

Full Length Research Paper

## Effect of the matrices and penetration enhancers in elemene transdermal drug delivery system

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The objective of this study is to develop an elemene transdermal drug delivery system by choosing suitable drug-loaded matrices and permeation enhancers and to observe their effects in this system. The *in vitro* transdermal release profiles were determined by Franz diffusion cells. The content of elemene in the collected samples was determined by gas chromatograph. Polyvinyl alcohol and sodium carboxymethyl cellulose were chosen as drug-loaded matrices. The optimal amount of polyvinyl alcohol and sodium carboxymethyl cellulose in matrices was 30% (w/w). It was found that azone was the best one among several penetration enhancers, compared with oleinic acid, eucalyptus oil and laurinol. Moreover, using azone together with propylene glycol, the permeation effect of elemene was even better. The appropriate ratio between azone and propylene glycol was 1:1. Moreover, 2% azone and 2% propylene glycol together as permeation enhancers achieved the best permeation effect for the elemene transdermal drug delivery system. The solution containing 20% saline, 50% ethanol and 30% PEG-400 was a good receiving media for -elemene in Franz diffusion cells. The amount of polyvinyl alcohol and sodium carboxymethyl cellulose in the matrices had obvious effects on the penetration ability of elemene. Types and concentration of the penetration enhancers had effect on the permeability of elemene through skin.

**Key words:** Transdermal, drug delivery system, penetration enhancers.

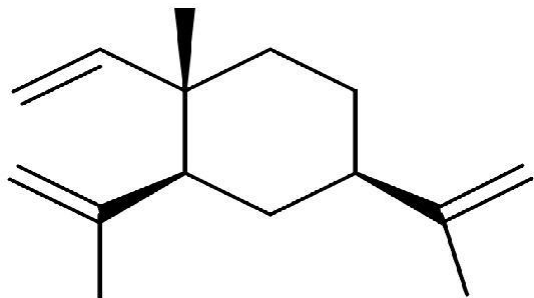
### INTRODUCTION

Elemene is chemical compounds that can be extracted from numerous plants (Chen, 2005). More than fifty different plants have been found to contain elemene. These plants include *Radix Inulae*, *Radix Ginseng*, *E. Wenyujin*, *G. Cymbopogon Winterianus Jowitt*, *Fuzhou Aglaia Odorata* flower, *Chunging Aglaia Odorata* leaves, *C. Wenyujin* Chen, etc., which grow in tropical areas around the world. Elemene has three isomers, -, - and -, with the -elemene as the main component (85%) (Guo, 1983). The chemical structure of -elemene can be seen in Figure 1. It has anti-tumor effects and low side effect. A large number of reports about elemene have been found in scientific magazines. In 1993, the -

elemene was officially approved as an anti-cancer drug in China. After that, injectable emulsions of -elemene has been used for the first line treatment of malignant pleural, chest and abdominal ascites, malignant brain tumors, cancers of respiratory and digestive tracts, as well as the second line treatment of cancers of female reproductive organs, breast cancer, metastatic bone cancer, skin cancer, lymphoma and leukemia (Li et al., 2005; Wang et al., 2005; Peng et al., 2006).

The present commercial dosage form elemene is oil-in-water emulsion (O/W) through injection or oral administration. Some problems occur in the emulsion, e.g., local phlebitis, fever, and decreased platelets after intravenously administration, as well as perfusion pain and throat burn after oral administration. In order to avoid these problems, new delivery systems for elemene are desired. So, a transdermal system for elemene is to be developed (Satturwar et al., 2005; Kawahara et al.,

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**Figure 1.** Chemical structure of elemene ( $C_{15}H_{24}$ , MW: 204.35).

2007). In this research, an elemene transdermal drug delivery system will be prepared and its percutaneous permeability *in vitro* through the mice dorsal skin will be studied.

