Division of Pharmaceutics, Department of Pharmacy, Shenyang Pharmaceutical University, Shenyang, P.R. China

Preparation and evaluation of microemulsion of vinpocetine for transdermal delivery

L. HUA, P. WEISAN, L. JIAYU, L. HONGFEI

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Hua Li, Shen Yang Pharmaceutical University, Department of Pharmacy, We Hua RD 103, 110016 Shen Yang, P.R. China
Lh7618@21cn.com

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Poorly soluble vinpocetine was selected as the model drug to prepare a microemulsion in order to increase solubility and *in vitro* transdermal delivery of the drug. Oleic acid was chosen as the oil phase due to its excellent solubilizing capacity. PEG-40 hydrogenated castor oil (Cremophor® RH40) was employed as a surfactant (S) and purified diethylene glycol monoethyl ether (Transcutol P®) was used as a cosurfactant (CoS). The effects of diverse types of oil, different weight ratios of surfactant to cosurfactant (S/CoS) on the solubility and permeation rate of vinpocetine were investigated. The optimized microemulsion consisted of 1% vinpocetine, 4% oleic acid, 20% Cremophor® RH40, 10% Transcutol P® and 65% distilled water (w/w), in which drug solubility was about 2,100 fold compared to that in water and the apparent permeation rate across the excised rat skin was 15.0 ± 2.5 μg/cm^2/h. Finally the physicochemical properties of the optimized microemulsion including pH, viscosity, refractive index, conductivity and particle size distribution were examined, which showed stable behavior after more than 12 months at ambient temperature. The irritation study showed that optimized microemulsion was a safe transdermal delivery system.

1. 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 (Miyazaki 1995; Szakall et al. 1998; Bowler et al. 1995; Bukanova et al. 1998). However, it undergoes a marked first-pass effect after oral administration, during which approximately 75% of the substance is metabolized, leading to an absolute bioavailability of 7% in man (Manuela et al. 1996). Efforts to avoid above-mentioned disadvantages should be attempted.

It has been reported that vinpocetine is a good candidate for transdermal delivery due to its appropriate partition coefficient ($\log K_{o/w} = 3.56$) and various transdermal formulations have been reported, including patch, gel and cream (Kobayashi et al. 1993; Hidaka et al. 1993; Kuniyoshi et al. 1993). Then how to further improve the permeability of the drug through the skin and widen its clinical use has been a focus. One way is to use skin penetration enhancers reported by Daisuke K and his coworkers (Kobayashi et al. 1993), another promising way is to develop appropriate vehicles to increase the solubility of the drug and then increase the permeation.

Microemulsions are clear dispersions of one immiscible liquid in another brought about by the presence of a suitable surfactant, usually in conjunction with a cosurfactant. It has several interesting characteristics, namely: enhanced drug solubility, simplicity of preparation and increased local or systemic delivery (Gasco 1997). 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 drugs due to the high solubilizing capacity (Ktistis et al. 1998). 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 the 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 (Elena et al. 2001). And the percutaneous absorption of drug will also be increased due to the hydration effect of the stratum corneum if the water content in microemulsion is high enough (Mohammed et al. 2000).

The aim of this study was to incorporate vinpocetine into microemulsions after screening oils and optimize the formulation to achieve high solubility and skin permeation rate. In addition, the physiochemical stability and skin irritation were evaluated.

2. Investigations, results and discussion

2.1. Solubility of vinpocetine in different oils

The solubility of vinpocetine was determined in 6 oils, respectively, 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). Fig. 1 shows that of the oils, the drug solubility was the highest in Mai-

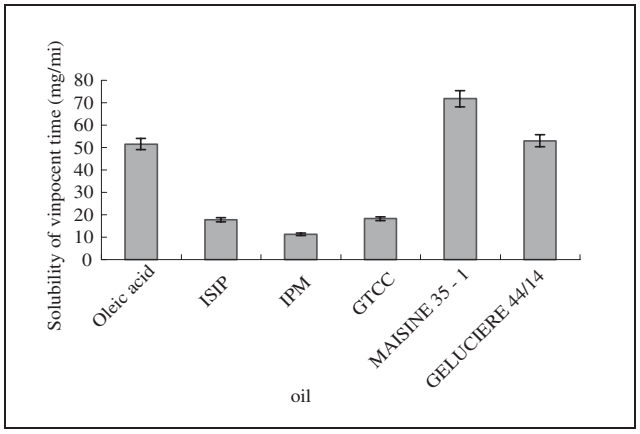

Fig. 1: The solubility of vinpocetine in various oils at 37 ± 1 °C (mg/ml, mean ± SD, n = 3)

sine 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), while solubility in other oils were relatively low. Oleic acid, Maisine 35-1 and Glucire 44/14, which showed high solubility of vinpocetine, were then used in the preparation of microemulsions.

