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Effect and mechanism of penetration enhancement of organic base and alcohol on Glycyrrhetinic acid *in vitro*

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ABSTRACT

The aim of the present study was to explore the effects of organic bases and alcohols on the percutaneous absorption of Glycyrrhetinic acid (GA). GA is a metabolite of Glycyrrhizic acid (GL), a major active ingredient of *Glycyrrhizae* (Gancao) Radices. Skin penetration parameters of GA were obtained via *in vitro* penetration experiments using intact and stripped mice back skin. Non-aqueous solvent comprising isopropyl myristate (IPM) and alcohols (ethanol, butanol, octanol and dodecanol) loaded with organic base (triethanolamine or triethylamine) were applied to improve the penetration of GA. In order to further confirm the mechanism by which the organic bases enhanced the penetration of GA, conductivity measurement, ^{1}H NMR spectroscopy and FT-IR analysis were used to observe the formation of ion pair between GA and organic base. The formation of ion pair increased the solubility of GA in the stratum corneum (SC) and its partition into the viable skin, and therefore enhanced the penetration of GA in skin.

1. Introduction

Glycyrrhizae (Gancao) Radices have many endocrine and biological properties (Armanini et al., 2002). Glycyrrhizic acid (GL), an active component of *Glycyrrhizae* Radices, has immunomodulatory property and is active against inflammation, virus, and tumors (Pompei et al., 1979; Yi et al., 1996; Chung et al., 2000; Raphael and Kuttan, 2003). Currently, GL is of clinical interest because of its potential use in the treatment of chronic hepatitis (Cinatl et al., 2003; Coon and Ernst, 2004).

The therapeutic use of GL is limited by its serious side effects such as hypertension, gastrointestinal irritation and pseudoaldosteronism disease, when administered orally (Wash and Bernard, 1975; Ploeger et al., 2000). Transdermal delivery offers an alternative route for administering GL that bypasses the gut, resulting in a more convenient and safer mean for its delivery. The molecular weight of GL is 822.92 and the preliminary study carried out in our laboratory demonstrated that GL could hardly permeate through the intact mouse skin. Glycyrrhetinic acid (GA) (Fig. 1) is the active aglycone of GL derived from hydrolysis after oral ingestion (Wang et al., 1994). It has been accepted that compound with $\log P_{o/w}$ ($\log P$) of 2–3 would be easier to penetrate through the skin (Ding et al., 1998). However, the $\log P$ of GA is 6.574, and therefore a strategy for permeation enhancement of GA will be necessary.

GA has a carboxyl group, and a compound which is acidic or alkaline possessing polar groups may not have the proper lipophilicity to enable it to penetrate through the skin. Oppositely charged ions could alter the lipophilicity of a compound with acidic or basic polar groups and improve its penetration by neutralizing the polar groups (Fang et al., 2003; Wang et al., 2005). The incorporation of mefenamic acid with alkanolamine (including monoethanolamine, diethanolamine, triethanolamine and propanolamine) increases the transdermal flux of mefenmic acid in a lipophilic enhancer system that consisted of isopropyl myristate (IPM) and ethanol (9:1; EI system). A series of fatty acids have been used to enhance the permeation of physostigmine in two solvents of opposing lipophilicity and the mechanism has been discussed. The objectives of this paper were to investigate the permeation enhancement for GA facilitated by organic base in non-aqueous solvent system and to further understand the enhancement mechanisms of organic base in skin permeation of GA *in vitro*.

2. Materials and methods

2.1. Materials

Glycyrhetic acid was purchased from Zelang Pharmaceutical Technology Co. Ltd. (Nanjing, China); triethanolamine (TEA), ethanol, butanol, octanol, monopotassium phosphate, sodium tetraborate and glycerin were purchased from Bodi Drug Manufacturing Co. Ltd. (Tianjin, China); triethylamine (TETN), sodium hydrate, sucrose, amidulin and acetonitrile which was of HPLC

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grade were purchased from Kemiou Technology Co. Ltd. (Tianjin, China); isopropyl myristate (IPM) was obtained from China National Medicines Co. Ltd. (Shenyang, China); dodecanol was purchased from No. 1 Reagent Co. Ltd. (Shanghai, China); sodium sulfide was purchased from Xinxing Reagent Co. Ltd. (Shenyang, China); orthophosphoric acid was purchased from Zhiao Chemical Reagents Research Institute (Anshan, China). All other chemicals and solvents were of reagent grade.

