Journal of Thermal Analysis and Calorimetry, Vol. 57 (1999) 313-322

THERMAL ANALYSIS STUDIES ON HUMAN SKIN AND SKIN BARRIER MODULATION BY FATTY ACIDS AND PROPYLENE GLYCOL

H. Tanojo[1], *J. A. Bouwstra*[2], *H. E. Junginger*[2*] *and H. E. Boddé*[2]

[1]Department Dermatology, University of California, San Francisco, USA
[2]Division of Pharmaceutical Technology, Leiden/Amsterdam Center for Drug Research Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Abstract

The thermal behaviour of human stratum corneum (SC) with various hydration levels was studied using differential thermal analysis DSC within the temperature range of –130 to 120°C. SC containing 20% water, resembling the intact condition, shows thermal transitions at around –20°C (representing water in skin), –10, 40, 70°C (representing skin lipids), 85°C (representing protein-associated lipids) and 100°C (representing skin protein). Dehydration of SC causes the transitions at –20 and 100°C to be invisible. Lipid extraction followed by dehydration eliminates all transitions. Further hydration produces a transition of water at around 0°C with a huge change in enthalpy. The perturbation effects of penetration enhancers fatty acids (FA) and propylene glycol (PG) were studied using DTA on SC after pretreatment with PG alone and FA/PG. The application of PG alone shifted the transitions at 70 and 85°C to lower temperatures. Additionally, the application to dehydrated stratum corneum removes the transitions at –10°C. Saturated fatty acids, e.g. nonanoic and decanoic acids, exert barely noticeable effects on the thermal behaviour of SC suggesting that they easily mix with the skin lipids. Thermal analysis also revealed that the *cis*-9- and 13-isomers of octadecenoic acid (monounsaturated fatty acids) form a separate domain containing mostly the pure fatty acids within the SC lipids and suppress the lipid transitions at 70/80°C. Polyunsaturated fatty acids linoleic and α-linolenic acids – form separate domains but do not completely suppress the SC lipid transitions at 70/80°C as monounsaturated acids do. This study suggests different ways of perturbation by various fatty acids.

Keywords: differential thermal analysis, fatty acids, human stratum corneum, propylene glycol, skin lipid, skin permeation enhancer

Introduction

Thermal analysis has been used for three decades to study the physico-chemical properties of the skin. The earliest thermal studies were aimed to investigate the part of skin which provides a barrier to the loss of water [1], and to calculate the amount of water bound in the stratum corneum at various hydration levels [2–5]. Subsequently, thermal analysis was utilized to find correlations between the endothermic transitions and the nature of the components of stratum corneum [6–9].

* Author to whom all correspondence should be addressed.

1418–2874/99/ $ 5.00

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

The major problem in administering drugs transdermally arises from the barrier capacity of the stratum corneum, the uppermost layer of the skin. This problem may be partially overcome by applying penetration enhancers, which are able to reversibly alter the barrier properties of stratum corneum and thus allow drugs to penetrate through the skin layers and enter the systemic circulation [10, 11]. Thermal analysis has been used in attempts to understand the mode of action of some enhancers on stratum corneum, such as dimethyl sulphoxide [12], azones [12–14], terpenes [8, 15], surfactants [16, 17], and other compounds [16]. In the case of a congeneric series of azones, the thermal analytical data could be nicely correlated with kinetic data, X-ray data and electron microscopic observations [14].

The present study consists of two parts. The first part is focussed on the investigation of the thermal behaviour of human stratum corneum, at various hydration levels, within a broad temperature range, from –130 to 120°C. In the second part, the analysis was carried out on human stratum corneum after pretreatments with a number of enhancers: fatty acids and propylene glycol.

Material and methods

Preparation of stratum corneum samples

Stratum corneum SC sheets were isolated by trypsination from fresh human breast or abdominal skin obtained by surgical operation. A detailed procedure is described elsewhere [9]. Prior to use, the sheets were dried and stored above silica gel in nitrogen atmosphere at room temperature.