## MATERIALS AND METHODS

Elemene was provided by DaLian Holleykingkong Co., Ltd. (Liaoning, China). Polyvinyl alcohol (AH-26) and Sodium carboxymethyl cellulose (300-800 mPa·s) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glycerine (chemical grade) and 1, 2-propylene glycol (chemical grade) were purchased from Jiangsu Shenhua Pharmaceutical Co., Ltd. (Jiangsu, China). Azone was purchased from Hubei tianmen kejie pharmacy Co., Ltd (Hubei, China). Oleinic acid and eucalyptus oil were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Lauryl alcohol was purchased from Tianjing Chemical Reagent Co., Ltd. (Tianjing, China). The animals were adult BALB/c mice of either sex, weighing 20 to 30 g, from Hangzhou Normal University; they were kept at  $25 \pm 1^\circ C$  and 45 to 55% relative humidity with a 12 h light/dark cycle. The *in vitro* experiments protocol was approved by the Institutional Animal Ethical Committee, Hangzhou Normal University, Hangzhou, China. The Franz cells used in this research was self-made, as shown in Figure 2. The surface area of permeation and the total volume of the Franz cell are  $4.5 \text{ cm}^2$  and 20 ml respectively.

### Preparation of elemene transdermal drug delivery system

Polyvinyl alcohol was fully wetted at  $60^\circ C$  and then mixed with sodium carboxymethyl cellulose. The mixture gelatin was transferred into a mortar followed by the addition of glycerin, penetration enhancer and a pressure-sensitive adhesive. Subsequently, elemene was added. All of the materials were ground sufficiently using a pestle, and then elemene transdermal drug delivery system was obtained. The system was a kind of cream that have smooth surface without the bullule in it. The content of elemene was 7.5% (w/w) in this system.

### Preparation of mice dorsal skins

8%  $\text{Na}_2\text{S}$  solution was daubed on the skin of BALB/c mice back for 3 to 4 min to shave the hair off, and then the mice were killed immediately. The dorsal skins of the mice were cut followed by removing subcutaneous fatty tissue, and then the skins were put in physiological saline, storage in  $4^\circ C$ .

## Transdermal release of elemene *ex-vivo*

The dorsal skin of mice was used as a skin model for the transdermal release experiments *ex-vivo* by using Franz diffusion cells. The experiment was conducted in a water bath at  $37^\circ C$ , while stirring at 100 rpm. At the following time points (1, 2, 3, 4, 12, 18, and 24 h), 0.5 ml of aliquot solution was taken out from the receptor cell and the same amount was replaced. The collected samples were extracted using n-hexane. Elemene concentration in the samples was determined by gas chromatograph. The cumulative permeation quantity (Q) of the drug per unit area ( $\text{cm}^2$ ) was calculated using the following equation.

$$\text{Equation 1: } Q = [C_n \times V + \sum_{i=1}^{n-1} C_i \times 0.5] / S$$

In this equation, Q is the cumulative permeation quantity, S is the area of permeation, V is the volume of the receiving media in receptor cell and C is the density of the drug in receiving media. All the permeation experiments were repeated three times and their mean values with standard deviation were calculated. The data of permeation rates were subjected to one-way analysis of variance (ANOVA) to determine the level of significance between various groups. The data were considered to have significant differences at  $p < 0.05$ .

### Standard curve

1 mg/ml elemene (purity 99.9%) solution in the n-hexane was diluted to 1, 2, 4, 8, 12, 16, 20, 30, 40, 60 and 80 g/ml. The analyses were carried out by gas chromatography. The expression of peak area and the concentration of elemene was established,  $Y=595.77X-118.01$ ,  $r=0.9998$ . The correlation coefficient of standard curve reached 0.9998 when the concentration ranged from 4 to 40 g/ml.

### Chromatographic parameters

The experiments were performed with a Shimadzu GS-2010 gas chromatograph equipped with a flame ionization detector. The column used is PEG-20M (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu\text{m}$ ). Nitrogen was used as a carrier gas. The GC analysis ramp consisted of a hold for 1 min at  $100^\circ C$ , and then a hold at  $230^\circ C$  for 22 min. 1 l of the sample liquid was detected.

### Passive cutaneous anaphylaxis (PCA) in mice

The cream of elemene transdermal delivery system was applied on the dorsal skin of mice alive for 24 h, 10 mice were used. The acute toxicity, dermal sensitivity and acute irritation of the transdermal delivery system were tested.

