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Formulation design of microemulsion for dermal delivery of penciclovir

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ABSTRACT

The purpose of the present study was to evaluate the potential application of microemulsions as a dermal drug delivery loading penciclovir. The pseudo-ternary phase diagrams were developed for various microemulsion formulations composed of oleic acid (oil phase), Cremorphor EL (surfactant) and ethanol (cosurfactant). Composition of microemulsion systems was optimized using simplex lattice mixture design including the concentrations of surfactant, cosurfactant and water (independent variables) and the solubility and the cumulative amount of penciclovir permeated through excised mouse skins per unit area (response variables). The physicochemical properties of the optimized microemulsion and the permeating ability of penciclovir from microemulsions were also investigated. The results showed that the optimized microemusion formulation was composed of oleic acid (5%, w/w), Cremorphor EL (20%, w/w), ethanol (30%, w/w) and water (45%, w/w). The mean particle diameter was 36.5 nm and solubility of penciclovir in the emulsion was 7.41 mg g^{-1}. The cumulative amount of penciclovir permeated through excised mouse skins from microemulsion was about 3.5 times that of the commercial cream. The conclusion was that the permeating ability of penciclovir was significantly increased from the microemulsion formulation compared with commercial cream.

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1. Introduction

Penciclovir, 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine, is a potent antiviral guanosine-type drug which maintains the antiviral activity against herpes simplex virus, varicella zoster virus, epstein-barr virus, hepatitis virus and cytomegalovirus (Smith et al., 2001; Abdel-Hag et al., 2006; Schmid-Wendtner and Korting, 2004; Andrei et al., 2004). However, the poor bioavailability (5–10% after oral administration) limited its clinical use. One promising route of penciclovir administration is dermal delivery. Various dermal formulations of penciclovir including cream and gel have been reported and appropriate vehicles in enhancing the permeation ability of penciclovir have attracted increasing attention in recent years. Yang et al. encapsulated penciclovir in liposome and then dispersed the liposome suspension into Carbopol gel (penciclovir content: 1%, w/w). The drug concentration penetrating into skins

of mice after liposome gel was administered to the abdominal skin of mice in vivo for 18 h was 1.94 times that of the commercial cream administrated at the same concentration and time (Yang and Wang, 2005).

Microemulsion is defined as an O/W or W/O emulsion producing a transparent product that has a droplet size from 10 to 100 nm and does not have the tendency to coalesce (Tenjarla, 1999; Kreilgaard, 2002; Lawrence and Rees, 2000). It is composed of oil phase, surfactant, cosurfactant and aqueous phase at appropriate ratios (Changez and Varshney, 2000). Microemulsion has several specific physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability (Lawrence and Rees, 2000; Changez and Varshney, 2000; Baroli et al., 2000). So it is promising for both transdermal and dermal delivery of drugs as an efficient route of drug administration (Kreilgaard, 2002; Rhee et al., 2001; Kreilgaard et al., 2000; Baboota et al., 2007; Kamal et al., 2007; Chen et al., 2007). Lee et al. reported that O/W microemulsion systems composed of Tween 80, n-methyl pyrrolidone (NMP) and oleyl alcohol could enhance the permeability of drugs. For example, lidocaine microemulsion can have 17-fold greater permeability than solution formulation. Permeability of estradiol microemulsion and diltiazem HCl microemulsion can be increased 58-fold and 520-fold, respectively, compared with the solution formulations (Lee et al., 2003).

Several mechanisms have been proposed to explain the advantages of microemulsion for the transdermal and dermal delivery of drugs (Gupta et al., 2005; Peltola et al., 2003; Changez et al., 2006a,b; Zhao et al., 2006; Chen et al., 2006). First, the thermodynamic towards the skin is increased due to large amount of a drug incorporated in the formulation. Second, the increased thermodynamic activity of the drug may favor its partitioning into the skin. Third, the ingredients of microemulsion may reduce the diffusional barrier of the stratum corneum and increase the permeation rate of drug via skin by acting as permeation enhancers. Also, the hydration effect of microemulsion on the stratum corneum may influence the permeation ability of formulations (Mohammed and Manoj, 2000). Although many drugs have been incorporated in microemulsion for transdermal and dermal delivery, penciclovir has not been evaluated.

In this study, O/W microemulsions containing 0.5% penciclovir have been developed after screening oils and surfactants. Pseudo-ternary phase diagrams were constructed to obtain the components and their concentration ranges, and the microemulsion formulations varied according to a simplex lattice design to find out the most suitable components ratio for the optimized formulation.

2. Materials and methods

2.1. Materials

Penciclovir (purity 99%) was procured from Lizhu Co., Ltd. (Changzhou, China). Cremorphor EL was purchased from Sigma Chemical Co. (St. Louis, USA). Ethanol and Tween 80 was purchased from Guangcheng chemical reagent Co. Ltd. (Tianjin, China). Oleic acid (OA) was purchased from Kemiou chemical reagent Co. Ltd. (Tianjin, China). Isopropyl myristate (IPM) was obtained from Fluka AG (Switzerland). Medium chain triglycerides (MCT) was obtained from Cognis Co. (Germany). Ethyl oleate (EO) was purchased from Shanghai chemical Co. (Shanghai, China). Triple distilled water was used for the preparation of microemulsions. All other chemicals and solvents were of analytical reagent grade.

