

# PHILIPPS - UNIVERSITÄT MARBURG

## Institut für Pharmazeutische Technologie und Biopharmazie



Prof. Dr. Alfred Fahr

Institut für Pharmazeutische Technologie und Biopharmazie  
Philipps-Universität Marburg, D-35032 Marburg (Deutschland)

Hausadresse (Paketanschrift)

**Ketzerbach 63  
D-35037 Marburg**

Telefon Sekretariat: 06421 / 28 25881  
Telefon Durchwahl: 06421 / 28 25884  
Telefax: 06421 / 870 391  
e-mail: [fahr@mail.uni-marburg.de](mailto:fahr@mail.uni-marburg.de)

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### **Investigation on the efficacy of a new device for substance deposition into deeper layers of the skin: Dermaroller®**

D.D. Verma and A. Fahr

Institut für Pharmazeutische Technologie und Biopharmazie,  
Philipps-Universität Marburg

#### **Summary**

The action of different types of Dermarollers® on skin penetration enhancement was investigated with a lipophilic (retinol) and a hydrophilic (flufenamic acid) model compound. The model compounds were administrated in a liposomal formulation on the surface of skin pieces, which were pre-treated with the appropriate Dermaroller. Drug penetration was investigated across human skin using a standard Franz diffusion cell. Six hours later the *stratum corneum* was removed layer-wise by tape-stripping. Drug amount was measured in the stripped layers by HPLC. Deeper skin was cut into thin slices using a cryotome; these slices were also analyzed for model compound content.

Three types of dermarollers were tested in this study. Dermaroller M8-1,5-15° showed the highest enrichment of the lipophilic compound retinol into the deeper skin layers by a factor of 40.72 (4072 % increase) compared to skin penetration without application of the dermaroller. This increase in penetration will be even higher, as the dermaroller will be usually applied in practice after application

of the drug formulation, whereas in this study the formulations had to be applied due to standardization reasons after the treatment with the dermaroller.

Similar arguments are valid for the penetration enhancement effect by the dermaroller for the hydrophilic compound flufenamic acid. Here we could observe with our standardized protocol a penetration increase of 240%. For this compound we made a pilot experiment by applying the dermaroller after the administration of the formulation. Under these circumstances we could find an increase in penetration into deeper skin layers by 1238%.

All investigated dermarollers caused also an increased deposition of model compounds into the *stratum corneum*.

## **Introduction**

Different physical and chemical methods are described for the deposition of active agents into the skin. Skin deposition of compounds by means of chemical penetration enhancers is very often strongly dependent on the structure of the active agent to be deposited, whilst smallest chemical variations of the active agent can already change the drug penetration behaviour drastically.

Physical methods permit at least a similar deposition in certain classes of active agents. Charged or ionisable active agents can be deposited into the skin by iontophoresis. However, this method is not very common since additionally the necessary expensive device also the dose delivered is difficult to maintain constant.

Ultrasound, well-known from the diagnostic field, is occasionally used for drug deposition. However, in this treatment a high ultrasound energy must be used (unlike in the cases for diagnostics uses), which under certain circumstances not only allows the topmost skin layer to become penetratable but also exerts tissue disturbing forces to deeper layers of the skin. The dose adjustment is problematic also here.

A reversible micro-perforation of the skin which puts smallest pores in the topmost skin barrier (*stratum corneum*) could allow a controllable deposition of substances at reproducible applicability.

The study described here examines the deposition enhancement of new skin micro-perforation devices (Dermaroller) for the two greatest classes of substances for the treatment of skin (lipophilic and hydrophilic ones). In addition, the reproducibility of the Dermaroller application was also tested.

For this purpose, human skin was treated with dermarollers, the formulation containing a model compound was administrated and the deposition of the drug in the different skin layers was estimated using acknowledged methods (Franz diffusion cell, HPLC).

Retinol as lipophilic active agent was used since this active agent is often used in skin treatment as vitamin A and a very sensitive, precise and reproducible HPLC method for the determination of the concentration in the skin could be developed.

Flufenamic acid, a non-steroidal anti-inflammatory drug was used as hydrophilic model active agent. For the analytics a reverse phase HPLC method on basis of an isocratic mobile phase had to be developed.