## RESULTS

### Effect of the drug-loaded matrices in the elemene transdermal drug delivery system

A good transdermal drug delivery system has the characteristics of favorable permeability, stable penetration rates and more drug-loading efficacy. The composition of matrices is very important in the preparation of elemene

**Table 1.** Effect of receiving media on the permeation of elemene through the mice skin.

Receiving medium	Elemene permeated in 12 h ( $\text{mg}\cdot\text{cm}^{-2}$ )
Saline	0
Saline : Ethanol = 1 : 1	$1.78 \pm 0.11$
Saline : Ethanol : PEG-400 = 2 : 5 : 3	$2.41 \pm 0.13$

It showed that when saline was used as the drug receiving media, elemene could not be detected in the receiving media. Elemene with low concentration could be detected when 50% ethanol was added to the receiving media containing saline. However, when PEG-400 was used to replace part of the saline in the receiving media, elemene of high concentration were detected.

**Table 2.** The mass ratio of polyvinyl alcohol and sodium carboxymethyl cellulose effect on the penetration rates of elemene transdermal drug delivery system.

The mass ratio of polyvinyl alcohol and sodium carboxymethyl cellulose	Penetration rates ( $\text{mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ )
0.25:1	$0.153 \pm 0.012$
0.5:1	$0.150 \pm 0.016$
1:1	$0.154 \pm 0.003$
2:1	$0.158 \pm 0.008$
3:1	$0.156 \pm 0.015$
4:1	$0.149 \pm 0.005$

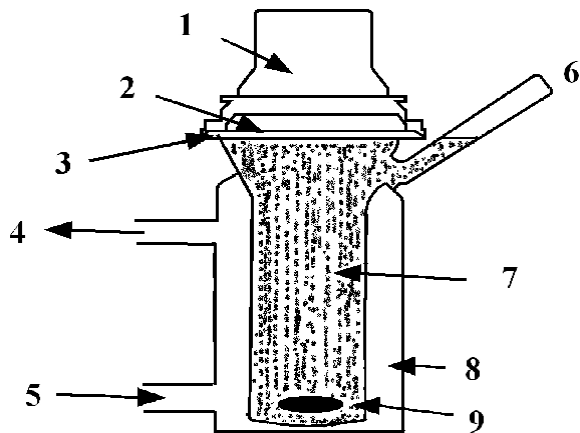
The data of permeation rates were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine the level of significance between various groups. The data were significantly different ( $p>0.05$ ). It showed that the effect of the mass ratio of polyvinyl alcohol and sodium carboxymethyl cellulose on elemene transdermal system was not very significant.

transdermal drug delivery system, not only because it has the effect on storing and loading the drug, but also affects the drug release from the system smoothly. In this research, polyvinyl alcohol and sodium carboxymethyl cellulose were used as the matrices skeleton due to good compatibility, and the resulted matrices had a high drug loading efficacy.

In this research, the drug receiving media in Franz diffusion cell were first investigated. Three different drug receiving media were used for the test. The first medium was saline. The second medium was saline and ethanol in a ratio of 1:1. The third one was saline, ethanol, and PEG-400 in a ratio of 2:5:3. The results of elemene concentration in the receiving cell were shown in Table 1. When saline was used as the drug receiving media, elemene could not be detected in the receiving media. This may be because the liposoluble elemene cannot be dissolved in the aqueous saline. Table 1 showed that elemene with low concentration could be detected when 50% ethanol was added to the receiving media containing saline. However, when PEG-400 was used to replace part of the saline in the receiving media, elemene of high concentration were detected. This may be due to the formation of the sink condition by enhancing the

dissolubility of elemene in the mixed solution.