2.2. Screening of oils and surfactants for microemulsions

To find out appropriate oil phase and surfactant in microemulsions, the solubility of penciclovir in various oils such as OA, IPM, MCT and EO and surfactants including Tween 80 and Cremorphor EL were measured. An excess amount of penciclovir was added to 5 ml of oil or surfactant and then the resulting mixture was shaken reciprocally at 37 °C for 72 h followed by centrifugation for 10 min at 12000 rpm. The supernatant was filtered through a membrane filter (0.45 μm) and the drug concentration in the filtrate was determined by high performance liquid chromatography (HPLC) analysis after the appropriate dilution with ethanol.

The oil and surfactant that showed high solubility of penciclovir were used in the preparation of microemulsions containing 0.5% penciclovir. Their effects on the skin permeation of penciclovir from the prepared microemulsions were evaluated.

2.3. Construction of phase diagrams and formulation of penciclovir microemulsions

2.3.1. Construction of pseudo-ternary diagrams

Pseudo-ternary phase diagrams were constructed in order to obtain the concentration range of components for the existing range of microemulsions. The weight ratio of surfactant to cosurfactant (Km) varied as 1:2, 1:3, 1:1, 2:1 and 3:1. For each pseudo-ternary phase diagram at a specific surfactant/cosurfactant weight ratio, the oily mixtures containing oil, surfactant and cosurfactant were prepared with the weight ratio of oil to the mixture composed of surfactant and cosurfactant at 5:95, 10:90, 15:85, 20:80, 25:75, 30:70, 35:65, 40:60, 45:55, 50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, 90:10 and 95:5, respectively. Water was added drop by drop to each oily mixture under proper magnetic stirring at 37 °C until the mixture became clear at a certain point. The concentrations of components were recorded in order to complete the pseudo-ternary phase diagrams, and then the contents of oil, surfactant, cosurfactant and water at appropriate weight ratios were selected based on these results.

2.3.2. Preparation of penciclovir-loaded microemulsion systems

According to the microemulsion areas in the phase diagrams, the penciclovir-loaded microemulsion formulations were selected at different component ratios. Microemulsion systems were obtained by mixing oil, surfactant and cosurfactant together, and adding precisely water drop by drop to these oily phases with magnetic stirring at ambient temperature. After the resulting systems were equilibrated with gently magnetic stirring for 30 min, appropriate amount of penciclovir was dissolved in them under ultrasonication. The final concentration of penciclovir in microemulsion systems was 0.5% (w/w).

2.4. Characterization of microemulsion

The morphology of penciclovir microemulsions was observed using transmission electron microscopy (JEM-100CXII, Japan). One drop of diluted samples was deposited on a film-coated copper grid and later stained with one drop of 2% aqueous solution of phosphotungstic acid (PTA) and allowed to dry before examined under the electron microscope. The average droplet diameters of the microemulsions were determined by photon correlation spectroscopy instrument (BI-200SM, Brookhaven Instrument, USA) at a temperature of 25 °C.

The viscosities of various microemulsion vehicles were measured at 25 °C, using the NDJ-8S digital viscometer (Shanghai Precision and Scientific Instrument, Shanghai, China) with a No. 1 rotor set at 60 rpm. Electrical conductivity was measured using a conductivity meter (DDS-11C, Shanghai Instrument, China). Based on electrical conductivity, the phase systems of the microemulsions were determined.

The pH values of microemulsions were determined at 25 °C using the Phs-3C digital acidimeter (Shanghai Rex Instruments Factory), and refractive indices were measured with a thermostated Abbe refractometer (Shijiazhuang Optical Instrument Factory, China).

The optimized microemulsions containing penciclovir were stored at ambient temperature for 6 months, and then the clarity, phase separation and concentration of penciclovir were investigated. The centrifuge tests at 12000 rpm for 30 min were carried out to assess the physical stability of microemulsions (Chen et al., 2004).

2.5. In vitro permeation experiments

2.5.1. Preparation of skin

Male Kunming mice weighing 20 ± 2 g (SCXK (Lu) 20030004) were purchased from Experimental Animal Center of Shandong University (Shandong, China) for the in vitro permeation studies. Skin from the abdominal region was excised after hair was removed with a depilatory, and then the subcutaneous fat and connective tissue were removed. The obtained skins were washed and examined for integrity, and then placed in a refrigerator at 4 °C overnight, and then used for the permeation experiments (Chen et al., 2004).

2.5.2. In vitro permeation study

Franz diffusion cell with an effective diffusion area of 3.8 cm² was used for in vitro permeation studies. The skin samples were mounted carefully on diffusion cells with the stratum corneum side up, donor solutions consisting of 0.8 g of test microemulsions containing 4 mg penciclovir or 0.8 g cream containing 8 mg penciclovir. The receiver compartment was filled with 15 ml normal saline to ensure sink condition and its temperature was maintained at $37 \pm 0.5\,^{\circ}C$ with magnetic stirring at 600 rpm throughout the experiment. For each experiment, 1 ml sample of the receiver medium was withdrawn at predetermined time and then the volume was made up with the equal volume of fresh receiver medium. All samples were filtered through a 0.45 μm pore size cellulose membrane filter and analyzed by HPLC.