2.2. HPLC conditions

A Shimadzu instrument (LC2010A, LC solution workstation) and a Diamonsil™ C18 column (5 μm, 4.6 mm × 150 mm) were used. The mobile phase was acetonitrile–purified water–orthophosphoric acid (71:29:0.03, v/v/v). The column was maintained at 30 °C and the flow rate was 1.0 ml/min while the UV detector was set at 254 nm. The volume of injection was 20 μl. A calibration curve was constructed to determine the concentration of GA in the range of 0.55–55 μg/ml and the R^2 were at least 0.999.

2.3. Skin preparation

Chinese Kun Ming (KM) male mice weighing 20–25 g used in all experiments were supplied by Dalian Medical University. Sodium sulfide 4 g, Amidulin 3.5 g, sucrose 2 g, sodium tetraborate 0.5 g, Glycerin 2.5 g and water 37.5 g were carefully weighed in a 50-ml beaker and stirred to give a homogeneous solution. The hairs on the back of the animal were gently rubbed with a pledget pre-wetted in this solution. After all the hairs were removed, the remaining solution on the back of the animal was washed away with warm water. The treated animal was kept for at least 48 h before the back skin was excised. The skin sample was checked to ensure that no visible defect was present. Intact skin without fat and sub-dermal tissue was used for *in vitro* permeation studies. The thickness of the skin used ranged from 0.25 to 0.45 mm. Tape-stripped skin was prepared by stripping the SC with an adhesive tape 15–20 times. The skin was stored at −20 °C for up to one week. Before starting the experiments, the skin was kept at room temperature for overnight.

2.4. GA solubility

The solubilities of GA in IPM, IPM–alcohol systems, water, and octanol with or without organic base (TEA or TETN, 6 μM) were determined at 37 °C. Excess GA was dispersed into 5 ml of each of the above solutions in a test tube. The mixture was mixed by vortexing at high speed and allowed to equilibrate in a water bath for more than 24 h. The saturated solution was then filtered through a 0.22 μm membrane and GA was assayed by HPLC after an appropriate dilution.

2.5. In vitro penetration experiment

The skin permeability of GA was evaluated *in vitro* using the modified Franz type horizontal diffusion cells consisting of two half-cells with a water jacket connected to a water bath at 37 ± 0.5 °C. Each half-cell had a volume of 5 ml. The SC was arranged to face the donor solution and the available skin area for permeation was 0.627 cm^2. For the determination of *in vitro* penetration parameters, both the donor and the receptor solutions consisted of 20% ethanol, but the donor solution was saturated with GA. In other *in vitro* penetration experiments, the receptor solution used was 40% ethanol in PBS (pH 7.4) in order to maintain sink condition. Both donor and receptor cells were placed on a magnetic stirrer and stirred at a speed of more than 700 rpm. During all the experiments, excess GA was maintained in the donor cell. Samples (4.0 ml) were withdrawn at 1, 3, 6, 9, 12 and 24 h, and 4.0 ml of fresh receptor fluid was immediately added to the cell. The concentration was determined by reversed phase HPLC with reference to a calibration curve.

2.6. Conductivity experiment

The conductivities of GA, organic base (TEA or TETN) and the corresponding 1:1 molar ratio of GA–organic base mixtures in octanol were measured. The conductivity of octanol alone was also measured for comparison. The electric conductivity was measured at room temperature with a conductivity meter (Leici DDSJ-308A, Shanghai precision & scientific instrument Co. Ltd., China). The unit of electric conductivity is μS/cm.

2.7. ^1H NMR spectroscopy

^1H NMR spectra of GA, TEA, TETN and their mixtures were measured in $CDCl_3$ with or without 5% alcohols, including ethanol, butanol and octanol with a Bruker Advance II400 spectrometer (Bruker, USA). Chemical shifts were recorded as units relative to tetramethylsilane (internal standard).

2.8. FT-IR analysis

FT-IR spectra of GA, TEA, TETN and their mixtures were recorded in the range of 400–3600 cm^{-1} using an Nexus™ FT-IR spectrometer (Nicolet, USA).

