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Investigation of the carbopol gel of solid lipid nanoparticles for the transdermal iontophoretic delivery of triamcinolone acetonide acetate

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ABSTRACT

The purpose of this study was to investigate solid lipid nanoparticles (SLN) hydrogel for transdermal iontophoretic drug delivery. Triamcinolone acetonide acetate (TAA), a glucocorticoids compound, was employed as the model drug. SLN containing the drug triamcinolone acetonide acetate (TAA-SLN) and their carbopol gel with stable physicochemical properties were prepared. The use of TAA-SLN carbopol gel as a vehicle for the transdermal iontophoretic delivery of TAA was evaluated *in vitro* using horizontal diffusion cells fitted with porcine ear skin. We found that the TAA-SLN gel possessed good stability, rheological properties, and high electric conductance. Transdermal penetration of TAA from TAA-SLN gel cross the skin tissue was significantly enhanced by iontophoresis. The enhancement of the cumulative penetration amount and the steady-state penetration flux of the penetrated drug were related to the particle size of TAA-SLN and the characteristics of the applied pulse electric current, such as density, frequency, and on/off interval ratio. These results indicated that SLN carbopol gel could be used as a vehicle for transdermal iontophoretic drug delivery under suitable electric conditions.

1. Introduction

The main challenge in transdermal drug delivery is to overcome the inherent barrier of the skin. There is evidence that the rate-limiting step in transdermal transport occurred at the stratum corneum, the outermost layer of the skin. Many approaches have been used to enhance the penetration of drugs through this layer of the skin. Iontophoresis is a non-invasive technique for transporting charged molecules into and through tissues by a mild electric field. It has been shown to effectively deliver a variety of drugs across the skin to the underlying tissue (Kalia et al., 2004). In addition to the enhanced continuous transport, iontophoresis allows dose titration by adjusting the electric field, which makes personalized dosing feasible. However, iontophoresis is more suited to hydrophilic, lower molecular weight substances (generally under 500 Da). Moreover, conventional iontophoresis of some drugs may result in lower plasma levels in a therapeutical range (Vutla et al., 1996). Therefore, it is necessary to improve the conventional iontophoretic system in order to realize its full potential in drug delivery.

The use of drug carriers as vehicles for topical and/or transdermal delivery is another strategy. Drug carrier could modify the physicochemical properties of the encapsulated molecule and offer a means to facilitate the percutaneous delivery of difficult-to-uptake substances. Recently, there are some reports about using liposomes (Vutla et al., 1996; Badkar et al., 1999; Fang et al., 1999; Essa et al., 2004), microemulsions (Kantaria et al., 1999) and polymeric nanoparticles (Nicoli et al., 2001) as vehicles for iontophoretic drug delivery.

The combined use of drug carriers and iontophoresis could offer many additional benefits. For example, charges could be imparted to neutral drugs by encapsulating them in charged drug carriers, which could be delivered by iontophoresis. Previous works have shown that encapsulation of neutral colchicines into positively charged liposomes significantly increased the iontophoretic flux of the drug (Kulkarni et al., 1996). For charged drugs such as peptides, encapsulation by drug carriers could prevent or minimize their proteolytic breakdown in the body during and after delivery, therefore enhancing their therapeutic efficacy (Vutla et al., 1996).

However, some of the above vehicles have the disadvantage of being rather complicated and time-consuming, and the intrinsic instability of liposomes and microemulsions may lead to the leaking of drugs (Fang et al., 1999). Solid lipid nanoparticles (SLN) have shown great potential as novel drug carriers for dermal/transdermal delivery (Muller et al., 2000; Mehnert and Mäder,

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2001; Wissing et al., 2004). The small particle size ensures close contact with the stratum corneum and increases the amount of encapsulated compounds penetrating into the skin (Jenning et al., 2000; Wissing and Muller, 2002; Muller et al., 2002). Because SLN is made of solid matrixes, they are also capable of protecting unstable active ingredients from degradation and releasing drugs in a controlled way (Dingler et al., 1999). The controlled drug release becomes important when the drug causes irritation of the skin at high concentrations and has to be delivered over a prolonged period of time (Souto et al., 2004). However, SLN suspension has very low viscosity; therefore, it is not convenient for use on the skin. Hydrogels are clinically acceptable systems that offer many advantages, such as suitable rheological properties, good tissue compatibility and convenience in handing and ease of application (Bentley et al., 1999).