2.5.3. Calculation of the in vitro data

Cumulative amount of drug (Q_n, μg cm⁻²) in the receiver compartment was plotted as a function of time (t, h), and the cumulative amount of penciclovir permeated through excised mouse skins was determined based on the following equation:

$$Q_n = \frac{C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_i}{S} \tag{1}$$

where C_n stands for the drug concentration of the receiver medium at each sampling time, C_i for the drug concentration of the ith sample, and V_0 and V_i stand for the volumes of the receiver solution and the sample, respectively, S for the effective diffusion area.

2.6. High performance liquid chromatography analysis of samples

The samples were analyzed using the HPLC system including a separations module (Waters 2695), a diode array detector (Waters 2996) and a reversed phase C_{18} column (5 μm, 4.6 mm × 250 mm, Dikma). The mobile phase was a mixture of 0.1% acetic acid solution/acetonitrile at a ratio 98:2 (v/v) with the flow rate at 1 ml/min and the detection wavelength was set at 253 nm. Aliquots of 20 μl of each sample were injected into the column, and all operations were carried out at ambient temperature.

The peak area correlated linearly with penciclovir concentration in the range of 5–50 μg/ml with the lowest detection limit at 0.5 μg/ml, and the average correlation coefficient was 0.9998.

2.7. Formulation optimization

A simplex lattice experiment design was adopted to optimize the composition of microemulsions (Jumaa et al., 1998; Subramanian et al., 2004; Li et al., 2004a,b; Ho et al., 1994). In this design, three factors were evaluated by changing their concentrations simultaneously and keeping their total concentration constant. The simplex lattice design for a three-component system is represented by an equilateral triangle in two-dimensional space (Fig. 1). Seven batches were prepared as follows: one at each vertex (A, B and C), one at the halfway point between vertices (AB, BC and AC), and another one at the center point (ABC). The concentrations of surfactant, cosurfactant and water were selected as independent variables. The solubility and the cumulative amount of penciclovir permeated through excised mice skins per unit area were taken as responses.

The responses for seven formulations were used to fit an equation for simplex lattice model (Hong and Wu, 2004; Mao et al., 2004; Ren, 2003) which then can predict the properties of all possible formulations. Graphs of these properties in the form of contour plots were constructed with Origin7.5 Software (Liu et al.,
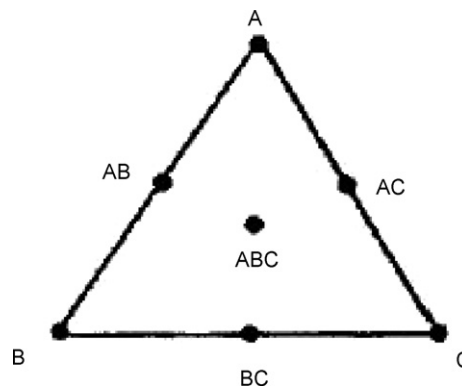

Fig. 1. Equilateral triangle representing simplex lattice design for three components.

1995; Van Kamp et al., 1987). With the aid of a computer program SPSS13.0 Software, the model equation was developed to represent the relationship between the solubility or permeation rate and the measured characteristics.

2.8. In vitro permeation behavior of the optimized microemulsion and the commercial cream

According to the method described in the Section 2.5, in vitro permeation behavior of the optimized microemulsion containing penciclovir (0.5%, w/w) was compared with the commercial cream (penciclovir content 1.0%, w/w) as a control one.

2.9. Statistical analysis

Data were shown as mean ± S.D. ($n = 5$). Statistical data were analyzed by the Student's t-test at the level of $p = 0.05$.

3. Results and discussion

3.1. Screening components for microemulsions

The solubility of penciclovir in various media was analyzed in order to screen components for microemulsions. In four oils, the solubility of penciclovir was highest in oleic acid (256 μg/g), followed by ethyl oleate (154.6 μg/g), and that in other two oils (IPM and MCT) was relatively low (70.0 and 47.5 μg/g). Previous reports indicated that the superior dermal flux appeared mainly due to the large solubilizing capacity of the microemulsions, which led to larger concentration gradient towards the skin (Kreilgaard et al., 2000; Sintov and Shapiro, 2004). It was also reported that oleic acid was a powerful enhancer for dermal delivery since it could increase fluidity of lipid portion of the stratum corneum which resulted in a permeation enhancing effect (Li et al., 2004a,b; Paolino et al., 2002; Kogan and Garti, 2006; Zhao et al., 2006; Kreilgaard et al., 2000). So oleic acid was chosen for the preparation of the microemulsions containing penciclovir.

