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Nail drug delivery using different formulations and different model systems

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6. Comparison of the penetration data
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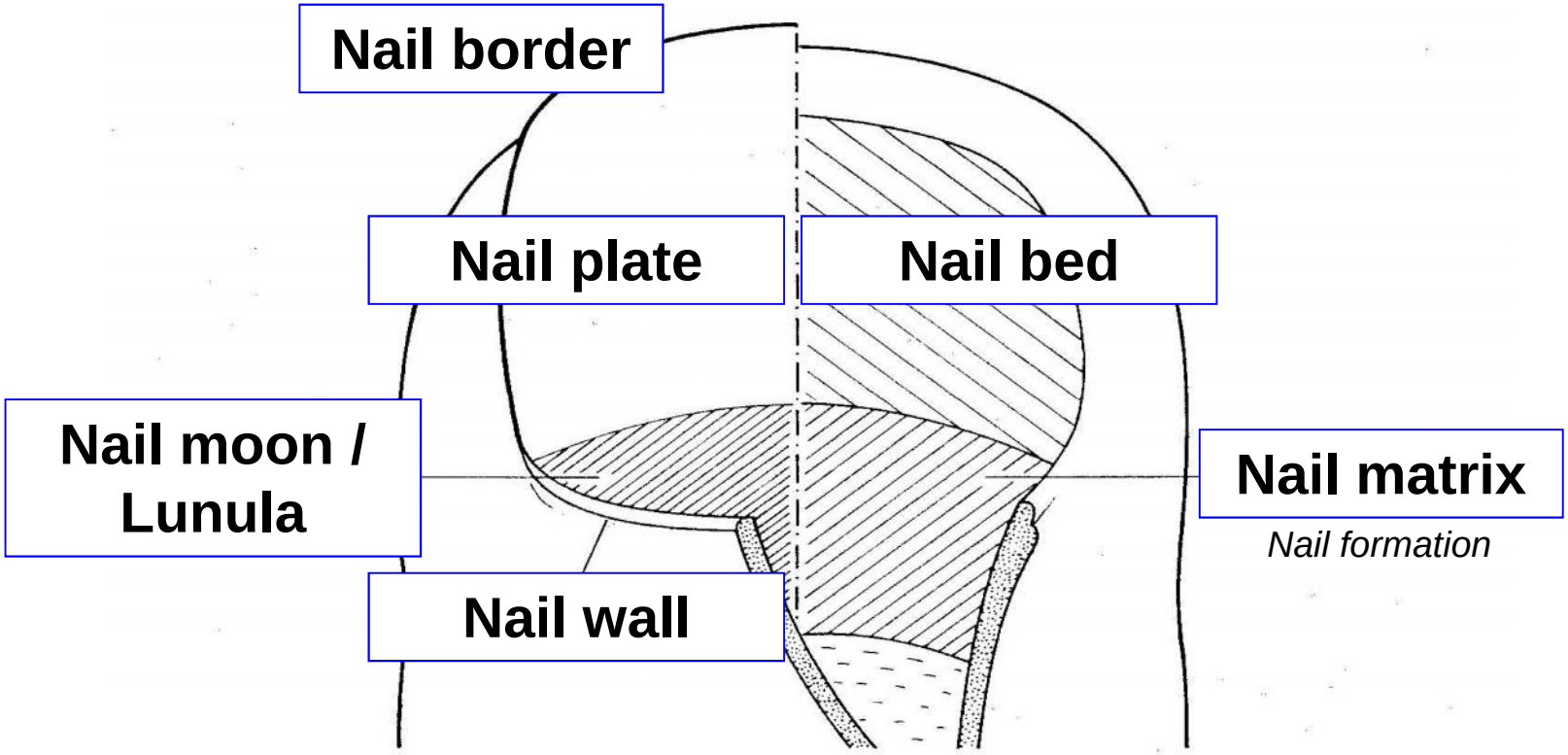
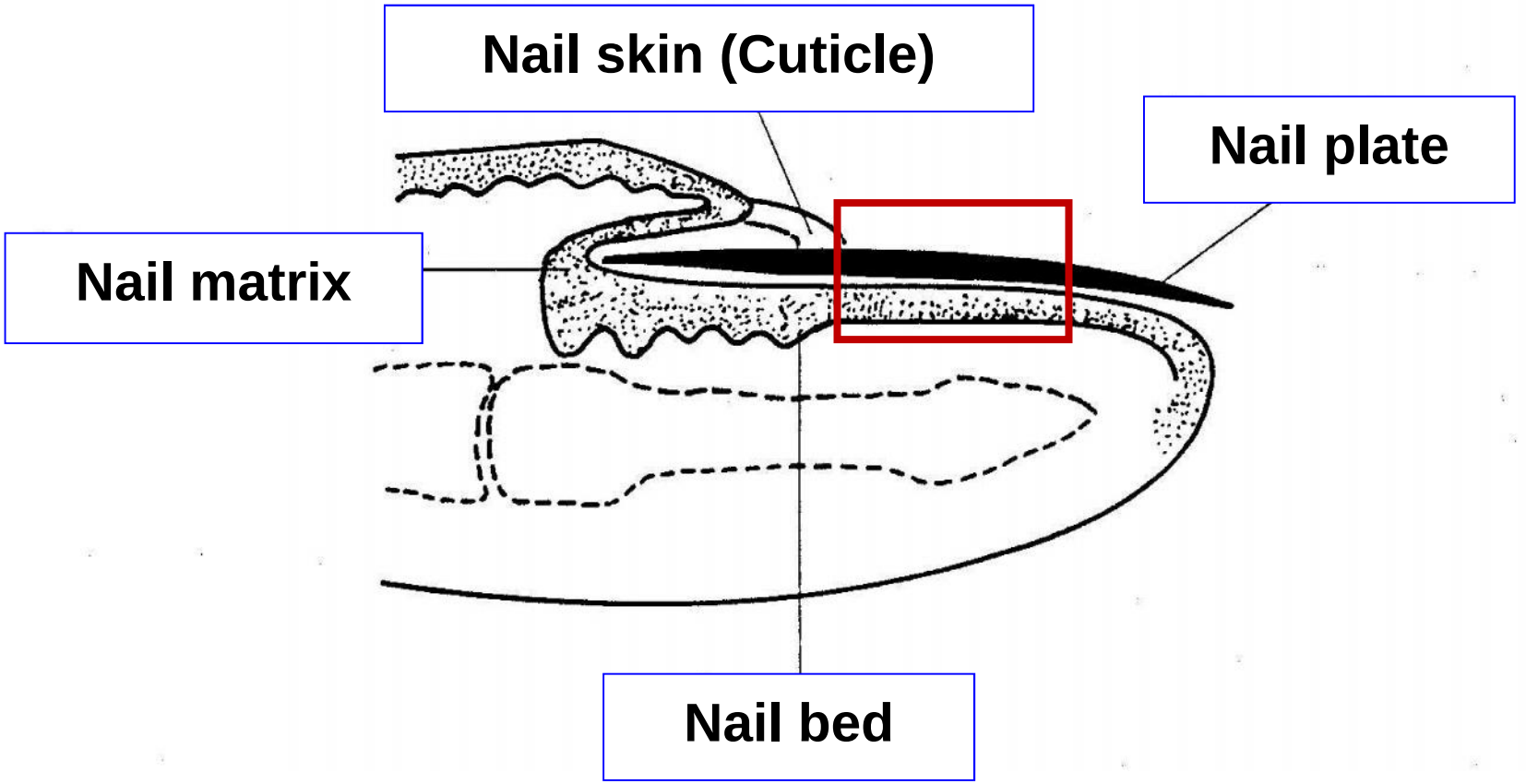
Introduction