The effects of the ratio of polyvinyl alcohol and sodium carboxymethyl cellulose in the matrices on the permeation ability of the elemene transdermal drug delivery system were also researched. All factors in this system were fixed except for the amount of polyvinyl alcohol and sodium carboxymethyl cellulose, their mass ratios were 1:0.25-4, and the content of elemene was 7.5% (w/w). The penetration rates of the elemene transdermal drug delivery system in 24 h were determined. The results were shown in Table 2. As shown in Table 2, it was found that the effect of the mass ratio of polyvinyl alcohol and sodium carboxymethyl cellulose on elemene transdermal system was not very significant. Meanwhile, the elemene transdermal drug delivery system had a good shape, such as smooth surface when the mass ratio of polyvinyl alcohol and sodium carboxymethyl cellulose was 3:1. However, more amount of sodium carboxymethyl cellulose could cause bubbles. The effects of the content of the matrices (polyvinyl alcohol and sodium carboxymethyl cellulose) on the percutaneous permeability of this system were also observed (Figure 3). The best content carrying matrices in the system is 30%.



**Figure 2.** Structure of self-made Franz Cell. (1) Cover, (2) Elemene transdermal patch, (3) Mice back's skin, (4) Outlet, (5) Inlet, (6) Sample tap, (7) Receptor cell, (8) Constant temperature water intercalated bed, (9) Stirrer.

### Effect of different penetration enhancers on transdermal permeation

Five different penetration enhancers had been investigated. Influence of 4% (w/w) oleinic acid, 4% (w/w) eucalyptus oil, 4% (w/w) laurinol, 4% (w/w) azone and a composite penetration enhancer (2% (w/w) propylene glycol plus 2% (w/w) azone) on the transdermal permeation have been compared with the system with no penetration enhancer (Figure 4). The effect of different penetration enhancers on transdermal permeation was as follows: 4% azone ( $0.119 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) > 4 % laurinol ( $0.081 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) > 4 % eucalyptus oil ( $0.073 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) > 4 % oleinic acid ( $0.04 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) > no penetration enhancer ( $0.038 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ). Azone is the best penetration enhancer. In contrast, the influence of 4% oleinic acid is little compared with other three penetration enhancers. Meanwhile, it was found that propylene glycol and azone had synergistic effect, which enhanced the permeation significantly. The rate of permeation reached  $0.156 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  when 2% propylene glycol plus 2% azone were used as permeation enhancers. The permeation amount per unit area of elemene increased from 0.12 to 0.94 mg in 4 h, while it increased from 0.12 to 3.74 mg in 24 h.

### Effect of composite penetration enhancers on transdermal permeation

In this research, different ratios of propylene glycol and azone influencing transdermal permeation were investigated (Figure 5). The results showed that when no propylene glycol was added in the elemene transdermal delivery system, the rate of permeation ( $0.119 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ ) was the slowest. When ratios of propylene glycol and

azone changed from 1:3 to 1:1, the rate of permeation increased from  $0.147$  to  $0.156 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ . However, the rate of permeation decreased to  $0.134 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$  when the ratio of propylene glycol and azone was 3:1. These results showed that content of the propylene glycol in the elemene transdermal drug delivery system had a great influence on the transdermal permeation. The best ratio of propylene glycol and azone was 1:1.

