



## Ungual application



### **Ex vivo unguinal models for characterizing topical dosage forms indicated to be applied on nails during product development, drug approval procedures, and safety evaluations**

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#### **Introduction**

Part of fingers and toes the human nail is not only important in practical issues allowing to pick and manipulate objects, or to scratch and groom, it also has a protecting function for the delicate tips of the fingers and toes. Last but not least it is of great cosmetic interest.

According to Chaudhari et al., 2006, each nail consists of the following parts:

- 1) A body, the attached uncovering of the nail,
- 2) a free edge, the anterior unattached extension of the body,

- 3) the nail root, the posterior or proximal part of the nail lying beneath a fold of the skin.

Being highly keratinized the nail plate consists of tightly packed dead cells. Nail disorders can be various. In most cases their treatment is difficult and needs a long time. Systemic treatment using orally applied medications has the disadvantage of side effects and interactions (Murdan, 2002). Therefore topical treatment becomes more and more attractive, and the development of topical formulations is increasing. Our starting point are the test compounds' physicochemical properties, which are relevant for the penetration into and through human nails (Neubert et al., 2006). Additionally by developing topical formulations for being used on human nails it has to be taken into consideration that the infection is normally deep-seated.

Furthermore other factors e. g. the nail's thickness or its compact construction are limiting the penetration (Hui et al., 2004). Hence it is of great importance to test the compounds penetration properties during the formulation development. Common formulations are e. g. lacquers, creams, solutions or even gels, mostly containing antifungal or antibacterial active ingredients.

To assist you in developing your new product Across Barriers GmbH has established an ex vivo ungual model providing up-to-date services in the fields of ungual permeation and penetration. In the course of the experiment we use bovine hoof membranes as their structure equates to that of the human nail.

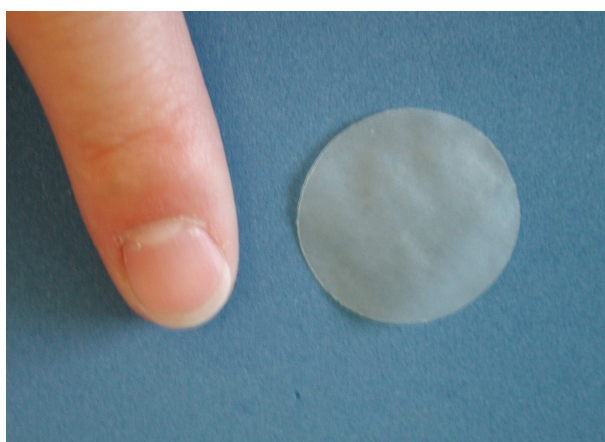


Fig. 1: Human finger nail and bovine nail plate

Our model is not only interesting for the pharmaceutical industry, it may also be used in developing cosmetics such as nail polish or remover. Additionally, by using our model we may e. g. prove your product's safety. Since animal testings are being more and more rejected in the cosmetics industry, testing with in vitro models features an acceptable alternative.

## Materials and Methods

### Materials

Across Barriers GmbH has established its bovine ungual in vitro model using the cylindrical glass Franz cell diffusion chamber. It is comprised of an upper and a lower part the bovine hoof membrane is clamped between. The two halves of the cell are held together by means of a ball-and-socket clamp. The diffusion area of nail in the Franz cell is approximately of  $1.77 \text{ cm}^2$ .

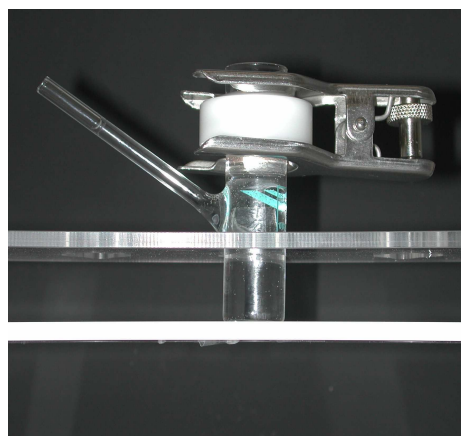


Fig. 2: Franz diffusion cell setup. Test formulation is applied in the donor compartment, the drug is released from it and diffuses through the inert membrane into the receiving medium.