Dehydrated SC samples were prepared by placing SC sheets for 24 h at 50°C in a closed vessel above phosphorus pentoxide (P_2O_5; Baker, Deventer, The Netherlands) 10 mg cm^{-3} vessel volume.

Hydrated SC samples were prepared by equilibrating SC sheets for 24 h at room temperature in a closed vessel above a 27% w/v sodium bromide solution in purified water or phosphate buffered saline (pH 7.4; according to Ph. Eur. II). All salts were purchased from Merck, Darmstadt, Germany.

Extraction of lipid from stratum corneum

Stratum corneum sheets were submerged and shaken continuously in a solution of chloroform:methanol (2:1 volume) for 48 h at room temperature. The sheets were then dried at room temperature above silica gel in nitrogen atmosphere, before further dehydration above phosphorus pentoxide. Chloroform, methanol and phosphorus pentoxide were obtained from Baker, Deventer, The Netherlands.

Treatment solutions

The solutions for the pre-treatment and co-treatment of SC were propylene glycol (PG; Baker, Deventer, The Netherlands), or 0.16 M fatty acid (FA) in PG. The following fatty acids were used in the study: (i) saturated (SFA): nonanoic (9:0) and decanoic (10:0); (ii) mono-unsaturated (MUFA): *cis*-octadecenoic acids with double

bonds at position: 9 or 13; (iii) poly-unsaturated (PUFA): linoleic (18:2) and α-linolenic (18:3). The concentration 0.16 M was chosen for every acid since it is below the lowest value of all the solubilities for the fatty acids used in this study. All acids were purchased from Aldrich Chemie, Bornem, Belgium and were of the highest purity.

Pre-treatments of stratum corneum

The SC sheet, either dehydrated or hydrated prior to pre-treatments, was submerged (without stirring) in penetration enhancer solutions (2 ml (10 μg)$^{-1}$ SC) for 24 h at 32°C. Then the SC sheet was pressed between two pieces of nylon wire-netting wrapped with tissue-paper repeatedly until the sheets did not wet the paper anymore.

Differential thermal analysis

SC samples (dehydrated, hydrated or penetration enhancer-pretreated), each weighing 10–30 mg, were placed into medium pressure stainless steel crucibles made by Mettler, Greifensee, Switzerland, and hermetically sealed to avoid water evaporation during the analysis. DTA was performed using Mettler TA 3000 Thermal Analysis System with a Low Temperature Cell, with an empty pan as reference. Samples were subjected to the following thermal analysis cycle: cooling from 20 to –130°C, then equilibrating isothermally for at least 5 min to achieve a stable condition at –130°C, followed by heating from –130 to 120°C. The rate for both cooling and heating was 2°C min^{-1}. The transition temperatures were determined by taking the temperature corresponding to the top of peaks on the heating curves. The heating curves were constructed by plotting the heat flow values, which have been normalised using sample weight (as mW mg^{-1}), against temperatures.

Results

Dehydration and hydration of stratum corneum

Figures 1 and 2 show the thermal profiles of stratum corneum following dehydration and hydration processes. The curves are arranged from the most dehydrated level (Fig. 1 curve A) to the highly hydrated level (Fig. 2). Curve D (Fig. 1) was obtained from the analysis of human stratum corneum after hydration above a sodium bromide solution. The hydration procedure added about 20% to the original dry mass of the stratum corneum sheet (after isolation). This hydration level approximates that of intact stratum corneum in human skin [18]. In the temperature range from –130 to +120°C this curve contains some endothermal transitions, i.e. at (approximately) –20, –10, 40, 70, 85 and 100°C, which have been reported in publications [1, 6, 7, 9, 16, 19, 20]. The last four transitions have been referred to by Goodman and Barry [16] as T_1, T_2, T_3 and T_4, respectively. These transitions, except the one at 100°C, can be found in curves C (Fig. 1), representing human stratum corneum after dehydration above silica gel. Curve B (Fig. 1), which is originated from human stratum corneum after dehydration above phosphorus pentoxide, also does not contain the peak at 100°C. Dehydration of SC reveals the lipid peak at –10°C