## **Material and methods**

### ***Materials***

All-trans retinol was bought from Sigma (Sigma-Aldrich Chemistry GmbH, Steinheim, Germany). Lipid for producing liposomes was obtained from Natterman Phospholipid GmbH (Cologne, Germany). HPLC degree methanol was obtained from Riedel de Haen (Seelze, Germany), the used water was demineralised by means of a Milli-Q plant (Millipore, Darmstadt, Germany). Flufenamic acid was donated by Lindopharm GmbH (Hilden, Germany). Trifluor acetic acid was bought by Merck (Darmstadt, Germany). All other chemicals were of the highest available purity degree (Merck, Darmstadt, Germany). The mini-extruder (Liposofast) for the production of liposomes was purchased from Avestin (Ottawa, Canada).

### ***Equipment***

Three different Dermarollers were delivered from Horst Liebl ETS (67860 Friesenheim, France):

a) model C 8 0,13-15°, for the deposition of dermatics and cosmetic active agents beyond the *stratum corneum*. This dermaroller has 192 needles with a length of  $0.13 \pm 0.02$  mm, 24 needles per row with a lateral distance of 2.5 mm in eight rows.

B) model M 8 1,5-15° dermaroller for deposition of dermatics and cosmetic active agents into deeper skin layers of approx. 1 mm: 192 needles with a length of  $1.5 \pm 0.02$  mm, 24 needles per row with a lateral distance of 2.5 mm in eight rows.

C) model M 8 1,5-30° for deposition of dermatics and cosmetic active agents in deeper skin layers of 1.5 mm: 96 needles with a length of  $1.5 \pm 0.02$  mm, 12 needles per row with a lateral distance of 2.5 mm in eight rows.

All Dermarollers have a diameter of 20 mm and a width of 21.5 mm. They run on an axis which is connected to a guiding fork. The forks are screwed together with a handle.

The Franz diffusion cells (max. 6 at the same time operated) had a nominal surface of 3.14 cm<sup>2</sup> and a receiver compartment of 12 mL volume. The Franz cells were kept at 37° ± 1° by means of water bath (Julabo, Germany).

The cryotome cuts were carried out with a Vogel Cryotome AS 620 (Anglia-Scientific, U.K.).

## **Methods**

### *Determination of retinol in the skin*

The used HPLC system consisted of a Merck-Hitachi HPLC 655 A-12 pump, a Kontron 360 auto sampler with a 20 µL-loop and a Merck Hitachi L-5000 LC controlling device. As column a Waters Xterra RP 18 (4.6 mm L.D. and 250 mm of length) was used, which contained spherically formed 5 µm silica gel. As detection system a Merck-Hitachi L4000 UV absorption detector was used at 322 nm wave length and as integrator a Merck Hitachi D 7500 was used. The mobile phase used for elution was methanol:water (95:5). The flow rate was 1.3 ml/min, the column was operated at ambient temperature. The pressure was kept between 117-119 bar. The retention time for retinol was 4.9 min in our system.

### *Determination of flufenamic acid in the skin*

The same equipment as for retinol determination was used, except the HPLC column, which was in these experiments a ET 250/3 Nucleosil 100-5 C18 (Macherey Nagel, Düren, Germany). As a mobile phase 0.1% v/v TFA in Acetonitril: 0.085% v/v in water (55:45) was used. The flow-rate was held at 0.7 ml/min, the pressure was kept between 180 and 182 bar and monitoring was done at 288 nm. The retention time of the compound was 11.1 ± 0.2 min.

### *Liposome production and size measuring*

Multilamellar vesicles were produced by means of a conventional method (see New, 1990). These multilamellar vesicles were extruded through polycarbonate membrane pores with 50 nm diameter by means of the Avestin-Miniextrusion device in order to get liposomes of the desired size. The diameter of the liposomes was determined by a Zetasizer IV instrument (Malvern Instrument, Herrenberg, Germany).

### *Formulations*

Both formulations contained a liposomal concentration of 100 mg/mL lipid. The Flufenamic acid formulation contained 0.5% flufenamic acid, the retinol liposomal formulation 2% retinol.

## **Skin**

Excised human skin from female patients, who had undergone abdominal plastic surgery, was used. Immediately after excision the subcutaneous fatty tissue was removed using a scalpel. The skin was wrapped in aluminum foil and stored in polyethylene bags at  $-25\text{ }^{\circ}\text{C}$  until use. For penetration experiments, skin disks of 35 mm in diameter were punched out, thawed, cleaned with cotton, which was soaked with ringer solution, and transferred on to the Franz diffusion cell. In this study skin of one donor was used for all the experiments in order to keep the variability as small as possible.