### Effect of penetration enhancer content on transdermal permeation

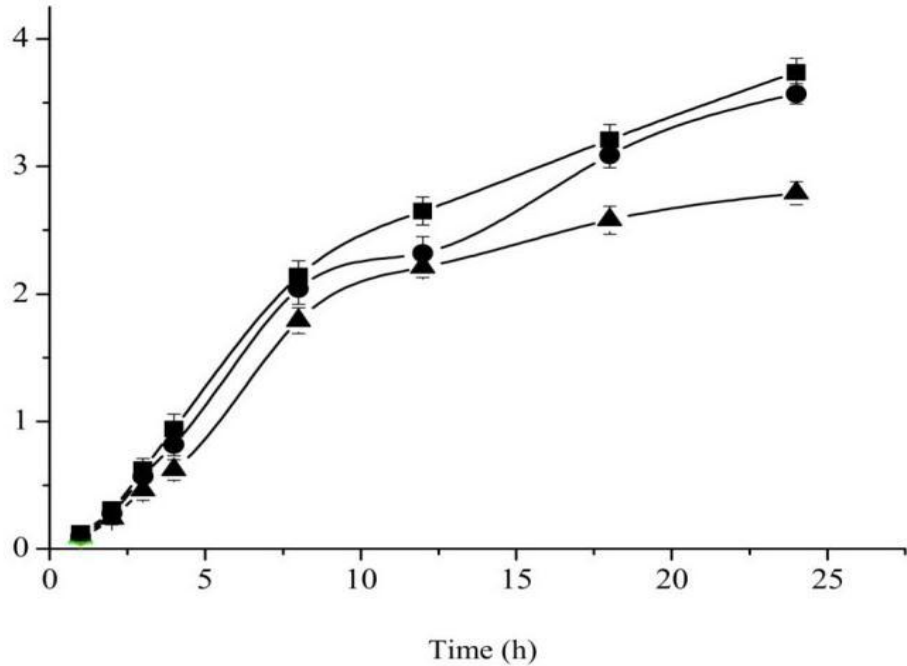
Composite penetration enhancer with the ratio of propylene glycol and azone as 1:1 was chosen to study the influence of penetration enhancer content on transdermal permeation (Figure 6). As shown in Figure 6, when the content of penetration enhancer increased from 2 to 4%, the rate of permeation increased greatly. Then, the increase of rate of permeation was very small when their content changed from 4 to 6%. However, the rate of permeation decreased when their content was continuously increased. The effect of azone on percutaneous permeability may be concentration-dependent at this concentration range, and hydrophobicity of high concentration of azone may prevent the diffusion of the drug.

### The safety of the system

It found no edema in the dorsal skins of mice. A little erythema was found on the dorsal skin of a mouse among the 10 mice, but the erythema was faded in 12 h. 10 mice lived normally after using the system for 24 h. Those demonstrated the safety of the elemene transdermal delivery system, suggesting it has mild skin allergy, low stimulatory and no toxicity.

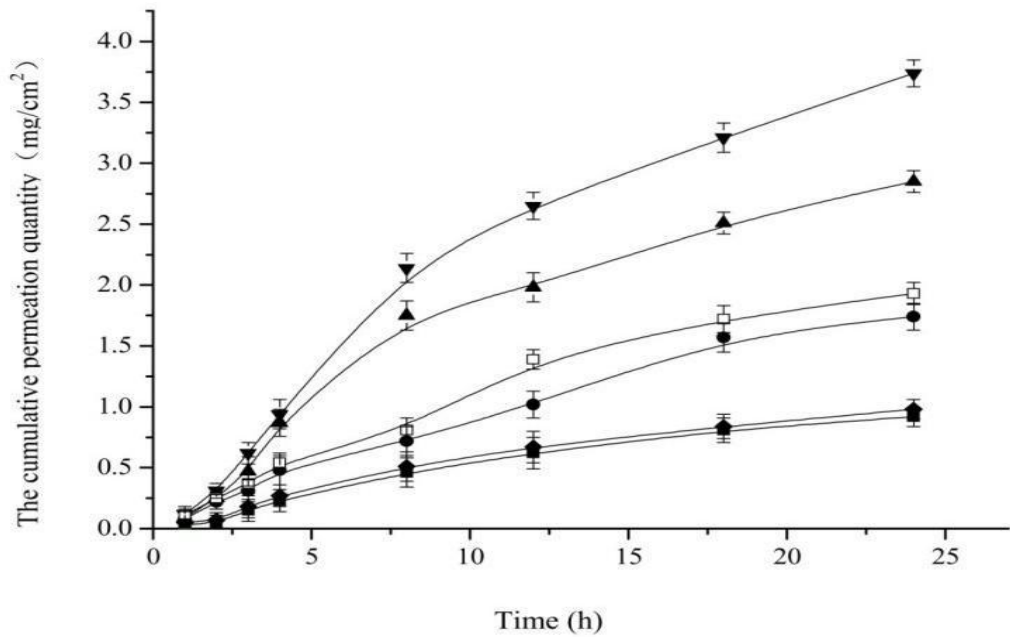
### DISCUSSION

Transdermal delivery of pharmacologically active agents has been extensively studied for the past 40 years. Despite the strong efforts, currently, only about 40 products are in market on about 20 drug molecules, due to the requirements that the patch area should be small enough for the patients to feel comfortable and due to the barrier properties of the stratum corneum. Various approaches to overcome the barrier function of skin through physical and chemical means have been broadly studied. The development of an effective transdermal drug delivery system is dictated by the unique physicochemical property of each drug molecule: (1) the transdermal drug candidate should possess both lipophilic and hydrophilic characteristics. Too hydrophilic molecules will not partition into the stratum corneum, and too lipophilic molecules will not move down to the



