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Research Paper

Penetration Profile of Taurine in the Human Skin and Its Distribution in Skin Layers

D. L. P. da Silva,[1] S. B. Thiago,[1] F. A. Pessôa,[1] Y. Mrestani,[2,4] H. H. Rüttinger,[1] J. Wohlrab,[3] and R. H. H. Neubert[1]

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Purpose. To measure *in vitro* release of taurine from a semisolid standard formulation (amphiphilic cream, DAC) containing 1% taurine, a multi-layer membrane system was used. The content and distribution of taurine in different healthy skin layers (stratum corneum, epidermis and dermis) before (native taurine) and after application of the DAC cream were determined using capillary electrophoresis.
Methods. The release of taurine from the DAC cream was studied using a multilayer membrane system. Due to the high hydrophilic properties of taurine, the artificial model membranes consisted of collodion as matrix and glycerol as the acceptor phase. In order to determine whether taurine shows the potential for dermal penetration a Franz diffusion cell system was used. The distribution of taurine in the skin layers was determined before and after application of the DAC cream followed by the incubation in a Franz diffusion cell. The excised skin sample was cut in horizontal sections using a cryomicrotome. In order to detect taurine, fluorescamine was used as a derivatization agent.
Results. Experiments with a multilayer membrane system were performed to verify the release of taurine at different times (1, 2 and 5 h). Approximately 42.5% taurine was released from the semisolid standard formulation, accumulating in the first membrane (17.63%). The native taurine content was quantified in human isolated skin layer before and after the application of the semisolid standard formulation followed by incubation in a Franz-type diffusion cell for 1 and 5 h. No statistically significant difference ($p<0.05$) of the taurine content in the skin layers existed between exposure times (1 and 5 h) studied. The highest taurine content was found in the epidermis both before (256.01 μg taurine/g skin layer) and after (555.5 μg taurine/g skin layer) the application of the DAC cream.
Conclusions. The distribution profile of taurine in the skin layers was very similar for the times studied, which suggests that taurine is accumulated in specific cells of the skin. The study suggests that taurine is effectively released from the semisolid standard formulation and can be used for topical application in dermatopharmaceutics.

KEY WORDS: capillary electrophoresis; Franz diffusion cell; multilayer membrane model; skin layers; taurine.

INTRODUCTION

Taurine, a 2-aminoethanesulfonic acid (MW, 125.48 g/mol), is one of the most abundant free amino acids in almost all mammalian tissues. It is synthesized in the liver from cysteine and methionine. The zwitterionic nature of taurine ($pK_1=1.5$, sulfonic acid group; $pK_2=9.08$, ammonium group) gives it high water solubility (104.8 g/l) and low lipophilicity (log $P=-1.02$, value between water und *n*-octanol) (1,2). Taurine melt at 305.11°C. Important physiological roles are attributed to taurine such as membrane stabilization, bile salt formation, antioxidation, modulation of calcium levels, neuromodulator and inhibitory neurotransmitter (3).

In the skin, taurine acts as an important osmolyte (4), and it shows a specific pattern of distribution in the skin. A light microscopic radioautography has been employed to demonstrate the distribution of taurine in adult and newborn mice after the injection of 3H-taurine (5). The study revealed a very high density of taurine over the epidermis and external root sheaths near the opening of the hair follicles of the adult mice. The external root sheath just below the insertion of the sebaceous glands and the peripheral part of sebaceous glands showed a high taurine content. Connective parts of tissue in the dermis, however, showed low content of taurine. The absence of taurine transporter (TAUT, a 69 kDa protein) in the dermis can be one main factor for low taurine level in this layer (6). The taurine accumulation in the epidermis, especially in the keratinocytes, has been investigated (6).