### *Determination of the thickness of the skin and the stratum corneum*

For determination of the thickness of the *stratum corneum* the tapes were accurately weighed before and after stripping, the resulting layer thickness being calculated according to the following equation (Michel et al 1992):

$$T = d / ap$$

where T represents the thickness of *stratum corneum* removed (in  $\mu\text{m}$ ), d is the difference in strip weight after and before stripping (in  $\mu\text{g}$ ), a denotes the area of the strip ( $\mu\text{m}^2$ ) and p is the density of the *stratum corneum* ( $1 \times 10^3 \mu\text{g}/1 \times 10^9 \mu\text{m}^3$ ).

### *Dosage regime and incubation times*

The dose applied was 20  $\mu\text{l}$  of loaded liposomes per  $\text{cm}^2$  of skin surface non-occlusively. The drug preparation was applied to the skin for 6 hrs on Franz diffusion cells.

### *Skin perforation by dermarollers*

The skin was taken out from the deep freezer, it was kept at room temperature for 5 minutes. The surface of the skin was washed with ringer solution. The dermaroller was then rolled 10 times with applying pressure with the hand with different sites. The skin was then placed on Franz diffusion cell and incubated for 3 hrs before applying the liposomal formulation.

### *Franz diffusion cell*

On the Franz diffusion cell (Gauer Glas, Püttlingen, Germany), the skin sections were mounted with nominal surface areas of  $3.14 \text{ cm}^2$  and receiver compartments with 12 mL capacities. The epidermal side of the skin was exposed to ambient conditions while the dermal side was bathed by a phosphate

buffer saline pH 7.4, in the case of flufenamic acid. The receptor fluid was mixed with a magnetic stirring bar at 500 rpm. Buffer was kept at  $37 \pm 1$  °C by a water jacket controlled by a water bath. Care was exercised to remove any bubbles between the under surface of the skin and the solution in the receiver compartment. To achieve higher reproducibility, the skin was pre-hydrated with the basolateral receptor medium for 3 hrs before applying the drug. The liposomal formulation was applied onto the skin surface area of  $3.14 \text{ cm}^2$  by the help of a micro-syringe. A minimum of three diffusion cells was used for each formulation. All experiments were carried out with non-occluded donor compartments. After 6 hrs the experiments were stopped and the diffusion set-up was dismantled. For retinol experiment the whole diffusion cell was covered by aluminum foil to avoid possible degradation of retinol. The receptor solution in the case of retinol was a mixture of phosphate buffer saline and ethanol (20:80) due to the solubility and stability of retinol in receptor compartment.

#### *Stripping of the skin*

After incubation for predetermined time period, the liposomal formulation was removed by wiping the skin with cotton. The skin was then transferred into a special apparatus where it was mounted on cork discs using small pins. The stretching of the skin, going along with that, helped to overcome problems of furrow in the subsequent tape-stripping procedure. For this procedure the surface of the skin was covered with a Teflon mask with a central hole of 15 mm in diameter. Proceeding from this hole the skin was stripped with 20 pieces of adhesive tape (size = 15 x 20 mm; Tesa<sup>®</sup>, Beiersdorf, Hamburg, Germany). The tape was of sufficient size to cover the full area of the skin which was in contact with the formulation. In a standardized procedure each tape was charged with a weight of 2 kg for 10 seconds and after that period quickly removed.

After the tape stripping, the skin was rapidly frozen by liquid nitrogen, and a specimen with a diameter of 13 mm was taken out of the stripped area and frozen on a metal bloc. The metal bloc was transferred into a cryomicrotome. The skin was cut into surface parallel sections and collected according to the following scheme: # 1 = first cut; # 2-5 = 6 x 20  $\mu\text{m}$  sections; # 6-9 = 12 x 20  $\mu\text{m}$  sections; # 10 = rest of the residual tissue.

#### *Extraction of retinol from human skin*

The extraction solvent used was a mixture of n-butanol and ethyl acetate (1:1). The retinol was extracted from the adhesive tape and the skin cuts with 2 ml extraction solvent. The skin samples were sonicated for 2 min followed by shaking at room temperature for 4 hrs. The samples were then centrifuged at 5000 rpm for 30 minutes and the supernatant was assayed directly with an HPLC-procedure. Care was taken to avoid exposure of retinol to light by wrapping all glass ware with aluminum foil.