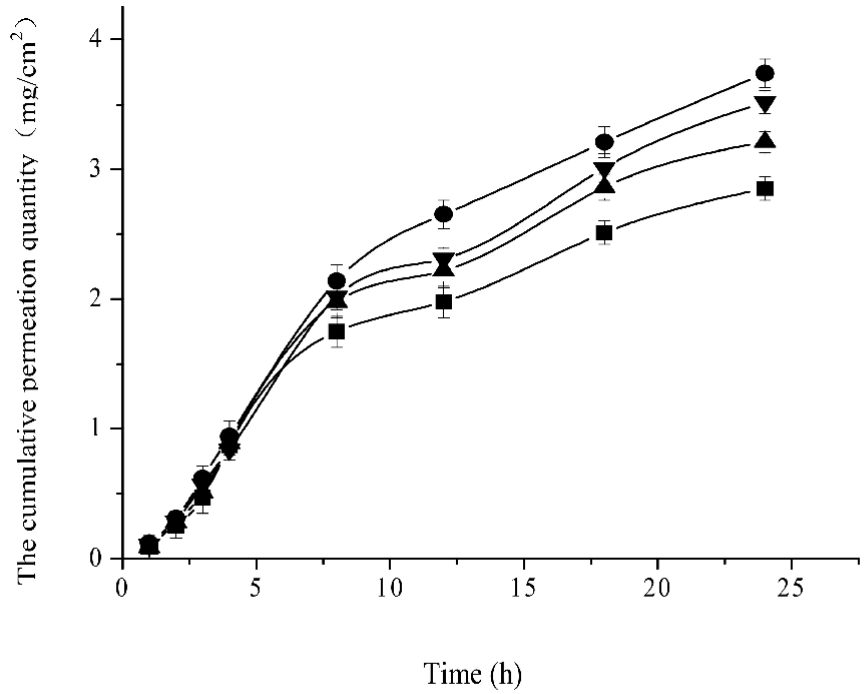
-▲- The content of carrying matrix is 20% -■- The content of carrying matrix is 30%  
 -●- The content of carrying matrix is 40%

**Figure 3.** The effect of the content of carrying matrix on the percutaneous permeability (the mass ratio of polyvinyl alcohol and sodium carboxymethyl cellulose is 3:1).



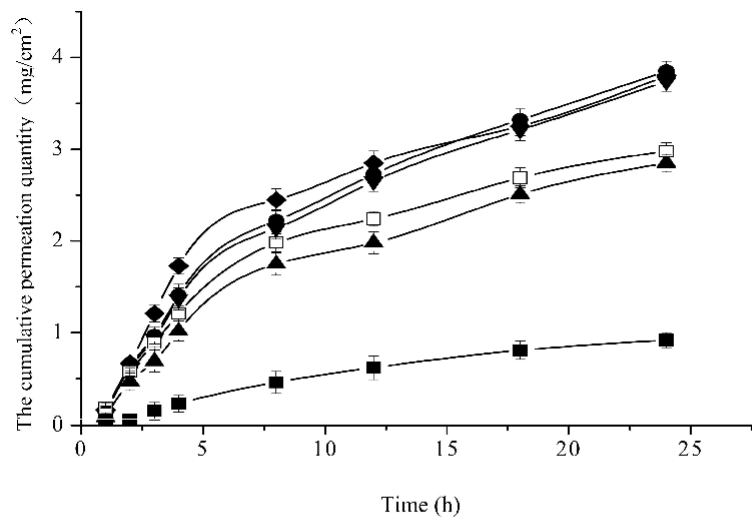
-▼- 2wt% propylene glycol + 2 wt% azone -▲- 4 wt% azone -□- 4 wt% laurinol -●- 4 wt% eucalyptus oil -◆- 4 wt% oleinic acid -■- no penetration enhancer

