ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 54 (2002) 143–150

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

The influence of dilution of topical semisolid preparations on hydrocortisone permeation through excised human stratum corneum

Hanan Refai, Christel C. Müller-Goymann*

Institut für Pharmazeutische Technologie, Braunschweig, Germany

Received 8 June 2001; accepted in revised form 4 April 2002

Abstract

Dilution of semisolid preparations, in order to tailor the formulations to the needs of the patients, was thought to be associated with a number of dangers, one of which is the unpredictable alteration of activity. In the present study the influence of dilution on hydrocortisone permeation through excised human stratum corneum was investigated. The permeation profiles of hydrocortisone from various cream bases (diluted and undiluted) were found to be very similar with no significant differences. This result was in accordance with the lack of interaction between the tested bases and the structure of stratum corneum as shown by differential scanning calorimetry experiments. Thus, the permeability of stratum corneum, which was not affected by the cream bases, is the rate limiting step for drug permeation. However, it could be shown that dilution of Soventol cream (placebo with 1% hydrocortisone) which is known to contain isopropyl myristate as permeation enhancer reduces drug permeation. The reduced hydrocortisone permeation is believed to be due to reduced enhancer concentration.

Keywords: Cream base dilution; Stratum corneum; Hydrocortisone; Permeation; Penetration enhancer

1. Introduction

The dilution of commercially available topical corticosteroid formulations is a common practice done in many countries in response to physicians' requests. The intuitive expectation is that dilution would reduce the activity of the corticosteroid formulations to meet the needs of the patients and at the same time enable the physician to restrict prescribing to well known corticosteroid molecules. However, predicting the extent to which activity is reduced when a topical corticosteroid formulation is diluted is no simple task, besides this practice is associated with a number of dangers. In particular, there is a risk of accelerating chemical and physical decomposition, facilitating microbial contamination and interfering with the biopharmaceutical profiles of the formulations [1].

The biopharmaceutical incompatibilities, which may arise from the inappropriate selection of the vehicle used for dilution, are of special importance as the sophisticated nature of the commercially available bases may imply that any changes in concentration or components of the base may affect the rate of release of the corticosteroid from the final preparation. It would therefore appear that extemporaneous dilution of proprietary topical corticosteroid formulations could result in a disturbance of the equilibrium of base components necessary for optimum release of active ingredients.

The ability of a drug in a topical formulation to exert its effect is dependent on two consecutive physical events. The drug must first diffuse out of the vehicle to the skin surface, and then it must permeate this natural barrier to the site of action. In a previous study [2] the influence of vehicle dilution on hydrocortisone release from topical semisolid preparations was investigated. It was found that the drug liberation was greatly affected by the type, composition and rheological properties of the vehicle used for dilution as well as the solubility of the drug in the final preparation.

The aim of the present study was to examine the influence of dilution on drug permeation through excised human stratum corneum. For this purpose water-containing hydrophilic ointment DAB 1998 (WHS) with 1% hydrocortisone was chosen as a model cream base. It was diluted with various vehicles selected from the German Pharmacopoeia, DAB 1998. The resulting formulations were then tested for hydrocortisone permeation through excised human stratum corneum. In addition, the interaction between the selected cream bases and stratum corneum structure was investigated

* Corresponding author. Institut für Pharmazeutische Technologie, Technische Universität Braunschweig, 38106 Braunschweig, Germany.

0939-6411/02/$ - see front matter
PII: S0939-6411(02)00054-1

via differential scanning calorimetry (DSC) in order to reveal its influence on permeation.

2. Materials and methods

2.1. Materials

Emulsifying cetostearyl alcohol (Henkel, D-Düsseldorf), cetostearyl alcohol (Caesar & Loretz GmbH, D-Hilden), liquid paraffin DAB 10 (Mainland, D-Frankfurt), white petrolatum DAB 10 (Hansen & Rosenthal, D-Hamburg), wool fat alcohol (Caesar & Loretz GmbH, D-Hilden), glycerol 85% (Henry Lamotte GmbH, D-Bremen), Tween® 60 (ICI, UK-Cleveland), isopropyl myristate (Merck, D-München), Soventol® Hydrocortison Creme placebo (Knoll Deutschland GmbH, D-Mannheim) were used as provided. Soventol Hydrocortson Creme placebo contains: Ammoniac-solution, carbomer, cera liquid, purified water, isopropyl myristate, macrogol 400, sodiumedetate, paraffin oil, parfume oil and 2-propanol. Micronized hydrocortisone (Synopharm, D-Hamburg) was used as a model drug in all permeation and DSC experiments. Doubly-distilled water was used in all of the experiments. For preparation of phosphate buffer pH 7.4, 2.38 g sodium monohydrogenphosphate and 0.19 g potassium dihydrogenphosphate were dissolved in 1000 ml water.

2.2. Methods

2.2.1. Preparation of creams

Creams were prepared according to the instructions of the German Pharmacopoeia DAB, 1998. In order to obtain homogeneous distribution of hydrocortisone within the base and homogeneously diluted formulations a Cito Unguator® [3] was used. The homogenization was done at 1000 rpm for 2 min at room temperature.