In two used surfactants, penciclovir had a higher solubility in Cremorphor EL (420.5 μg/g) than that in Tween 80 (199.1 μg/g). Ethanol has a good ability in forming microemulsions with oleic acid and Cremorphor EL, and its aqueous solution has a good solubility of penciclovir which can form a concentration gradient. Also, ethanol can be able to act as a permeation enhancer (Kogan and Garti, 2006; Biruss et al., 2007; Yuan et al., 2006). So oleic acid, Cremorphor EL and ethanol were subsequently used as the oil phase, surfactant and cosurfactant for the formulation of microemulsions containing penciclovir in this study.
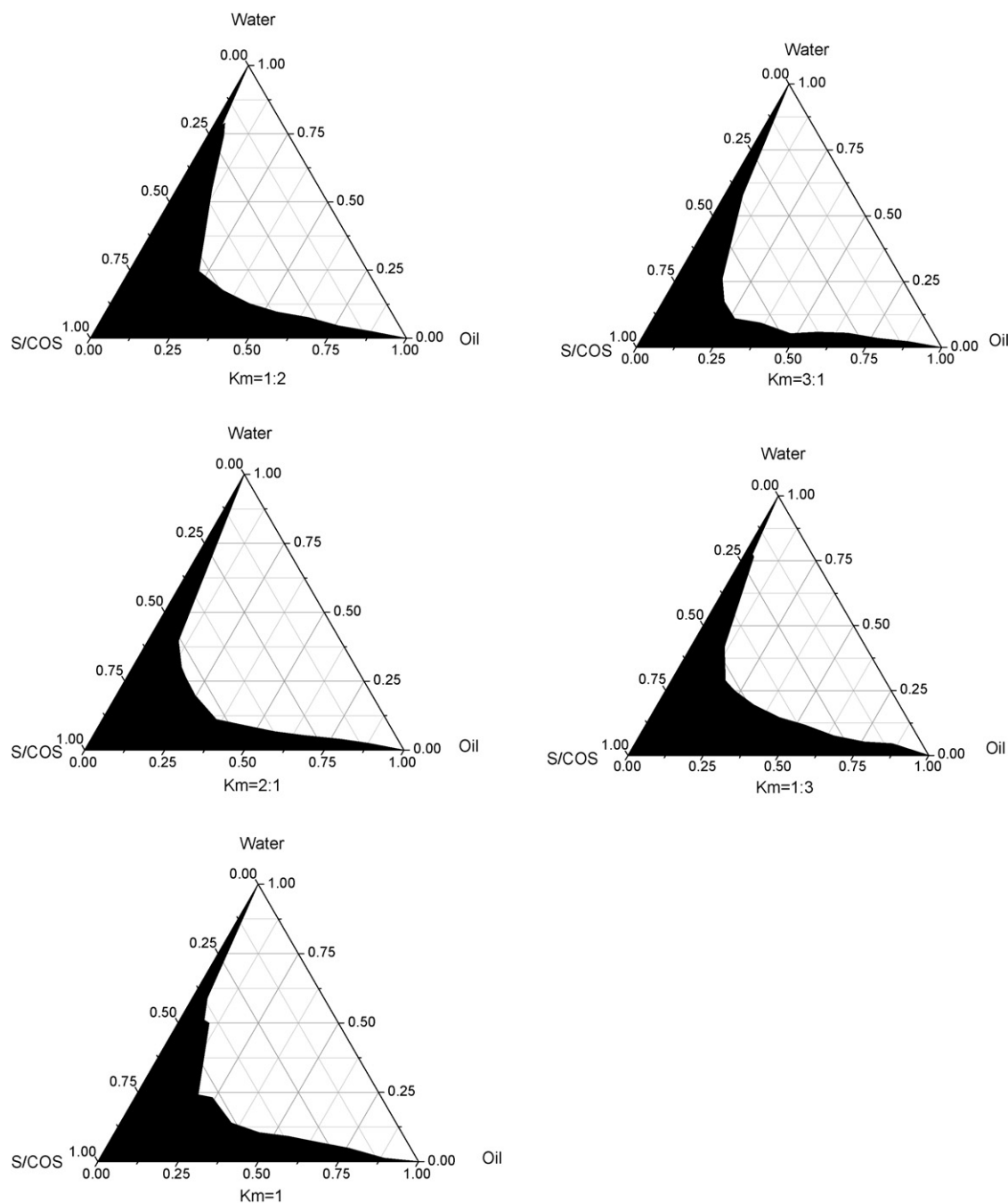
Fig. 2. Pseudo-ternary phase diagrams of microemulsions composed of oil (oleic acid), surfactant (Cremorphor EL), cosurfactant (ethanol) and water at various Km values.

3.2. Construction of pseudo-ternary diagrams

The construction of pseudo-ternary phase diagrams was used to obtain appropriate concentration ranges of components in the areas of forming microemulsions. The pseudo-ternary phase diagrams of O/W microemulsions composed of oleic acid, Cremorphor EL, ethanol and distilled water with various Km values were shown in Fig. 2. The area region of microemulsions became enlarged as Km decreased, reaching the maximum point at Km of 1:2.

3.3. Formulation optimization

Simplex lattice method was used to optimize the formulation of microemulsions. The concentrations of water (X_1), surfactant (X_2)

and cosurfactant (X_3) were chosen as the independent variables. The solubility or the cumulative amount of penciclovir permeated through excised mouse skins per unit area was taken as responses (Y), respectively. The equation for simplex lattice model is described as follows:

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3$$
$$+ b_{23}X_2X_3 + b_{123}X_1X_2X_3$$

where Y is the dependent variable and b_i is the estimated coefficient for the factor X_i. The main effects (X_1, X_2, and X_3) represent the average results of changing one factor at a time from its low to high value, and the interactions X_1X_2, X_2X_3, X_1X_3, and $X_1X_2X_3$
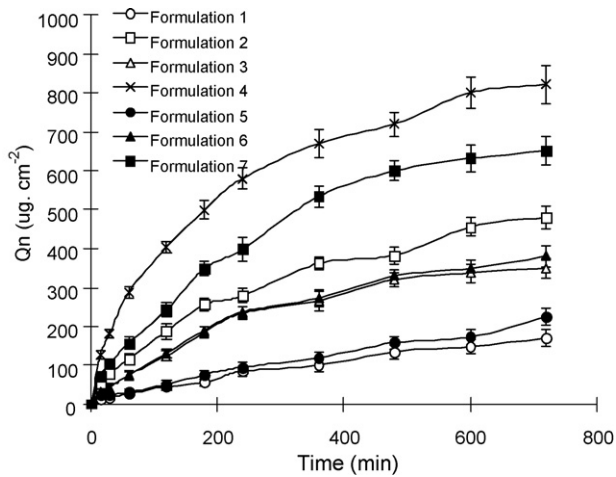
Fig. 3. Percutaneous permeation profiles of the tested microemulsion formulations (mean ± S.D.; $n = 5$).