2.9. Data analysis

Parameters of drug penetration through the intact and stripped skin *in vitro* were calculated according to the literature using the following equations (Tojo, 2003):

$$\eta = \frac{(dQ/dt)_w}{(dQ/dt)_v} \quad (1)$$

$$\tau = \frac{t_{dv}}{t_{dw}} \quad (2)$$

$$C_s = \frac{(1 - 3\tau + 2\eta\tau)}{(1 + 2\eta)(1 - \eta)} \frac{6t_{dw}}{h} \left(\frac{dQ}{dt}\right)_w \quad (3)$$

$$D_s = \frac{1}{1 - \eta} \frac{h}{C} \left(\frac{dQ}{dt}\right)_w \quad (4)$$

$$D_v = \frac{{H_v}^2}{6t_{dv}} \quad (5)$$

$$D_w = \frac{{H_w}^2}{6t_{dw}} \quad (6)$$

$$K_v = \left(\frac{dQ}{dt}\right)_v \frac{H_v}{C \cdot D_v} \quad (7)$$

$$K_w = \left(\frac{dQ}{dt}\right)_w \frac{H_w}{C \cdot D_w} \quad (8)$$

$$K_{s/v} = \frac{1}{(K_v/K_w) - 1} \quad (9)$$

where v represents viable skin, w represents intact skin, s represents SC, D is the diffusion coefficient of drugs, K is the partition coefficient, $K_{s/v}$ represents the partition coefficient between SC and viable skin, Q represents the cumulative amount penetrated, and C_s is the concentration of the drug on the surface of skin. Steady state flux (Flux) and lag time (t_{lag}) were obtained from the slope and x-intercept of the steady state portion of the permeation profile, respectively. The permeation coefficient (P) was calculated by

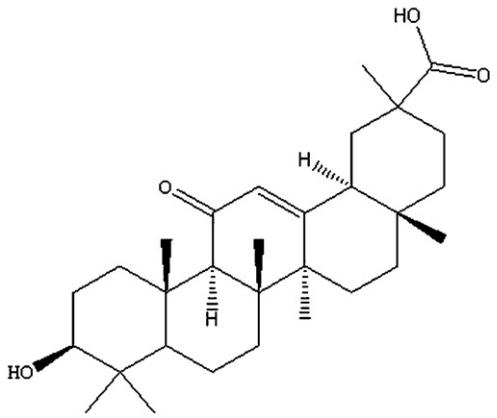

Fig. 1. Chemical structure of Glycyrrhetinic acid.

dividing J_s by the solubility of GA in the individual donor vehicles. The enhancement ratio (ER) was calculated by dividing Flux of GA suspension (with enhancer) by that of GA suspension without enhancer. All experiments were replicated at least three times. All data were calculated and presented as mean ± SE.

3. Results and discussion

3.1. Determination of penetration parameters in vitro

In the experiment that determined the *in vitro* penetration parameters of GA, the effect of solvent on SC should be as small as possible so that the calculated parameters can be closer to the true value and more informative to the subsequent experiments. Though water is often used as the solvent, the extremely poor solubility of GA in water has limited its application. Water containing 20% ethanol could effectively increase the solubility of GA and was therefore used as the donor and receptor solutions to keep the sink condition. *In vitro* penetration experiments through intact and stripped skin were carried out and the penetration profiles obtained are shown in Fig. 2. The calculated parameters are shown in Table 1.

Some drugs may bind to the skin tissue. Both the SC and the viable skin are responsible for the binding of drugs by the skin. Preliminary results (unpublished data) indicated that no binding occurred between GA and the skin. Linear regression was used to evaluate the steady state, and the corresponding correlation coefficient of GA obtained with intact or stripped skin after 6 h was over 0.9 in both cases. All the permeation parameters were calculated after the steady state had been reached.

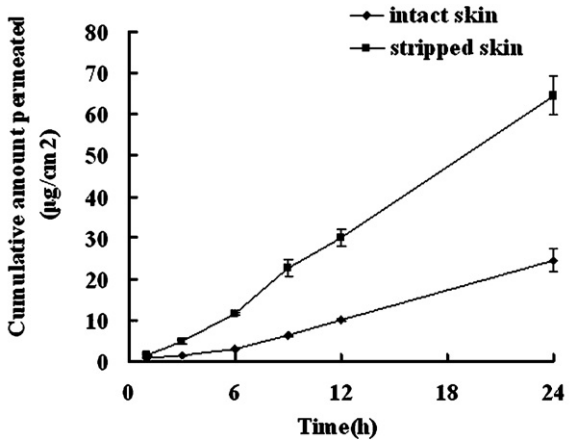

Fig. 2. Penetration of GA through intact and stripped skin ($n = 3$, mean ± SD).