[1] Institute of Pharmacy, Martin Luther University, Wolfgang Langenbeck Str. 4, 06120 Halle–Wittenberg, Germany.
[2] Institute of Applied Dermatopharmacy, Martin Luther University of Halle–Wittenberg, Halle (Saale), Germany.
[3] Department of Dermatology, Martin Luther University, Ernst-Grube Str. 40, 06097 Halle–Wittenberg, Germany.
[4] To whom correspondence should be addressed. (e-mail: mrestani@pharmazie.uni-halle.de)

Keratinocyte uptake of taurine, which has shown to be strongly dependent on extracellular sodium and chloride (7), is caused by the presence of the TAUT. In the epidermis, a gradient was evident with maximal levels of TAUT in the outermost granular keratinocyte layer and lower levels in the stratum spinosum, but no TAUT was found in the basal layer. Taurine has shown to play an important role not only in the keratinocyte hydration but also to act as a protective agent against transforming growth factor (TGF)-β1, an inhibitor of *in vitro* hair growth (8). The survival rate *in vitro* was increased when isolated hair follicles are grown in the presence of taurine. These findings strongly suggest that taurine could be involved in the maintenance of human hair bulb.

Quantitative analysis of taurine in squamous epithelia has been performed by HPLC after derivatisation with *o*-phthalaldehyde in the presence of 2-mercaptoethanol using immunohistochemical methods (9). While in the epidermis of the hairless skin of the hind footpad of the rat was found at a concentration of 5.49 μmol/g fresh tissue, the values in isolated stratum corneum were extremely low (<0.073 μmol/g in the horny layer of the same skin area).

Taurine has antioxidant effects on cell proliferation, inflammation and collagenogenesis. Taurine has also been used to prevent damage by oxidation in many tissues and on incisional skin wounds (10–12). Due to its antioxidant property, topical formulation containing taurine has been developed to improve wound healing on the skin after exposure to irradiation (13). An extraordinary number of patents of new topical formulations and shampoos for hair treatment containing taurine have been published in the recent years. However, no study about its permeation into the skin has been performed.

The aim of this study was to analyze the release profile of taurine using a multilayer membrane model (MLMM), which was developed by Neubert *et al.* (14–17), and to quantify the taurine content in isolated human skin layers before (native taurine content) and after the application of a standard formulation containing 1% taurine followed by incubation in the Franz diffusion cell (FD-C) (18).

MATERIAL AND METHODS

Materials

Taurine and fluorescamine were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol, ether, and ethanol (HPLC grade) were obtained from J.T. Baker (Deventer, Netherlands). Beta-cyclodextrin was purchased from Fluka (Buchs, Switzerland). Collodium (4%) and amphiphilic cream (AC) were obtained from Caelo (Hilden, Germany), and glycerol from KMF (Lohmar, Germany).

Semisolid Standard Formulation

A weighted amount of taurine was carefully added to the amphiphilic cream (AC according to the DAC—German Drug Codex (19)) and gently stirred to assure the total dissolution of taurine in the AC (the resulting concentration was 1% taurine). The taurine stability in the AC was tested over at least 12 months. No change in the taurine content was observed. The compounds of the cream are shown in Table I.

Table I. Ingredients of the Amphiphilic Cream (AC) used in the Experiments

Ingredients of the formulation (g)	
Glycerol monostearate 60	4.0
Cetyl alcohol	6.0
Medium-chain triglyceride	7.5
White vaseline	25.5
Macrogol 20 glycerol monostearate	7.0
Propylene glycol	10.0
Water	40.0

Release Studies—Multilayer Membrane Model (MLMM)

The membrane preparation was performed as follows: to a mixture of 96 g absolute ethanol and ether (1.5:8.5) 8 g glycerol were added and then followed by collodium until a total weight of 200 g. The resulting mixture was placed on a glass surface of a film-forming apparatus. The membrane formed was dried for 12 h at room temperature and cut into disks of 4 cm diameter (corresponding to the base plate on the model system, see Fig. 1). The glycerol membranes (thickness 2 μm) were used immediately after drying.