Bovine hoof membranes are a commonly accepted model for human nails and in vitro testing (Monti et al., 2005). Compared to human nails, which can only be obtained from dead bodies, bovine plates being approximately  $100 \mu\text{m}$  thick are thinner as human ones, and therefore more permeable and less selective than human nails toward large permeants (Mertin and Lippold, 1997a, 1997b, 1997c).

### Permeability studies

The test products can be applied on the plates as finite dose (pharmacologic relevant concentration, limited amount of test compound) or infinite dose (unlimited amount of test compound, oversized donor, entailing that the amount of active agent has no influence on its transport velocity into the acceptor). During an incubation time of e. g. 24 hours samples are withdrawn at distinct time points. After completion of the incubation phase the nails are washed to obtain the amount of compound stayed on the nail's surface. To extract the compound from the nail, the membrane treated is cut into small fragments, and collected into a glass vial adding an extraction medium.

### Permeability calculation

The apparent permeability coefficient ( $P_{app}$ ) is calculated according to the following equation:

$$P_{app} = \frac{\Delta Q}{\Delta t} \cdot \frac{1}{m_0} \cdot \frac{1}{A} \cdot V_D \quad [\text{cm} \cdot \text{s}^{-1}]$$

$\Delta Q/\Delta t$  permeability rate (steady state transport rate) obtained from the profile of the transported amount of substrate versus time [ $\mu\text{g} \cdot \text{s}^{-1}$ ]. Calculated by the linear regression of time and concentration

A area of the exposed nail plate [ $\text{cm}^2$ ]

$m_0$  initial mass of test compound in the donor compartment [ $\mu\text{g}$ ]

$V_D$  donor volume [ $\text{cm}^3$ ]

## Results

Results from two different case studies are shown in the following figures.

### Case study 1:

In case study 1 the cumulative transport of one lipophilic compound from four different formulations was assessed. The  $P_{app}$  was calculated.

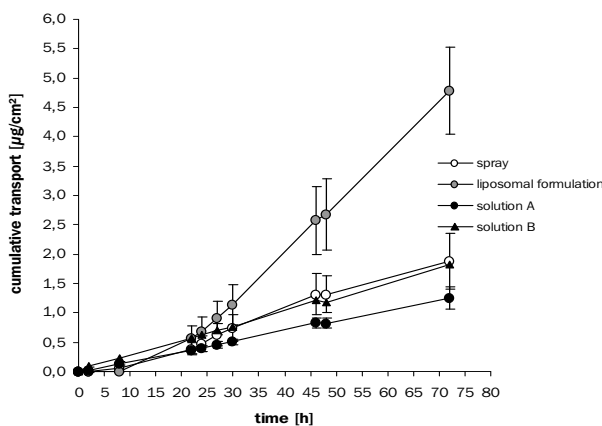


Fig. 3: Cumulative transport of a lipophilic compound from different formulations: spray, liposomal preparation and two solutions. The cumulative transport into the acceptor compartment is expressed in  $\mu\text{g}$  per  $\text{cm}^2$  of nail plate area.

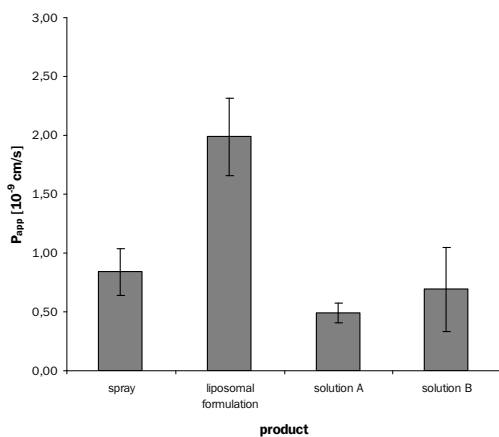


Fig. 4: Apparent permeability coefficients for the transport of a lipophilic compound from the different test formulations: spray, liposomal preparation and two solutions.