Table 1
Penetration parameters of GA through intact and stripped skin using 20% ethanol as donor and receptor solution ($n = 3$).

Skin	t_{lag} (h)	D (cm²/s)	K
Intact skin	3.54	6.14×10^{-7}	3.23
Stripped skin	1.46	1.45×10^{-6}	3.57
SC		2.45×10^{-9}	9.62[a]

[a] K of SC stands for $K_{s/v}$.

Diffusion and partition are two impacting factors of penetration. As shown in Fig. 2, the cumulative amount of GA penetrated within 24 h (Q_{24}) through intact skin was as low as 24.45 μg/cm². The lag time of stripped skin was 1.46 h, 2 h shorter than that of intact skin (3.54 h), which was consistent with the literature, since the lag time for most drugs without skin binding is usually 3–5 h in hairless mouse skin (Tojo, 1987). This also showed that a faster steady state could be achieved in viable skin. The diffusion coefficients of viable intact skin and SC were 1.45×10^{-6}, 6.14×10^{-7} and 2.45×10^{-9} cm²/s, respectively, which suggested the low Q_{24} could not be attributed to the diffusion of GA across the skin. The poor solubility of GA in 20% ethanol (30.91 μg/ml) led to its poor solubility in SC (521.71 μg/cm³, as calculated according to Eq. (3) in Section 2.9) and therefore resulted in the low Q_{24}. On the other hand, the Q_{24} for stripped skin was increased by only 2.6-fold. The calculated $K_{s/v}$ was about 9.6, which showed that GA was a compound with strong lipophilicity. Thus not only SC, but viable skin also acted as a barrier to the penetration of GA. Hence, non-aqueous solvent system was introduced to increase the solubility of GA, and organic bases were used to increase the partition of GA in viable skin in order to improve its penetration.

3.2. Effects of organic bases and alcohols

The effects of organic bases and alcohols on the permeation of GA through mouse back skin *in vitro* were examined, and the results are summarized in Table 2 and Fig. 3.

In the absence of organic base, the solubility of GA was increased from 1.18 mg/ml to 4.85, 3.53, 3.15 and 2.02 mg/ml after addition of 5% ethanol, butanol, octanol and dodecanol, respectively, with the corresponding Q_{24} values of 272.52, 626.29, 427.05, 588.55 and 337.83 μg/cm². Increased solubility of GA could therefore lead to greater Q_{24}. However, P, which had eliminated the effect of solubility, did not vary significantly in some extent, indicating that

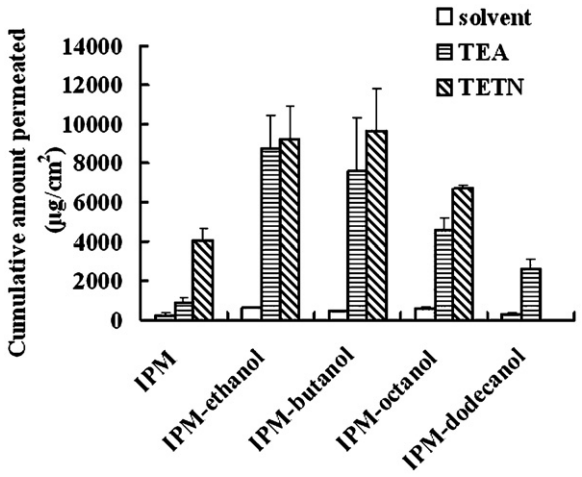

Fig. 3. Effects of organic bases and alcohols on the permeation of GA (IPM:alcohols = 95:5 (v:v); final concentration of organic base = 6 μM) ($n = 3$, mean ± SD).

Table 2
Parameters for the penetration of GA through mice back skin using different donor phases consisting of IPM; IPM:alcohols = 95:5 (v/v) with or without organic base including TEA and TETN ($n = 3$, mean ± SD).