### *Extraction of flufenamic acid from human skin*

The flufenamic acid was extracted from the adhesive tape and the skin cuts with 2 ml 0.05 N NaOH. After 2 hrs shaking at room temperature and centrifugation of the skin cuts at 5000 rpm for 30 minutes the supernatant was assayed directly with an HPLC-procedure.

## **Results and discussion**

### *Analytics*

The developed HPLC method for Flufenamic acid content is suitable for the quantitative determination of the flufenamic acid in liposomes and skin tissue. The calibration curve (not presented) had a linear behaviour in the concentration area of 0.035  $\mu$  g/mL, 5  $\mu$  g/mL with a correlation coefficient of 0.99987 at wavelength 288 nm. The method described here is quick, simple, selective and for routine analysis of penetration studies suitable in skin tests. Both extraction and HPLC methods show 100 % recovery as well as a great reliability what is particularly noticeable by the means of the column "complete amount found" in table 1 and 2. These values are not distinguishable from 100%.

### *Flufenamic acid deposition in the skin*

All the 3 dermarollers used in this study showed significant penetration enhancement in deeper layers of the skin (see Table 1).

The dermaroller M-8 1,5-30° exhibited the largest increase (237% in comparison to control), not showing an increased deposition into *stratum corneum*, which was not the intention of the inventor (Horst Liebl ETS, France, personal communication). In contrast, the dermaroller C-8 0,13-15° showed the highest enrichment of flufenamic acid in *stratum corneum* (162 %).

<b>Dermaroller</b>	<b>Amount on skin surface %</b>	<b>Amount in stratum corneum %</b>	<b>Amount in deeper skin layer %</b>	<b>Receiver-compartment %</b>	<b>Total amount recovered %</b>
<b>Control</b>	61,54 $\pm$ 5,21	38,94 $\pm$ 5,21	1,572 $\pm$ 0,286	0	102,01 $\pm$ 2,69
<b>C8-0.13-15°</b>	38,13 $\pm$ 10,06	63,16 $\pm$ 8,88	2,268 $\pm$ 0,094	0	103,56 $\pm$ 1,76
<b>M8-1,5-15°</b>	39,6 $\pm$ 2,95	58,12 $\pm$ 2,02	2,32 $\pm$ 0,367	0,799 $\pm$ 0,13	100,89 $\pm$ 0,78
<b>M8-1,5-30°</b>	63,41 $\pm$ 8,1	33,50 $\pm$ 8,08	3,69 $\pm$ 0,548	0,289 $\pm$ 0,051	100,89 $\pm$ 3,13

*Table 1: Amount of flufenamic acid from a liposomal formulation found in different skin layers after use of the dermaroller and the application of the formulation after 6 hr in the different skin layers (applied dose = 100%). The results are represented as mean average value  $\pm$  standard deviation of the mean average value (n = 3). Control: measurement done without dermaroller application.*

No significant amount of drug could to be detected in the receiver compartment (see Table 1). From

this finding it can be inferred that no significant amount will be found in the blood circulation system in vivo.

The skin depth profiles for flufenamic acid for the different treatments is depicted in Fig. 1.