Table 2
Actual and transformed values of six different formulations

Formulations	Formulation components (%)			Transformed fraction		
	Water	Surfactant	Cosurfactant	Water	Surfactant	Cosurfactant
1	45	20	30	0.2	0.4	0.4
2	60	10	25	0.8	0	0.2
3	45	30	20	0.2	0.8	0
4	50	15	30	0.4	0.2	0.4
5	55	20	20	0.6	0.4	0
6	45	25	25	0.2	0.6	0.2

Table 3
Comparison of responses between experimental results and predicted values (mean ± S.D.; $n = 5$)

Formulations	Solubility (mg g^{-1})		Q_n (μg cm^{-2})	
	Experimental	Predicted	Experimental	Predicted
1	7.411 ± 0.378	7.393	589.502 ± 33.2	601.401
2	9.492 ± 0.370	9.523	192.242 ± 21.6	184.216
3	6.301 ± 0.233	6.323	746.295 ± 44.5	735.654
4	8.404 ± 0.513	8.376	537.584 ± 37.3	539.344
5	6.725 ± 0.276	6.744	755.327 ± 41.1	769.865
6	6.796 ± 0.387	6.747	702.274 ± 37.7	690.432

show how the responses change when two or three factors change simultaneously.

Based on the results of pseudo-ternary diagrams, appropriate ranges of the components were chosen. Because a high content of oleic acid could cause serious skin irritation (Boelsma et al., 1996), 5% was chosen as the content of oil phase in this study. It was reported that there was a really tight relationship between the hydration effect of the stratum corneum and the dermal permeation (Mohammed and Manoj, 2000), and the thermodynamic activity of drug in microemulsions was a significant driving force for the release and penetration of the drug into skin (Changez and Varshney, 2000; Mohammed and Manoj, 2000). So the water content was determined between 40 and 65%. The ranges of Cremorpor EL and ethanol were 10–35% and 20–45%, respectively.

In order to simplify the computations, the actual concentrations of surfactant, cosurfactant and water were transformed based on the simplex lattice method so that the minimum concentration corresponds to zero and the maximum concentration corresponds to one (Table 1). Solubility of the seven formulations was measured as described above and the results were also presented in Table 1.

As shown in Fig. 3, the percutaneous permeation profiles of the tested microemulsion formulations demonstrated that penetration through skin was a passive diffusion process. With the help of SPSS13.0 Software, the results of analysis were shown in Eqs. (2) and (3).

$$Y_S = 8.564X_1 + 6.637X_2 + 8.278X_3 - 4.371X_1X_2 + 6.354X_1X_3$$
$$- 1.120X_2X_3 - 8.284X_1X_2X_3 \quad (2)$$

$$Y_{Q_n} = 170.586X_1 + 480.989X_2 + 350.077X_3 + 1979.660X_1X_2$$
$$- 139.175 X_1X_3 - 139.469X_2X_3 + 3435.433X_1X_2X_3 \quad (3)$$

Eqs. (2) and (3) may be used to calculate the predicted values for other formulations in the design space. Six formulations showed in Table 2 were chosen to test the agreement between observed and predicted values, and as shown in Table 3 the predicted values of solubility and Q_n from simplex lattice system were close to that of the experiment.

According to the above results, when the ratio of S/Cos was close to 1:1 (w/w), the skin permeation of penciclovir in microemulsion was significantly increased, while the solubility of penciclovir in microemulsion was usually decreased, which is consistent with the results of Li et al. (2004a,b). The possible reason is that dermal drug permeation is influenced primarily by the solubility of drug in vehicle and the partition coefficient of skin/vehicle. If the vehicle can significantly raise the solubility of the drug, then the drug itself would be retained in the vehicle after administration at the surface of skin, which resulted in reduced partition into the skin (Ceschel et al., 2005).

Graphics of the solubility and the cumulative amount of penciclovir permeated through excised mouse skin per unit area were constructed in the form of contour plots, and the optimized formulation was chosen by superimposing the contour plots of the two responses, which were shown in Fig. 4.

Table 1
Actual and transformed values, solubility and Q_n of seven different formulations as per simplex lattice design

Formulation number	Formulation components (%)/transformed fraction			Solubility (mg g^{-1})	Q_n (μg cm^{-2})
	Water	Surfactant	Cosurfactant		
1	65/1	10/0	20/0	8.564 ± 0.745	170.586 ± 20.8
2	40/0	35/1	20/0	6.637 ± 0.212	480.989 ± 29.5
3	40/0	10/0	45/1	8.278 ± 0.099	350.077 ± 25.8
4	52.5/0.5	22.5/0.5	20/0	6.508 ± 0.208	820.702 ± 50.2
5	52.5/0.5	10/0	32.5/0.5	10.01 ± 0.350	225.538 ± 21.8
6	40/0	22.5/0.5	32.5/0.5	7.178 ± 0.215	380.666 ± 27.6
7	48.3/0.33	18.4/0.33	28.3/0.33	7.615 ± 0.213	650.124 ± 36.7

Data of solubility and Q_n were shown as mean ± S.D. ($n = 5$).