Carbopol gels are approved for pharmaceutical use in several different administration routes. They also represent an interesting media for iontophoresis delivery. Cutaneous use of these gels is advantageous as they possess good rheological properties resulting in long residue times at the site of administration. They offer good alternatives to oil based ointment formulations. Moreover, carbopol gels are anionic hydrogels with good buffering capacity, which may contribute to the maintenance of the desired pH in the process of iontophoresis (Merclin et al., 2004).

To our knowledge, there is still no report on the study of combined use of SLN hydrogel and iontophoresis for transdermal drug delivery. In this work, triamcinolone acetonide acetate (TAA, $M_W = 476$, $\log P \approx 3.2$), a glucocorticoids compound, was employed as the model drug to investigate the transdermal delivery by combined use of SLN hydrogel and iontophoresis. TAA has poor water solubility and high lipophilicity, which make it suitable for SLN encapsulation. We prepared TAA-loaded SLN (TAA-SLN) carbopol gel with stable physicochemical properties and evaluated the use of SLN carbopol gel as a vehicle for transdermal iontophoretic delivery of TAA.

2. Materials and methods

2.1. Materials

TAA was provided by Tianjin Pharmaceuticals Group Corporation (Tianjin, China). Precirol ATO 5 (ATO-5), a mixture of mono-, di- and triglycerides of palmitic acid (C_{16}) and stearic acid (C_{18}), was used as lipid matrix of SLN. It was a gift from Gattefosse S.A. (Saint-Priest, France). Carbopol ETD 2020, a copolymer of acrylic acid and alkyl acrylate with allyl pentaeritrithol ether, was used as gel matrix. It was obtained from Noveon Asia Pacific Ltd. (Hong Kong, China). Soybean lecithin (SLT), sodium cholate (SC), Tween-80, propylene glycol, triethanolamine, silver and silver chloride (purity >99.9%) were purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). Other reagents were analytical or HPLC grade. Double-distilled water was prepared in our laboratory.

2.2. Preparation of TAA-SLN and TAA-SLN carbopol gel

TAA-SLN formulations (Table 1) were prepared by high-pressure homogenization technique using APV 2000 (APV System, Albertslund, Denmark). Briefly, the lipid matrix was melted at 75 °C, TAA and SLT was added to obtain a melting lipid phase. The hot lipid phase was dispersed into a hot water–surfactant solution at the same temperature, and a pre-emulsion was formed using high shear homogenizer (FA-25, Fluko Equipment, China). The hot pre-emulsion was homogenized for 3–6 cycles at 600–1000 bars to produce nanoemulsion. This nanoemulsion was then cooled under refrigerated temperature to form SLN suspension.

Carbopol ETD 2020 was added into the solution of double-distilled water and propylene glycol (4:1, wt./wt.) at a concentration of 1.5% (wt.). The resulting mixture was stirred for 3–5 h at room temperature with magnetic stirrer (85-2, Shanghai Shiluo Instruments, China), and neutralized with triethanolamine to give a carbopol gel matrix with a pH value of 7.0. SLN suspension was mixed with an equal volume of the carbopol gel with continuing stirring to form SLN carbopol gel. The final concentration of Carbopol ETD 2020 was 0.75% (wt.).

2.3. Characterization of TAA-SLN and TAA-SLN carbopol gel

2.3.1. Particle size and zeta potential

Particle size and polydispersity index (PI) of various SLN formulations were measured by photon correlation spectroscopy (Nano-ZS90 zetasizer, Malvern Instruments Corp., UK) at 25 °C under a fixed angle of 90° in disposable polystyrene cuvettes. The measurements were obtained using a He-Ne laser of 633 nm. Zeta potential was measured in folded capillary cells using the Nano-ZS90 zetasizer. The PCS sample was prepared by diluting the sample with double-distilled water. And the conductivity of the same solution was adjusted to 50 μS/cm by 0.1 mmol/l sodium chloride solution for zeta potential measurement. The zeta potential values were calculated using the Smoluchowski equation.