Vehicle	Solubility (mg/ml)	Q_{24} (μg/cm^2)	$P \times 10^3$ (cm/h)	ER
Without organic base				
IPM	1.18	272.52 ± 77.63	9.99 ± 2.74	1.00
IPM–ethanol	4.85	626.29 ± 7.84	6.59 ± 0.03	2.71
IPM–butanol	3.53	427.05 ± 34.76	5.51 ± 0.41	1.65
IPM–octanol	3.15	588.55 ± 88.29	9.94 ± 1.17	2.66
IPM–dodecanol	2.02	337.83 ± 34.76	10.36 ± 0.57	1.78
With TEA				
IPM	1.33	880.85 ± 244.37	37.80 ± 7.65	4.26
IPM–ethanol	5.73	8779.71 ± 1688.40	65.25 ± 12.28	31.71
IPM–butanol	3.98	7630.17 ± 2666.85	94.20 ± 31.48	28.20
IPM–octanol	3.12	4562.10 ± 646.96	81.66 ± 8.64	21.61
IPM–dodecanol	2.43	2624.75 ± 492.87	61.51 ± 8.45	12.67
With TETN				
IPM	1.56	4061.60 ± 633.99	123.38 ± 16.94	16.33
IPM–ethanol	5.85	9194.66 ± 1713.36	72.36 ± 12.20	35.91
IPM–butanol	4.37	9600.37 ± 2213.76	97.95 ± 21.11	36.30
IPM–octanol	3.11	6672.72 ± 201.74	104.72 ± 25.86	27.62

addition of alcohols in such a small amount only had negligible effect on the SC.

It has been accepted that compound with log P of 2–3 could easily penetrate through the skin (Ding et al., 1998). SC is mainly composed of keratinocytes and intercellular space, including sterols, fatty acids, as well as a variety of lipid and other components. The overall nature of SC is hydrophobic. The density and conductivity of viable skin is only slightly larger than that of water. Viable skin in which molecules could diffuse easily is hydrophilic (Benson, 2005). The log P of GA is 6.574, which is much bigger than the value of 2–3 for effective penetration. Even if GA could easily penetrate through SC, it could not partition into viable skin. After the addition of TEA or TETN, there was a slight improvement in solubility. However, Q_{24} increased significantly in the presence of TEA or TETN compared to that of GA in the single solvent system (IPM only). As shown in Table 3, after adding TEA or TETN (6 μM), the solubility of GA in water reached 104.98 or 730.37 μg/ml compared to 2.75×10^4 or 3.02×10^4 μg/ml in octanol. According to these data, the calculated apparent log P was 2.42 for TEA–GA or 1.62 for TETN–GA ion pair. The use of an organic base as an organic modifier could change the characteristic of the original solvent, which would then cause a change in solubility of a compound. Organic bases might also form ion pairs with GA. The formation of ion pair on the one hand could neutralize the polar group of a compound and make the whole ion pair more inclined to electric neutrality, leading to lower polarity and higher lipotropy. On the other hand, the formation could change the equilibrium distribution of polar groups within a compound, leading to increased polarity. From the data of log P, the latter was the major factor. Therefore, the increased amount of GA permeated can be attributed to an increase in solubility, and it mainly depended on the formation of ion pairs, which could improve the partition of GA into viable skin and reduce the barrier of skin to increased penetration.

TETN improved the penetration of GA more effectively than TEA. This could be explained by TETN being a stronger base, and thus would form a stronger ion pair with GA. In the IPM–alcohol system with TEA, ethanol varied the penetration of GA significantly and the order of Q_{24} was ethanol > butanol > octanol > dodecanol. In these systems with TETN, the order was ethanol ≈ butanol > octanol. The effect of enhancement had some relationship with the length of the carbon chain; the longer the chain, the weaker the enhancement effect. The bigger Q_{24} and smaller P (65.25 and 72.36) of GA in the IPM–ethanol system indicated that the most significant enhancement could mostly be attributed to the increase in GA solubility compared to other systems. The enhancement ratio with ethanol was 2.71 while that with TETN was 4.26. However, the enhancement ratio in the solvent system with both ethanol and TETN was 31.7, which was much larger than the sum of 2.71 and 4.26. Similar results could be observed in other groups with other alcohols or TEA. This suggested that alcohols and organic bases had synergistic enhancement effect.

Table 3
Solubility of GA in the vehicle of water, octanol with TEA and TETN.