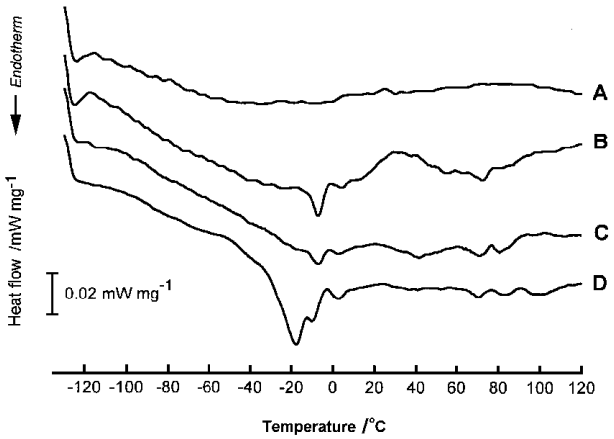

Fig. 1 Thermal profiles of human stratum corneum after (A) lipid extraction followed by dehydration above P_2O_5; (B) dehydration above P_2O_5; (C) dehydration above silica gel; and (D) hydration above 27% NaBr solution

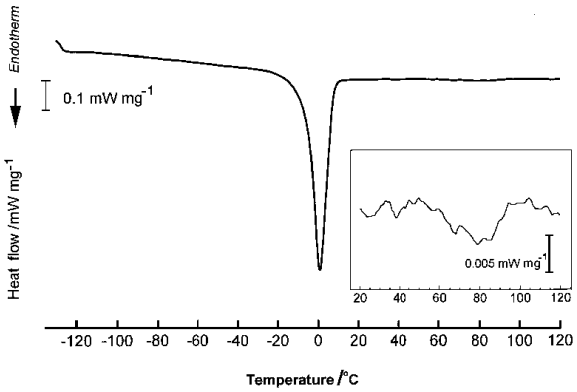

Fig. 2 Thermal profiles of human stratum corneum after hydration above phosphate buffered saline. Inset: the same curve on different scale, showing the region between 20 and 120°C

which tends to hide under the water transition in samples with high water content. This is the case in the curve of Fig. 2, that is collected from stratum corneum after hydration above phosphate buffered saline. This condition will set the water content to about 100% the dry mass of stratum corneum sheet. The obviously huge thermal transition near 0°C covers a wide region from –20 to 0°C.

Curve A (Fig. 1) is obtained from the stratum corneum sheets, which have undergone a lipid extraction following the similar procedure as described by other investigators [6, 20] and, additionally, a *dehydration* procedure above phosphorus pentoxide. This resulted in the absence of peaks at the temperature range between –120 to 100°C. Golden *et al*. reported the presence of transitions at temperature range 85 to 100°C, but when they cooled and reheated the extracted samples, subsequent analy-

ses confirmed the disappearance of any peaks [20]. In all experiments it was verified that the transitions at 40 and 70°C were absence after lipid extraction and dehydration of stratum corneum. In addition, the subzero peak at –10°C.was also not present.

Pre-treatment of stratum corneum with propylene glycol and fatty acids in propylene glycol

Dehydrated samples

The temperature changes of the thermal transitions after the treatments of dehydrated SC with fatty acids dissolved in PG (0.16 M) are presented qualitatively in Fig. 3A. After the pre-treatment of PG the lipid transition at –10°C disappeared. Furthermore, the transitions which are localized at 70 and 80°C in untreated SC were shifted to lower temperatures as reported before [9,16]. The total enthalpy changes involved in these two transitions was reduced by 25% (data not shown). Applications of SFA, nonanoic and decanoic acid, in PG to stratum corneum revived the subzero peak, which disappeared by the pre-treatment of PG alone. Dehydrated SC pretreated by nonanoic acid/PG showed two transitions in the low temperature region, namely at –8 and +2°C, whereas SC treatment by decanoic acid/PG resulted in only a single peak at –14°C. The enthalpy involved in this transition after the treatment by decanoic acid was higher than after the treatment with nonanoic acid. Additionally,

