid as oil phase was significantly higher than that in microemulsions containing other oils ($P < 0.05$) shown in Fig. 4. Previous reports indicated that the superior transdermal flux appeared to be mainly due to the large solubilizing capacity of the microemulsion, which led to larger concentration gradients towards the skin.[14] It was also reported that oleic acid was a powerful enhancer for transdermal delivery.[25,26] Based on these findings, oleic acid was chosen as the oil phase for the preparation of the microemulsion containing vinpocetine.

In order to evaluate oleic acid as oil phase, nine microemulsions (referred to as system A1, A2, A3, B1, B2, B3, C1, C2, and C3 in Table 1) were selected from the shaded regions. The solubility of vinpocetine in different microemulsions increased with the decreasing

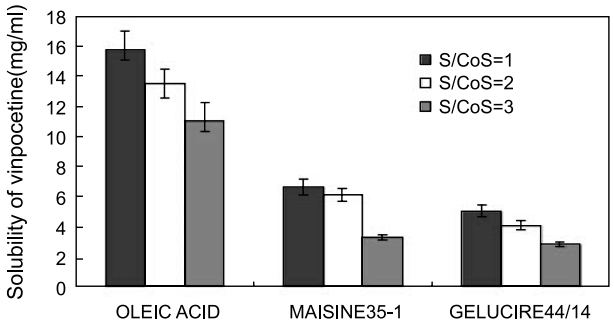


***Figure 4.*** The solubility of vinpocetine in various microemulsion at 25±1°C (mean±SD, n=3). The test microemulsions consist of 4% oil (oleic acid, Maisine 35-1, or Gelucire 44/14), 41% Labrasol-Transcutol P mixture with different S/CoS ratio (1:1, represented by black bar; 2:1, represented by white bar; 3:1, represented by gray bar), and 55% distilled water.

ratio of S/CoS from 3:1 to 1:1 when the Labrasol-Transcutol P mixture ($S_{mix}$) was fixed at 41%, 62%, and 70%, respectively (seen from Table 1 and Fig. 4). These results could be explained that the increase of Transcutol P content from 3:1 to 1:1, in which drug was readily soluble, would increase the solubilizing capacity of microemulsion. It has been reported that the different composition of microemulsion resulted in different solubility, and the permeation rate of drug was different from the different microemulsion with equal drug concentration.[26] Therefore, the efficient methods should be chosen to determine if there was a relationship between the microemulsion structure and in vitro permeation rate. The following experiments were performed to further elucidate the relationship between drug self-diffusion coefficients in various microemulsions and the in vitro skin permeation rate of the drug.

## Determination of Self-Diffusion Coefficient

Nine microemulsions (referred to as system A1, A2, A3, B1, B2, B3, C1, C2, and C3 in Table 1) were used for the preparation of the drug microemulsion containing 1% vinpocetine. The previously reported Gradient Compensated Stimulated Echo Spin Lock (GCSTESL) pulse sequence was employed to determine the self-diffusion coefficients. The self-diffusion coefficients of different components in these microemulsions are presented in Table 2 and Fig. 5. The signals of the methylene groups ($-CH_2$) corresponded to the multiple species, including Labrasol, oleic acid, and Transcutol P. The self-diffusion coefficients for the unique signal of Labrasol were determined from the resonance of the ethylenedioxy group ($-OCH_2CH_2O-$) of the polyethylene glycol (PEG) moiety. As seen from Table 2 and Fig. 5, the self-diffusion coefficients of Labrasol, oleic acid, and Transcutol P in all microemulsion systems were very slow ($10^{-11}$ $m^2s^{-1}$ range), while the water diffusion in all microemulsions was much faster than that of oil and $S_{mix}$. This suggested that water constituted a continuous free phase in these systems.[14] The diffusion of vinpocetine was very slow in all systems, which indicated that the drug was associated with the inner phase (lipophilic phase) due to its high solubility in the oil-$S_{mix}$ mixture and low solublity in water. Table 2 and Fig. 5 show that the drug diffusion decreases with the increase of the amount of the oil-$S_{mix}$ mixture and the viscosity of the systems. This result is in accordance with the previously reported result that the self-diffusion

***Table 1.*** Solubility of the drug in the different microemulsions with oleic acid as oil phase, Labrasol as surfactant, Transcutol P as cosurfactant, and distilled water at 25±1°C (mean ± SD, n=3).