2.2.2. Isolation of stratum corneum

Healthy skin gained in plastic surgeries from the abdominal region of female donors was used. Immediately following excision the skin was cooled and the subcutaneous fat tissue and part of the dermis were mechanically removed by a scalpel. Stratum corneum sheets were then isolated by trypsination [4]. This was done by spreading the skin sheet with its dermal side on filter paper, which was wetted with 2% aqueous trypsin solution and incubated for 24 h at 37°C. After this time the stratum corneum was carefully peeled off from the underlying cells using a blunt forceps. To prevent further enzymatic degradation, the stratum corneum was bathed for several minutes in a 0.01% aqueous solution of trypsin inhibitor, then washed several times in water and subsequently dried and stored at room temperature in a desiccator over blue gel.

2.2.3. Permeation experiments through excised human stratum corneum

Permeation experiments were performed in modified Franz diffusion cells [5]. The experiments were carried out in triplicate. The donor compartment was filled with the formulation and phosphate buffer pH 7.4 was used as acceptor. A proper homogenization of the released drug in the acceptor throughout the experiment was achieved by a rotating magnet (400 rpm). Prior to the experiment, the stratum corneum sheets were completely hydrated in water. Before mounting the stratum corneum pieces on the diffusion cells, the sheets were placed on polycarbonate filter (5 μm) for higher mechanical stability. The acceptor compartments of the Franz cells were mounted in a water bath at 37°C, whereby the membranes have a temperature of 32°C which is comparable to the physiological temperature of the skin surface. Samples of 250 μl were taken from the acceptor compartment over 29 h and replaced by fresh buffer. The concentration of hydrocortisone was determined by high performance liquid chromatography (HPLC). The permeation coefficient and the persol-coefficient were calculated from the flux (slope of the graph of the permeation curve in g/cm^2 per s) according to the Eqs. (2.1) and (2.2), respectively:

$$P = J/C_0 \tag{2.1}$$

$$Z = J/C_S \tag{2.2}$$

P	permeation coefficient (cm/s)
Z	persol-coefficient (cm/s)
J	flux (g/cm^2 per s)
C_0	starting concentration (g/cm^3)
C_S	saturation concentration (g/cm^3).

2.2.4. HPLC

Analysis was performed by reversed phase chromatography using a column of Hypersil® ODS 5 μm 250 × 4 mm (Grom, D-Herrenberg), the mobile phase was methanol/

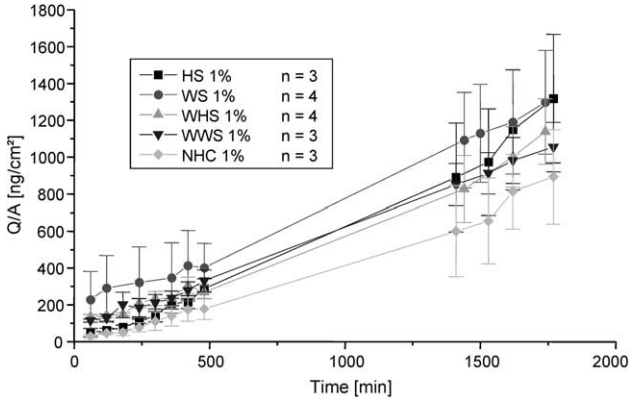

Fig. 1. Permeation of hydrocortisone from 1% WHS, WWS, NHC, HS and WS through excised human stratum corneum. The data represent the mean (n = 3–4) ± SD.

Table 1
Hydrocortisone flux, permeation coefficient and per-sol coefficient of 1% WHS, WWS, NHC, WS and HS[a]

Vehicle (1% hydrocortisone)	Flux J (g/cm^2 per s) × 10^{-12}	Permeation coefficient P (cm/s) × 10^{-9}	Persol-coefficient Z (cm/s) × 10^{-9}
HS	12.35 ± 3.2	1.42 ± 0.37	273.72 ± 71.0
WS	11.38 ± 1.89	1.34 ± 0.22	1335.29 ± 222.4
WHS	9.72 ± 1.83	1.01 ± 0.19	84.6 ± 15.9
WWS	9.6 ± 0.75	1.06 ± 0.08	529.28 ± 41.6
NHC	7.34 ± 3.31	0.76 ± 0.35	63.82 ± 28.8

[a] Each value represent the mean ($n = 3$–4) ± SD.

water (60:40) with a flow rate of 1.1 ml/min. The HPLC system consisted of a Beckman System Gold Solvent Delivery System 126 and UV detector Beckman System Gold Detector Module 166 (Beckman, D-München). Linear correlation between peak area and hydrocortisone concentrations was obtained within the concentration range of 10–1000 ng/ml.

2.2.5. Pretreatment of excised stratum corneum for DSC experiments

In order to obtain sharper transitions stratum corneum was hydrated to 20% water content by placing it in a desiccator with saturated sodium chloride solution (rel. humidity: 75.2%) for 48 h. Afterwards, the stratum corneum was immersed in the respective cream bases for 30 min at 37°C. After this time the rests of the cream bases were removed carefully, the stratum corneum sheets were folded in an aluminium crucible and analyzed by DSC.