The model apparatus, as shown in Fig. 1, consists of polyacrylate (Piacryl®, Piesteritz, Germany) cells. Six cells, each containing four membranes used as acceptor, were fitted together and placed in a chamber maintained at 32±0.5°C during the experimental period. The AC (10 mg containing 1% taurine) was applied to an exposed membrane area (4 cm^2, see Fig. 1). At selected time intervals (60, 120 and 300 min) the model apparatus was removed from the thermostating chamber. After removal, the release cells were separated and the amount of applied formulation remaining on the first acceptor layer was removed. Taurine was extracted from the separated membranes with 2 ml of bidistilled water by shaking for 20 min. The solution was filtered through a 0.45 μm nylon filter (Roth, Karlsruhe, Germany), and then analysed by CE.

Penetration Studies in the Skin—Franz Type Diffusion Cell (FD-C)

Skins from the breast were obtained from healthy women aged between 21 and 45 years during plastic surgery. Upon receipt, the fresh tissue samples were cut into pieces using a standard cutter of 2 cm diameter (Biopsy punch Stiefel®, Offenbach am Main, Germany). After cleaning with 0.9% sodium chloride solution and removal of the subcutaneous fat, the skin samples were stored at −40°C until use (7 days). Before the experiments the skin samples were thawed and placed onto filter gauze in the diffusion cells. The dermal side of the skin was in contact with the acceptor solution (20 ml of a physiological solution—NaCl, pH 7.4) which was stirred continuously. The AC containing 1% taurine was used as donor. A defined amount of the formulation (20 mg) was applied onto the skin surface (3.14 cm^2). After incubation for 1 or 5 h at 37±1°C, the remaining formulation was wiped by a cotton wool tip. The skin was removed and rinsed with bidistilled water. Three punch biopsies (each 0.2827 cm^2) were excided from each skin sample and cut in horizontal sections using a cryomicrotome

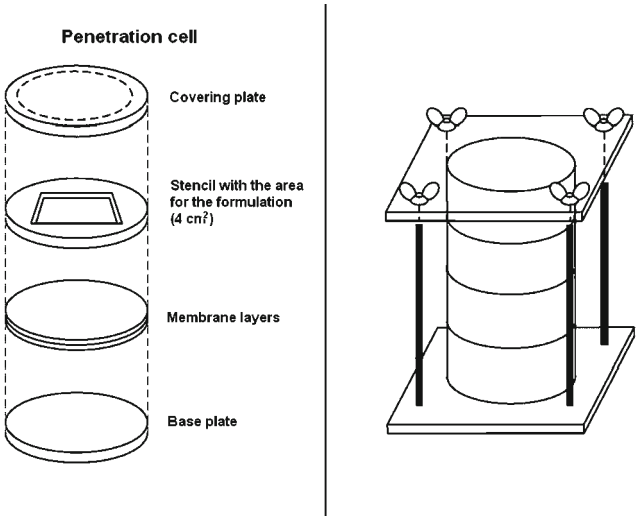

Fig. 1. Multilayer membrane model used.

(Jung, Heidelberg, Germany) as described in Table II. Several sections were pooled to one sample to guarantee the detection of small taurine amounts in the skin. The collected cuts were placed in Eppendorf tubes, carefully weighted and extracted with definite amounts of a solution of methanol/water (1:1, *v/v*) for 1 h. Afterwards the supernatant was centrifuged at 7,000×*g* for 10 min (Labnet, Woodbridge, USA). The taurine amount in the different skin layers, in the cotton wool tips and in the acceptor solution was analysed by capillary electrophoresis. The taurine concentration was expressed as μg of taurine per gram skin layer.

Analytical Method

The samples were analysed using capillary electrophoresis apparatus (Clinical Systems Division, Models Prince, Crystal 300 Series Part Number, Emmen, The Netherlands) equipped with a fluorescence detector (Model ARGOS 25OB, Basel, Switzerland). A capillary (CS Chromatography Service, Langerwehe, Germany) with an internal diameter of 75 μm, a total length of 120 cm, and 92 cm until detector was employed. The software, Borwin® version 1.21.07 (JMBS Developpements; Grenoble, France), was used for acquisition of the data. The samples were derivatized as follows: a 10 μl aliquot of sample was transferred to a tube containing a mixture of 150 μl of acetonitrile, 90 μl water, and 10 μl borate buffer (pH 10, 50 mM). After 20 μl fluorescamine solution (5 g/l in acetonitrile) was added and vigorously agitated for 1 min. The resulting mixture was analyzed by CE. This method satisfies the necessary requirements with respect to sensibility for taurine with a detection limit of 7.5 pM.