Fig. 5. Percutaneous permeation profiles of the optimized microemulsions and the commercial cream (mean ± S.D.; $n = 5$).

3.4. Characterization of optimized microemulsions

With the measurement of transmission electron microscope, the optimized microemulsion vesicles appeared as perfect round shape without aggregation. The parameters for physicochemical characters of the optimized formulation were as follows: 36.5 nm for mean particle size, 7.11 mm^2 s^{-1} for viscosity value, 5.33 for pH value, 1.38 for refractive indices, and 134.5 μs cm^{-1} for conductivity, respectively. The solubility of penciclovir in the optimized microemulsion was 7.41 mg g^{-1}. The results of conductivity showed that the microemulsions were O/W system.

All microemulsion formulations were stable at ambient temperature in the presence or absence of penciclovir. No changes of particle size, phase separation and degradation of penciclovir were observed during 6 months. The centrifuge tests showed that all microemulsion systems had good physical stability.

3.5. Comparison of percutaneous permeation between cream and microemulsions

The results of percutaneous permeation of the commercial cream containing 1% penciclovir and O/W microemulsions containing 0.5% penciclovir were shown in Fig. 5.

Q_ns of the optimized microemulsions and the commercial cream in 12 h after applications were 589.50 and 168.98 μg cm^{-2}, respectively ($p < 0.05$).

As shown in Fig. 5, microemulsions could improve the skin permeation of penciclovir over the commercial creams. As reported previously, the thermodynamic activity which can be described as viscosity is important to the permeation into skin. It is known that the viscosity of microemulsions is much lower than that of cream, so the mobility of drugs in microemulsions is more facile. Furthermore, the microemulsions may affect the stratum corneum structure and reduce the diffusional barrier by acting as a permeation enhancer (Mei et al., 2003; Chang et al., 2005; Changez et al., 2006a,b).

4. Conclusion

In this paper, the application of microemulsion systems for percutaneous delivery of penciclovir was investigated and simplex lattice was used to optimize the formulations. The results suggested that the microemulsion played a role in permeation enhancing effect. Compared with commercial cream, the skin permeation ability of penciclovir was significantly increased by microemulsions, which might result from the special characteristics of microemulsions. It is promising that the concentration of penciclovir used to treat relative skin illness could be decreased due to the high permeation ability of penciclovir microemulsion and side effects of
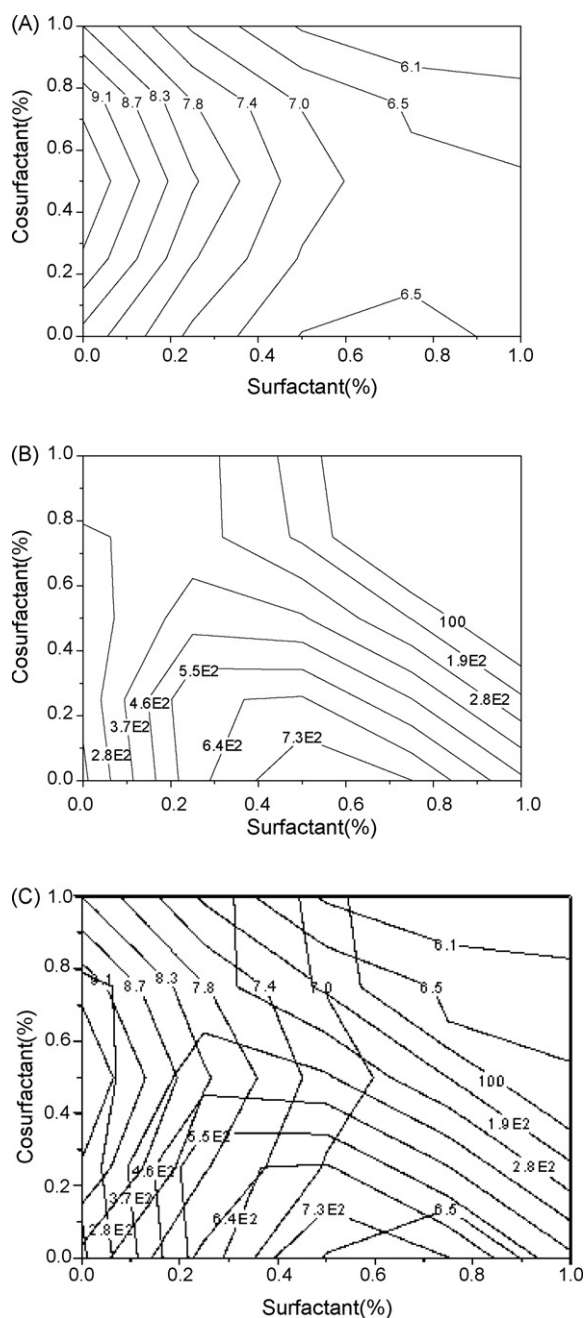

Fig. 4. Contour plots (A) contour plots for solubility of penciclovir; (B) contour plots for the cumulative amount of penciclovir permeated through excised mice skins per unit area after 12 h; (C) superimposed contour plots of the two responses.