Vehicle	Solubility (μg/ml)	
	With TEA	With TETN
Water	104.98	730.37
Octanol	2.75×10^4	3.02×10^4

3.3. Conductivity measurement

Conductivity of GA with or without organic base in octanol was studied to mimic *in vivo* modality of GA in the SC. The conductivity in octanol reflected the number and mobility of ions in the SC. Fig. 4 illustrates the changes in conductivity after the addition of GA to octanol with or without organic base. The conductivities of octanol with and without organic bases were almost the same (about 0.02 μS/cm), which indicated that in octanol (a non-aqueous

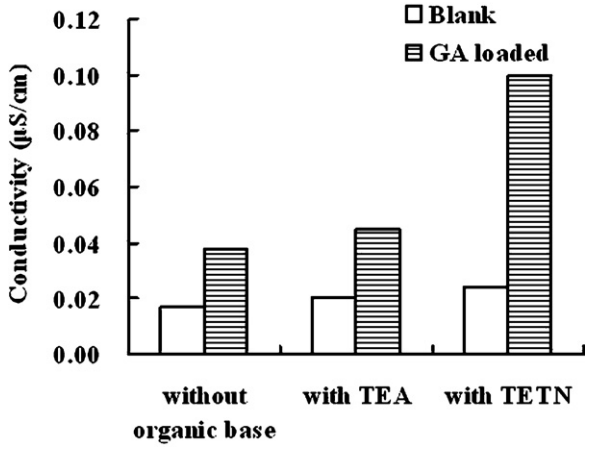

Fig. 4. Conductivity measurements of octanol with and without 6 μM organic base before and after the addition of GA ($n = 3$, mean ± SD).

solvent) the two organic bases ionized weakly. After GA was loaded, the conductivities in all systems increased, but the systems containing organic bases showed higher increases in conductivity, with the highest increase (about fourfold) occurred in the TETN system. The higher increases in conductivity observed for TETN and TEA systems caused by the addition of GA suggested that more GA and organic base ionized, forming ion pairs. The higher conductivity of TETN system compared to TEA system showed that the ion pair formed between GA and TETN was stronger and having higher polarity, consistent with the log *P* of the ion pairs described above.

3.4. ^{1}H NMR spectroscopy

The interaction between organic base and GA was evaluated by ^{1}H NMR spectroscopy (Fig. 5). There was no peak belonging to the active hydrogen atom of the carboxyl group in GA and the chemical shifts of GA with the addition of TEA or TETN were similar to that of GA alone. However, the characteristic peaks of TEA and TETN varied after the addition of GA. The characteristic peaks of TEA consisted of multiplet (2.59 → 2.89, belonging to $-CH_2-$ bonded with nitrogen atom) and triplet (3.63 → 3.75, belonging to $-CH_2-$ bonded to –OH). The characteristic peaks of TETN consisted of quartet (2.52 → 2.89, belonging to $-CH_2-$ bonded with nitrogen atom) and triplet (1.03 → 1.18, belonging to $-CH_3$).

The increased chemical shifts of the characteristic peaks belonging to TEA and TETN showed the change in chemical environment of the hydrogen atoms. This may be due to electrostatic interaction between the nitrogen atom of TEA as well as TETN and the carboxyl group of GA, as a result of ion pair formation. Formation of N^+ from nitrogen atom increased its ability to withdraw electron and deshield, leading to the increased chemical shift. The effect was more significant on the hydrogen atom that was nearer to the nitrogen atom. In the presence of TEA, the extent of shift was 0.30 whereas in the presence of TETN it was 0.37. The difference between the two shifts was 0.07 ppm. Since the alkalinity of TETN was higher than that of TEA, the ion pair between GA and TETN was expected to be stronger as reflected by its greater chemical shift.

As alcohols could form hydrogen bond with the carboxyl group of GA and the nitrogen atom and hydroxyl group of TEA as well as the nitrogen atom of TETN, the effect produced by the presence of alcohol in the solvent system on the formation of ion pair was evaluated. The effects of alcohol on the chemical shifts of TEA and TETN are shown in Table 4. All alcohols caused an increase in chemical shift compared to the organic base alone, implying the formation of ion pair. On the contrary, the addition of alcohol caused a decrease in chemical shift compared to the system with GA but with no alcohol. This suggested that the presence of alcohol reduced the intensity of ion pairs via hydrogen bond effect.