2.2.6. DSC

Human stratum corneum as well as the cream bases were thermally analyzed using a Differential Scanning Calorimeter DSC 220 C with a disc station 5200 H (Seiko, J-Tokyo). Stratum corneum pieces were folded in an aluminium crucible and fused on cold. The probes were measured against empty reference crucible over a temperature range of −20–140°C with a heating rate of 5°C/min. DSC experiments for all tested bases with and without the drug as well as for the drug itself were done as preexperiments to insure the absence of any interfering peaks.

2.2.7. Determination of the solubility of the drug in the vehicle

The base was prepared with a definite concentration of hydrocortisone; after having been left for 3 days at room temperature, the preparation was examined for the presence of hydrocortisone crystals using a polarizing microscope, Zeiss Photomicroscope III (Zeiss, D-Oberkochen). If no crystals were detected, higher concentrations were examined, until crystals could be found. The first concentration at which crystals could be detected was taken as the concentration at saturation [6].

3. Results and discussion

3.1. Permeation of hydrocortisone from different cream bases

In order to examine the influence of base type on permeation of hydrocortisone through excised human stratum corneum various bases were chosen from the German Pharmacopoeia, DAB 1998; taking in consideration that these bases cover a wide range of base types regarding the lipophilicity, hydrophilicity and water content. This enabled the investigation of most possible factors concerning vehicle composition that may exhibit an effect on drug permeation. The selected bases can be classified into the following types:

- anhydrous hydrophilic base: hydrophilic ointment (HS), DAB 1998;
- hydrous hydrophilic bases: WHS, DAB 1998 and non-ionic hydrophilic cream (NHC), DAB 1998;
- anhydrous lipophilic base: wool fat ointment (WS), DAB 1998;
- hydrous lipophilic base: water-containing wool fat ointment (WWS), DAB 1998.

The above mentioned cream bases were prepared with 1% hydrocortisone and examined for drug permeation. The amount of drug permeated per unit area (ng/cm^2) was plotted versus time (min).

From Fig. 1 it is evident that there is no significant differ-

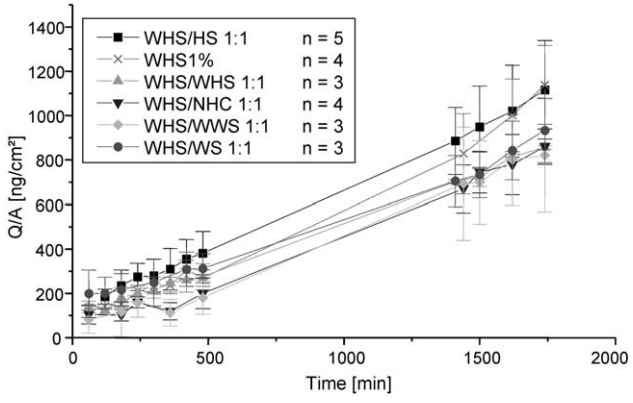

Fig. 2. Permeation of hydrocortisone from WHS 1% diluted 1:1 with WHS, WWS, NHC, WS and HS. The data represent the mean ($n = 3$–5) ± SD.

Table 2
Hydrocortisone flux, permeation coefficient and per-sol coefficient of WHS 1% diluted in the ratio of 1:1 with the various cream bases[a]

Vehicle	Flux J (g/cm^2 per s) $\times 10^{-12}$	Permeation coefficient P (cm/s) $\times 10^{-9}$	Persol-coefficient Z (cm/s) $\times 10^{-9}$
WHS 1%	9.72 ± 1.83	1.01 ± 0.19	84.6 ± 15.9
WHS/HS 1:1	9.28 ± 1.56	2.02 ± 0.35	154.63 ± 26.5
WHS/WS 1:1	7.8 ± 0.98	1.67 ± 0.21	433.33 ± 54.6
WHS/WHS 1:1	7.6 ± 0.57	1.58 ± 0.12	66.09 ± 4.9
WHS/WWS 1:1	7.68 ± 1.97	1.56 ± 0.40	236.31 ± 60.6
WHS/NHC 1:1	7.77 ± 1.32	1.66 ± 0.28	69.38 ± 11.78

[a] Each value represents the mean ($n = 3$–5) ± SD.

ence between the various cream bases regarding the permeation of hydrocortisone through excised human stratum corneum. From Table 1 it is obvious that WS 1% and HS 1% have greater fluxes and permeation coefficients than the other bases, but this observation is not significant as the confidence intervals overlap. The great differences in the per-sol coefficient of the various bases varying from 1335.29×10^{-9} cm/s for WS 1% to 84.6×10^{-9} cm/s for WHS 1% (Table 1) though having similar permeation rates indicate the negligible role of the amount of drug dissolved in the bases on permeation through stratum corneum. This finding allows the conclusion that the factors affecting drug release such as vehicle composition, drug solubility in the base and viscosity of the preparation do not play a role in drug permeation.

