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In vivo infrared spectroscopy studies of alkanol effects on human skin

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Abstract

Many studies investigate the permeation of actives through the skin and ignore the role of excipients. The solvents used in formulations will undoubtedly penetrate the skin where they can have a number of effects. They can extract skin lipids, they can alter the fluidity of the lipids and they can alter the polarity of the skin. The degree to which they do this and the depth into the skin where this occurs will depend on the uptake kinetics. The problem is to distinguish the different effects. Using ATR-FTIR and deuterated materials this can be achieved in vivo. The aim of the present study was to study the higher alkanols (hexanol, octanol, decanol) in vivo using a combination of ATR-FTIR spectroscopy and tape stripping. Studies conducted in vivo using deuterated vehicles confirmed the lipid extraction effects of D-hexanol and D-octanol, whereas D-decanol did not change skin lipid content. The uptake of D-decanol was higher than for the other vehicles consistent with previous observations on mouse skin for alkanols of increasing chain length. In general, solvent uptake was proportional to the induced shift in the C–H stretching frequency. Lipid disorder was induced by all vehicles studied in vivo and was proportional to the amount of vehicle present in the skin.

Keywords: Infrared spectroscopy; Skin; Alkanols; Lipid extraction; Lipid disorder; Penetration enhancement

1. Introduction

Fourier transform infrared (FTIR) spectroscopy and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy are useful tools with which to characterize the lipids, proteins and water content of the stratum corneum (SC) [1–3]. These techniques are also useful to investigate the effects of water and other vehicles, possessing a characteristic IR signature distinct from the SC components, on the barrier itself [4]. The methylene group (CH_2) stretching vibrations in the IR spectrum of the SC (2950–2850 cm^{-1}) arise primarily from the alkyl chains of the lipids in intercellular spaces [5]. Golden et al. [6] used the complementary techniques of thermal perturbation IR and differential scanning calorimetry (DSC) to investigate the effects of hydration on the skin.

The mechanisms of action of other vehicles and penetration enhancers on skin structure may also be probed using IR spectroscopy [7,8]. For example, Kai et al. [8] investigated the impact of a homologous series of *n*-alkanols (C_2–C_{12}) on nicotinamide absorption. Hairless mouse skin was pretreated with the alkanols resulting in significant penetration enhancement of nicotinamide; the extent of the enhancement varied parabolically with the alkanol chain length. Alkanol uptake was also evaluated and increased with increasing alkyl chain length. In contrast, alkanol flux mirrored the parabolic profile observed for the influence of alkyl chain length on nicotinamide flux enhancement. The spectroscopic results suggested, there-

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fore, that the major action of ethanol was lipid extraction; significant lipid depletion by butanol and octanol was also implicated. Lipid disordering effects could not be deduced because the absorbances from the remaining lipids were too small to allow for unambiguous interpretation.

Kurihara-Bergstrom et al. [9] evaluated the effect of ethanol on human skin using stratum corneum samples equilibrated with perdeuterated ethanol (EtDO) and D_2O solutions of varying EtDO content. As the volume fraction (V_f) of EtDO increased from 0.4 to 1.0, a corresponding decrease in the ratio of CH_2 stretching relative to NH stretching was observed confirming lipid extraction. This disruption of skin barrier function was correlated with the permeation of salicylate, which was greatest when the vehicle contained an ethanol V_f of 0.63.

Bommannan et al. [10] conducted in vivo experiments on the human forearm to further investigate the permeation-enhancement effects of ethanol. In this study, the human forearm was first tape stripped four times and ATR-FTIR measurements were made before and after this procedure. This resulted in a red shift (shift to lower wavenumbers) in the peak CH_2 stretching frequency of about $2\ cm^{-1}$. Subsequently, treatment with ethanol induced a further shift of $\sim 1\ cm^{-1}$. The peak CH_2 stretching frequency from ethanol alone is $\sim 10\ cm^{-1}$ higher than that of the skin lipids, suggesting that the change observed following treatment may be an underestimation of the increased lipid order. The bandwidth at half height of the CH_2 stretching frequency also implied a lipid-ordering effect of the solvent. Treatment with ethanol also decreased the SC lipid content to a significant extent ($p < 0.05$). To ensure that this extraction was not masking any possible lipid disordering effects, the same experiment was performed by exposing the SC to ethanol-saturated vapour rather than to the pure liquid. Under these conditions, ethanol uptake into the SC was evident but no lipid disordering was observed.