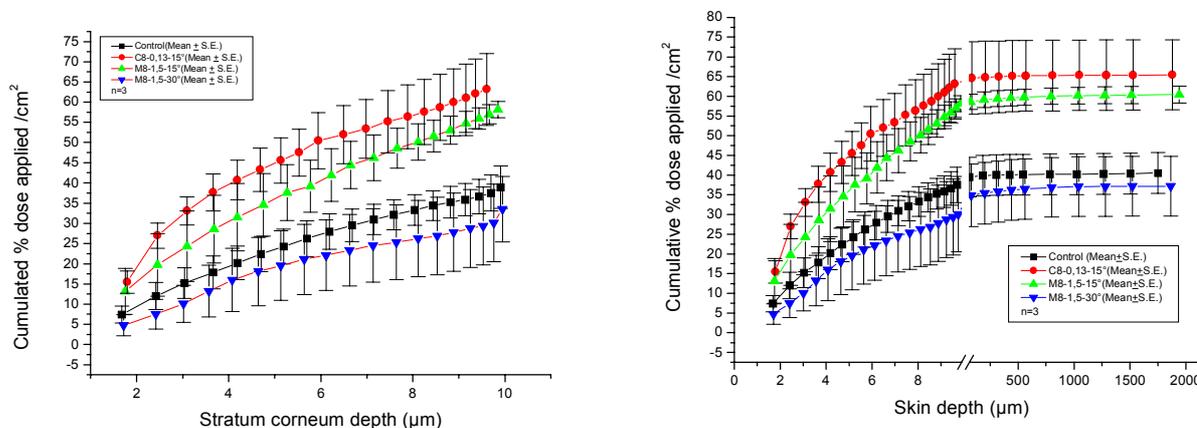


Fig. 1: Penetration of the active agent flufenamic acid in a liposomal formulation into the skin: Dependence of the skin depth. Left picture: Depth profile of the stratum corneum, right picture: Depth profile of the complete examined skin. Details see "material and methods".

Flufenamic acid deposition into the skin at conditions, were the application of the formulation was done prior to the treatment with the dermaroller.

A drastic increase of the deposition can be seen, if the formulation is applied before the treatment with the dermaroller took place (see Table 2). However, under this experimental conditions, there was no complete recovery of the applied dose. This can be explained at least in part by the fact, that part of the dose is incorporated in areas of the skin outside the diffusional area and was therefore not analysed.

Dermaroller	Found in Derma-roller %	Amount on skin surface %	Amount in stratum corneum %	Amount in deeper skin %	Receiver-compartment %	Total amount found %
Control (1)	-	61,54 ± 5,21	38,94 ± 5,21	1,572 ± 0,286	0	102,01 ± 2,69
M8-1,5-15°	37,385 ± 2,8	5,7 ± 0,24	3,31 ± 0,45	19,5 ± 4,0	0,45 ± 0,07	66,4 ± 6,3

Table 2: Amount of flufenamic acid from a liposomal formulation after administration on the skin and subsequent treatment by the dermaroller, which has been recovered from different parts of the skin after 6 hr of administration (applied dose = 100%). The results are represented as mean average value ± standard deviation of the mean average value (n = 2). (1) results for control from table 1.

The steep rise in the depth profile of the substance from *stratum corneum* to the deeper skin layers is here particularly interesting (Fig. 2). Obviously, the formulation is pushed by the dermaroller treatment to a greater share directly into the lower skin layers. The very small quantity found in the *stratum*

*corneum* is at variance to the results from the control group and may have its cause in the methodology which was changed. Unfortunately, this effect could not be examined further in the framework of the study described here.

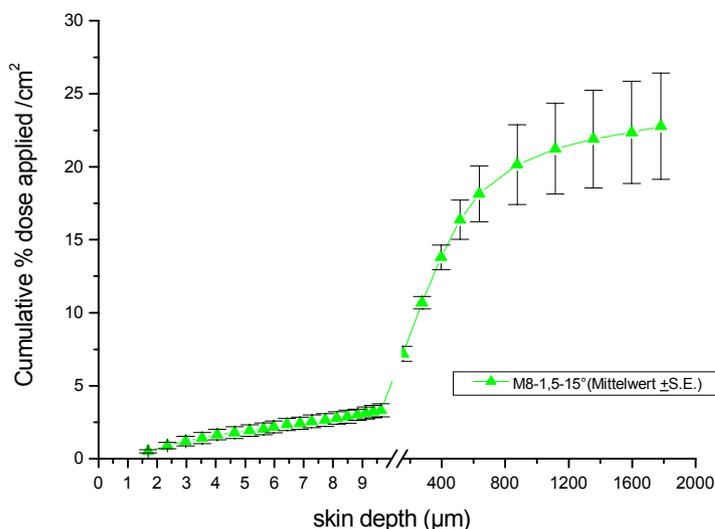


Fig 2: Penetration of the active agent flufenamic acid in a liposomal formulation into the skin: Dependence of the skin depth. Depth profile of the complete examined skin for the experimental situation “administration of the formulation before the treatment by dermaroller takes place”. Details see “material and methods” for details.

#### Retinol deposition in the skin

All the three dermarollers used in this study exerted a good penetration enhancement for retinol both in *stratum corneum* as well as in deeper layers of the skin as compared to control experiment for the lipophilic model drug retinol.

In the control group (i.e. without applying the dermaroller) 13.7 % of total applied drug was found in the *stratum corneum* and 0,291 % in deeper skin layers (see Table 3). No drug was detectable in receiver compartment of the Franz diffusion cells after 6 hrs of incubation. This showed that retinol has lower penetration than flufenamic acid under the given conditions.