Anatomy nail (top view)



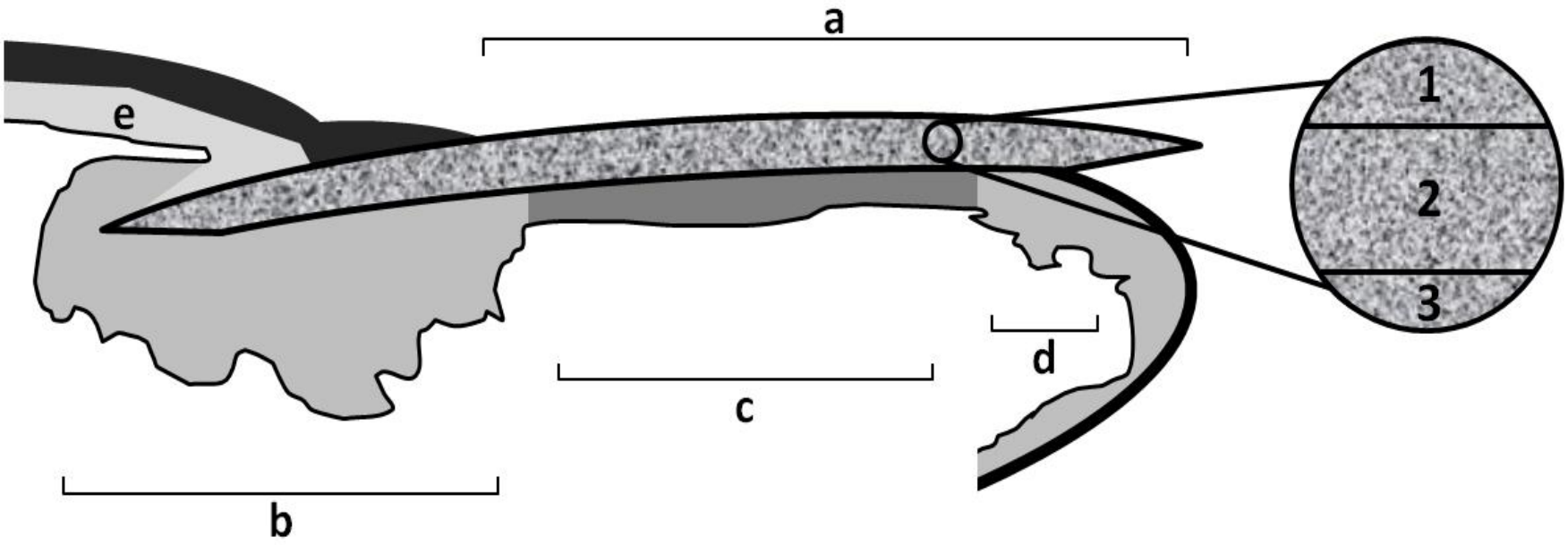
Introduction

Nail Anatomy (lateral view)



Introduction

Nail Anatomy (lateral)



a = nail plate (1 = dorsal, 2 = intermediate, 3 = ventral nail plate)
b = nail matrix, c = nail bed, d = hyponychium, e = proximal nail fold

Physiologic conditions of the nails

Properties	SC	Nail
Thickness	10-40 µm	50 – 1000µm
Disulfide linkages	1.2%	10.6%
Lipid content	**10-20%**	**0.1-1%**
Water content	**1-2% (up to 5%)**	**10-20% (up to 25%)**
TEWL/TOWL	1.17-3.35 mg/cm²/h	1.3-1.9 mg/cm²/h
Behaviour	Lipophilic partition membrane	Concentrated hydrogel

Physiologic conditions of the nails

Nails: Hydrophilic gel membrane

- **Thermal movement of the keratine fibers create wholes (pores) which would used as pathway for drugs as diffusion way**
  → small molecules diffuses faster compared to larger ones

- **Molecule size much smaller than pore size – convection and diffusion**

Aqueous pathway dominant

- **For extremely lipophilic substances: lipophilic pathway is discussed but special formulations are necessary**

Onychomycosis

- Most relevant desease of nails: **Onychomycosis**
- Most relevant topical formulations: **Lacquers**
- **Scientific defiencies:**
  - Studies according to drug liberation from lacquers and drug penetration/diffusion into the nails.
- **Problems:**
  - Model systems are needed to optimise drug liberation and penetration !

Onychomycosis

- **Onychomycosis** = chronic fungal infection of the nail apparatus, particularly the nail plate and the nail bed

- European Achilles foot screening project (n = 13486):
  Prevalence in the population **8 to 23 %**