| | Phase composition (%, w/w) | | | | Solubility |
|---|---|---|---|---|---|
| Microemulsion | Oleic acid (O) | Labrasol (S) | Transcutol P (CoS) | Water (W) | (mg/mL) |
| A1 | 10.0 | 35.0 | 35.0 | 20.0 | 25.0±2.8 |
| A2 | 10.0 | 46.7 | 23.3 | 20.0 | 24.0±2.1 |
| A3 | 10.0 | 52.5 | 17.5 | 20.0 | 19.5±1.8 |
| B1 | 8.0 | 31.0 | 31.0 | 30.0 | 23.0±2.0 |
| B2 | 8.0 | 41.3 | 20.7 | 30.0 | 20.5±1.6 |
| B3 | 8.0 | 46.5 | 15.5 | 30.0 | 19.1±1.1 |
| C1 | 4.0 | 20.5 | 20.5 | 55.0 | 15.8±0.8 |
| C2 | 4.0 | 27.3 | 13.7 | 55.0 | 13.5±0.9 |
| C3 | 4.0 | 30.8 | 10.3 | 55.0 | 11.0±0.7 |

***Table 2.*** Viscosity of different microemulsions containing 1% vinpocetine, self-diffusion coefficient (d) of vinpocetine, and single constituents (oleic acid, Labrasol, Transcutol P, and water) in microemulsion systems at 25°C (mean ± SD, n=3).

| | | D ($m^2 \times \cdot s^{-1} \times 10^{11}$) | | | |
|---|---|---|---|---|---|
| Microemulsion | Viscosity (mpa·s) | Labrasol±oleic acid±Transcutol ($-CH_2$) | Labrasol ($-OCH_2CH_2O-$) | Vinpocetine | Water |
| A1 | 25.0±0.2 | 1.54±0.02 | 1.41±0.01 | 1.24±0.02 | 9.92±0.17 |
| A2 | 34.5±0.3 | 1.39±0.01 | 1.23±0.01 | 1.12±0.01 | 9.19±0.15 |
| A3 | 39.0±0.3 | 1.28±0.01 | 1.53±0.02 | 1.02±0.01 | 9.01±0.13 |
| B1 | 22.5±0.2 | 1.61±0.02 | 1.21±0.01 | 2.33±0.03 | 12.10±0.21 |
| B2 | 31.5±0.2 | 1.40±0.02 | 1.17±0.01 | 1.84±0.02 | 11.79±0.22 |
| B3 | 35.5±0.3 | 1.25±0.02 | 1.55±0.02 | 1.77±0.02 | 11.59±0.19 |
| C1 | 14.5±0.2 | 1.03±0.01 | 1.02±0.01 | 6.92±0.05 | 32.51±0.28 |
| C2 | 19.0±0.2 | 1.15±0.01 | 1.15±0.01 | 4.99±0.04 | 31.50±0.27 |
| C3 | 22.0±0.2 | 1.18±0.01 | 1.09±0.01 | 4.75±0.03 | 30.21±0.27 |

coefficient of a compound is inversely related to the viscosity of the medium and the obstruction effect arises from the increasing volume fractions of the aggregates when the oil content increases.[14] In order to elucidate the relationship between the transdermal permeation rate and NMR characteristics of microemulsion at an equal drug concentration (1% vinpocetine), in vitro skin permeation of the drug in nine microemulsions of different compositions was studied as follows.

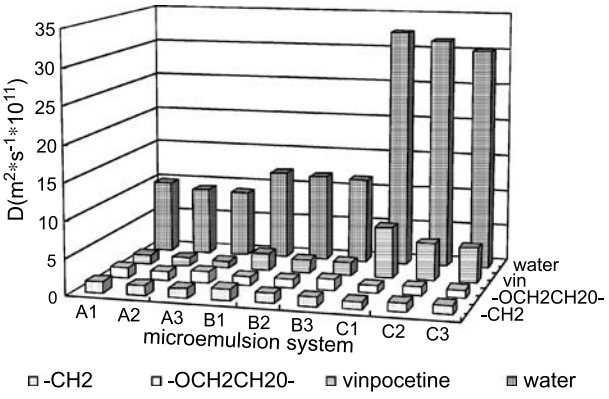


***Figure 5.*** The self-diffusion coefficients of different components ($-CH_2$, $-OCH_2CH_2O-$, vinpocetine, water) in microemulsions (A1, A2, A3, B1, B2, B3, C1, C2, C3) containing 1% vinpocetine at 25°C. The signals of the methylene groups ($-CH_2$) corresponded to multiple species, including Labrasol, oleic acid, and Transcutol P. The self-diffusion coefficients for the unique signal of Labrasol were determined from the resonance of the ethylenedioxy group ($OCH_2CH_2O$) of the polyethylene glycol (PEG) moiety.

## In Vitro Skin Permeation Study

Figure 6 indicates that microemulsions containing 1% vinpocetine had excellent zero-order permeation character in vitro.