For all the preparations being suspension vehicles the variations in drug solubility in the formulation through changing the vehicle is always compensated by the reverse change in the partition coefficient of the drug between vehicle and stratum corneum.

From Eq. (3.1) [7],

$$Q = \frac{D \times F}{d} f\mathrm{B} \times C\mathrm{sB} \times t \tag{3.1}$$

D diffusion coefficient of the drug in stratum corneum;
F permeation area;
f_B fraction of the drug in stratum corneum;
c_{sB} saturation concentration of the drug in stratum corneum;
d thickness of stratum corneum;
t time.

It is evident that the main factor affecting drug transport through stratum corneum is the saturation concentration of the drug in stratum corneum c_{sB} which is specific to the substance and not the solubility of the drug in the vehicle as long as there is enough free (dissolved) substance able to saturate the barrier. This finding was also observed by Schwarb et al. [8], who reported that in contrast to the in vitro release testing for fluocinonide, the in vivo human skin blanching assay was found to be independent of the degree of saturation.

3.2. Permeation of hydrocortisone from diluted cream bases

In order to investigate the influence of dilution on hydrocortisone permeation through excised human stratum corneum WHS 1% was diluted in the ratio 1:1 with the same base, WHS, and the different chosen cream bases WWS, WS, NHC and HS.

From Fig. 2, it is clear that no noticeable differences can be detected between the permeation rates of all diluted formulations. This was expected as the permeation profiles of the different undiluted bases were similar as shown in Fig. 1. Furthermore, the permeation rates of the diluted as well as the undiluted (WHS 1%) preparations cannot be distinguished from each other, which reveals the negligible influence of dilution on permeation. The flux, permeation coefficient and the per-sol coefficient were again calculated and demonstrated in Table 2.

In order to confirm this result, higher dilutions of WHS 1% were performed. WHS 1% was diluted with a lipophilic base, WWS, as well as with a hydrophilic base, NHC, in the ratio 1:3 and examined for permeation of hydrocortisone. Figs. 3 and 4 show that even the 1:3 dilution of WHS 1% with either of the two chosen bases does not lead to a reduced permeation of hydrocortisone. It must be considered that hydrocortisone in all diluted formulations is still suspended in the vehicle to a great excess [2], i.e. the dilution only reduced the amount of the drug suspended in the base, whereas the vehicle is still saturated with the drug.

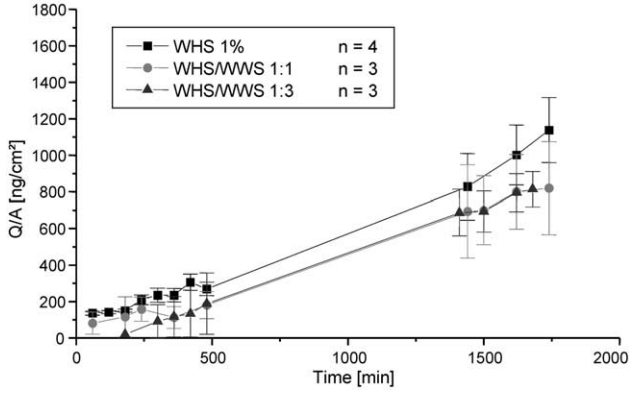

Fig. 3. Permeation of hydrocortisone from WHS 1% diluted 1:1 and 1:3 with WWS. The data represent the mean ($n = 3$–4) ± SD.

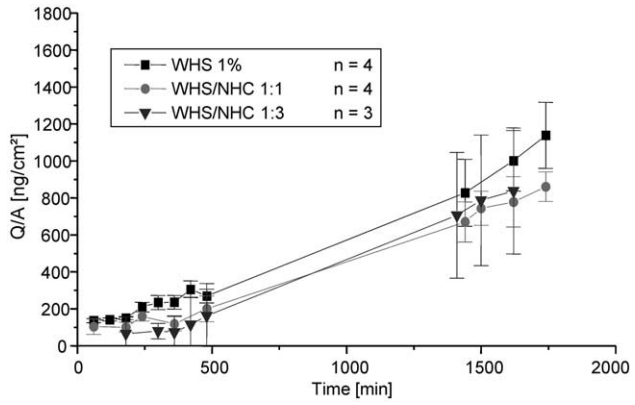

Fig. 4. Permeation of hydrocortisone from WHS 1% diluted 1:1 and 1:3 with NHC. The data represent the mean ($n = 3$–4) ± SD.

Therefore, for all diluted preparations enough free drug was available to saturate the stratum corneum having thereby no influence on hydrocortisone permeation. Even in an in vivo study carried out by Gao and Li Wan Po [9] the (1 in 4) and (1 in 10) diluted Synalar® creams were found to be of the same potency as the full strength cream. The authors suggested that for all tested preparations sufficient corticosteroid is present to saturate the receptors of corticosteroids in the skin, so that an increase in concentration will not produce any enhancement in activity. According to these considerations in order to influence drug permeation very high dilutions (up to 1:100) should be performed to reduce the drug content in the vehicle below its saturation concentration so that it would not be able to saturate the barrier. These dilutions were not carried out in this work as they are not done in practice.