2.3.2. Entrapment efficiency (EE)

TAA-SLN was separated using Sephadex-G50 column (200 mm × 15 mm), washing with double-distilled water at a flow rate of 1.0 ml/min. The entrapped and free TAA was respectively collected at a volume interval of 2.0 ml. The collected samples were diluted using a mixed solvent of methanol and chloroform (1:2, v/v). EE was calculated according to the following equation:

$$\mathrm{EE} = \frac{\text{the amount of entrapped TAA in SLN}}{\text{the amount of entrapped TAA in SLN and free TAA in dispersion}}$$

2.3.3. Surface morphology

The surface morphology of TAA-SLN gel was studied by transmission electron microscopy (TEM). TAA-SLN gel was diluted 10-fold with double-distilled water before measurement. Before TEM measurement, one drop of sample was placed on the copper grid coated with carbon film and dried. The air-dried samples were then directly examined under a TECNAI G2-20 transmission electron microscope (FEI Co., Netherlands) at 200 kV.

2.3.4. Storage stability

TAA-SLN carbopol gel was stored at room temperature for 3 months. Clarity, particle size and zeta potential were determined after 3 months of storage to evaluate their stabilities.

2.3.5. Rheological behavior

NDJ-8S rotating cylinder viscometer (Shanghai, China) was used to study the flow curve of carbopol gel matrix and TAA-SLN gel with

Table 1
The composition of the investigated TAA-SLN

TAA (wt.%)	ATO-5 (wt.%)	SLT (wt.%)	SC (wt.%)	Tween-80 (wt.%)	Water (wt.%)
0.12	3.6	1.8	0.8	3.0	90.68

the alteration of shear rate. The viscosity of the gel matrix and TAA-SLN gel were measured under the temperature of 25 °C, at shear rates of 0.3, 0.6, 1.5, 3.0, 6.0, 12.0, 30.0 and 60.0 r/min with the rotor of 2# or 3#.

2.3.6. Electrical conductivity

The electrical conductivity of SLN gel is a key factor for its use in iontophoresis. The electrical conductivity (Ψ) of the carbopol gel matrix and TAA-SLN gel, and the difference between SLN gel and gel matrix ($\Delta\Psi$) were determined. DJS-1 platinized platinum electrode (Shanghai, China) was selected to determine the electrical conductivity under the temperature of 25 °C.

2.4. In vitro drug release kinetics

The *in vitro* drug release study using horizontal glass diffusion cells and cellulose dialysis membrane (M_W cut-off 12 000, Sigma), which performed for 32 h by using pulse electric current with current density of 0.50 mA/cm^2, frequency of 2000 Hz, and on/off interval ratio of 1:1. The other conditions were the same as described in Section 2.5.

2.5. Transdermal iontophoretic delivery studies

Porcine ear skin was used for the permeation experiment. Fresh porcine ears were obtained from the local slaughterhouse and were cleaned under the cold running water. The whole skin was removed carefully from the outer region of the ear and separated from the underlying cartilage with a scalpel. The skin samples were used immediately.

The iontophoretic penetration test was performed using horizontal glass diffusion cells. The porcine ear skin was soaked in physiological saline for more than 0.5 h before mounted into the diffusion cell. Three g of SLN formulation was placed in the donor compartment. The receptor compartment was filled with medium solution (physiological saline:ethanol = 80:20, v/v), kept in a water bath at 32 °C, and continuously agitated by a magnetic stirrer at 400 r/min (TK-12A, China). Silver–silver chloride reversible electrodes were used for the study because they do not cause water electrolysis and the shift of pH. A pulse electric current with square waveform was supplied from the iontophoretic device (GZ-IIIC, China). At different periods of time, 0.5 ml medium solution was removed from the receptor compartment for HPLC determination and replaced with the same volume of fresh medium solution immediately. The samples were filtrated using micro-porous membrane (0.45 μm) and analyzed with HPLC.

The cumulative amount of TAA permeated through porcine ear skin (Q, nmol/cm^2) was plotted as a function of time. The permeation flux of TAA at steady-state (J_s, nmol/cm^2 h) through porcine ear skin was calculated from the slope of linear portion of the cumulative amount permeated through the skin per unit area versus time plot.

2.5.1. Effect of particle size

The effect of particle size on iontophoretic flux of TAA-SLN gel was studied at pulse current with current density of 0.50 mA/cm^2, pulse frequency of 2000 Hz, and a 1:1 pulse on/off interval ratio.

2.5.2. Effect of current density

The effect of pulse current density (current intensity per unit area) on iontophoretic flux of TAA-SLN gel was studied at current density ranging from 0.20, 0.30, 0.50 to 0.60 mA/cm^2 with pulse frequency of 2000 Hz and pulse on/off interval of 1:1.