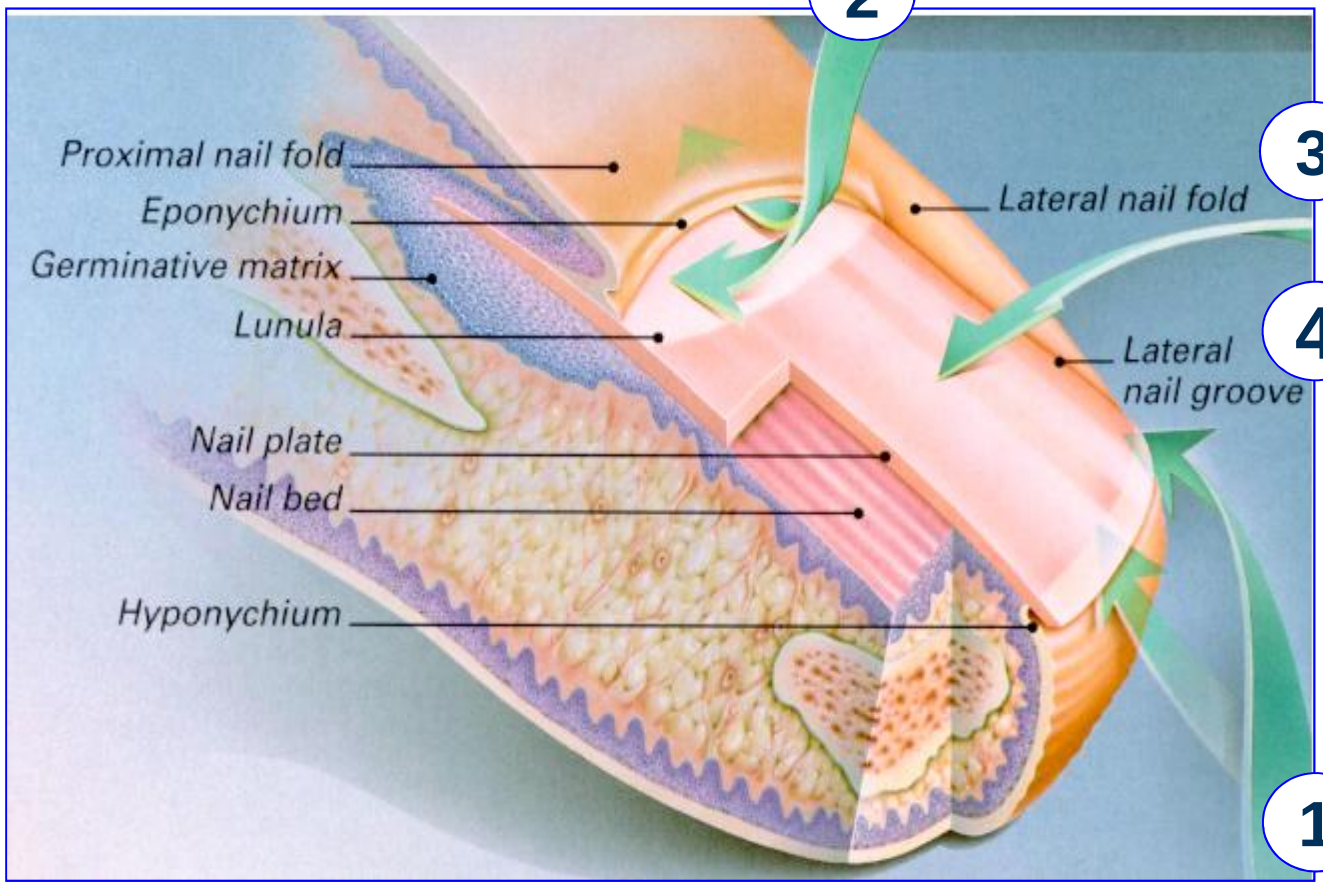
- **Problems:**
  - Long term treatment
  - High recurrence rate
  - Low patient compliance

- **Standard therapy:**
  - Terbinafine systemically
  - Amorolfine and ciclopirox topically (lacquers)

Onychomycosis

Proximal subungual Onychomycosis

(Matrix involved)



3 **Surface Onycho-mycosis**

4 **Endonyx**

Distal and/or lateral subungual Onychomycosis ***(most frequently)*** **1**

Diffusion/Penetration set up

- **Online FTIR-ATR diffusion cell:**
  - ***Bovine hoof***
  - ***Equine hoof***
  - sliced into sections (approx. 60 – 150 µm)
- **Modified Franz diffusion cell:**
  - ***Human cadaver nail***
  - slicing of the nail plate using microtome

FRANZ & FTIR-ATR diffusion cell

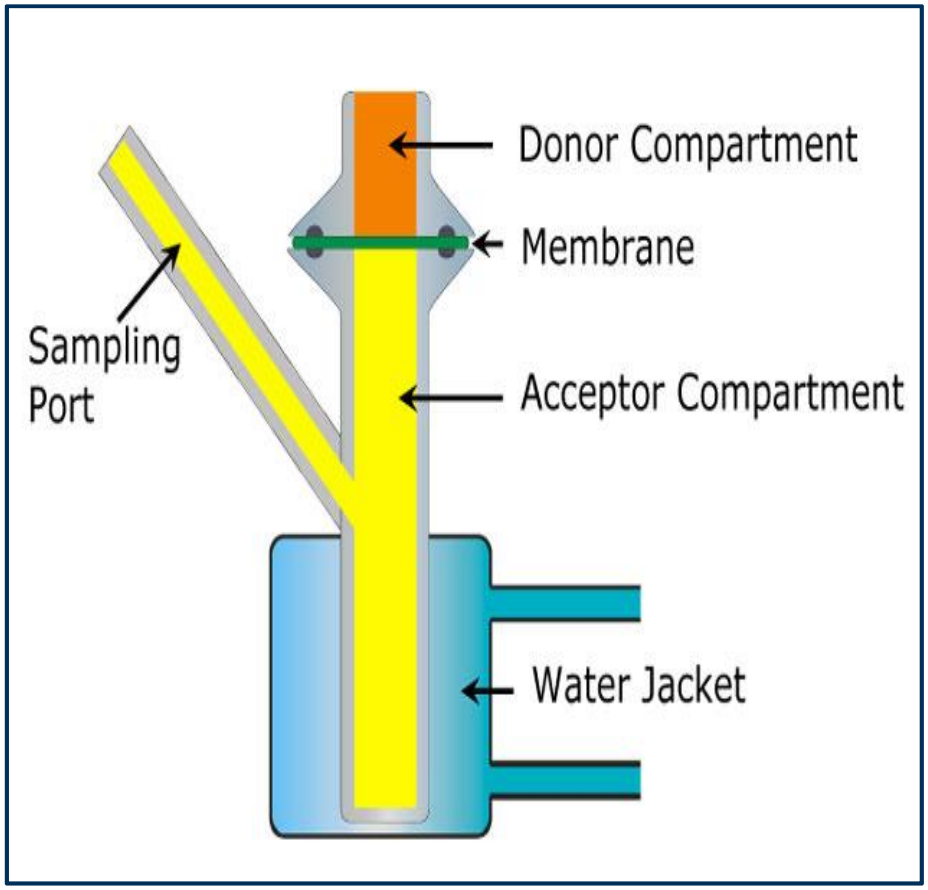
Franz diffusion cell:

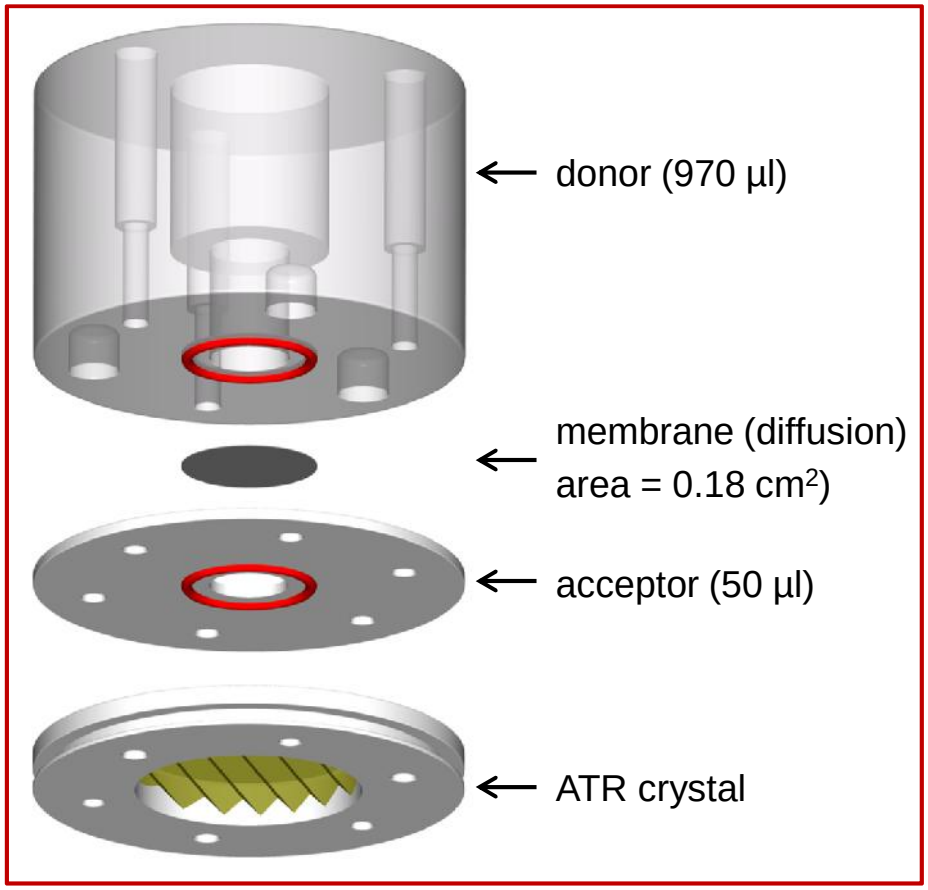
- human skin ex vivo

FTIR-ATR diffusion cell:

- for online measurements

SN2



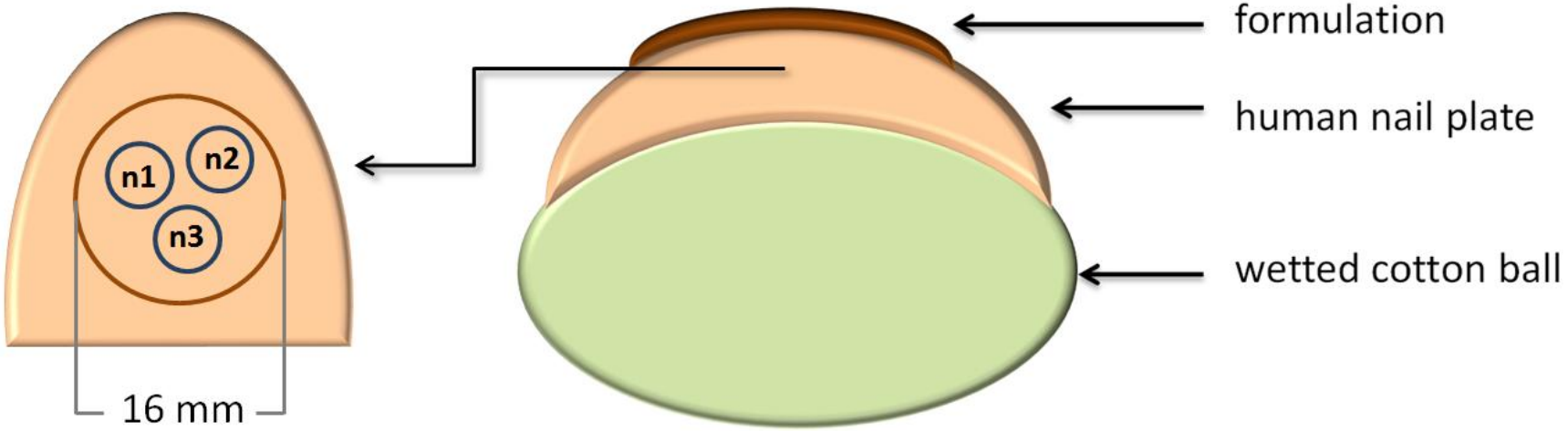


SN2 Ich hatte eine Diffusionsfläche von 0.0962 cm² ausgerechnet...der Durchmesser der Donatoröffnung bzw. des Akzeptors beträgt 3,5 mm
Sandy Naumann; 26.03.2014

Diffusion/Penetration set up

Modified Franz diffusion cell:

- ***Human cadaver nail***
- Slicing of the nail plate using microtome



Diffusion/Penetration set up

Modified Franz diffusion cell:

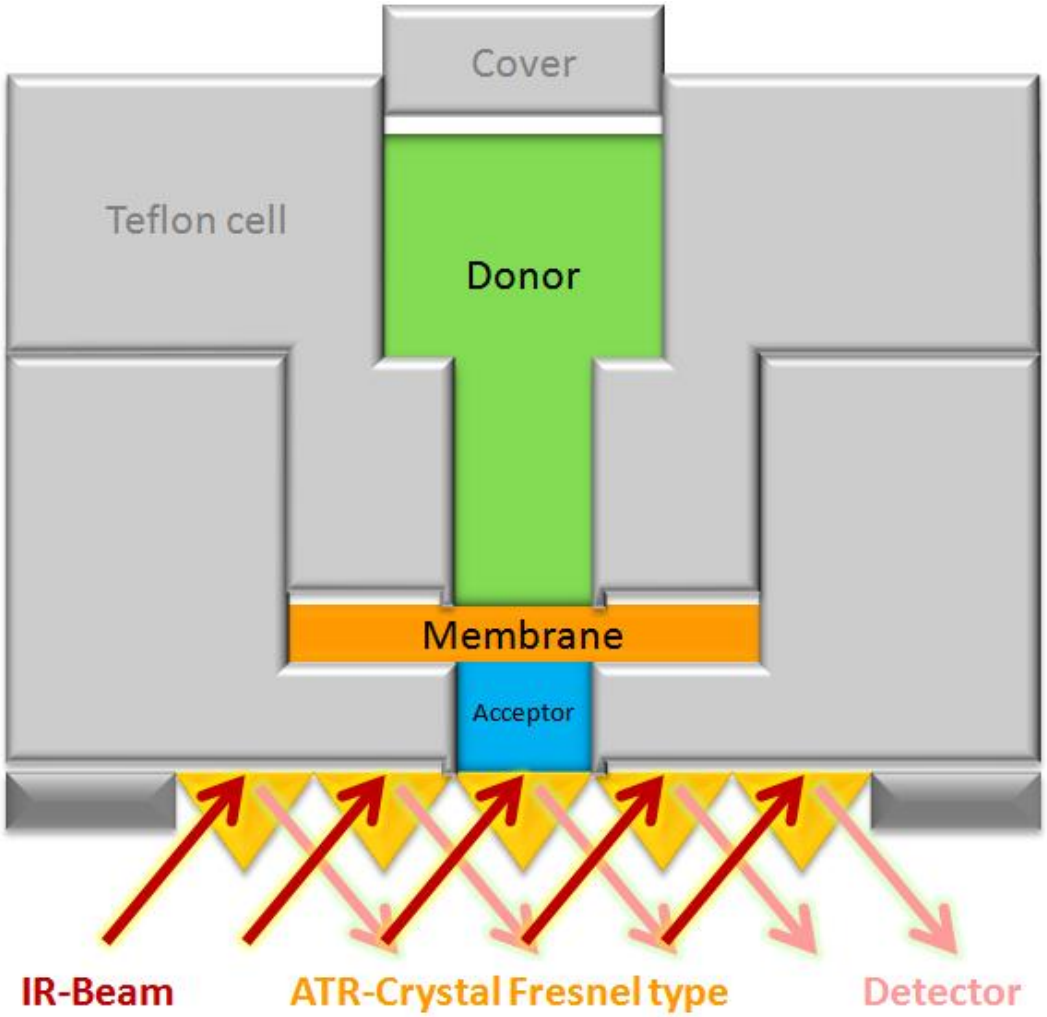
- ***Human cadaver nail***
- Slicing of the nail plate using microtome

Number of sections and thickness of nail samples of the penetration experiments.