**Figure 4.** Effect of different penetration enhancers on transcutaneous permeation.



-●-2 wt% propylene glycol +2 wt% azone    -▲-3 wt% propylene glycol +1 wt% azone  
 -▼-1 wt% propylene glycol +3 wt% azone    -■-4 wt% azone

**Figure 5.** Effect of different ratio of propylene glycol and azone on transcutaneous permeation.



-□-10 wt% complicate penetration enhancer    -◆-8 wt% complicate penetration enhancer    -●-6  
 wt% complicate penetration enhancer    -▼-4 wt% complicate penetration enhancer    -▲-2 wt%  
 complicate penetration enhancer    -■-no penetration enhancer

**Figure 6.** Effect of penetration enhancer content on transcutaneous permeation (propylene glycol: azone is = 1:1).

subsequent aqueous layer in the epidermis (Potts et al., 1992); (2) some drugs are not appropriate for transdermal administration because of their physico-chemical properties, they may be too large, charged, or have insufficient lipid solubility; (3) drug may also have unfavorable pharmacokinetic or pharmacodynamic behavior such as too rapid clearance relative to achievable rate of skin delivery (Gwak et al., 2001). As shown in Figure 1, elemene as small and lipophilic molecule belongs to the terpene family. The elemental components of elemene were carbon and hydrogen only. Physically, terpenes behave like volatile oils with a high vapor pressure at room temperature. Being small molecules with a high vapor pressure, elemene oil could be very "invasive" and capable of penetrating into skin. Hence the potentiality of elemene transdermal delivery is feasible. In this research, an elemene transdermal drug delivery system had been developed. The transdermal drug delivery system may overcome the adverse effect caused by "tissue compatibility", such as pain, irritation, inflammation or other adverse reactions at the injection site. This transdermal drug delivery system of elemene has not been studied previously. So, this research pioneered the study of elemene transdermal drug delivery system for the future. It was found in this study that the cumulative permeation quantity of elemene was the lowest when there was no penetration enhancer in the system. It may be due to the low hydrophilic characteristics of elemene, resulting in the aqueous layer of the skin preventing the penetration of the drug. So, several penetration enhancers that may increase the permeability of the drug had been examined (Barry et al., 1987; Sinha et al., 2000). It was found that azone was the best one among several penetration enhancers, compared with oleinic acid, eucalyptus oil, and laurinol. Moreover, using azone together with propylene glycol, the permeation effect of elemene was even better. The optimum ratio of azone and propylene glycol was 1:1. Moreover, 2% azone plus 2% propylene glycol together as permeation enhancers achieved the best permeation effect for the elemene transdermal drug delivery system. Penetration enhancers may either disrupt lipid organization and increase drug diffusion coefficient or interact with keratin in corneocytes, opening up the dense protein structure (Goldberg-Cettina et al., 1995; Kim et al., 1992). Alteration of the chemical environment could also favor the partitioning of drug in the stratum corneum. The penetration enhancer azone could create gaps in the packed lipid structure and thus reduced diffusional resistance (Zhu et al., 1996). Meanwhile, propylene glycol usually took part in transdermal drug delivery system as solvent, co-solvent, humectant, and preservative. Researchers also found that, it could promote the permeation, when it synergistically acted with penetration enhancers (Zhao et al., 2009). The possible reasons were: (1) the ability of the transport and the distribution in corneum layer of the penetration

enhancers was increased by propylene glycol; (2) the solubilization of lipid bilayer could be promoted by propylene glycol. When more penetration enhancer plugged in the lipid bilayer, more drugs passed through the skin; (3) holding period of penetration enhancers in corneum layer could be prolonged by propylene glycol; (4) liposolubility penetration enhancers such as azone also could help propylene glycol reach the intercellular space of corneum layer (Thong et al., 2007).

## Conclusion

The amount of polyvinyl alcohol and sodium carboxymethyl cellulose in the matrices had obvious effects on the penetration ability of elemene. Types and concentration of the penetration enhancers had effect on the permeability of elemene through skin.

## ACKNOWLEDGEMENTS

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