2.5.3. Effect of pulse frequency

The effect of pulse current frequency on iontophoretic flux of TAA-SLN gel was studied at pulse frequency varied from 50, 1000, 2000, 4000 to 9000 Hz under current density of 0.50 mA/cm^2 and pulse on/off interval of 1:1.

2.5.4. Effect of on/off interval ratio

The effect of pulse on/off interval on iontophoretic flux of TAA-SLN gel was studied at on/off interval ratio varied from 1:5, 1:3, 1:1, 3:1 to 5:1 under current density of 0.50 mA/cm^2 and pulse frequency of 2000 Hz.

2.6. HPLC analysis

TAA was determined by HPLC using an Agilent G1310A pump (Agilent 1100 Series, USA), an Agilent G1314A Variable Wavelength Detector set at 240 nm and a Hypersil C_{18} column (5 μm, 250 mm × 4.6 mm). The mobile phase was a mixture of methanol, water and ether with a ratio of 65:35:4 (v/v/v). The HPLC analysis was performed at 25 °C with a mobile phase flow rate of 1 ml/min.

2.7. Statistical analysis

Statistical analysis was performed with SPSS 11.0 software package. Results are expressed as the mean ± standard deviation ($\bar{x} \pm s$). Statistically significant differences were determined using the Student's t-test and analysis of variance (ANOVA) with $p < 0.05$ as a minimal level of significance.

3. Results and discussion

3.1. Characterization of TAA-SLN

By controlling the parameters of high-pressure homogenization (pressure and cycle times of homogenization), we obtained TAA-SLN with different particle sizes. The physicochemical properties of TAA-SLN with different particle sizes were shown in Table 2.

TAA-SLN-1 with the smallest particle size had the most narrow size distribution of 0.183 compared with TAA-SLN-2 and TAA-SLN-3, likely because when the pressure and cycle times of homogenization was decreased, the produced TAA-SLN was less homogeneous. The zeta potential of TAA-SLN was decreased with the increase of particle size, and zeta potentials of all TAA-SLN were above −40 mV. A high zeta potential can provide an electric repulsion, which might be beneficial to iontophoresis. The EE of all TAA-SLN were higher than 90%. The low concentration of TAA in SLN and the high compatibility between drug and lipid might result in the high EE.

TEM image of TAA-SLN-1 in carbopol gel was presented in Fig. 1. The mean value of TAA-SLN diameter determined by TEM was in agreement with that determined by PCS. TEM image showed that TAA-SLN was distributed homogeneously in carbopol gel matrix.

3.2. Storage stability

The results showed that all TAA-SLN carbopol gel was stable and still transparent after 3 months with little change in particle size and zeta potential. No significant change of clarity and phase separation was observed, which indicated that TAA-SLN gel had a good physical stability. Jenning et al. had used the wide angle X-ray diffraction to study the polymorphic transitions of SLN made of retinyl palmitate (Compritol 888 ATO) in oil/water cream (Jenning et al., 2000). They found that after 6 months SLN still remained in the nanoparticulate state and maintained the β′-polymorphism.

Table 2
The physicochemical properties of TAA-SLN ($\bar{x} \pm s$, $n = 3$)

Formulation	Particle size (nm)	PI	Zeta potential (mV)	EE (%)
TAA-SLN-1	114.7 ± 2.1	0.183 ± 0.015	−45.8 ± 1.4	91.2 ± 1.3
TAA-SLN-2	263.1 ± 4.4	0.220 ± 0.021	−44.2 ± 2.7	90.7 ± 2.6
TAA-SLN-3	842.0 ± 10.6	0.279 ± 0.034	−41.6 ± 2.3	93.4 ± 0.8

The authors argued that the network of the gel hampered the polymorphic SLN transitions and thus enhanced the stability of SLN. Under these conditions, the contact of nanoparticles was decreased and aggregation was avoided.

3.3. Rheological behavior

The rheological property of a semisolid drug carrier is a very important physical parameter for its percutaneous application (Lippacher et al., 2001, 2002). Therefore, rheological behavior of the SLN gel for transdermal iontophoretic drug delivery was studied. The flow curves of carbopol gel matrix and TAA-SLN-1 gel were shown in Fig. 2.