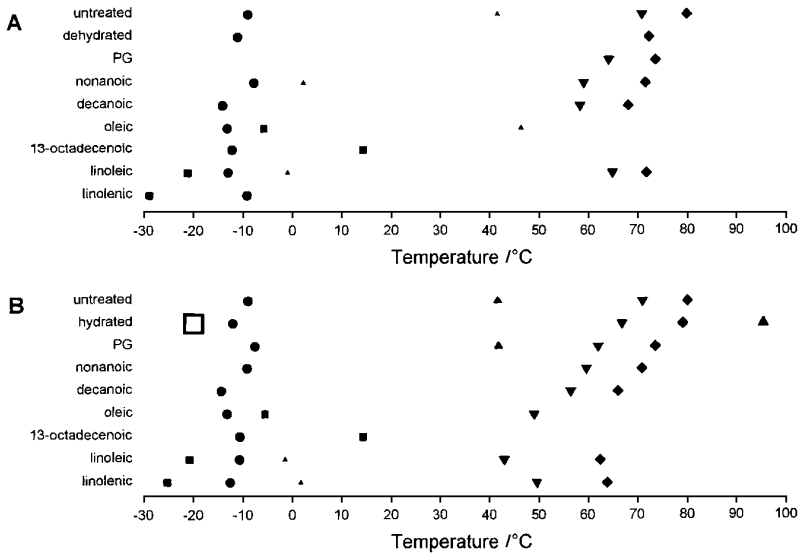

Fig. 3 Transition temperatures of (A) dehydrated and (B) hydrated stratum corneum after pre-treatments with fatty acids dissolved in propylene glycol (PG) and pure PG as reference. Different symbols show the classification of transition temperatures: at around (●) –10°C, (▼) 70°C, (◆) 80°C; (□) of water; (■) of native solution; (▲) additional peak. The size of symbols reflect the enthalpy of the transitions

both fatty acid treatments shifted the transitions at 70 and 80°C to slightly lower temperatures at the same extent. However, in comparison to untreated samples, decanoic acid decreased the sum of the enthalpies of these transitions, whereas nonanoic increased it.

In contrast to nonanoic and decanoic acids, the native thermal transition of MUFA, oleic acid (*cis*-9-octadecenoic acid) and *cis*-13-octadecenoic acid, in PG was also present in the thermal profile of treated SC. Furthermore, the subzero peak at –10°C, which was absent by the application of PG alone, was present after these treatments. The temperature of this transition was shifted to –14 and –13°C after the treatment of oleic acid/PG and of 13-octadecenoic acid/PG, respectively. Both treatments tremendously reduced the enthalpy of the transitions at 70 and 80°C.

After the treatment of PUFA, linoleic and linolenic acids, in PG the transition at –10°C remained present. However, it has been shifted to –13°C for linoleic and –9°C for linolenic acid. Additionally, a transition (melting) peak was observed at a lower temperature, –21 and –29°C for linoleic and linolenic acid, respectively. These temperatures correspond to the transition of the respective pure acid solutions in PG. The DTA scan of SC treated with linoleic acid in PG also produced an additional peak at –1°C, which was not visible after the α-linolenic acid in PG treatment. The transition temperatures at 70 and 80°C were shifted to lower temperatures by linoleic acid in PG to the same extent as by PG alone, but unlike with PG, the enthalpy of the transitions did not change in comparison to the untreated control. After the treatment with α-linolenic acid in PG both transitions almost completely disappeared.