As shown in Table 1 and Fig. 4, with the restriction of the above chosen concentrations, an inverse relationship exists between the permeation ability and the drug solubility in the penciclovir microemulsions. Although a high solubility can lead to slightly lower permeation ability within the range of chosen concentrations, the higher solubility can increase the loading efficiency to produce a high concentration gradient, maintain a longer acting time, and improve the thermodynamic activity of drug in vesicles, which can make the microemulsion systems more stable (Zhao et al., 2006; Li et al., 2004a,b). In order to obtain both high solubility and high permeation ability, the appropriate ratio of the components was chosen for the optimized formulation, which consisting of oil (5%), surfactant (20%), cosurfactant (30%) and water (45%).
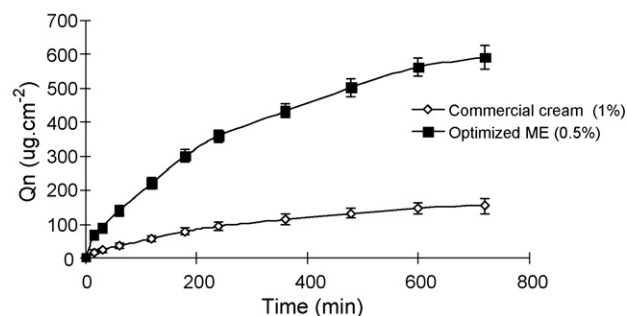
penciclovir might be reduced. Further investigation is needed for in vivo studies.

Acknowledgements

References

Abdel-Hag, N., Chearskul, P., Al-Tatari, H., Asmar, B., 2006. New antiviral agents. Indian J. Pediatr. 73, 313–321.

Andrei, G., De Clercq, E., Snoeck, R., 2004. In vitro selection of drug-resistant varicella-zoster virus (VZV) mutants (OKA strain): differences between acyclovir and penciclovir? Antiviral Res. 61, 181–187.

Baboota, S., Al-Azaki, A., Kohli, K., Ali, J., Dixit, N., Shakeel, F., 2007. Development and evaluation of a microemulsion formulation for transdermal delivery of terbinafine PDA. J. Pharm. Sci. Technol. 61, 276–285.

Baroli, B., López-Quintela, M.A., Delgado-Charro, M.B., Fadda, A.M., Blanco-Méndez, J., 2000. Microemulsions for topical delivery of 8-methoxsalen. J. Control Rel. 69, 209–218.

Biruss, B., Kählig, H., Valenta, C., 2007. Evaluation of an eucalyptus oil containing topical drug delivery system for selected steroid hormones. Int. J. Pharm. 328, 142–151.

Boelsma, E., Tanojo, H., Boddé, H.E., Ponec, M., 1996. Assessment of the potential irritancy of oleic acid on human skin: Evaluation in vitro and in vivo. Toxicol. In Vitro 10, 729–742.

Ceschel, G., Bergamante, V., Maffei, P., Lombardi Borgia, S., Calabrese, V., Biserni, S., Ronchi, C., 2005. Solubility and transdermal permeation properties of a dehydroepiandrosterone cyclodextrin complex from hydrophilic and lipophilic vehicles. Drug Deliv. 12, 275–280.

Chang, X.L., Chen, H.B., Zhao, X.Z., Gao, Z.H., Xu, H.B., Yang, X.L., 2005. High-performance liquid chromatography determination of triptolide in vitro permeation studies. Anal. Chim. Acta 534, 215–221.

Changez, M., Varshney, M., 2000. Aerosol-OT microemulsions as transdermal carriers of tetracaine hydrochloride. Drug Dev. Ind. Pharm. 26, 507–512.

Changez, M., Chander, J., Dinda, A.K., 2006a. Transdermal permeation of tetracaine hydrochloride by lecithin microemulsion: in vivo. Colloids Surf. B: Biointerf. 48, 58–66.

Changez, M., Varshney, M., Chander, J., Dinda, A.K., 2006b. Effect of the composition of lecithin/n-propanol/isopropyl myristate/water microemulsions on barrier properties of mice skin for transdermal permeation of tetracaine hydrochloride: in vitro. Colloids Surf. B: Biointerf. 50, 18–25.

Chen, H.B., Chang, X.L., Weng, T., Zhao, X.Z., Gao, Z.H., Yang, Y.J., Xu, H.B., Yang, X.L., 2004. A study of microemulsion systems for transdermal delivery of triptolide. J. Control. Rel. 98, 427–436.

Chen, H.B., Chang, X.L., Du, D.R., Li, J., Xu, H.B., Yang, X.L., 2006. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int. J. Pharm. 315, 52–58.

Chen, H., Mou, D., Du, D., Chang, X., Zhu, D., Liu, J., Xu, H., Yang, X., 2007. Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. Int. J. Pharm. 341, 78–84.

Gupta, R.R., Jain, S.K., Varshney, M., 2005. AOT water-in-oil microemulsions as a penetration enhancer in transdermal drug delivery of 5-fluorouracil. Colloids Surf. B: Biointerf. 41, 25–32.