Table II. Number of Sections and Thickness of Skin Samples after Penetration Experiments

Skin sample	Number of sections and thickness
Stratum corneum	1 section (10 μm)
Viable epidermis	4 sections (20 μm each)
Dermis 1 (DR1)	5 sections (40 μm each)
Dermis 2 (DR2)	5 sections (40 μm each)
Dermis 3 (DR3)	5 sections (40 μm each)
Dermis 4 (DR4)	5 sections (40 μm each)
Dermis 5 (DR5)	5 sections (40 μm each)
Remaining corium[a]	

[a] Not included in the calculations.

Standard Addition

Calibration curves of taurine in standard solution (borate buffer) and in skin layer extract were performed and compared. The taurine quantification in aqueous solution (50 mM borate buffer, pH 10) yielded parallel results to those obtained by interpolation on the calibration curve of the skin extract. This allows the use of a calibration curve constructed in aqueous medium for the determination of taurine in this biological sample.

Statistics

All data are presented as arithmetic mean values± standard error (SE). Significant differences were analysed using Student's *t*-tests, $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

The MLMM model was performed for simulating the permeation of taurine in the skin. This *in vitro* model consists of membranes with collodion as a matrix acceptor and can be adapted to a broad range of experimental conditions by variation of the content and type of the membrane lipid and by addition of hydrophilic substances. In our studies glycerol was used as acceptor medium due to the highly hydrophilic property of taurine, which is diffusing through the stratum corneum via the polar route. The *in vitro* release of taurine from the AC was monitored for 60, 120 and 300 min, as shown in Fig. 2. It was determined that approximately 45% of the applied taurine penetrated within 60 min, of which 17.63± 2.17% was found in the first membrane. No significant difference ($p > 0.05$) was found for longer times. This result indicates that taurine is diffusing very fast via solvent drag diffusion dissolved in the aqueous solvents (water and

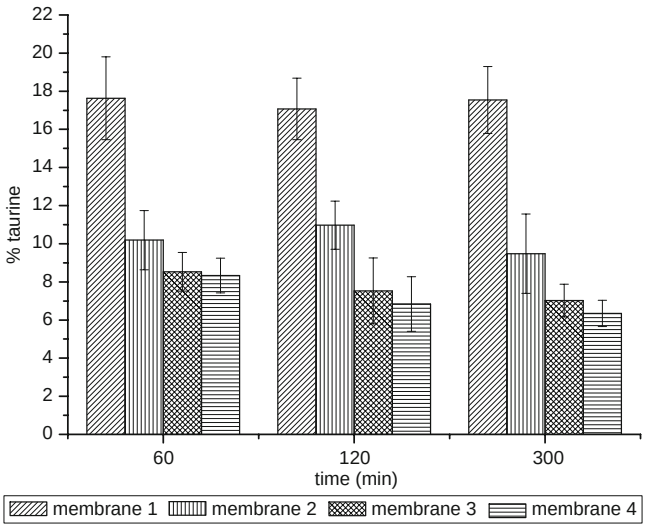

Fig. 2. Penetration of taurine from a standard vehicle (AC) containing 1% of taurine into a four-layer membrane system.

propylene glycol) of the AC as described by Bendas *et al.* (20) when the drug (e.g. taurine) is highly soluble in the solvent and when the solvent penetration is fast (e.g. propylene glycol, PG). The passive movement of taurine dissolved in the solvents occurs across the hydrophilic pores (glycerol) of the membranes. According to the solvent drag diffusion mechanism the solvent flux is coupled to the flux of taurine that also penetrates through this channel.