Ethanol induced alterations of hairless mouse SC were investigated further by Krill et al. [11]. Contrary to the anticipated fluidisation effect, there was a decrease in the SC intercellular lipid chain mobility implying an increase in the rigidity of the lipids. The effects of isopropanol, *n*-propanol and *n*-butanol on hairless mouse skin were also considered [12]. Isopropanol and *n*-butanol did not alter the SC lipid interchain interaction and, below 45 °C, *n*-butanol decreased the alkyl chain freedom of motion.

As a systematic study of the effects of the higher alkanols (hexanol, octanol, decanol) on the barrier properties of human skin has not previously been reported, the aim of the present study was to investigate the effect of these solvents and other vehicles on SC in vivo. By using perdeuterated materials it has been possible to separate the different effects of the alcohols. Lipid extraction as seen by a decrease in the skin lipid peak and skin lipid fluidisation as manifest by a change in the stretch frequency can be monitored. In addition, the degree to which the alcohol permeates into the skin can be seen as an increase in the CD stretch intensity. Since tape stripping was also employed it is possible to see how far into the skin these effects take place. The experimental design and the choice of the deuterated materials have allowed a unique opportunity to study the effects of the alcohols in vivo using minimally invasive procedures.

2. Materials and methods

2.1. Materials

The perdeuterated solvents, D-decanol, D-octanol and D-hexanol, were purchased from Cambridge Isotope Laboratories (Woburn, MA, USA). The adhesive tape used was Scotch Booktape (845, France).

2.2. Spectroscopy

The in vivo experiments were conducted on a Nicolet 730 FTIR spectrometer and the spectra were analyzed using SpectraCalc software. The internal reflection element was a ZnSe crystal with a trapezoidal cut at 45° and 7 cm^2 surface area, and the spectrometer was equipped with a liquid nitrogen cooled mercury–cadmium–telluride detector.

2.3. In vivo experiments

IR spectra from human skin were obtained after treatment with D-octanol, D-decanol and D-hexanol. The studies were performed under a protocol approved by the University of Geneva Hospital research Ethics Committee (Commission centrale d'éthique de la recherche des HUG). The experimental subjects, from whom informed consent was obtained, were eight healthy volunteers (4 males and 4 females), aged between 25 and 35 with no history of dermatological disease. For each subject, two sites ($\sim 8\ cm^2$ in area) on the ventral aspect of both forearms were delineated using a medical adhesive. The skin site was cleaned with a dry cotton swab. Each of the vehicles studied was applied on three skin sites (50 μl); the fourth site served as an untreated control. The treated and untreated sites were randomly assigned for each volunteer. Each site was occluded during the application period to prevent evaporation of the vehicle. After 30 min of treatment, the occlusive patches were removed and excess vehicle on the skin sites was gently removed with a dry cotton swab. IR spectra were immediately recorded (64 scans were taken with a resolution of $2\ cm^{-1}$). The skin was left non-occluded for a further 25 min, after which time another spectrum of the application area was recorded.

The skin was then stripped 10 times recording an IR spectrum after each tape strip. To quantify the amount of stratum corneum removed, and hence the depth into the barrier membrane by each tape strip, the tape was weighed before and after SC removal as described previ-

ously [13]. The amount of SC removed was determined by individually weighing the tapes before and after stripping on a semi-microbalance (precision, 10 μg; Mettler-Toledo AT 261, Greifensee, Switzerland). Assuming that the SC density is constant (1 g cm^{-3}) across its thickness [14], the SC weights can be converted to volumes and, given that the area stripped is constant, to the SC thickness removed. In this way, the spectral profile of the skin was derived as a function of position in the SC.

3. Results and discussion

3.1. In vivo results

3.1.1. Lipid content

Deuterated alcohols were used so that their action and presence in the skin could be distinguished from endogenous lipids. Their appearance in the skin could be observed from an increase in the CD peak. Their effect on the extraction of skin lipids was seen from the decrease in the CH stretch peaks, and their effect on the fluidity of the skin lipids was seen from the shift in the frequency (both symmetric and asymmetric) of the CH skin lipids. The principal aim of these experiments was to evaluate the impact and uptake of a series of deuterated *n*-alkanols on, and into human SC in vivo. The effects of these solvents on human skin have not been studied using this approach. IR spectral observations were made as a function of depth into the SC via sequential tape stripping to expose progressively deeper regions of the barrier. The use of deuterated solvents allowed their clear spectroscopic differentiation, and their effects on the endogenous, intercellular lipids to be observed, as has been previously described for oleic acid [15]. Because the IR spectra were obtained in a reflectance mode, such that the intensity of absorption is dependent on the degree of contact between the skin and the internal refection element of the spectrophotometer, all absorbances (peak areas) originating from either the SC lipids or the exogenously applied deuterated solvents were normalized with respect to the combined areas under the amide I and amide II peaks predominantly due to SC protein [16].