3.5. FT-IR analysis

In order to confirm the interaction between GA and organic base, FT-IR analysis was performed. A peak at 1705 cm^{-1}, which is attributed to carbonyl stretching, appeared in the spectrum of GA (Fig. 6). This characteristic peak appeared weaker in the mixture of GA and organic base, but another peak appearing at 1560 cm^{-1} was detected in the mixture, and this peak can be attributed to carboxylate salt, which tends to have a lower frequency than that of the carboxylic acid due to resonance. Carboxylate anion usually shows an absorption peak at 1650–1550 cm^{-1} due to strong asymmetric carboxylate stretching. This indicated that interaction between GA and organic base led to the formation of a structure that belongs to a carboxylate.

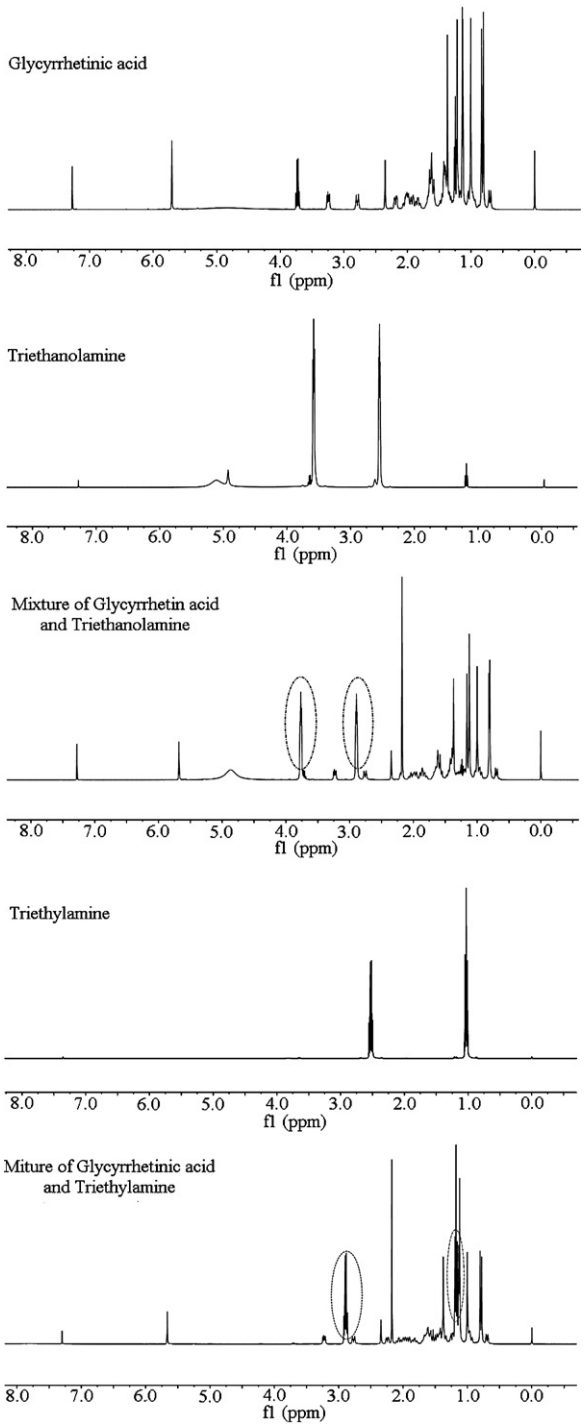

Fig. 5. ^{1}H NMR spectra of GA, TEA, TETN and their mixtures.

3.6. Effect of ethanol on the permeation of GA

The percentage of alcohol in previous experiments was 5% and ethanol yielded the most efficient permeation enhancement. The effect of ethanol concentration was evaluated to further understand the effect of ion pair formation.

In the presence of TEA or TETN, the addition of ethanol (up to a maximum of 20%) increased the solubility of GA in a concentration dependent manner. At 20% ethanol, the solubility of GA in TEA system was 16-fold more than that without ethanol. At the same concentration of ethanol in the TETN system, the solubility of GA increased further reaching 25-fold higher than that without

Table 4
Chemical shift of hydrogen atoms near the nitrogen atom of TEA and TETN alone or in combination with GA in the $CDCl_3$ with or without alcohols.