Table 3 shows the effects of the different compositions of microemulsion with equal drug concentration on the skin permeation rate of vinpocetine. As the ratio of S/CoS decreased from 3:1 to 1:1 at the fixed concentration of Labrasol-Transcutol P mixture ($S_{mix}$), the skin permeation rate was significantly increased ($P < 0.05$). One explanation might be that the enhancing effect of Transcutol P, a strong permeation enhancer, had been weakened with lowering its concentration in the

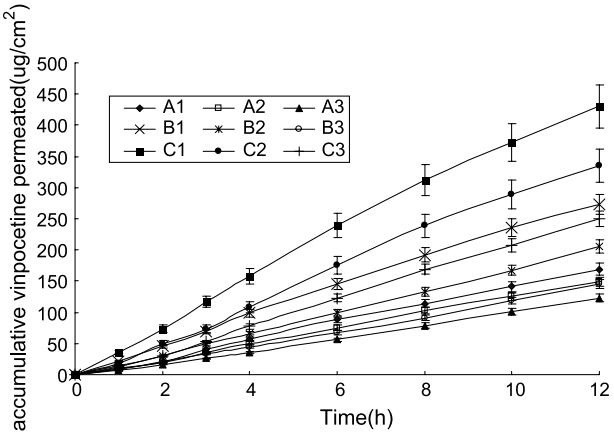


***Figure 6.*** The permeation profiles of vinpocetine through excised rat skin from microemulsions (A1, A2, A3, B1, B2, B3, C1, C2, C3) with 1% vinpocetine (mean ± SD, n = 3). Vertical bars indicate SD. (*View this art in color at www.dekker.com.*)

***Table 3.*** The permeation parameters of vinpocetine across the excised rat skin and human skin from different microemulsions containing 1% vinpocetine (mean ± SD, n = 3).

| Microemulsion | $J_s$ (μg/cm$^2$/h) across $J_s$ the rat skin | (μg/cm$^2$/h) Across the human skin |
|---|---|---|
| A1 | 14.7±1.2 | 2.76±0.56 |
| A2 | 12.8±1.1 | 2.41±0.54 |
| A3 | 10.6±0.6 | 1.98±0.49 |
| B1 | 23.2±1.8 | 4.29±0.38 |
| B2 | 17.3±1.1 | 3.65±0.82 |
| B3 | 12.3±0.7 | 2.37±0.49 |
| C1 | 36.4±2.1 | 7.05±0.67 |
| C2 | 29.6±1.9 | 5.81±0.61 |
| C3 | 22.1±1.7 | 4.61±0.53 |

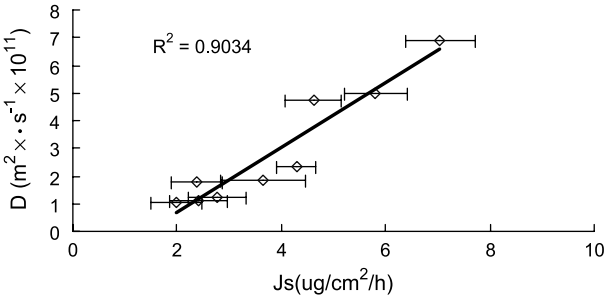


***Figure 8.*** Correlation between self-diffusion coefficient (D) and transdermal permeation rate (Js) across the human skin of vinpocetine in microemulsions (A1, A2, A3, B1, B2, B3, C1, C2, C3) with 1% vinpocetine (mean ± SD, n = 3). Vertical bars indicate SD. (*View this art in color at www.dekker.com.*)

microemulsion when the ratio of S/CoS increased from 1:1 to 3:1. Another explanation could be that the self-diffusion coefficient of the drug decreased with the increasing ratio of S/CoS from 1:1 to 3:1, which was directly related to the transdermal delivery potential reported by Kreilgaard, Erik, and Jaroszewski.[14] As shown in Figs. 7 and 8, the correlation between transdermal permeation rate and vinpocetine self-diffusion coefficient has a linear relationship (R = 0.9348 when across the rat skin, R = 0.9505 when across the human skin). From this relationship, the fractional composition of a given microemulsion vehicle could be optimized using the values of drug self-diffusion coefficient, which could be determined more easily than transdermal permeation rate. Certainly, further studies are required to elaborate the above hypothesis.

Since microemulsions may act as penetration enhancers and this activity is dependent on the dissociation of the microemulsion on the skin, the diffusion coefficients of other components (Labrasol, oleic acid, and Transcutol P) and the permeability were correlated. A partial linear relationship was found (R = 0.7982 when across the rat skin, R = 0.8267 when across the human skin).