2.2. Preparation of pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were constructed to obtain the components and their concentration ranges of the microemulsions. Fig. 2 showed the phase diagram of an o/w microemulsion consisting of oleic acid, Cremophor® RH 40, Transcutol P® and water. The staining method was used to identify if the transparent system was an o/w microemulsion (Hsiu et al. 1996). Similar results were obtained from phase diagrams composed of Maisine 35-1 and Glucire 44/14 as oils. From the diagram, we can see that the areas of microemulsion increased with the increasing ratio of surfactant to cosurfactant (S/CoS). When S/CoS was 0.5 : 1, the region of the microemulsion was too narrow to form a stable formulation. This agreed with the results reported by Zhong-Gao Gao et al. who prepared a microemulsion using caprylic/capric triglyceride as oil, polyoxyethylated castor oil (Cremophor EL) as a surfactant, Transcutol P as a cosurfactant and saline (Zhong et al. 1998). Then different microemulsions within the shaded region were prepared to evaluate the effects of microemulsion composition with various oils on the drug solubility.

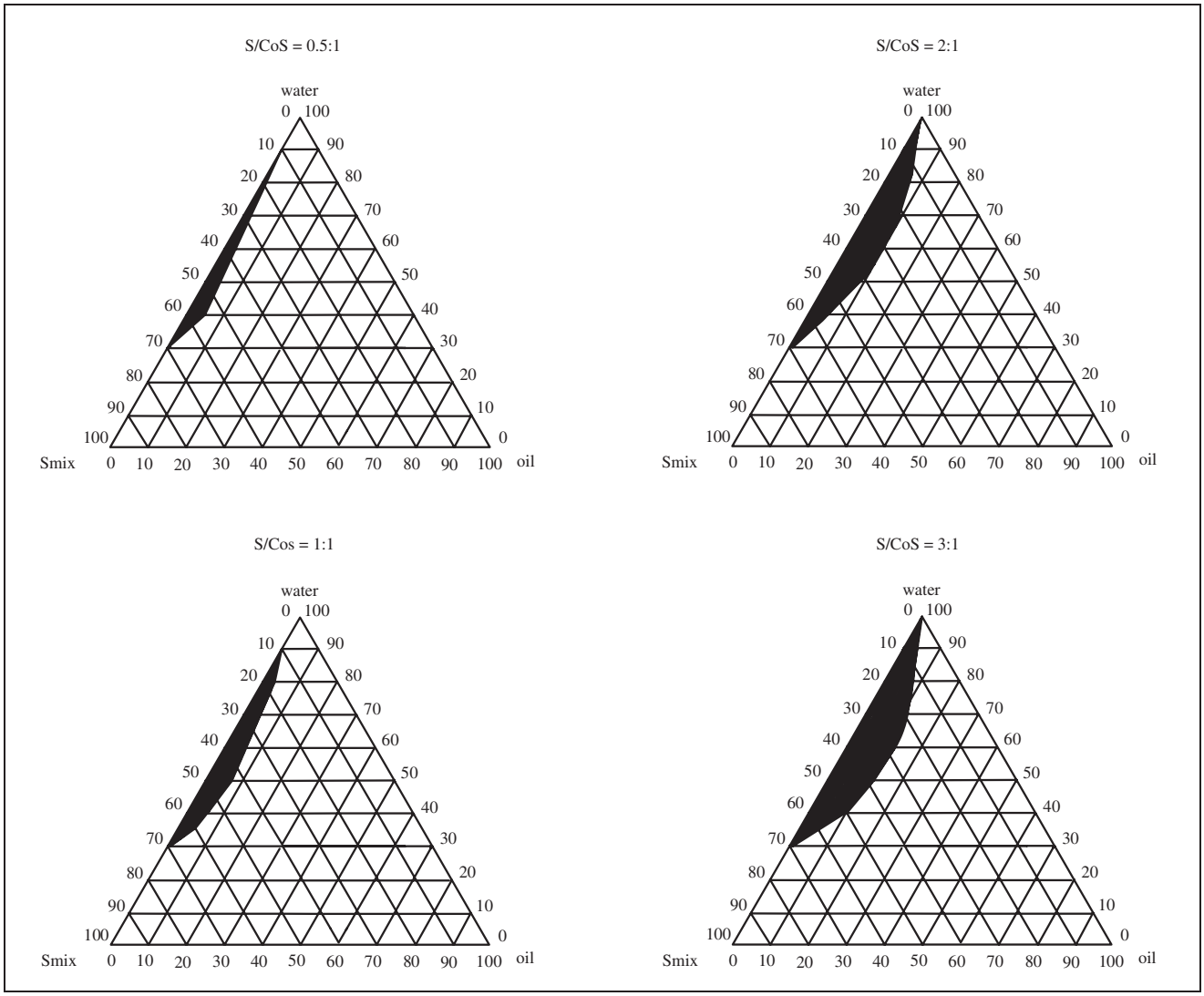

Fig. 2: Pseudo-ternary phase diagram composed of oleic acid, Cremophor® RH40-Transcutol P® mixture (S_{mix}) (S/CoS = 0.5 : 1, 1 : 1, 2 : 1, 3 : 1) and distilled water. One axis representing water, one representing oil and the third representing the S_{mix}. The concentration of each component is in weight percent (w/w). The shaded region represents the area of o/w microemulsion existence

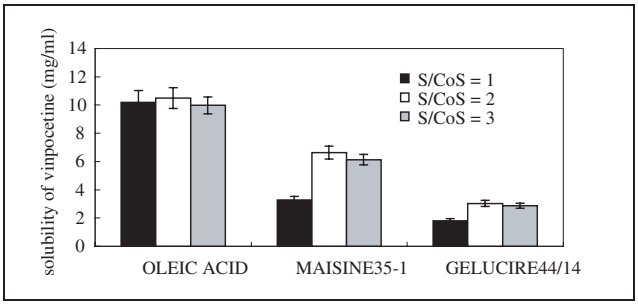

Fig. 3: The solubility of vinpocetine in various microemulsion at 25 ± 1 °C (mean ± SD, n = 3). The test microemulsions consist of 4% oil content (oils employed are oleic acid, Maisine® 35-1 and Gelucire® 44/14) and 40% Cremophor®RH40-Transcutol P® mixture with different S/CoS ratio (1 : 1, represented by black bar; 2 : 1, represented by white bar; 3 : 1, represented by gray bar) and 56% distilled water

2.3. Screening of oils for microemulsion