The solvent drag effect of PG can also be applied to a real skin. Routinely PG is used to solubilize lipophilic drugs in aqueous vehicles (20). Taurine is highly soluble in water (104.8 g/l, at 25°C) and shows a good solubility in PG (7.44 g/l, at 25°C), which correspond to 40 and 10% of the formulation (AC), respectively. For polar drug like taurine the stratum corneum barrier is formidable and completely rate limiting (21), and its penetration in the skin is reached by the stratum corneum uptake of PG. A non-specific mechanism, i.e. the solvent drag effect is thought to participate in skin permeation enhancement of PG (22,23).

Taurine distribution into human skin before and after the topical administration of the AC containing 1% taurine for 1 and 5 h of incubation was performed by horizontal slicing of the skin and its quantification was determined by capillary electrophoresis. The amount of the penetrated and the native taurine (expressed as µg of taurine/g skin layer) in different skin layers is demonstrated in Fig. 3.

A significant increase ($p<0.05$) of taurine content in all skin layers was observed after the FD-C experiment. No statistic difference ($p>0.05$) of taurine content in the skin layers was found for the incubation times studied (1 and 5 h). Approximately 27% taurine was released from the semisolid standard formulation. Moreover; it can be observed a similar and specific distribution profile of taurine in skin layers for the different times studied. As expected, the highest taurine concentration (555.56±19.9 µg/g skin layer, after 5 h incubation) was found in the epidermis, due to the presence of TAUT specially in this layer and cells such as keratinocytes, which are considered a depot of taurine in the skin (6). Lower

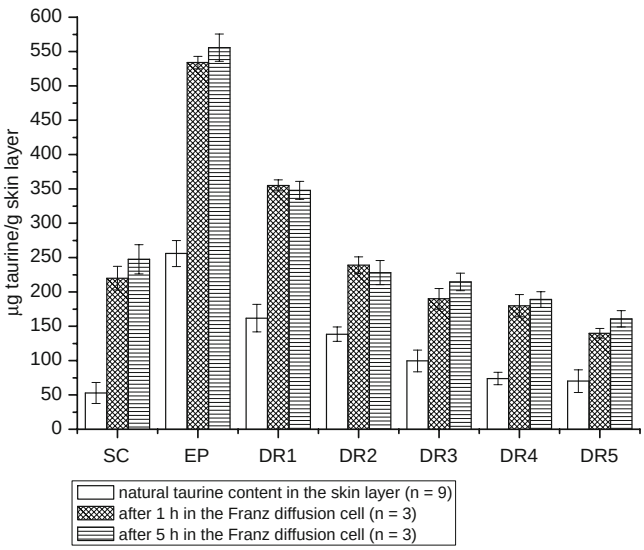

Fig. 3. Distribution of penetrated taurine from a semi solid standard vehicle (AC) containing 1% taurine in comparison to the distribution of natural taurine into human skin.

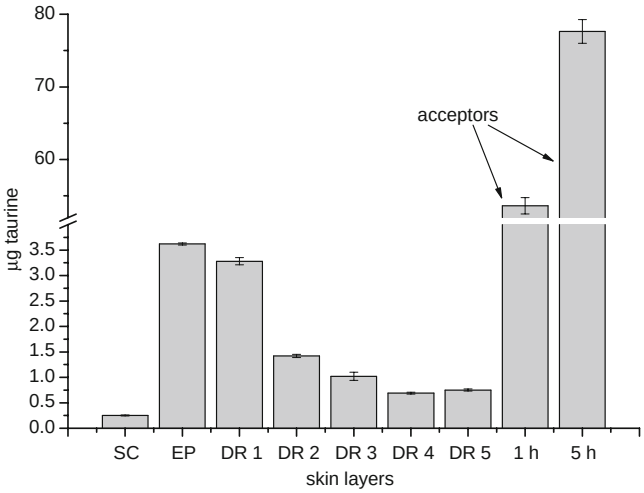

Fig. 4. Absolute taurine content (µg) in each skin layer and acceptor solution after 1 and 5 h using the Franz diffusion cell.

taurine contents were found in the SC, which derives directly from taurine-containing granular cells, and in the deeper layers of the dermis (DR2, DR3, DR4 and DR5). Absolute contents (expressed in µg) of taurine not only in the skin layers but also in the acceptor solution (buffer) are demonstrated in Fig. 4.