Ho, H.O., Huang, F.C., Sokoloski, T.D., Sheu, M.T., 1994. The influence of cosolvents on the in-vitro percutaneous penetration of diclofenac sodium from a gel system. J. Pharm. Pharmacol. 46, 636–642.

Hong, W., Wu, C.Z., 2004. Experiment Design and Analysis-principles, Operation and Cases. China Forestry Press, Beijing.

Jumaa, M., Kleinebudde, P., Müller, B.W., 1998. Mixture experiments with the oil phase of parenteral emulsions. Eur. J. Pharm. Biopharm. 46, 161–167.

Kamal, M.A., Iimura, N., Nabekura, T., Kitagawa, S., 2007. Enhanced skin permeation of diclofenac by ion-pair formation and further enhancement by microemulsion. Chem. Pharm. Bull (Tokyo). 55, 368–371.

Kogan, A., Garti, N., 2006. Microemulsions as transdermal drug delivery vehicles. Adv. Colloid Interf. Sci. 123–126, 369–385.

Kreilgaard, M., 2002. Influence of microemulsions on cutaneous drug delivery. Adv. Drug Deliv. Rev. 54, 77–98.

Kreilgaard, M., Pedersen, E.J., Jaroszewski, J.W., 2000. NMR characterisation and transdermal drug delivery potential of microemulsion systems. J. Control. Rel. 69, 421–433.

Lawrence, M.J., Rees, G.D., 2000. Microemulsion-based media as novel drug delivery systems. Adv. Drug Deliv. Rev. 45, 89–121.

Lee, P.J., Langer, R., Shastri, V.P., 2003. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm. Res. 20, 264–269.

Li, H., Pan, W.S., Li, J.Y., 2004a. Preparation, evaluation, and NMR characterization of vinpocetine microemulsion for transdermal delivery. Drug Dev. Ind. Pharm. 30, 657–666.

Li, H., Pan, W.S., Wu, Z., Li, J.Y., Xia, L.X., 2004b. Optimization of microemulsion containing vinpocetine and its physicochemical properties. Yao Xue Xue Bao 39, 681–685.

Liu, C.H., Ho, H.O., Hsieh, M.C., Sokoloski, T.D., Sheu, M.T., 1995. Studies on the in-vitro percutaneous penetration of indomethacin from gel systems in hairless mice. J. Pharm. Pharmacol. 47, 365–372.

Mao, S.S., Zhou, J.X., Chen, Y., 2004. Design of Experiment. China Statistics Press, Beijing.

Mei, Z.N., Chen, H.B., Weng, T., Yang, Y.J., Yang, X.L., 2003. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur. J. Pharm. Biopharm. 56, 189–196.

Mohammed, C., Manoj, V., 2000. Aerosol-OT microemulsions as transdermal carriers of tatracaine hydrochloride. Drug Dev. Ind. Pharm. 26, 507–512.

Paolino, D., Ventura, C.A., Nisticò, S., Puglisi, G., Fresta, M., 2002. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. Int. J. Pharm. 244, 21–31.

Peltola, S., Saarinen-Savolainen, P., Kiesvaara, J., Suhonen, T.M., Urtti, A., 2003. Microemulsions for topical delivery of estradiol. Int. J. Pharm. 254, 99–107.

Ren, L.Q., 2003. Optimum Design and Analysis of Experiments, 2nd ed. High Education Press, Beijing.

Rhee, Y.S., Choi, J.G., Park, E.S., Chi, S.C., 2001. Transdermal delivery of ketoprofen using microemulsions. Int. J. Pharm. 228, 161–170.

Schmid-Wendtner, M.H., Korting, H.C., 2004. Penciclovir cream-improved topical treatment for herpes simplex infections. Skin Pharmacol. Physiol. 17, 214–218.

Sintov, A.C., Shapiro, L., 2004. New microemulsion vehicle facilitates percutaneous penetration in vitro and cutaneous drug bioavailability in vivo. J. Control. Rel. 95, 173–183.

Smith, R.L., Morroni, J., Wilcox, C.L., 2001. Lack of effect of treatment with penciclovir oracyclovir on the transdermal establishment of latent HSV-1 in primary sensory neurons in culture. Antiviral Res. 52, 19–24.

Subramanian, N., Ray, S., Ghosal, S.K., Bhadra, R., Moulik, S.P., 2004. Formulation design of self-microemulsifying drug delivery systems for improved oral bioavailability of celecoxib. Biol. Pharm. Bull. 27, 1993–1999.

Tenjarla, S., 1999. Microemulsions: an overview and pharmaceutical applications. Crit. Rev. Ther. Drug Carrier Syst. 16, 461–521.

Van Kamp, H.V., Bolhuis, G.K., Lerk, C.F., 1987. Optimization of a formulation for direct compression using a simplex lattice design. Pharm. Weekbl. Sci. 9, 265–273.

Yang, L.P., Wang, W.H., 2005. The influence of liposome on penciclovir penetration through mouse skin. Chin. Pharm. J. 40, 289–291.

Yuan, Y., Li, S.M., Mo, F.K., Zhang, D.F., 2006. Investigation of microemulsion system for transdermal delivery of meloxicam. Int. J. Pharm. 321, 117–123.

Zhao, X., Liu, J.P., Zhang, X., Li, Y., 2006. Enhancement of transdermal delivery of theophylline using microemulsion vehicle. Int. J. Pharm. 327, 58–64.