The solubility of vinpocetine determined in various microemulsions is shown in Fig. 3. The test microemulsions consisted of 4% oil content (oils employed are oleic acid, Maisine 35-1 and Gelucire 44/14) and 40% Cremophor RH40-Transcutol P mixture with different S/CoS ratio (1 : 1, 2 : 1, 3 : 1) and 56% water. Although the drug solubility in oleic acid was lower than that in Maisine 35-1 and Gelucire 44/14, Fig. 3 shows that the drug solubility in the microemulsion containing oleic acid as oil phase was significantly higher than that in other microemulsions ($P < 0.05$).

With each oil, we found that the solubility of vinpocetine in the microemulsion increased with the increasing ratio of S/CoS from 1 : 1 to 2 : 1 and then decreased when the ratio was 3 : 1. When the Cremophor RH40-Transcutol P mixture (S_{mix}) was fixed at 30% and 50% respectively, the same tendency could be seen. This results might be explained by the fact that increasing the content of Transcutol P from 3 : 1 to 2 : 1, in which the drug was very soluble, would increase the solubilizing capacity of the microemulsion. However, drug solubility decreased at a ratio of 1 : 1 due to the formation of larger droplet sizes than that at a ratio of 2 : 1. Previous reports indicated that the superior transdermal flux appears to be mainly due to the large solubility capacity of the microemulsion, which leads to larger concentration gradients towards the skin (Kreilgaard et al. 2000). Previous reports also confirmed that oleic acid was a powerful enhancer for transdermal delivery (Kanikkanan et al. 2000; Yun et al. 2001). Considering these rules, oleic acid was selected as oil phase and the composition of microemulsion was further optimized according to the *in vitro* skin permeation study.

2.4 In vitro skin permeation measurement

The Table and Fig. 4 show the effects of the different composition of microemulsions on the skin permeation of vinpocetine. The test microemulsions were prepared with oleic acid as oil phase, Cremophor RH 40 as surfactant, Transcutol P as cosurfactant and distilled water. Fig. 4 indicates that test microemulsions containing vinpocetine had excellent zero-order release characteristics *in vitro*.