As shown by the flow curves in Fig. 2, the gel matrix and TAA-SLN gel possessed good thixotropy and its viscosity decreased with the increase of shear rate. Thixotropy indicates that the viscosity of the fluid decreases with the increase of shear stress. After the shear stress is removed, the viscosity slowly returns to the former state under isothermal conditions (Chen and Dai, 1984). The characteristic thixotropy flow curve descends moving toward left compared with the ascending curve. The figure represents a counter-clockwise hysteresis curve, namely hysteresis loop. Thixotropy was affected by the regeneration of hydrogen bonding and the time needed to restructure the three-dimensional network structure. The negatively charged TAA-SLN may affect the recovery of hydrogen bonding in the gel. It therefore takes longer time to restructure the three-dimensional network structure.

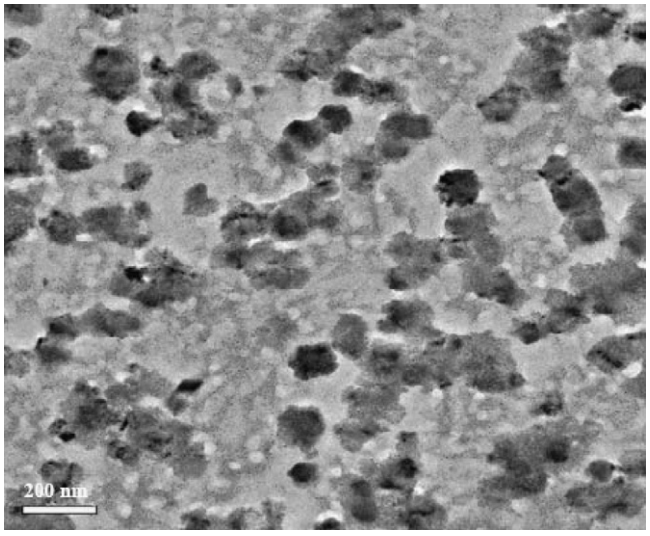

Fig. 1. TEM image of TAA-SLN in carbopol gel.

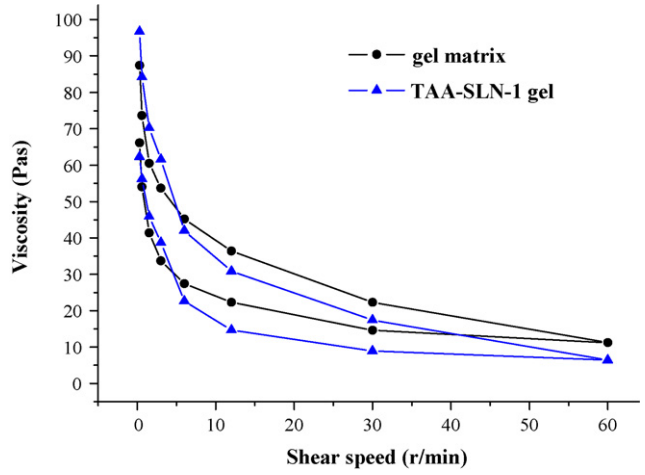

Fig. 2. The flow curves of carbopol gel matrix and TAA-SLN-1 gel.

The rheological behavior of thixotropic fluid system is analyzed by Ostwade power equation (Battista, 1985):

$$\eta = KS^{-n}$$

η is the apparent viscosity, K is the constant, S is the shear rate, and n is the thixotropic degree.

The logarithmic transformation of the equation is as follows:

$$\ln \eta = \ln K - n \ln S$$

When $\ln \eta$ is plotted against $\ln S$, the slope of the curve is thixotropic degree. Thixotropic degree of carbopol gel matrix and TAA-SLN gel was presented in Table 3.

As shown in Table 3, the thixotropic degree of TAA-SLN gel increased compared with the gel matrix, presenting better thixotropy. The thixotropic degree of TAA-SLN gel increased slightly with the increase of particle size of TAA-SLN.

Thixotropy is important for the percutaneous application of a drug. When the preparation is subjected to a shear force, its network structure breaks down leading to a gradual decrease in viscosity in order to spread on the skin. When the shear force is removed, the viscosity recovers slowly and the increased viscosity keeps the preparation staying on the skin. Our results indicated that carbopol gel and its SLN gel possessed favorable thixotropy and viscosity, suggesting that they are suitable for transdermal iontophoretic drug delivery.