Hydrated samples

Figure 3B displays the effects of fatty acid treatments on hydrated SC. The thermal behaviour of hydrated SC is characterized by the presence of an additional transition at –20°C caused by the water in skin and an additional transition assigned to protein at +100°C (Fig. 2). These transitions were reduced after a 24 h treatment of PG alone. In contrast to dehydrated SC, PG did not suppress the lipid transition at –10°C in hydrated SC, showing the higher affinity of PG towards water than towards the lipids involved in this transition [9]. The shift of the transitions at 70 and 80°C to lower temperatures was similar to that observed in the dehydrated samples. Following the treatments of nonanoic and decanoic acids the transition at –10°C was still visible and the transitions at 70 and 80°C were slightly shifted to lower temperatures compared to the effects of PG alone. Decanoic acid increased the enthalpy of transition at -10°C and decreased the enthalpy of transitions at 70 and 80°C, whereas nonanoic acid resulted in an opposite effect on the transition enthalpies, just as in case of dehydrated SC.

Treatments of 9- and 13-octadecenoic acids showed consistently the subzero transition at –10° and the peak based on the transition of pure solutions of these acids at –5 and +14°C, respectively. The transitions at 70 and 80°C were almost entirely suppressed. This is consistent with the observations on dehydrated samples.

Next to the transition at –10°C, the transition of native linoleic (at –21°C) or linolenic acid (at –29°C) in PG was also visible. Additionally, a transition at around 0°C was observed in both treatments. The transitions at 70 and 80°C were shifted to

lower temperatures, accompanied with a high enthalpy reduction by α-linolenic acid in comparison to linoleic acid; this was also observed in the dehydrated samples. In general, the results on the hydrated SC are in line with those observed in previous experiments on dehydrated SC.

Discussion

Effects of hydration on stratum corneum

It is generally in accord that the transitions at –10, 40 and 70°C are assigned to the SC lipids, whereas the one at 80°C originates from protein-associated lipids. The transition at 100°C belongs to the denaturation of protein and can only be observed when the hydration level of SC is high enough (more than 10%) [7]. The summary of the interpretation of these transitions is given in Table 1.

Table 1 Endothermic transitions of human stratum corneum

Average temperature/°C	Assigned to	Reference
–10	lipid with low melting points	[9]
40	lateral lipid packing phase transition from orthorhombic to hexagonal	[6, 22]
70	lipid structure transformation from lamellar to disordered; lateral packing: hexagonal to liquid	[6, 19]
80	protein-associated lipid transition from gel to liquid	[16, 19, 20]
100	irreversible protein denaturation	[6]

All endothermic transitions in the range of –20 to 0°C so far reported for SC have been related to the presence of water, particularly bound water [2–5]. Thermal experiments for measuring the amount of bound water in SC have generally been performed according to the following procedure: drying the SC sheet to a 'zero' water content, hydrating it to a certain water content, and then analyzing it. Walkley assigned the minimum water content to samples, which had undergone drying over a molecular sieve [2], but in more recent papers SC samples were said to have 'zero' water content values when stored over phosphorus pentoxide [3, 4] or silica gel [5] for 24 h under reduced pressure. Inoue *et al.* [3] reported the subzero transitions of SC containing 26 wt% bound water (with respect to dry mass) at –14 and –9°C. Imokawa *et al.* [4] observed in isolated SC lipids with 17 to 60% water content a single peak between –6 and –2°C, but in intact human SC sheets with 30 to 90% water content multiple peaks between –17 and –6°C. They found that lowering the water content decreased the magnitude of the peaks (calculated as the transition enthalpy). The same phenomenon has also been observed by Takenouchi *et al.* [5]. From the data presented it is also apparent that lowering the water content decreased the tran-

Table 2 Changes in enthalpy of transitions $\Delta_r(\Delta H)$ compared to control samples

Treatment	A. Dehydrated stratum corneum		B. Hydrated stratum corneum	
	$T\approx$–10°C	$T\approx$70 +80°C	$T\approx$–10°C	$T\approx$70 +80°C
PG	–100	–26.1	–96.8	–25.8
nonanoic/PG	–22.4	38.7	–96.1	8.4
decanoic/PG	26.0	–15.4	–93.6	–57.1
oleic/PG	–8.4	–100	–95.4	–100
13-octadecenoic/PG	–27.4	–100	–96.8	–100
linoleic/PG	–49.6	9.5	–97.0	–25.0
α-linolenic/PG	2.1	–100	–95.9	–72.4