As the ratio of S/CoS increased from 1 : 1 to 2 : 1 when the content of Cremophor RH40-Transcutol P mixture (S_{mix}) was fixed, the skin permeation rate was significantly increased ($P < 0.05$), while decreased when the ratio was 3 : 1. A similar result was obtained by Kreilgaard et al. (2000) who confirmed that this result could be explained by the fact that the drug delivery potential of the microemulsions was greatly dependent on the internal structure/fractional composition of the system. More studies would be desirable to determine the relationship indicated by this study. Combining these results, the ratio of S/CoS was ensured as 2 : 1.

Table: Composition and permeation parameters of the microemulsion with 1% vinpocetine, 4% oleic acid as oil phase, Cremophor® RH 40 as surfactant, Transcutol P® as cosurfactant and distilled water

Microemulsion	Phase composition (%, w/w)				Parameter	
	Oil	S	CoS	Water	J_S (μg/cm²/h)	T_{lag} (h)
A1	4	15.0	15.0	65	4.4 ± 0.5	0.7 ± 0.2
A2	4	20.0	10.0	65	15.0 ± 2.5	1.4 ± 0.5
A3	4	22.5	7.5	65	10.5 ± 1.8	1.1 ± 0.4
B1	4	20.0	20.0	55	6.3 ± 1.0	1.9 ± 0.8
B2	4	26.7	13.3	55	9.4 ± 1.1	0.6 ± 0.2
B3	4	30.0	10.0	55	7.5 ± 0.8	2.4 ± 1.2
C1	4	25.0	25.0	45	5.8 ± 0.5	0.3 ± 0.1
C2	4	33.3	16.7	45	8.0 ± 1.2	1.9 ± 0.9
C3	4	37.5	12.5	45	6.8 ± 1.2	0.9 ± 0.1

(Mean ± S.D, n = 3)

As the ratio of S/CoS was 2 : 1, the drug solubility increased when the content of S_{mix} increased from 30% to 40%, while the skin permeation rate decreased (found from formulation B2 to A2 in the Table). According to the literature (Mohammed et al. 2000), this result might be owing to the hydration effect of water. When the content of water increased from 55% in formulation B2 to 65% in formulation A2, the hydration of the stratum corneum was increased and a higher permeation was then obtained. Another explanation was found in the paper of Yun et al. (2001) who thought this might due to the increased thermodynamic activity of the drug in the microemulsion at the lower content of the surfactant, as vinpocetine is poorly water-soluble, but soluble in the surfactant mixture.

Data were analyzed synthetically and the optimized composition of the microemulsion containing 1% vinpocetine was confirmed as 4% oleic acid, 20% Cremophor RH40, 10% Transcutol P and 65% distilled water (formulation A2 in the Table, % presented as w/w).

The drug solubility at 25 ± 1 °C in the optimized microemulsion is 10.5 ± 1.5 mg/ml, which was about 2100 fold compared to the solubility of vinpocetine in water (5 μg/ml)

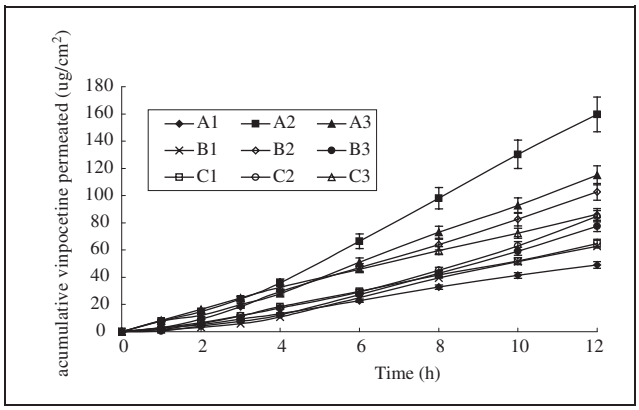

Fig. 4: The permeation profiles of vinpocetine from microemulsion (see Table) A1, A2, A3, B1, B2, B3, C1, C2, C3 (mean ± SD, n = 3), Vertical bars indicate S.D

(Kobayashi et al. 1993). The *in vitro* permeation study across the excised rat skin resulted in an apparent permeation rate of 15.0 ± 2.5 μg/cm^2/h.

2.5. Physicochemical characterization

The physicochemical characterization data indicated that the optimized microemulsion containing 1% vinpocetine with oleic acid/Cremophor RH 40/Transcutol P/distilled water (4/20/10/65) showed ideal viscosity (20.2 ± 1.2 mpa · s), appropriate pH values (6.3 ± 0.6) and homogeneous particle size (32.3 ± 1.3 nm) with lower polydispersity (0.30), high conductivity (49.2 ± 0.8 μs/cm) and refractive index (1.38 ± 0.08) at 25 ± 1 °C. The microemulsion had good shelf stability, as no changes in these physicochemical parameters were observed over 12 months of storage at 25 ± 1 °C. No phase separation as shown by cloudiness or the formation of two distinct layers was seen at ambient temperature.