3.4. Electrical conductivity

Hydrogel has good electrical conductivity because water medium is filled in its capillary space, which might be beneficial to iontophoresis (Nadia et al., 2000). As shown in Table 4, the electrical conductivity of TAA-SLN gel is higher than the gel matrix,

Table 3
Thixotropic degree of carbopol gel matrix and TAA-SLN gel

Formulation	Gel matrix	TAA-SLN-1 gel	TAA-SLN-2 gel	TAA-SLN-3 gel
n	0.3479	0.4113	0.3984	0.3920

Table 4
Electrical conductivity of carbopol gel matrix and TAA-SLN gel

Formulation	Gel matrix	TAA-SLN-1 gel	TAA-SLN-2 gel	TAA-SLN-3 gel
Ψ ($\times 10^{-4}$ S/cm)	25.7	32.0	31.1	29.6
$\Delta\Psi$	/	+6.3	+5.4	+3.9

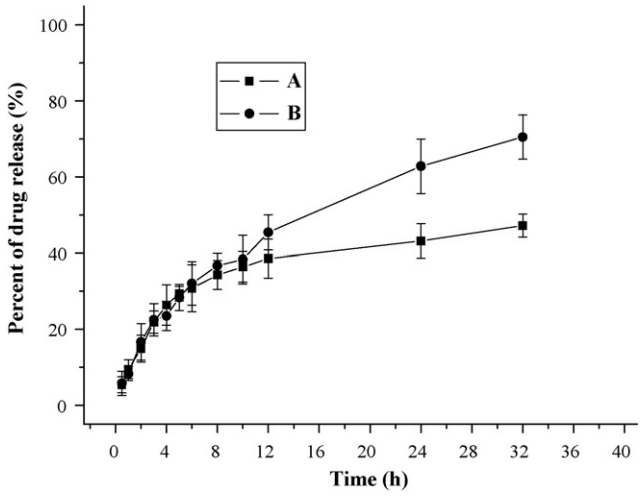

Fig. 3. *In vitro* release kinetics of TAA-SLN-1 gel (A: no electric field; B: pulse electric current) ($\bar{x} \pm s$, $n = 3$).

likely because the negatively charged TAA-SLN had electric repulsion with carboxylate anion and enhanced the movement of SLN under electric field, leading to the increase in the electrical conductivity. Table 4 also showed that the zeta potential of TAA-SLN correlated with its electrical conductivity. The electrical conductivity of TAA-SLN gel decreased slightly with the decrease of zeta potential of TAA-SLN. The results in our study indicated that TAA-SLN gel possessed high electric conductance that had good potential for transdermal iontophoretic application.

3.5. In vitro drug release kinetics

The *in vitro* drug release kinetics of TAA-SLN gel with and without applying electric current was shown in Fig. 3. Fig. 3 showed that the release rate of TAA from SLN gel was steady; there is no indication of drug release with and without applying electric current for 10 h. After 10 h, the drug release became slightly faster. The amount of the released drug reached 70.5% after 32 h, which was significantly higher than that without applying electric current (44.6%). The electric field enhanced drug releases from TAA-SLN gel may be based on the general principle of charge repel (Kalia et al., 2004). The increased drug release may be the long time effect of the electric field. When an electric field force is applied, the charged TAA-SLN is propelled through the gel towards the surface of dialysis membrane. The drug concentration on the membrane increases and the concentration gradient becomes higher, which promotes the drug transportation into the receptor compartment. The experiment also showed that the carbopol gel matrix was perfectly stable in electric field after the drug was released for 32 h.

3.6. Transdermal iontophoretic delivery studies

3.6.1. Effect of particle size

Summarized results of the assays of iontophoretic delivery of TAA from TAA-SLN gel and passive delivery of TAA from carbopol gel after 24 h porcine ear skin penetration were shown in Table 5. Passive penetration of TAA from carbopol gel was the control, which had the same drug concentration (0.06%, wt.%) as that in TAA-SLN gel. The enhancement ratio (ER) was treated as the iontophoretic penetration flux of TAA from TAA-SLN gel versus passive penetration flux of TAA from carbopol gel.

As shown in Table 5, all iontophoretic delivery of TAA from TAA-SLN gel was more effective than passive delivery of TAA from carbopol gel, and the particle sizes of TAA-SLN significantly affected the iontophoretic delivery of TAA. The larger the particle sizes of TAA-SLN, the lower the cumulative penetration amount and the steady-state penetration flux of TAA.

The relation between particle size of charged particles and iontophoresis permeability is not well studied. Previous studies have shown that podophyllotoxin-loaded SLN with small diameters penetrate into stratum corneum along the surface furrows on the skin and high accumulative amount of the drug in skin from SLN could be obtained (Chen et al., 2006). Similarly, the small diameter and increased drug release under electric field that might lead to the more effective iontophoretic delivery of TAA.