Fig. 1 shows that the amount of D-decanol applied did not significantly remove SC lipids from the barrier. In contrast, both D-octanol and D-hexanol significantly depleted these lipids (Fig. 2), especially from the outermost few microns of the SC.

Absorbances arising from the deuterated solvents taken up into the SC were easily visualized at 2200 cm^{-1}, a spectral region where the SC is IR-transparent. The uptake of D-hexanol, D-octanol and D-decanol after 30 min of exposure is summarized in Fig. 3. Hexanol was present to the lowest degree and was no longer seen at 55 min. The latter probably also accounted for the variability in hexanol uptake (Fig. 4). The results obtained for D-octanol and D-decanol were consistent with those obtained by Kai et al. [8], who observed increased solvent uptake with increasing alkanol chain length in hairless mouse skin. While the presence of octanol in the SC had disappeared after 55 min, decanol remained clearly and could be seen further into the barrier.

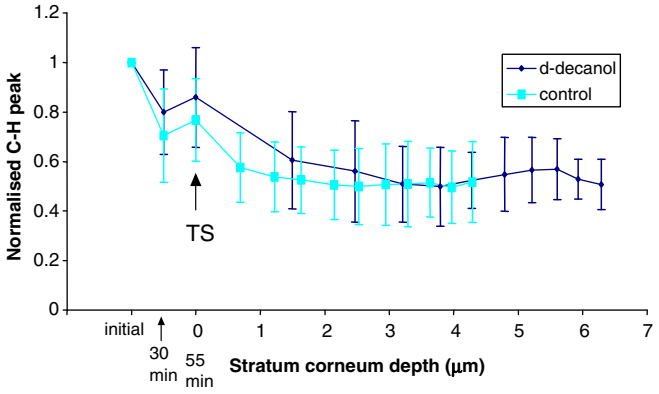

Fig. 1. Relative amount of spectroscopically detected SC lipids (as assessed by the normalized area of the $-CH_2$ stretching absorbances) as a function of time and depth into the barrier (means ± SD; $n = 8$): untreated control values are compared to those obtained after a 30-min treatment with decanol.

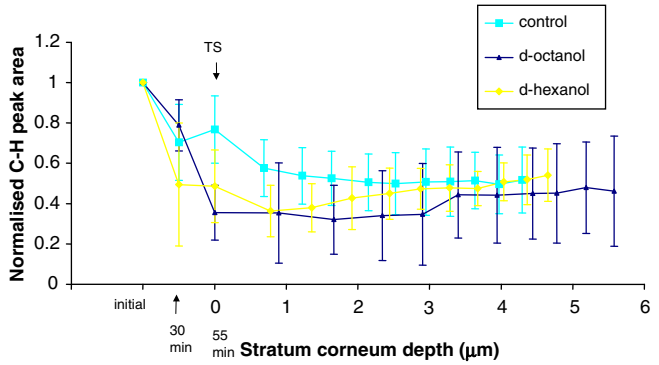

Fig. 2. Relative amount of spectroscopically detected SC lipids (as assessed by the normalized area of the $-CH_2$ stretching absorbances) as a function of time and depth into the barrier (means ± SD; $n = 8$): untreated control values are compared to those obtained after a 30-min treatment with either octanol or hexanol.

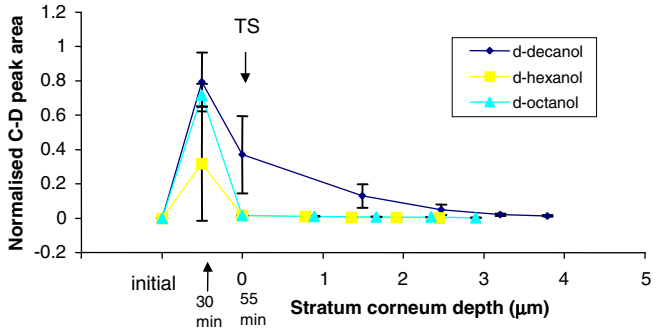

Fig. 3. Spectroscopically assessed uptake of deuterated alkanols into human SC in vivo following a 30-min treatment period and subsequent tape stripping (means ± SD; $n = 8$ for hexanol and decanol, $n = 3$ for octanol).