Dermaroller	Amount on skin Surface %	Amount in stratum corneum %	Amount in deeper skin layers %	Receiver-compartment %	Total dose Recovered %
Control	81,40 ± 3,26	13,69 ± 0,152	0,291 ± 0,058	0	94,88 ± 1,82
C8-0.13-15°	69,55 ± 1,63	28,47 ± 2,61	3,330 ± 0,994	0,511 ± 0,04	101,59 ± 3,87
M8-1,5-15°	57,81 ± 1,63	32,62 ± 1,39	11,85 ± 0,984	1,792 ± 0,22	104,52 ± 2,87
M8-1,5-30°	68,64 ± 0,68	22,62 ± 0,48	7,491 ± 0,491	0,162 ± 0,017	100,38 ± 2,49

Table 3: Amount of retinol from a liposomal formulation, after application of the dermarollers, which was found 6 hr after administration in the different skin layers (applied dose = 100%). The results are given as mean average value ± standard deviation of the mean value (n = 3).

The effect of the micro-perforation by means of the Dermaroller M8 1,5-15°, were the increase of deposition into the deeper layers of the skin by 4070% is particularly impressive. Similar values have been observed for the Dermaroller M8 1,5-30°. Both dermarollers have been developed for the penetration of compound into deeper layers of the skin (Horst Liebl ETS, France, personal communication).

The skin depth profiles of retinol for the different treatments are shown in Fig. 3.

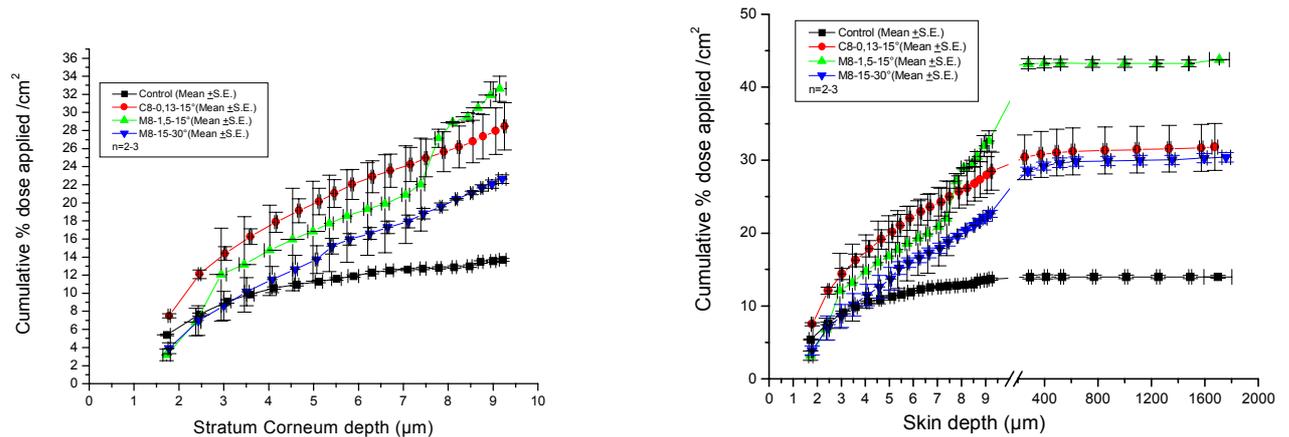


Fig. 3: Penetration of the active agent retinol in a liposomal formulation into skin: Dependence of the skin depth. Left figure: Depth profile of the stratum corneums, right figure: Depth profile of the complete examined skin. Details see "material and methods" for details.

The effect of the different dermarollers on penetration enhancement in summary (see Fig. 4 and 5):

The dermaroller C8 0,13-15° showed 1.62 times more drug in *stratum corneum* and 1.48 times more drug in deeper skin as compared to control experiment for the hydrophilic model compound. The values for the lipophilic compound are 2.078 (*stratum corneum*) and 11.34 (deeper skin layer), respectively. This dermaroller was obviously designed to improve the deposition of drug in *stratum corneum*. The 1.62 times higher deposition in *stratum corneum* as compared to control, indicated that the needle length was enough to make pores inside the *stratum corneum*, but was not able to make pores through the whole *stratum corneum* due to the length of the needles and the flexibility and lipids composition of *stratum corneum*. In the case of the lipophilic drug, retinol may penetrate deeper into the lipophilic environment, whereas the hydrophilic compound will remain at its deposition site.