Differences concerning transport rates and lag phases have been demonstrated for the four formulations tested: The lag phases for the spray and liposomal formulation were at least ten times longer than for the solutions. After 30 hours of incubation the greatest amount of the test compound permeated through the bovine nail plates from the liposomal preparation. The transported amount from spray and solutions was in the same

range but at least two times lower in comparison to the liposomal preparation.

### Case study 2:

In case study 2 the transport of one lipophilic compound from three different recipes of nail lacquers was investigated. The  $P_{app}$  was calculated.

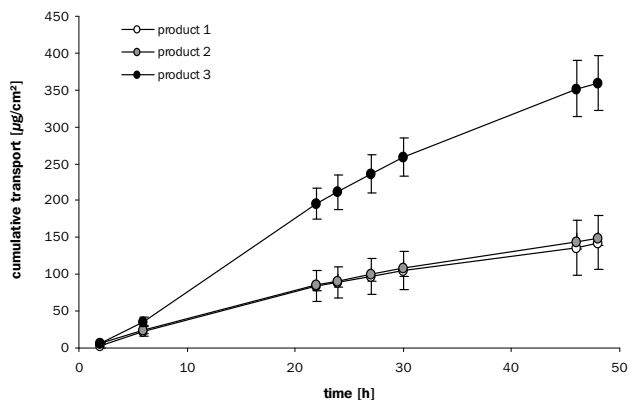


Fig. 5: Transport of one compound from three test formulations through nail plates. The values shown are arithmetic mean values from experiments performed ( $n=6$ )  $\pm$  SD. The cumulative transport into the acceptor compartment is expressed in  $\mu\text{g}$  per  $\text{cm}^2$  of nail plate area.

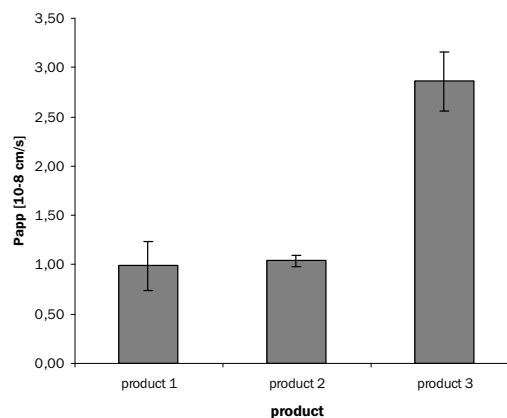


Fig. 6: Apparent permeability coefficients for the transport of one compound from three test formulations through nail plates. The values shown are arithmetic mean values from experiments performed ( $n=6$ )  $\pm$  SD.

In the beginning (after 6 hours of incubation) the transport rates of the test compound have still been in a comparable range with all test lacquers. But after an incubation time of 48 hours, only two of the nail lacquers tested lead to comparable transport rates and  $P_{app}$  values, one product showing a higher cumulative transport and a higher  $P_{app}$  value. In this case study no lag phase was observed.

## Conclusion

Both case studies showed that our in vitro ungual permeation model has the ability to characterize the transport of test compounds from different formulations.

## Services of Across Barriers GmbH - when to use our unguinal model

With our knowledge and experience we are your competent partner regarding all absorption questions during the life cycle of your product.

Our unguinal in vitro model is used to investigate and characterize the unguinal permeability of pharmaceutical relevant compounds:

- Is your antifungal or antibacterial compound low permeable or high permeable?
- Is your compound systemically or only locally available? Does it reach the deep-seated infection?