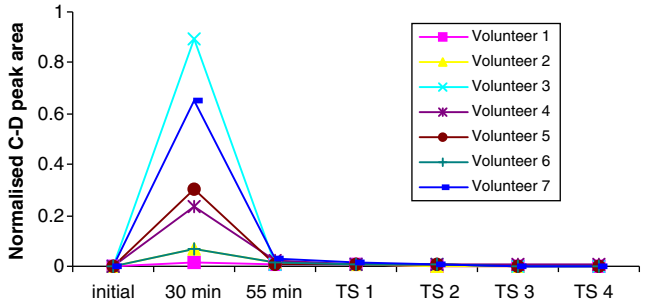

Fig. 4. Individual variability of the results for D-hexanol presented in Fig. 3.

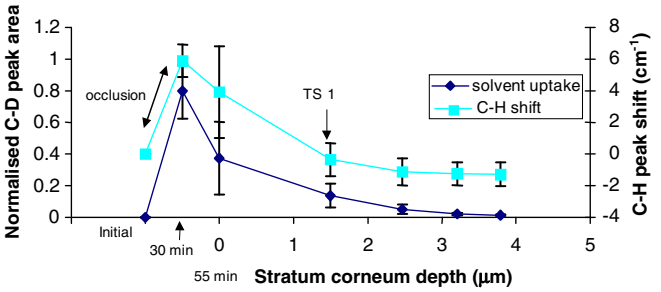

Fig. 6. D-Decanol uptake into human SC in vivo and frequency shift in the CH_2 asymmetric stretching absorbance peak following a 30-min treatment (means ± SD; $n = 8$).

SC lipid disordering is associated with a 'blue' shift to a higher wavenumber of the $-CH_2$ symmetric and asymmetric stretching vibrations [17]. It has been reported that the vibrations at 2920 cm^{-1} can shift to a higher frequency by topical application of oleic acid [7] and PG [18]. To confirm that the applied alcohol induces conformational changes in skin lipid organization, perdeuterated chemicals are preferred as they do not absorb at the $-CH_2$ stretching vibration.

Our previous work indicates that while ethanol appears to extract appreciable amounts of lipid from the stratum corneum, it does not appear to induce lipid disorder [10]. This is not the case for the longer chain alkanols as can be seen from the results of the present study. Fig. 5 shows the shift in the CH_2 asymmetric stretching frequency after a 30-min application, at $t = 55$ min into the experiment, and as a function of depth into the SC. The initial measurement (before treatment and tape stripping) was assigned a zero shift and all subsequent measurements are reported relative to this initial value. For the control, after 30 min occlusion, there was a negative shift of about 1.5 cm^{-1}. In contrast, D-octanol, D-decanol and D-hexanol induced positive shifts of 6 cm^{-1}, 6.5 cm^{-1} and 2.7 cm^{-1}, respectively, after the same period of exposure indicating significant disordering of the SC lipids at the surface. At 55 min post-treatment, only the effect of D-decanol remained visible in the outer layers of the SC (up to ~1.5 μm), whereas those of D-octanol and D-hexanol had disappeared. The disruption in SC lipid organization induced by D-decanol is also apparent from the fact that the initial tape strips post-treatment with this alkanol removed significantly more SC than the shorter-chain homologues (Fig. 5). There are fewer tape strips required in the presence of D-decanol to get to a similar depth into the SC than for either D-hexanol or D-octanol.

From the areas of the $-CD_2$ stretching absorbances, the relative uptake of the alkanols into the outer SC could be estimated and correlated with the lipid disordering effects discussed above. Fig. 6 presents the data for decanol.