3.6.2. Effect of current density

Summarized results of the iontophoretic delivery of TAA from TAA-SLN-1 gel cross porcine ear skin at different current density after 24 h were shown in Table 6. Passive penetration of TAA from TAA-SLN-1 gel was used as the control.

From Table 6, it was apparent that the ER was linearly correlated with the applied current density, suggesting that the current density contributed to the iontophoretic penetration flux.

The dependence of iontophoretic penetration on the applied current is consistent with the Faraday's law and the equation is shown below (Yamamoto and Yamamoto, 1976; Singh and Maibach,

Table 5
Permeation kinetic parameters of TAA-SLN with different particle sizes in iontophoresis ($\bar{x} \pm s$, $n = 3$–5)

Formulation	24 h cumulative penetration amount, Q (nmol/cm^2)	Steady-state penetration flux, J_s (nmol/cm^2 h)	ER
Control	6.42 ± 1.36	0.52 ± 0.06	/
TAA-SLN-1	33.64 ± 2.80**	3.56 ± 0.37**	6.85
TAA-SLN-2	27.25 ± 3.04**	2.36 ± 0.55**	4.54
TAA-SLN-3	16.82 ± 3.17**	1.83 ± 0.62**	3.52

$^{**}p < 0.01$. ER = iontophoretic penetration flux of TAA from TAA-SLN gel/passive penetration flux of TAA from carbopol gel (control).

Table 6
Permeation kinetic parameters of TAA-SLN-1 in the iontophoresis at different current density ($\bar{x} \pm s$, $n = 3$–5)

Current density (mA/cm^2)	24 h cumulative penetration amount (nmol cm^2)	Steady-state penetration flux (nmol/cm^2 h)	ER
Control	13.74 ± 0.59	0.94 ± 0.22	/
0.2	21.64 ± 1.85**	1.56 ± 0.29*	1.70
0.3	24.56 ± 3.71**	2.06 ± 0.41**	2.19
0.5	33.64 ± 2.80**	3.56 ± 0.37**	3.79
0.6	39.80 ± 4.46**	4.23 ± 0.52**	4.50

$^{*}p < 0.05$, $^{**}p < 0.01$. ER = iontophoretic penetration flux of TAA from TAA-SLN-1 gel/passive penetration flux of TAA from TAA-SLN-1 gel (control).

Table 7
Permeation kinetic parameters of TAA-SLN-1 in the iontophoresis at different pulse frequency ($\bar{x} \pm s$, $n = 3–5$)

Frequency (Hz)	24 h cumulative penetration amount (nmol cm^2)	Steady-state penetration flux (nmol/cm^2 h)	ER
Control	13.74 ± 0.59	0.94 ± 0.22	/
50	23.60 ± 2.50**	2.12 ± 0.44**	2.26
1000	30.34 ± 1.84**	2.85 ± 0.32**	3.04
2000	33.64 ± 2.80**	3.56 ± 0.37**	3.79
4000	34.25 ± 3.63**	3.67 ± 0.56**	3.90
9000	34.86 ± 4.51**	3.98 ± 0.70**	4.23

** $p < 0.01$. ER = iontophoretic penetration flux of TAA from TAA-SLN-1 gel/passive penetration flux of TAA from TAA-SLN-1 gel (control).

Table 8
Permeation kinetic parameters of TAA-SLN in the iontophoresis at different on/off ratio ($\bar{x} \pm s$, $n = 3–5$)

On/off ratio	24 h cumulative penetration amount (nmol cm^2)	Steady-state penetration flux (nmol/cm^2 h)	ER
Control	13.74 ± 0.59	0.94 ± 0.22	/
1:5	17.42 ± 0.33*	1.79 ± 0.15**	1.90
1:3	21.61 ± 0.74**	2.42 ± 0.29**	2.58
1:1	33.64 ± 2.80**	3.56 ± 0.37**	3.79
3:1	30.50 ± 0.95**	3.03 ± 0.24**	3.22
5:1	28.39 ± 3.22**	2.59 ± 0.73**	2.76

* $p < 0.05$, ** $p < 0.01$. ER = iontophoretic penetration flux of TAA from TAA-SLN-1 gel/passive penetration flux of TAA from TAA-SLN-1 gel (control).