In order to find other factors correlated with the permeability, the viscosity of the microemulsion was taken into consideration. The correlation between viscosity and permeability was good (R = 0.9224 when across the rat skin, R = 0.9159 when across the human skin). The permeability increased with the decrease of the viscosity of the microemulsion.

Combining the results of Table 3 and Fig. 6, the ratio of S/CoS was ensured as 1:1.

At the 1:1 ratio of S/CoS, the drug solubility increased when the content of $S_{mix}$ increased from 41% to 70%, while the skin permeation rate significantly decreased ($P < 0.05$). Concerning the diffusivities of water in the NMR determination, we found good linear relationship between water diffusivity and permeability (R = 0.8615 when across the rat skin, R = 0.8899 when across the human skin). According to the literature,[13] this result might be due to the hydration effect of water. When the water content was increased from 20% in formulation A1 to 55% in formulation C1, the diffusivity of water increased and then the hydration of stratum corneum increased. As a result, the permeation rate also increased. Another explanation could be found in the paper of Yun et al., who concluded this might be due to the increased thermodynamic activity of the drug in the microemulsion at the lower content of the surfactant, in which vinpocetine could be more soluble than in water.[26]

Finally, the optimized composition of microemulsion containing 1% vinpocetine was confirmed as 4%

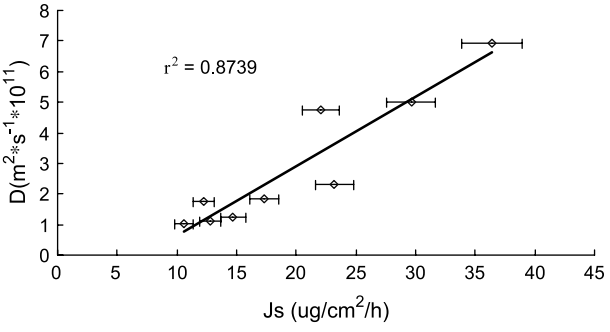


***Figure 7.*** Correlation between self-diffusion coefficient (D) and transdermal permeation rate (Js) across the rat skin of vinpocetine in microemulsions (A1, A2, A3, B1, B2, B3, C1, C2, C3) with 1% vinpocetine (mean ± SD, n = 3). Vertical bars indicate SD. (*View this art in color at www.dekker.com.*)

oleic acid, 20.5% Labrasol, 20.5%Transcutol P, and 55% distilled water base on the systematical analysis.

The drug solubility in the optimized microemulsion was 15.8±0.8 mg/mL at 25±1°C, which was about 3160-fold higher compared to the solubility of vinpocetine in water (5 μg/mL).[7] The apparent permeation rate of vinpocetine was determined to be 36.4±2.1 μg/cm$^2$/h across the rat skin and 7.05±0.67 μg/cm$^2$/h across the human skin by the in vitro permeation study. The vinpocetine clearance was about 43.0 L/h, the steady state concentration was then obtained as about 3.14 ng/mL. The known limited steady state concentration that is needed in the treatment was about 0.8 ng/mL.[7] So the transdermal delivery of vinpocetine from microemlusion is valuable in practical application.

### Physicochemical Characterization

The optimized microemulsion containing 1% vinpocetine of oleic acid/ Labrasol/Transcutol P/distilled water (4%/20.5%/20.5%/55%) had ideal viscosity (14.5 ± 1.3 mPa·s), appropriate pH values (6.3 ± 0.6) and homogeneous particle size (36.0 ± 4.4 nm) with low polydispersity (0.30), relatively high conductivity (50.2 ± 0.8 μs/cm), and refractive index (1.39 ± 0.08) at 25 ± 1°C. The microemulsion had good shelf stability, showing no changes in these physicochemical parameters over 12 months of storage at 25 ± 1°C. No phase separation, either cloudiness or two distinct layers formation, was seen at ambient temperature.

### Skin Irritancy Test

The skin irritancy test of the optimized microemulsion showed that the mean value of the resultant indices is 0. Utely and Van Abbe mentioned that a value between 0 and 9 indicates that the applied formulation probably would not be irritant to human skin.[24] Thus the optimized microemulsion is considered to be safe for the use of transdermal drug delivery.

## CONCLUSION

The microemulsion prepared with oleic acid as oil phase, Labrasol as surfactant, Transcutol P as cosurfactant, and double-distilled water showed a high solubility potential and transdermal permeation ability for poorly water-soluble vinpocetine. A linear correlation was found between the self-diffusion coefficient and transdermal permeation rate of drug in the microemulsion. This could be used to optimize the composition of a given microemulsion vehicle in order to maximize the transdermal delivery of the drug. The results of physicochemical property and skin irritancy tests showed that the optimized microemulsion was a promising vehicle for transdermal application.

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