System			Chemical shift (ppm)	Difference from base alone (ppm)
With TEA	–	–	2.59	0.00
	GA loaded	–	2.89	0.30
		Ethanol	2.71	0.12
		Butanol	2.75	0.16
		Octanol	2.73	0.14
With TETN	–	–	2.52	0.00
	GA loaded	–	2.89	0.37
		Ethanol	2.84	0.32
		Butanol	2.63	0.11
		Octanol	2.67	0.15

"–" means without.

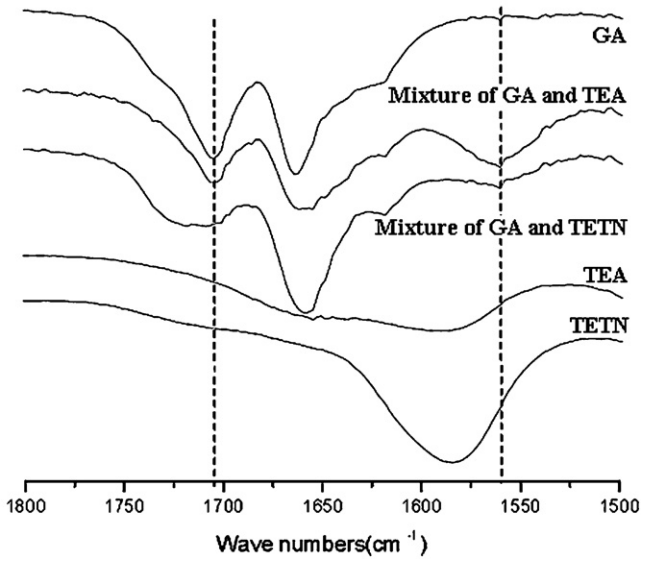

Fig. 6. FT-IR spectroscopy of GA, TEA, TETN and their mixture.

ethanol. The variations in the trend of Q_{24} after addition of ethanol were different from those of solubility in both systems. Increases in Q_{24} reached a maximum at around 5% ethanol, and the peak appeared rather broad. Contrary to our expectation Q_{24} did not increase with higher ethanol concentration (Table 5).

The concentration of organic base in the system was 6 μM, and the mass concentration of GA that was equivalent to the molar concentration of organic base was 3 mg/ml. If the added organic base formed ion pairs with GA entirely, the required concentration of GA would be at least 3 mg/ml without consideration of equilibrium constant. The increased amount of ethanol added in the system led to an increased solubility of GA, and the ion pair concentration increased until it reached a maximum value. Q_{24} also increased with increasing ion pair concentrations, and reached a maximum when the ion pair concentration was at a maximum. The solubility of GA further increased when the amount of ethanol was increased. As the concentration of the organic base was limited, excess GA that did not form ion pair was less likely to penetrate through the skin. The amount of ion pair was constant while its activity was reduced, which resulted in a decrease in Q_{24}. As for the lower amount of ethanol needed to achieve a maximum Q_{24} in the TETN system compared to TEA system, the explanation could be that TETN is a stronger base than TEA, and GA has a higher solubility in the presence of TEA in a solvent system with the same composition. Q_{24} therefore largely depended on the concentration and activity of GA–organic base ion pair.

Table 5
Penetration parameters of GA using IPM–ethanol system with organic base and different ethanol percent ($n = 3$, mean ± SD).

	Ethanol (%)	Solubility (mg/ml)	Q_{24} (μg/cm^2)
TEA	0	1.33	880.85 ± 244.37
	3	3.79	2137.89 ± 828.40
	5	5.73	8779.71 ± 1688.40
	8	10.22	8032.61 ± 2484.72
	12	16.78	8720.00 ± 3818.64
	16	21.41	5100.42 ± 1216.56
	20	27.53	5793.73 ± 1079.52
TETN	0	1.56	4061.60 ± 634.08
	3	4.02	10872.73 ± 4002.87
	5	5.85	9194.66 ± 1713.36
	12	22.10	9015.32 ± 2839.57
	20	39.48	7287.30 ± 2837.81

4. Conclusions

According to the results of this study, it can be concluded that organic base and non-aqueous solvent system is a useful method to increase the penetration of acidic compound with strong lipophilicity such as GA. The solubility of GA increased significantly in a non-aqueous system, especially in the IPM–ethanol system. Organic base could form ion pair with GA and improve the partition of GA between SC and viable skin, and further increase the cumulative amount of GA permeated. TETN was a more effective permeation enhancer than TEA, due mainly to its stronger alkaline property and greater ability to form ion pair.

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