2.6 Skin irritation test

The skin irritation test was carried out to investigate the potential irritancy of the optimized microemulsion containing 1% vinpocetine with oleic acid/Cremophor RH® 40/ Transcutol P®/distilled Water (4/20/10/65). The mean value of the resultant indices is 0. According to Utely and Van Abbe, values between 0 and 9 indicate that the applied formulation probably would not irritate human skin. Thus, the optimized microemulsion is safe for transdermal drug delivery.

3. Experimental

3.1. Materials

Vinpocetine was supplied by Shenyang Dongbei Pharmaceutical Corporation, China. Isopropyl isostearate (ISIP) was supplied by Shanghai Yangjia Chemical Ltd. Oleic acid was from Shenyang Chemical Plant. Isopropyl myristate (IPM) and caprylic/capric triglyceride (GTCC) were kindly donated by Croda, UK, glyceryl monolinoleate (Maisine 35-1), lauroyl macrogolglycerides (Glucire 44/14) and diethyleneglycol monoethyl ether (Transcutol P) by Gattefosse, France, PEG-40, hydrogenated castor oil (Cremophor RH 40) by BASF, Germany, respectively. All other chemicals were of analytical grade.

Skin samples were obtained from male Wistar rats weighting 250 ± 20 g. After hair was shaved carefully, a patch of skin was excised from the dorsal portion from each sacrificed rat. Then subcutaneous fat was trimmed. The excised skin samples were stored at −20 °C far one week.

3.2. Preparation of the microemulsions

Cremophor RH40 (S) was gently heated before use and then mixed with 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 an isotropic and transparent mixture was obtained. The mixture was kept at ambient temperature (25 °C) to get to equilibrium. The dilution and dye solubility test was performed to identify the type of the microemulsion (Hsiu et al. 1996). 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 weigh ratio of S/CoS on the area of O/W microemulsion region was investigated.

Within the microemulsion region in the pseudo-ternary phase diagram, the microemulsions containing vinpocetine were produced by adding drug into the oil-S_{mix} mixture and then mixed with double-distilled water under gentle agitation.

3.3. Solubility of vinpocetine

The solubility of vinpocetine was determined in 6 oils and also in prepared microemulsions. An excess amount of vinpocetine was added to each dissolute medium and the mixture was stirred for 72 h at 37 ± 1 °C in oils and 25 ± 1 °C in microemulsions for the determination of drug solubility in different media. Triplicate samples were centrifuged at 10,000 rpm for 10 min. Then, aliquots of supernatant were filtered through 0.45 μm membrane filters to obtain clear fluid and the solubility of vinpocetine was determined by HPLC after dilution with methanol of HPLC grade. For each sample, three replicate assays were performed.

3.4. In vitro skin permeation of vinpocetine

Vertical Franz cells were used and the previously excised rat skin was employed. The area of the cell surface was 2.5 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 30% ethanol aqueous solution and magnetically stirred. The diffusion cells were thermostated at 25 ± 1 °C using a re-circulating water bath (79HW-1, Zhejiang, China). At hourly intervals, the entire contents of the receptor cell were withdrawn and the cell was refilled with fresh receiving solution. The samples were filtered (0.45 μ m) and analyzed by HPLC. At least three samples of each formulation were evaluated.

3.5. Physicochemical characterization of study microemulsion

Physicochemical parameters were measured at 25 ± 1 °C. pH was determined with a pH meter (pH S-2C, Shanghai, China). Viscosity was measured with a capillary viscometer. (Tianjing, China). Conductivity was measured with a conductivity meter (DDS-11C, Tianjing, China). Refractivity index was measured with 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 the microemulsions. Light scattering was monitored at 90° angle and 25 ± 1 °C.

3.6. Irritancy test

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 considered as a control. The development of erythema was monitored daily for 6 days using the method of Utelly and Van Abbe.

3.7. HPLC analysis of vinpocetine

Vinpocetine was assayed by reversed HPLC (Shimadzu LC-10AT vp). The mobile phase consisted of methanol/0.1 mol · 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 · min^{-1} and the UV detector (Shimadzu, SPD 10-AV) was set at $\lambda = 268$ nm.

3.8. Data analysis and statistics

The cumulative amount of vinpocetine permeated through the skin was plotted as a function of time. The slope of the linear portion of the curve and the intercept values (T_{lag}, h) were derived by regression. The permeation rate at steady-state (Js, μg/cm^2/h) was calculated as the slope.

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

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