The individual data for each volunteer for decanol treatment were analyzed and the shift in the $-CH_2$ asymmetric stretching frequency (ν_{CH2}) was plotted against relative decanol uptake into the SC (Fig. 7). A linear correlation was obtained with an apparent maximum shift in ν_{CH2} of ~7 cm^{-1} (Fig. 7). Although the duration of the lipid disorder induced by hexanol was short-lived, a similar correlation with uptake was also observed for this alkanol (Fig. 8). For both solvents the linear relationship indicates

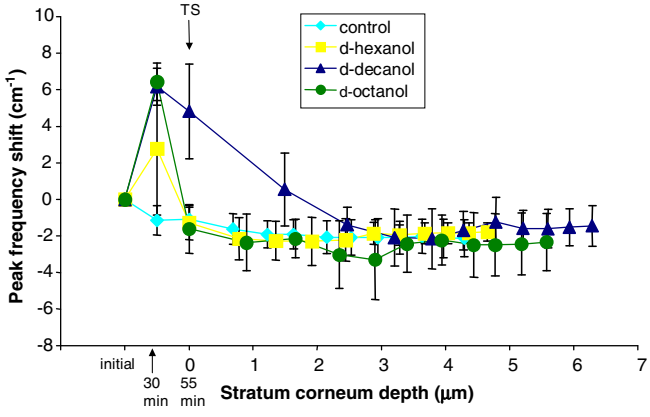

Fig. 5. Frequency shift in the $-CH_2$ asymmetric stretching absorbance peak following a 30-min treatment with deuterated alkanols (means ± SD; $n = 8$ for hexanol and decanol, $n = 3$ for octanol).

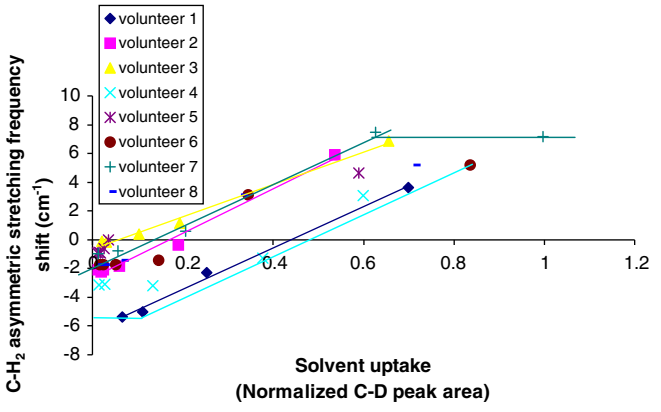

Fig. 7. Relationship between the relative amount of decanol present in the outer layers of human SC in vivo and the intercellular lipid disordering effect of the alkanol (as measured by the shift in the maximum $-CH_2$ asymmetric stretching frequency); individual data from eight volunteers.

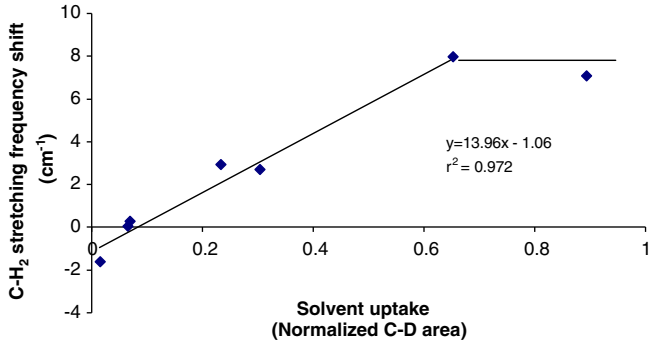

Fig. 8. Corresponding relationship to that in Fig. 7 for one volunteer treated with D-hexanol.

that the amplitude of the shift is proportional to the amount of vehicle present in the skin.

4. Conclusions

ATR-FTIR spectroscopy combined with the use of per-deuterated solvents can be used successfully to deduce the effect of topical formulation excipients on the barrier function of human SC in vivo. Although ATR-FTIR has previously been used to study the interaction of the shorter-chain alkanols and in particular ethanol, with human and animal skin, no investigations have been conducted on the effects of the longer chain alkanols on human skin structure. From the results of this study, it was clearly evident that C_6–C_{10} alkanols were able (i) to reduce the presence of lipids at the skin surface and (ii) increase the conformational disorder of the intercellular lipids of the SC. The relative impact was hexanol > octanol > decanol in the first instance, the reverse for the second. Additionally, uptake of the alkanols into the outer SC was correlated significantly with the lipid disordering observed. As alkanols are commonly used as permeation enhancers in (trans)dermal delivery, further studies will be conducted to elucidate the relative importance of the present findings for drug permeation and/or partition into the stratum corneum.