Although no significant difference has been detected on the taurine content in the skin layers for the times studied (1 and 5 h), the taurine content increased significantly in the acceptor solution (from 53.62 µg taurine at 1 h to 77.62 µg taurine at 5 h), see Fig. 4. These results suggest that taurine can effectively reach deeper skin layers.

The FD-C is a model which is already established for dermal penetration studies. The acceptor medium may help to simulate the blood flow by maintaining sink conditions in the skin during the whole experimental time. The success of this practice depends on the solubility of the drug in the acceptor medium. At the same time, there will always be an interaction between the acceptor medium and the skin, which may affect its barrier properties. Decisive for the drug penetration using the FD-C is only the solubility in the different skin layers. The metabolism and the taurine transport in the bloodstream, which is of importance concerning the results of the experiment, could influence the taurine penetration. Under *in vivo* conditions there is a high tissue clearance by the bloodstream. Using the FD-C model, this clearance might be simulated by the buffer (acceptor solution).

Once the buffer substances penetrate into the dermis, a change of the solubility of the penetrating drug can take place. A change in the buffer capacity of the skin and its pH can also occur. Depending on the drug properties, its solubility could increase and changes in the distribution balance may take place as a result of the dissociation of the drug molecules. Wagner *et al.* (24) measured the thickness of the skin before and after the end of the experiments. The data indicated that in the FD-C experiments a water uptake occurs at up to 3 h. The skin is fully hydrated after 3 h, which is in accordance with the very slow penetration rate at the short incubation times (hindrance by the water), but the penetration rate increases with longer incubation times

(diffusion without any counter current). In relation to taurine, its distribution was not influenced by the incubation time or skin hydration. Its accumulation in the skin did not seem to depend on a simple diffusion process. In fact, there is a specific distribution pattern of taurine in the skin.

CONCLUSIONS

This study shows that taurine is not evenly distributed into the human skin layers, accumulating specially in the viable epidermis before and after administration of a semisolid standard formulation using the Franz diffusion cell. The taurine content in the skin layer was significantly increased after administration of the AC containing 1% of taurine; however its distribution profile was maintained. This suggests that taurine has a specific uptake in the different skin cells. The incubation times studied (1 and 5 h) seem to have no effect on the taurine accumulation in the different skin layers. The release of taurine from a semisolid standard formulation was studied using a four-layer membrane model and the penetration into the human excised skin of taurine from this formulation using Franz diffusion cell was determined. The results obtained using both test systems did not seem to be influenced by long exposure time. Using both *in vitro* systems a very fast penetration both into the artificial membrane system of the MLMM and into the skin was observed.