sition temperature, although no explanation about this was given. In fact, the data from previous investigators always displayed more than one peak of different magnitudes in the subzero temperature range –20 to 0°C [3–5]. This is consistent with the data from this study comparing the effects of hydration using 27% sodium bromide solution (about 20% water content) and phosphate buffered saline (about 100% water content). Increase in water content of SC resulted in an increase in this water-related transition enthalpy. This also shifted the transition temperature from –20°C towards 0°C. The high transition enthalpy due to high water content obscured the lipid peak at –10°C. In absence of water, the water peak in the temperature region of –20 and 0°C disappears and so does the peak at 100°C (of skin protein). The absence of the transition at 100°C in SC after dehydration has been related to the irreversible denaturation of proteins or intercellular keratin [12], which is dependent on the water content of the SC [6, 19]. The transition is observed at high water contents, while it is disappeared at low water contents [7, 16]. The decrease in water content of SC resulted in a more apparent lipid transition at –10°C as can be concluded after comparing the curve of samples after the dehydration by silica gel to those by phosphorus pentoxide. Lipid extraction followed by dehydration eliminates all transitions from SC sheets.

Effects of propylene glycol

The treatment of SC by PG is known to affect the thermal transitions of the components of this skin layer. The transitions above 0°C from SC pre-treated with PG have been discussed by Bouwstra *et al.* [7, 13]. The disappearance of the peak at 100°C was thought to be due to the extraction of water from the protein regions by PG [7]. The protein denaturation would have turned the two clearly separated lipid transition peaks into one peak [13, 19].

The curves from the hydrated SC treated with PG (Fig. 3B) contained the subzero peak at –10°C, but on the curves of dehydrated samples this peak can hardly be noticed (Fig. 3A). These phenomena can be explained by the affinity of PG to water

molecules and by taking into account the nature of lipids of the –10°C peak. Apparently, PG has more affinity towards water than lipids. In the absence of water, as in the case of dehydrated samples, PG might interact with lipids, especially those with low melting points, causing a depression in their phase transition temperatures. In the hydrated SC samples, i.e. in the presence of water and lipids together, PG might interact strongly with water and proteins. The interaction of PG with water and proteins is clearly demonstrated by the depression of the transition peaks at 100°C as well as the shift of lipid peaks at 70 and 85°C to 63 and 73°C, respectively. However, the interaction with water and proteins seems to inhibit the interaction with lipids, so that the peak at –10°C is less affected and can be clearly observed.

Effects of fatty acids

It was observed by thermal analysis, that human SC treated by saturated fatty acids (SFA) dissolved in PG does not produce a distinctive extra transition of pure solution to the lipid phase transitions of SC. This suggests that these fatty acids are not present in large separated domains in SC or that they are dissolved in the present system. The temperature decrease of the SC phase transitions at 70 and 80°C induced by these fatty acids suggests that these acids predominantly modulate the gel-liquid transformation properties and/or lamellar ordering of SC lipids. The increase in transition enthalpies induced by nonanoic acid and the decrease in enthalpy induced by decanoic acid cannot be satisfactorily explained yet. This may be clarified by more studies using other members containing less or more carbon atoms.

There is a large difference of physical properties among the monounsaturated fatty acids (MUFA), e.g. the difference of melting points between oleic acid (*cis*-9-octadecenoic acid) and *cis-13-octadecenoic acid is 20°C. However, thermal analysis shows that these acids exhibit the same changes in the thermal behaviour of SC. The results indicate that the presence of a double bond affects the interaction of the fatty acids with SC. However, the position of a double bond apparently does not play a major role. Both oleic and 13-octadecenoic acids form separate domains resulting in a transition peak of pure fatty acid solution (in PG) and reduce the enthalpy change of the lipid transitions at 70 and 80°C suggesting that the lipid organization is strongly perturbed. The formation of separate domains by oleic acid within SC lipids has been reported before and used to explain the mode of action of oleic acid as penetration enhancers [21].*

Thermal analysis of hydrated human SC following the treatment of polyunsaturated fatty acids (PUFA) – linoleic and linolenic acid – shows a moderate suppression of the lipid phase transitions at 70 and 80°C, i.e. in a lesser degree compared to MUFA. This suggests a limited extent of lipid perturbation. Additionally, the appearance of the transition peak of the native acids in PG solution can be noticed. The dissimilarity of the effects on the enthalpy of transitions at 70 and 80°C between these two acids indicates that the PUFA might not work in the same way, although the permeation studies showed a similar enhancement capacity.