3.3. Influence of diluting a cream base containing penetration enhancers

Soventol® cream was found to significantly promote the permeation of hydrocortisone acetate through stratum corneum when compared with other market products [10].

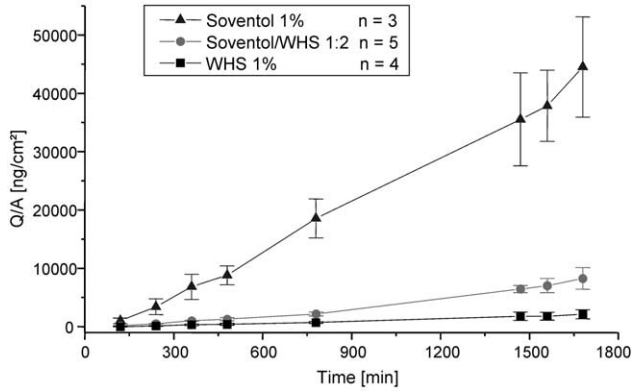

Fig. 5. Permeation of hydrocortisone from 1% Soventol cream, Soventol cream diluted with WHS 1:2 and 1% WHS. The data represent the mean ($n = 3$–5) ± SD.

Table 3
Hydrocortisone flux and permeation coefficient of 1% Soventol cream, Soventol cream diluted with WHS 1:2 and 1% WHS[a]

Vehicle	Flux J (g/cm^2 per s) × 10^{-10}	Perm.coef. P (cm/s) × 10^{-9}
Soventol 1%	4.5 ± 0.8	43.06 ± 7.65
Soventol/WHS 1:2	0.85 ± 0.15	25.87 ± 4.61
WHS 1%	0.23 ± 0.07	2.39 ± 0.71

[a] Each value represents the mean ($n = 3$–5) ± SD.

It is to be noted that Soventol® cream contains isopropyl myristate which is a pronounced penetration enhancer [11,12] as well as isopropyl alcohol which is known to possess also some enhancing properties. Therefore, Soventol cream placebo prepared with 1% hydrocortisone and its 1:2 dilution with WHS were tested for permeation of hydrocortisone in comparison to WHS 1% [13]. From Fig. 5 it is to be noted that hydrocortisone is permeating significantly better from Soventol cream than from WHS. As shown in Table 3 the permeation of hydrocortisone from Soventol cream is about 20 times greater than that of WHS proving thereby the influence of the permeation enhancers. Furthermore, the diluted formulation of Soventol cream/WHS 1:2 (Fig. 5) revealed a considerable reduction of hydrocortisone permeation flux which was found to be about five times lower than that of Soventol 1% (Table 3) (the permeation

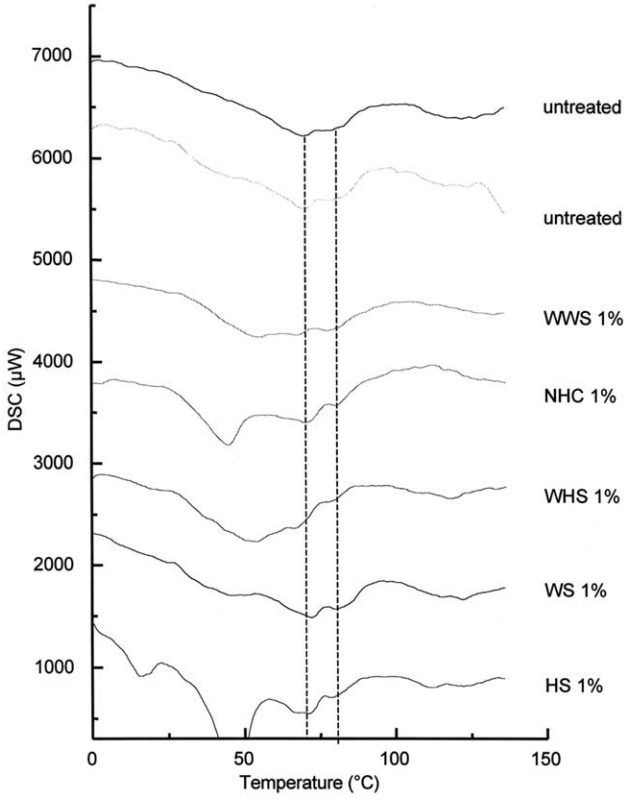

Fig. 6. DSC thermogram for untreated and vehicle (1% hydrocortisone) pretreated stratum corneum. Donor: male, abdomen, 26 years.

coefficient is relatively high because the starting concentration is reduced to the third).

Considering the fact that hydrocortisone is suspended in the base these results strongly suggest that the reduced permeation profile of hydrocortisone from Soventol cream upon dilution is probably due to the reduced concentration of the enhancer and not that of the drug.

This finding allows the conclusion that dilution of cream bases could indeed alter drug permeation depending on the vehicle composition in a manner which could not be predictable, i.e. the 1:2 dilution of Soventol cream resulted in a 1:5 reduction in drug permeation.