The dermaroller M8-1,5-15°, which has the same number of needles but a much larger needle length (1,5 mm in comparison to 0,13 mm in the case of C8-0,13-15°), was designed to deliver the drug in deeper layers of the skin by perforating the whole *stratum corneum*. This roller showed a 1.49fold and 1.48times higher deposition in *stratum corneum* and in deeper skin, respectively, as compared to the

control experiment. The results from the pilot experiment, where this dermaroller was applied after deposition of the formulation on the skin surface, showed a much higher deposition of flufenamic acid (12.4fold higher compared to control). The lipophilic compound retinol is deposited into deeper layers of the skin by a factor of 40.7 and the increase of retinol deposition into the *stratum corneum* by a factor of 2.38 compared to the control. The deposition into the deeper skin layers using the dermaroller shows such a high increase, that under these conditions also a significant part of the compound can be monitored in the receiver compartment (1.8 % of total dose).

In the case of the dermaroller M8-1,5-30° possesses only half the number of needles as the other dermarollers. Because of the different rolling characteristics and due to the higher pressure of a single needle at similar pressure on the dermaroller, the penetration depth of a single needle will be higher. This notion is supported by the experimental findings: the drug deposition in *stratum corneum* was less as compared to the other derma roller as the number of needles are only half and hence also the half numbers holes in this case. However, the drug deposition in the deeper layer of the skin was at maximum of all the dermarollers (i.e. 2.35 times more as compared to the control experiment), because the complete perforation of the *stratum corneum* eases the drug diffusion through this barrier. The lipophilic compound retinol does not need this enhancer effect, as this compound can diffuse through the rest of this barrier after depositioning into the *stratum corneum*.

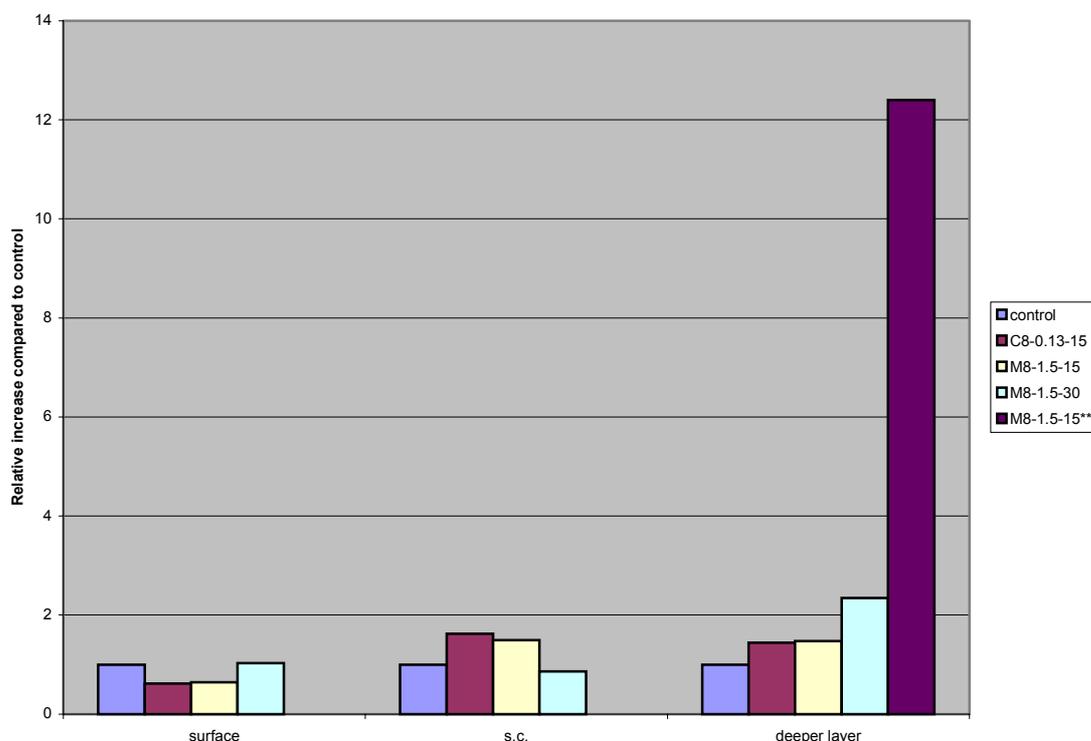


Fig. 4: Values from table 1 and 2 (hydrophilic active agent in different skin layers after 6 hrs administration) normalised on the control results (control = 1). Values from the receptor compartment have not been added, as they contain values of 0. \*\*: Values from the pilot study (application of dermaroller after administration of formulation).