Our model can be used for formulation optimization:

- You are developing a new nail lacquer? Choose our model to compare your different formulations or excipients.
- Use different excipients to see the influence on the permeability of your compound and decide which one to have the best effect (Walters et al., 1985a and b).
- Optimize your formulation by using penetration enhancer (Khengar et al., 2007; Brown et al., 2009).
- What kind of formulation do you want to develop? Compare your topical formulation to the originator products.
- How your compound has to be applied? Test single and multiple dosing using our model.

You are active in the cosmetics industry? Our model may also be used to characterize nail polish or remover.

- Prove your product being secure.
- Show that your product ingredients do not penetrate the nail or that they are systemically available either.

- Show that the nail is not affected by your product.
- Investigate drying or film forming properties of your product.

## Choosing the Across Barriers GmbH unguinal in vitro model you minimize time consuming and expensive in vivo studies.

Across Barriers GmbH does not only provide the in vitro model, we also design the optimized in vitro study including preliminary tests and analytics perfectly adjusted according to your needs. Therefore preliminary characterizations of your compound in terms of physicochemical properties, such as solubility or stability parameters, and also your compound's quantification via e. g. LC-UV or LC-MS or scintillation counting in the samples generated are part of the service package Across Barriers GmbH is providing you.

## Let Across Barriers GmbH be your competent partner during the life cycle management of your API and product.

## References

- Brown MB, Khengar RH, Turner RB, Forbes B, Traynor MJ, Evans CR, Jones SA. Overcoming the nail barrier: A systematic investigation of unguinal chemical penetration enhancement. *Int J Pharm.* 2009; 370(1-2): 61-7.
- Hui X, Wester RC, Barbadillo S, Lee C, Patel B, Wortzman M, Gans EH, Maibach HI. Ciclopirox delivery into the human nail plate. *J Pharm Sci.* 2004; 93(10): 2545-8.
- Khengar RH, Jones SA, Turner RB, Forbes B, Brown MB. Nail swelling as a pre-formulation screen for the selection and optimisation of unguinal penetration enhancers. *Pharm Res.* 2007; 24(12): 2207-12.
- Chaudhari PD, Chaudhari SP, Kolsure PK, Bothiraja C. Drug Delivery Through Nail - A Review. <http://www.pharmainfo.net/reviews/drug-delivery-through-nail-review/>; 2006.
- Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: influence of the partition coefficient octanol/water and the water solubility of drugs on their permeability and maximum flux. *J Pharm Pharmacol.* 49(1), 30-34.
- Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: penetration of chloramphenicol from lipophilic vehicles and a nail lacquer. *J Pharm Pharmacol.* 49(3), 241-245.
- Mertin D, Lippold BC. In-vitro permeability of the human nail and of a keratin membrane from bovine hooves: prediction of the penetration rate of antimicrobics through the nail plate and their efficacy. *J Pharm Pharmacol.* 49(9), 866-872.
- Monti D, Saccomani L, Chetoni P, Burgalassi S, Saettoni MF, Mailland F. In vitro transungual permeation of ciclopirox from a hydroxypropyl chitosan-based, water-soluble nail lacquer. *Drug Dev Ind Pharm.* 2005; 31(1): 11-7.
- Murdan S. Drug delivery to the nail following topical application. *Int. J. Pharm.* 2002, 236, 1-26.
- Neubert RH, Gensbügel C, Jäckel A, Wartewig S. Different physicochemical properties of antimyco-tic agents are relevant for penetration into and through human nails. *Pharmazie.* 2006; 61(7): 604-7.
- Walters KA, Flynn GL, Marvel JR. Physicochemical characterization of the human nail: solvent effects on the permeation of homologous alcohols. *J Pharm Pharmacol.* 1985; 37(11): 771-5.
- Walters KA, Flynn GL, Marvel JR. Penetration of the human nail plate: the effects of vehicle pH on the permeation of miconazole. *J Pharm Pharmacol.* 1985; 37(7): 498-9.