3.4. Influence of vehicle on structure of stratum corneum

It is well known that the barrier function of the stratum corneum is related to the unique composition of its lipids and their complex structural arrangement. Alteration in the lipoidal matrix of the stratum corneum, therefore, increases its permeability. In order to find further explanations for the results obtained in the permeation experiments, the interactions between vehicles used for permeation and the structure of stratum corneum were investigated using differential scanning calorimetry.

Isolated sheets of human stratum corneum show normally four characteristic endothermic transitions at about 40, 75, 85 and 105°C, respectively [14–16]. The first two transitions are lipid-based. The third transition at 85°C is considered by several investigators to represent a phase transition of lipids which are associated with proteins [15,17]. The fourth transition at 105°C represents the denaturation of the protein portion of the stratum corneum, i.e. the α proteins [14].

It has to be mentioned that the first peak, which is not easily detectable, as well as the α protein denaturation peak, which requires a certain water content of the sample in order to become apparent, could not be detected in the present work. Therefore, all DSC curves were evaluated with regard to the peak maximum temperatures of the distinct lipid-phase transitions at about 70 and 80°C. These two transitions give the required information about any possible interaction between the vehicle and the lipoidal structure of the stratum corneum, which is responsible for its barrier function.

Table 4
Peak maximum temperatures of the second and third phase transitions of untreated and 1% vehicle pretreated stratum corneum[a]

Stratum corneum	Peak 1 (°C)	Peak 2 (°C)
Untreated	69.9	80.4
WWS 1%	68.3	79.8
NHC 1%	70.5	80.4
WHS 1%	68.8	80.4
WS 1%	71.4	80.7
HS 1%	70.7	80.1

[a] Donor: male.

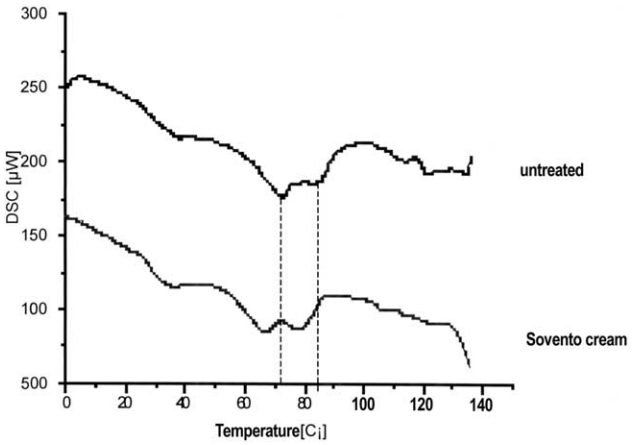

Fig. 7. DSC thermogram for human stratum corneum after pretreatment with Soventol cream. Donor: male, breast, 26 years.

3.4.1. Differential scanning calorimetry of stratum corneum pretreated with WHS, WWS, WS, NHC and HS

Fig. 6 and Table 4 show that none of the tested bases produced significant changes in the lipid-phase transitions of the stratum corneum, as the maximum peak shift observed was 1.6°C which is considered to be insignificant. In conclusion, the vehicles investigated do not interact with stratum corneum structure; therefore, the barrier properties and subsequently the permeability of the stratum corneum remain unchanged. These results give further explanation why all tested vehicles showed similar permeation profiles.

The peaks detected at temperatures below 70°C were proven to be produced from the base which remain adhered to the stratum corneum after pretreatment.

3.4.2. Differential scanning calorimetry of stratum corneum pretreated with Soventol cream, isopropyl myristate and isopropyl alcohol

The pretreatment of human stratum corneum with Soventol cream as well as isopropyl myristate revealed a significant shift of about 5°C to lower temperatures in both peaks (Fig. 7 and Table 5). This shift indicates an alteration in the lipoidal structure of stratum corneum which is known to be responsible for its barrier properties subsequently leading to an increase in its permeability.

Isopropyl myristate is believed to increase the fluidity of the lipid bilayers leading to a less ordered state of the intercellular lipids [16].

This fluidizing action is interpreted either by the devel-

Table 5
Peak maximum temperatures of the second and third phase transitions of untreated and Soventol cream pretreated stratum corneum[a]

Stratum corneum	Peak 1 (°C)	Peak 2 (°C)
Untreated	72.68	84.53
Pretreated with Soventol cream	66.86	78.97

[a] Donor: male, breast, 26 years.

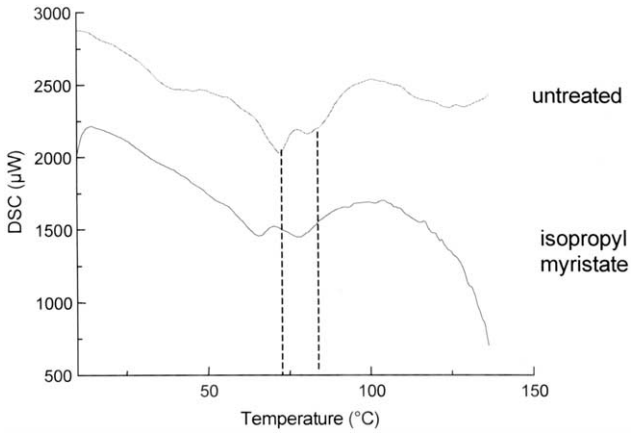

Fig. 8. DSC thermogram for human stratum corneum after pretreatment with isopropyl myristate. Donor: male, breast, 26 years.

opment of arrangements with periodic undulations [18] or the formation of solid solutions (lamellar gel states consisting of a homogeneous mixture of the lipids and the vehicle molecules).