REFERENCES

1. A. Albert. Quantitative studies of the avidity of naturally occurring substances for trace metal. I. Amino acids having only two ionizing groups. *Biochem. J*. **47**:531–535 (1950).
2. J. D. Madura, J. B. Lombardini, J. M. Briggs, D. L. Minor, and A. Wierzbicki. Physical and structural properties of taurine and taurine analogues. *Amino Acids*. **13**:131–139 (1997).
3. R. J. Huxtable. Physiological actions of taurine. *Physiol. Rev*. **72**:101–163 (1992).
4. B. Anderheggen, C. Jassoy, M. Waldmann-Lane, T. Forste, A. Wadler, and T. Doering. Taurine improves epidermal barrier properties stressed by surfactants—a role for osmolytes in barrier homeostasis. *Int. J. Cosmet. Sci*. **28**:386–387 (2006).
5. H. Watanabe, M. Watanabe, N. Jo, K. Kiyokane, and M. Shimada. Distribution of [1,2-3H]taurine in the skin of adult and newborn mice studied by microradioautography. *Cell. Mol. Biol*. **41**:49–55 (1995).
6. G. Janeke, W. Siefken, S. Carstensen, G. Springmann, O. Bleck, H. Steinhart, P. Höger, K. P. Wittern, H. Wenck, F. Stäb, G. Sauermann, V. Schreiner, and T. Doering. Role of taurine accumulation in keratinocyte hydration. *J. Invest. Dermatol*. **121**:354–361 (2003).
7. F. Grafe, W. Wohlrab, R. H. Neubert, and M. Brandsch. Functional characterization of sodium- and chloride-dependent taurine transport in human keratinocytes. *Eur. J. Pharm. Biopharm*. **57**:337–341 (2004).
8. C. Collin, B. Gautier, O. Gaillard, P. Hallegot, S. Chabane, P. Bastien, M. Peyron, M. Bouleau, S. Thibaut, F. Pruche, A. Duranton, and B. A. Bernard. Protective effects of taurine on human hair follicle grown *in vitro*. *Int. J. Cosmet. Sci*. **28**:289–298 (2006).
9. M. V. T. Lobo, F. J. M. Alonso, A. Latorre, and R. M. Rio. Taurine levels and localization in the stratified squamous epithelia. *Histochem. Cell Biol*. **115**:341–347 (2001).
10. H. Högstrom. Mechanism and prevention of decrease in wound margin strength. *Acta Chir. Scand*. **539**:5–63 (1987).
11. S. T. Bergren, J. H. Bodzin, and J. A. Cortes. Improved survival using oxygen free radical scavengers in the presence of ischemic bowel anastomosis. *Am. Surg*. **54**:333–336 (1988).
12. S. Dinçer, A. Babül, D. Erdoğan, C. Özoğul, and S. L. Dinçer. Effect of taurine on wound healing. *Amino Acids*. **10**:59–71 (1996).
13. Z. Değim, N. Çelebi, H. Sayan, A. Babül, D. Erdoğan, and G. Take. An investigation on skin wound healing in mice with a taurine–chitosan gel formulation. *Amino Acids*. **22**:187–198 (2002).
14. R. H. H. Neubert, and W. Wohlrab. *In vitro* methods for the biopharmaceutical evaluation of topical formulations. *Acta Pharm. Technol*. **36**:197–206 (1990).
15. R. H. H. Neubert, C. Bendas, W. Wohlrab, B. Gienau, and W. Fürst. A multilayer membrane system for modelling drug penetration into skin. *Int. J. Pharm*. **75**:89–94 (1991).
16. R. H. H. Neubert, K. Winter, and B. Bendas. Bestimmung der Arzneistoffverfügbarkeit aus kommerziellen topischen Zubereitungen mit dem Mehrschichtmembranmodell. *Pharmazie*. **48**:54–56 (1993).
17. R. H. H. Neubert, W. Wohlrab, and C. Bendas. Modelling of drug penetration into human skin using a multulayer membrane system. *Skin Pharmacol*. **8**:119–129 (1995).
18. T. J. Franz. Percutaneous absorption. On the relevance of *in vitro* data. *J. Invest. Dermatol*. **64**:190–195 (1975).
19. Neues Rezeptur-Formularium (NRF). Bundesvereinigung Deutscher Apoteker Verbände. Govi, Eschborn, 2005.
20. B. Bendas, U. Schmalfuß, and R. H. H. Neubert. Influence of propylene glycol as cosolvent on mechanisms of drug transport from hydrogels. *Int. J. Pharm*. **116**:19–30 (1995).
21. I. P. Dick, and R. C. Scott. Pig ear skin as an *in-vitro* model for human skin permeability. *J. Pharm. Pharmacol*. **44**:640–645 (1992).
22. K. Moser, K. Kriwet, A. Naik, Y. N. Kalia, and R. H. Guy. Passive skin penetration enhancement and its quantification *in vitro*. *Eur. J. Pharm. Biopharm*. **52**:103–112 (2001).
23. I. M. Schneider, B. Dobner, R. Neubert, and W. Wohlrab. Evaluation of drug penetration into human skin *ex vivo* using branched fatty acids and propylene glycol. *Int. J. Pharm*. **145**:187–196 (1996).
24. H. Wagner, K. H. Kostka, C. M. Lehr, and U. F. Schaefer. Drug distribution in human skin using two different *in vitro* test systems: comparison with *in vivo* data. *Pharm. Res*. **17**:1475–1481 (2000).