References

1 J. J. Bulgin and L. J. Vinson, Biochim. Biophys. Acta, 136 (1967) 551.
2 K. Walkley, J. Invest. Dermatol., 59 (1972) 225.
3 T. Inoue, K. Tsujii, K. Okamoto and K. Toda, J. Invest. Dermatol., 86 (1986) 689.
4 G. Imokawa, H. Kuno and M. Kawai, J. Invest. Dermatol., 96 (1991) 845.
5 M. Takenouchi, H. Suzuki and H. Tagami, J. Invest. Dermatol., 87 (1986) 574.
6 B. F. Van Duzee, J. Invest. Dermatol., 65 (1975) 404.
7 J. A. Bouwstra, M. A. de Vries, G. S. Gooris, W. Bras, J. Brussee and M. Ponec, J. Controlled Rel., 15 (1991) 209.
8 B. W. Barry and A. C. Williams, in K. A. Walters and J. Hadgraft (Eds.), Pharmaceutical Skin Penetration Enhancement, Marcel Dekker, New York 1993, Chap. 4, p. 95.
9 H. Tanojo, J. A. Bouwstra, H. E. Junginger and H. E. Boddé, Pharm. Res., 11 (1994) 1610.
10 B. W. Barry, Dermatological Formulations. Percutaneous Absorption, Marcel Dekker, New York 1983.
11 K. A. Walters, in J. Hadgraft and R. H. Guy (Eds), Transdermal drug delivery: developmental issues and research initiatives, Marcel Dekker, New York 1989, Chap. 10, p. 197.
12 M. Goodman and B. W. Barry, Anal. Proc., 23 (1986) 397.
13 J. A. Bouwstra, L. J. C. Peschier, J. Brussee and H. E. Boddé, Int. J. Pharm., 52 (1989) 47.
14 H. E. Boddé, M. Ponec, A. P. IJzerman, A. J. Hoogstraate, M. A. I. Salomons-de Vries and J. A. Bouwstra, in K. A. Walters and J. Hadgraft (Eds), Pharmaceutical Skin Penetration Enhancement, Marcel Dekker, New York 1993, Chap. 8, p. 199.
15 P. A. Cornwell and B. W. Barry, in R. C. Scott, R. H. Guy, J. Hadgraft and H. E. Boddé (Eds), Prediction of Percutaneous Penetration, Vol. 2, IBC Technical Services, London 1991, p. 394.
16 M. Goodman and B. W. Barry, in R. L. Bronaugh and H. I. Maibach (Eds), Percutaneous Absorption, Marcel Dekker, New York, 2nd edn, 1989, p. 567.
17 P. Ashton, K. A. Walters, K. R. Brain and J. Hadgraft, Int. J. Pharm., 87 (1992) 265.
18 R. O. Potts, J. Soc. Cosmet. Chem., 37 (1986) 9.
19 K. Knutson, R. O. Potts, D. B. Guzek, G. M. Golden, J. E. McKie, W. J. Lambert and W.I. Higuchi, J. Controlled Rel., 2 (1985) 67.
20 G. M. Golden, D. B. Guzek, R. R. Harris, J. E. McKie and R. O. Potts, J. Invest. Dermatol., 86 (1986) 255.
21 B. Ongpipattanakul, R. R. Burnette, R. O. Potts and M. L. Francoeur, Pharm. Res., 8 (1991) 350.
22 G. L. Wilkes, A. L. Nguyen and R. H. Wildnauer, Biochim. Biophys. Acta, 304 (1973) 267.