The reason for the fluidizing effect of isopropyl myristate might be its branched structure [19]. Isopropyl myristate is believed to operate in a similar way to oleic acid which penetrates into the lipid structure with its polar end close to the lipid polar heads [16]. Because of the branched structure of isopropyl myristate, it then disrupts the packing of the intercellular lipids and increases their fluidity. Drug mobility in this less tightly packed arrangement will then increase [20].

Isopropyl alcohol is known to act mainly via extraction of stratum corneum lipids [21], therefore the DSC curves were evaluated with regard to the peak maximum temperatures of the lipid-phase transitions as well as the phase transition enthalpies. Figs. 8 and 9 and Tables 6 and 7 show that the pretreatment of stratum corneum with isopropyl alcohol did not reveal a significant alteration in the phase transition

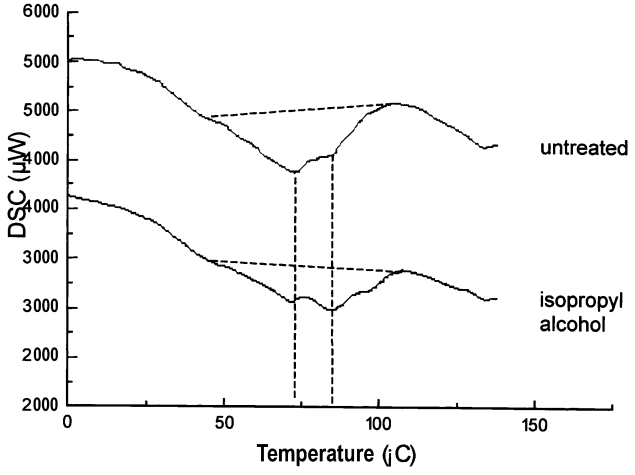

Fig. 9. DSC thermogram for human stratum corneum after pretreatment with isopropyl alcohol. Donor: female, breast, 57 years.

Table 6
Peak maximum temperatures of the second and third phase transitions of untreated and isopropyl myristate pretreated stratum corneum[a]

Stratum corneum	Peak 1 (°C)	Peak 2 (°C)
Untreated	72.44	82.84
Pretreated with isopropyl myristate	65.71	77.94

[a] Donor: male, breast, 26 years.

temperatures. Moreover, the effect observed in the phase transition enthalpies (~12.2%) was considered to be also insignificant (Leopold and Lippold [16] reported a statistically significant effect for an enthalpy change of more than 17%). This finding proves that isopropyl myristate is alone responsible for the permeation profile of hydrocortisone from Soventol cream.

4. Conclusion

Taken together, these results suggest that permeation is controlled alone by structure integrity and degree of saturation of stratum corneum. Thus dilution would affect drug permeation only if one of these two factors or both are altered. Accordingly, dilution may result in a reduction, an increase or no change in permeation depending on the type of the final preparation (whether suspension or solution vehicle) and its influence on stratum corneum structure in comparison to the original formulation. For example, if the original preparation is a suspension vehicle containing no permeation enhancer then dilution with an enhancer free base may result either in suspension type vehicle with no change in permeation, or in solution type vehicle with a probable reduction in permeation. But if the diluent contains an enhancer the final product probably shows an increased drug permeation.

Nevertheless, it must be taken in consideration that in the case of in vitro testing such as that performed in this study the effect of occlusion can not be examined. The application of an ointment on the skin surface prevents evaporation of water and develops a state of increased hydration of the skin, which results in increased drug penetration. Application of creams and lotions under normal conditions (non-occluded) allows

Table 7
Peak maximum temperatures of the second and third phase transitions and the enthalpy of both peaks of untreated and isopropyl alcohol pretreated stratum corneum[a]

Stratum corneum	Peak 1 (°C)	Peak 2 (°C)	Enthalpy of both peaks in a temperature range of ~47–105°C (mJ/mg)
Untreated	73.09	84.42	18.0
Pretreated with isopropyl alcohol	72.67	84.42	15.8

[a] Donor: female, breast, 57 years.

free exchange of air and water, and the skin does not achieve the state of higher hydration. Thus different formulations may result in different amounts of drug permeation into the skin and may, thus, exhibit different intensities of activity [22]. Dilution may therefore change drug permeation by altering the occlusive properties of the base.

However, all the above mentioned facts are true for intact skin. In the case of damaged skin and mucosa the relations are completely different. Here the release is the rate limiting step for the drug uptake due to the absence or leakage of stratum corneum. Hence, dilution would greatly influence drug release depending on the properties (composition, water content, viscosity, etc.) of the formulation used for dilution [2].