References

[1] N.A. Puttnam, Attenuated total reflectance studies of skin, J. Soc. Cosmet. Chem. 23 (1979) 209–226.
[2] R.O. Potts, D.B. Guzek, R.R. Harris, J.E. McKie, A noninvasive in vivo technique to quantitatively measure water concentrations of the stratum corneum using attenuated total reflectance Fourier transform infrared spectroscopy, Arch. Dermatol. Res. 277 (1985) 489–495.
[3] D. Bommannan, R.O. Potts, R.H. Guy, Examination of the intercellular lipids of the stratum corneum, J. Invest. Dermatol. 92 (1989) 405.
[4] C.L. Gay, R.H. Guy, G.M. Golden, V.H.W. Mak, M.L. Francoeur, Characterization of low-temperature (i.e. <65 °C) lipid transitions in human stratum corneum, J. Invest. Dermatol. 103 (1994) 233–239.
[5] A.C. Park, C.B. Baddiel, Rheology of stratum corneum II: a physicochemical investigation of factors influencing the water content of the stratum corneum, J. Soc. Cosmet. Chem. 23 (1972) 13–21.
[6] G.M. Golden, D.B. Guzek, R.R. Harris, J.E. McKie, R.O. Potts, Lipid thermotropic transitions in human stratum corneum, J. Invest. Dermatol. 86 (1986) 255–259.
[7] G.M. Golden, J.E. McKie, R.O. Potts, Role of stratum corneum lipid fluidity in transdermal drug flux, J. Pharm. Sci. 76 (1987) 25–28.
[8] T. Kai, V. Mak, R.O. Potts, R.H. Guy, Mechanism of percutaneous penetration enhancement: effect of *n*-alkanols on the permeability barrier of hairless mouse skin, J. Control. Release 12 (1990) 103–112.
[9] T. Kurihara-Bergstrom, K. Knutson, L.J. DeNoble, C.Y. Goates, Percutaneous absorption enhancement of an ionic molecule by ethanol–water systems in human skin, Pharm. Res. 7 (1990) 762–766.
[10] D. Bommannan, R.O. Potts, R.H. Guy, Examination of the effect of ethanol on human stratum corneum in vivo using infrared spectroscopy, J. Control. Release 16 (1991) 299–304.
[11] S.L. Krill, K. Knutson, W.I. Higuchi, Ethanol effects on the stratum corneum lipid phase behaviour, Biochim. Biophys. Acta 1112 (1992) 273–280.
[12] S.L. Krill, K. Knutson, W.I. Higuchi, The influence of iso-propanol, *n*-propanol and *n*-butanol on stratum corneum lipid phase behaviour, J. Control. Release 25 (1993) 31–42.
[13] I. Alberti, Y.N. Kalia, A. Naik, J.D. Bonny, R.H. Guy, In vivo assessment of enhanced topical delivery of terbinafine to human stratum corneum, J. Control. Release 71 (2001) 319–327.
[14] R.L. Anderson, J.M. Cassidy, Variation in physical dimensions and chemical composition of human stratum corneum, J. Invest. Dermatol. 61 (1973) 30–32.
[15] B. Ongpipattanakul, R.R. Burnette, R.O. Potts, M.L. Francoeur, Evidence that oleic acid exists in a separate phase within stratum corneum lipids, Pharm. Res. 8 (1991) 350–354.
[16] K. Knutson, R.O. Potts, D.B. Guzek, G.M. Golden, J.E. McKie, W.J. Lambert, W.I. Higuchi, Macro- and molecular physical–chemical considerations in understanding drug transport in the stratum corneum, J. Control. Release 2 (1985) 67–87.
[17] J.E. Harrison, A.C. Watkinson, D.M. Green, J. Hadgraft, K. Brain, The relative effect of Azone and Transcutol on permeant diffusivity and solubility in human stratum corneum, Pharm. Res. 13 (1996) 542–546.
[18] Y. Takeuchi, H. Yasukawa, Y. Yamaoka, Y. Kato, Y. Morimoto, Y. Fukumori, T. Fukuda, Effects of fatty acids, fatty amines and propylene glycol on rat stratum corneum lipids and proteins in vitro measured by Fourier transform infrared/attenuated total reflection (FT-IR/ATR) spectroscopy, Chem. Pharm. Bull. 40 (1992) 1887–1892.