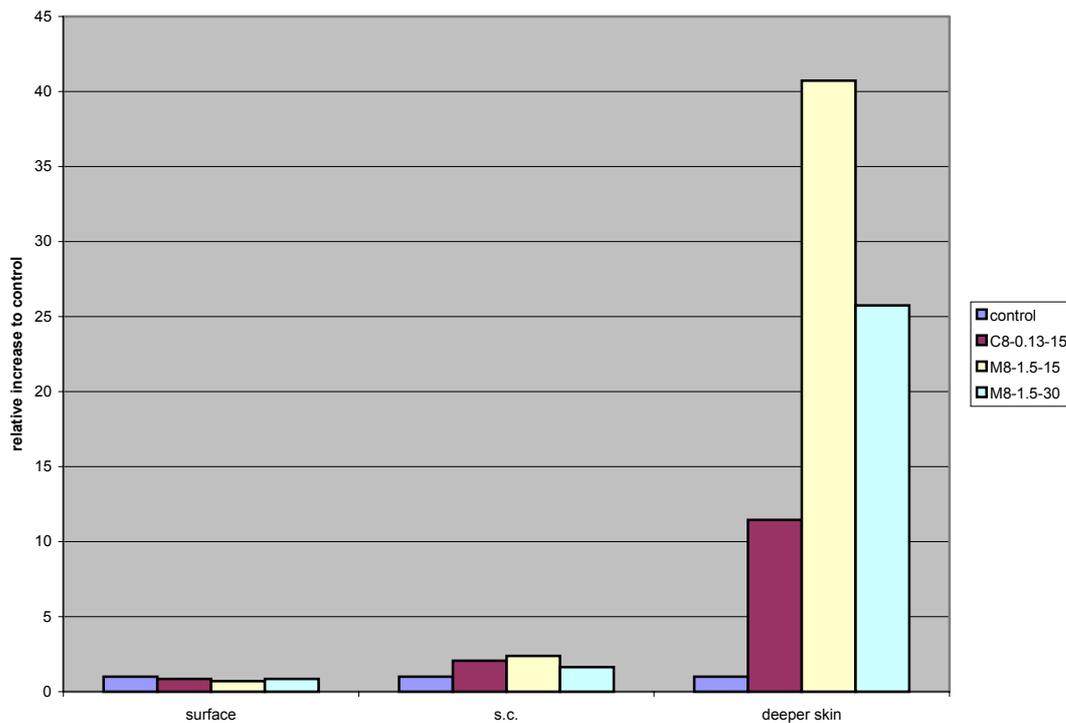


Fig. 5: Values from Table 3 (lipophilic active agent in different skin layers after 6 hrs administration) normalised on the control results (control = 1). Values from the receptor compartment have not been added, as they contain values of 0.

## Conclusions.

The examined Dermaroller are able to deposit hydrophilic and hydrophobic compounds into the *stratum corneum* and into deeper skin layers. This could be shown by the Franz diffusion cell model.

The scientific results support the intentions of the development protocols for the dermarollers. The Dermaroller C8-0,13-15° is particularly qualified to deposit substances into the *stratum corneum*, whereas the Dermaroller M8-1,5-15° promotes very well the deposition of hydrophilic and hydrophobic compounds into deeper layers of the skin. The third Dermaroller M8-1.5-30° showed the best results for depositing hydrophilic substances in deeper skin layers, if the dermaroller is used before the compound is applied. The presented results can be interpreted on physical ground by the geometry of the different dermarollers.

It is also worth mentioning, that the reproducibility of the application procedure is very good.

The enhanced deposition found with the model system (Franz diffusion cell) used here will be even higher in the practice, because for reproducibility and comparability reasons the following sequence was chosen in most of the experiments: “dermaroller application – administration of formulation on the skin”. In practical applications the sequence “administration of formulation – dermaroller application” will be chosen, which will *per se* deliver more substance to the different skin layers, as

could be shown in an exploratory study using a hydrophilic compound and the dermaroller M8-1,5-15°.

The examined dermarollers are pieces of equipment with a simple but effective operation, which shows surprisingly high deposition effects for both hydrophilic and lipophilic compounds into the *stratum corneum* and into deeper skin layers.

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