In conclusion, it is very difficult for the physician who prescribes the dilution as well as for the pharmacist to predict the effect of dilution on activity, as there are different factors concerning the skin of the patient and the preparation itself affecting drug permeation. Therefore additional studies should be conducted in order to establish rules to control this practice.

References

[1] M.J. Busse, Dangers of dilution of topical steroids, Pharm. J. 14 (1978) 25–26.
[2] H. Refai, C.C. Müller-Goymann, Larvated incompatibilities of hydrocortisone cream preparations upon dilution with different cream bases, Pharmazie 54 (1999) 754–758.
[3] H.P. Zobel, I. Brinkmann, B. Fröstl, S. Heim, C. MüllerVan, T.H. Nguyen, K. Prescher, A. Zimmer, Alternativen zum Salbenrühren im Vergleich, Pharm. Ztg. 35 (1997) 2944–2951.
[4] A.M. Kligman, E. Christophers, Preparation of isolated sheets of human stratum corneum, Arch. Dermatol. 88 (1964) 702–705.
[5] T.J. Franz, Percutaneous absorption. On the relevance of in vitro data, J. Invest. Dermatol. 64 (1975) 190–195.
[6] H. Loth, A. Holla-Benninger, M. Hailer, Untersuchungen der Arzneistoffliberation aus Salben, Pharm. Ind. 41 (1979) 789–796.
[7] B.C. Lippold, Kutane Resorption-Möglichkeiten, Modelle, Beeinflussung, Acta Pharm. Technol. 27 (1981) 1.
[8] F.P. Schwarb, G. Imanidis, E.W. Smith, J.M. Haigh, C. Surber, Effect of concentration and degree of saturation of topical fluocinonide formulations on in vitro membrane transport and in vivo availability on human skin, Pharm. Res. 16 (6) (1999) 909–915.
[9] A.Y. Gao, A. Li Wan Po, Topical formulations of Fluocinolone acetonide: are creams, gels and ointments bioequivalent and does dilution affect activity? Eur. J. Clin. Pharmacol. 46 (1994) 71–75.
[10] U. Alberg, Wasserhaltige Hydrophile Salbe DAB mit suspendiertem Hydrocrtisonacetat – Einfluβ von Ethanol auf die Mikrostruktur der Cremes, Arzneistofffreigabe und Arzneistoffpermeation durch humanes Stratum corneum. Thesis TU Braunschweig, 1998.
[11] S.R. Gorukani, L. Li, K.H. Kim, Trandermal delivery of antiparkinsonian agent, benztropine. I. Effect of vehicles on skin permeation, Int. J. Pharm. 192 (1999) 159–172.
[12] J.Y. Fang, P.C. Wu, Y.B. Huang, Y.H. Tsai, Percutaneous absorption of capsaicin, nonovamide and sodium nonivamide acetate from gel and ointment bases: in vitro formulation evaluations in pigs and in vivo bioengineering methods in humans, Int. J. Pharm. 130 (1996) 121–135.
[13] C.C. Müller Goymann, H. Refai, Tücken im apothekenlabor, Pharm. Ztg. 145 (2000) 11–16.
[14] B.F. Van Duzee, Thermal analysis of human stratum corneum, J. Invest. Dermatol. 65 (1975) 404–408.
[15] G.M. Golden, D.B. Guzek, A.H. Kennedy, J.E. McKie, R.O. Potts, Stratum corneum lipid phase transitions and water barrier properties, Biochemistry 26 (1987) 2382–2388.
[16] C.S. Leopold, B.C. Lippold, An attempt to clarify the mechanism of the penetration enhancing effects of lipophilic vehicles with differential scanning calorimetry (DSC), J. Pharm. Pharmocol. 47 (1995) 276–281.
[17] B.W. Barry, Mode of action of penetration enhancers in human skin, J. Controlled Release 6 (1987) 85–97.
[18] A. Rolland, A. Brzokewicz, B. Shroot, J.C. Jamoulle, Effect of penetration enhancers on the phase transition of multilamellar liposomes of dipamitoylphosphatidylcholine. A study by differential scanning calorimetry, Int. J. Pharm. 76 (1991) 217–224.
[19] E.W. Smith, H.I. Maibach, Percutaneous Penetration Enhancers, Chapter 9.1, CRC Press, Inc, FL, USA, 1995.
[20] B.W. Barry, Lipid-protein-partitioning theory of skin penetration enhancement, J. Controlled Release 15 (1991) 237–248.
[21] R.H. Guy, V.H.W. Mak, T. Kai, D. Bommannan, R.O. Potts, Percutaneous penetration enhancers: mode of action, in: R.C. Scott (Ed.), Prediction of percutaneous absorption, IBC Terminal Service Ltd, London, 1989, pp. 213–223.
[22] V.P. Shah, J. Elkins, J. Hanus, C. Noorizadeh, J.P. Skelly, In vitro release from topical preparations and automated procedure, Pharm. Res. 8 (1) (1991) 55–59.