1994):

$$\frac{Q}{t} = \frac{\lambda I}{|Z|F}$$

where Q is the amount of iontophoretic penetration, t is the time, I is the current intensity (Amperes), and Z is the valence of the charged ion (or particulate). The transport number (λ) is the fraction of the total current transported by a specific ion (or particulate) and is a measure of its efficiency as a charge carrier, and F is Faraday's constant (Coulombs/mol).

Previous studies have shown that ER of iontophoretic penetration is proportional to the current intensity applied (Hinsberg et al., 1994; Gupta et al., 1998). Gupta et al. investigated the iontophoretic transport of fentanyl in rabbit under low current intensity and high current intensity. After 12 h, the AUC under high current intensity was linearly proportional to the current intensity, but was not linear under the low current intensity. Hinsberg et al. used DGAVP as the model drug and found that the relationship between steady-state ion iontophoretic transport and current density were not liner but parabola, indicating that the linear relationship between iontophoretic flux and current density is conditional. However, the higher current density (more than 0.6 mA/cm^2) might damage skin in the process of iontophoresis. The suitable current density should be selected to reduce the damage of high current intensity on skin, while keeping the efficiency of iontophoresis sufficient.

3.6.3. Effect of pulse frequency

Effect of pulse frequency applied on iontophoretic delivery of TAA from TAA-SLN-1 gel was shown in Table 7.

Results in Table 7 indicated that the cumulative penetration amount and steady-state penetration flux increased with the increase of current frequency in a certain range. But when the current frequency reached 2000 Hz, the penetration increase became slower.

When the electric current is constant, the skin impedance decreases with the increase of pulse frequency. The effective current intensity and the amount of iontophoretic transport will increase accordingly (Johnson and Gallo, 1998; Shibaji and Yasuhara, 2001). However, some adverse effects including electric burn and erythema have been reported in clinical situations. Furthermore, the operating time of direct current (DC) iontophoresis is limited by the polarization effect of the skin. The skin acts as a capacitor in electric circuit, and the effective current decreases with the time of continuous DC application. In order to avoid this polarization, pulse square DC iontophoresis has been used.

In our experiment, the penetration promotion effect at 2000 and 4000 Hz were similar to that at 9000 Hz. Therefore, we could regulate the electric parameters, such as current density and on/off interval ratio, to reduce the damage of high frequency DC on skin while keeping the efficiency of iontophores is high.

3.6.4. Effect of on/off interval ratio

Effect of on/off interval ratio applied on iontophoretic delivery of TAA from TAA-SLN-1 gel was shown in Table 8. The stratum corneum of the skin is composed of keratinocytes, which is a poor conductor because it contains high intercellular lipids and its water content is only approximately 20% (Yamamoto and Yamamoto, 1976). When continuous DC is applied on the skin, this layer of the skin may act as resistance and thus result in magnetic polarization currents. This polarization operates against the applied electrical field and greatly decreases the magnitude of effective current across the skin and the efficiency of transdermal delivery of drugs by iontophoresis. To avoid this polarization, the current should be applied in a periodic manner (pulsed current) (Koizum et al., 1999; Wu and Jin, 2004). With pulsed current, the electrical field is switched on and off periodically. In the "on" state, drugs are delivered by the iontophoretic diffusion process into the skin channels, and they could be blocked and accumulated in the skin reservoir. In the "off" state, electrical field is removed to permit the skin to depolarize and the drugs slowly diffused out of the skin and the current is discharged. Therefore, every new cycle could start with no residual polarization remaining in the skin from the previous cycle.

In our results, when the on/off interval ratio was 1:1, the efficiency of iontophoresis was high; when the on/off interval ratio was too low, like 1:5 or 1:3, the skin had long depolarization time and the effective iontophoresis time was short; when the on/off interval ratio was high, like 3:1 or 5:1, the skin was depolarized. Therefore, in the iontophoretic process, suitable on/off interval ratio of the current should be selected to enhance the iontophoretic efficiency.

4. Conclusion

In conclusion, TAA-SLN gel possessed good stability, rheological properties, and high electric conductance. Transdermal penetration

of TAA from TAA-SLN gel was significantly enhanced by iontophoresis. The enhancement in cumulative penetration and steady-state penetration flux of TAA were related to the particle size of TAA-SLN and the properties of the pulse electric current applied, such as density, frequency, and on/off interval ratio. The synergistic effect of iontophoresis and carbopol gel allowed the use of SLN carbopol gel as a vehicle for transdermal iontophoretic drug delivery.

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