Sections	***CCS***	***S***	***NL***
punch 1	18 x 40 µm	21 x 40 µm	21 x 40 µm
punch 2	23 x 40 µm	26 x 40 µm	26 x 40 µm
punch 3	30 x 40 µm	23 x 40 µm	29 x 40 µm
non-penetrated amount	small filter paper + swab	small filter paper + swab	swab + 60 µl water
acceptor compartment	wetted cotton ball	wetted cotton ball	wetted cotton ball
mean thickness of the nail	947 µm	933 µm	1013 µm

FTIR-ATR diffusion cell

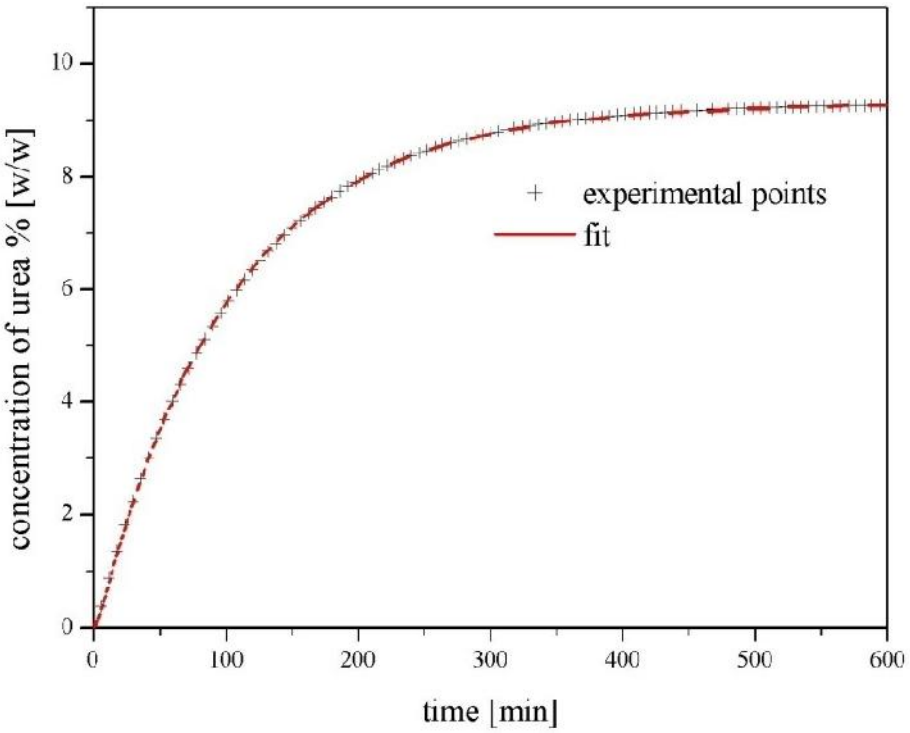
Online determination:



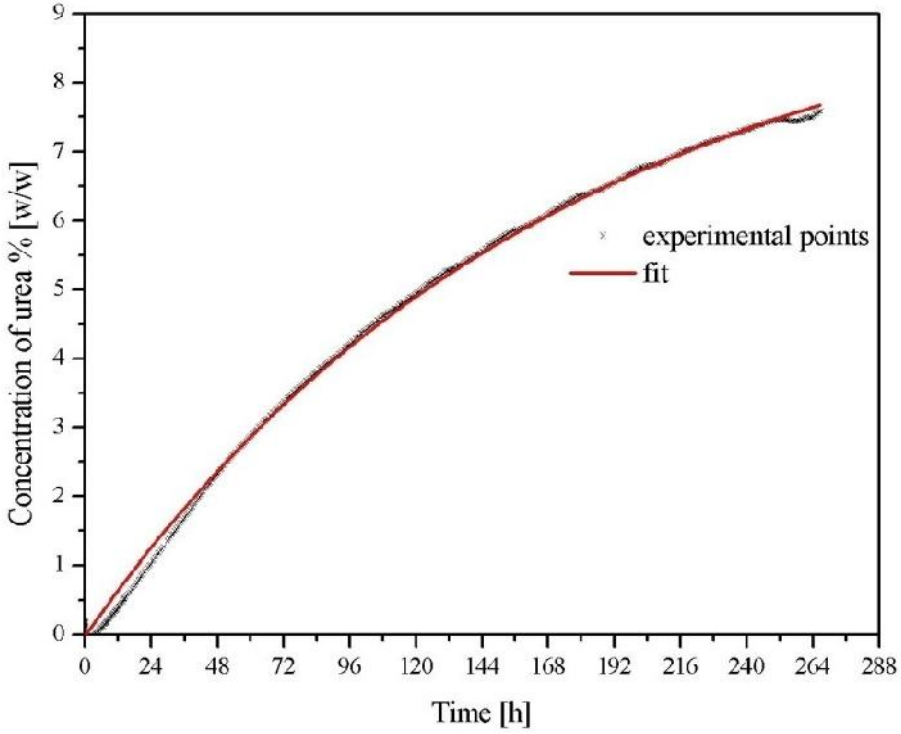
FTIR-ATR cell: Urea diffusion

- **FTIR-ATR diffusion cell**: Fourier transformed IR attenuated total reflection
- **Diffusion of urea**: Hartmann et al. Analyst 129 (2004) 902

Bovine hoof



Isolated Stratum corneum



Neubert and Naumann

18/07/2014

Urea diffusion

Diffusion coefficients of urea in human **stratum corneum** and **bovine hoof:**

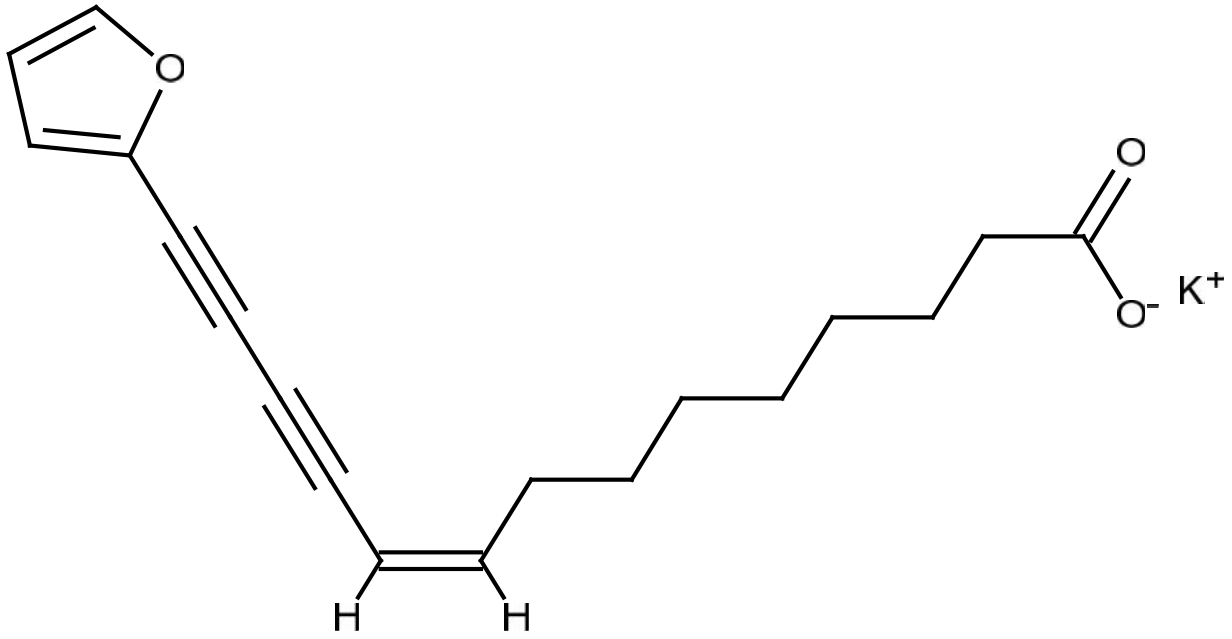
SC [µm]	D [10^{-10} cm^2/s]	Rel. SD [%]	Experiment time [h]
24 ± 2	6.04 ± 0.04	0.7	278
30 ± 2	0.63 ± 0.05	7.9	640
36 ± 4	1.88 ± 0.08	4.3	615

Hoof [µm]	D [10^{-7} cm^2/s]	Rel. SD [%]	Experiment time [min]
137 ± 9	2.98 ± 0.02	0.67	600
139 ± 5	2.95 ± 0.02	0.68	600
110 ± 4	3.43 ± 0.02	0.58	600

Model drug

Chemical structure of the antifungal EV-086K; M_w = 322.44 g/mol

(Naumann et al. J Control Rel 180 (2014) 60 – 70, Poster xxxx)



SN3 Vielleicht möchten Sie noch etwas zum Wirkmechanismus sagen? Ich kann Ihnen gern meine Aufzeichnungen aus der Dissertation zukommen lassen.
Sandy Naumann; 26.03.2014

Model drug

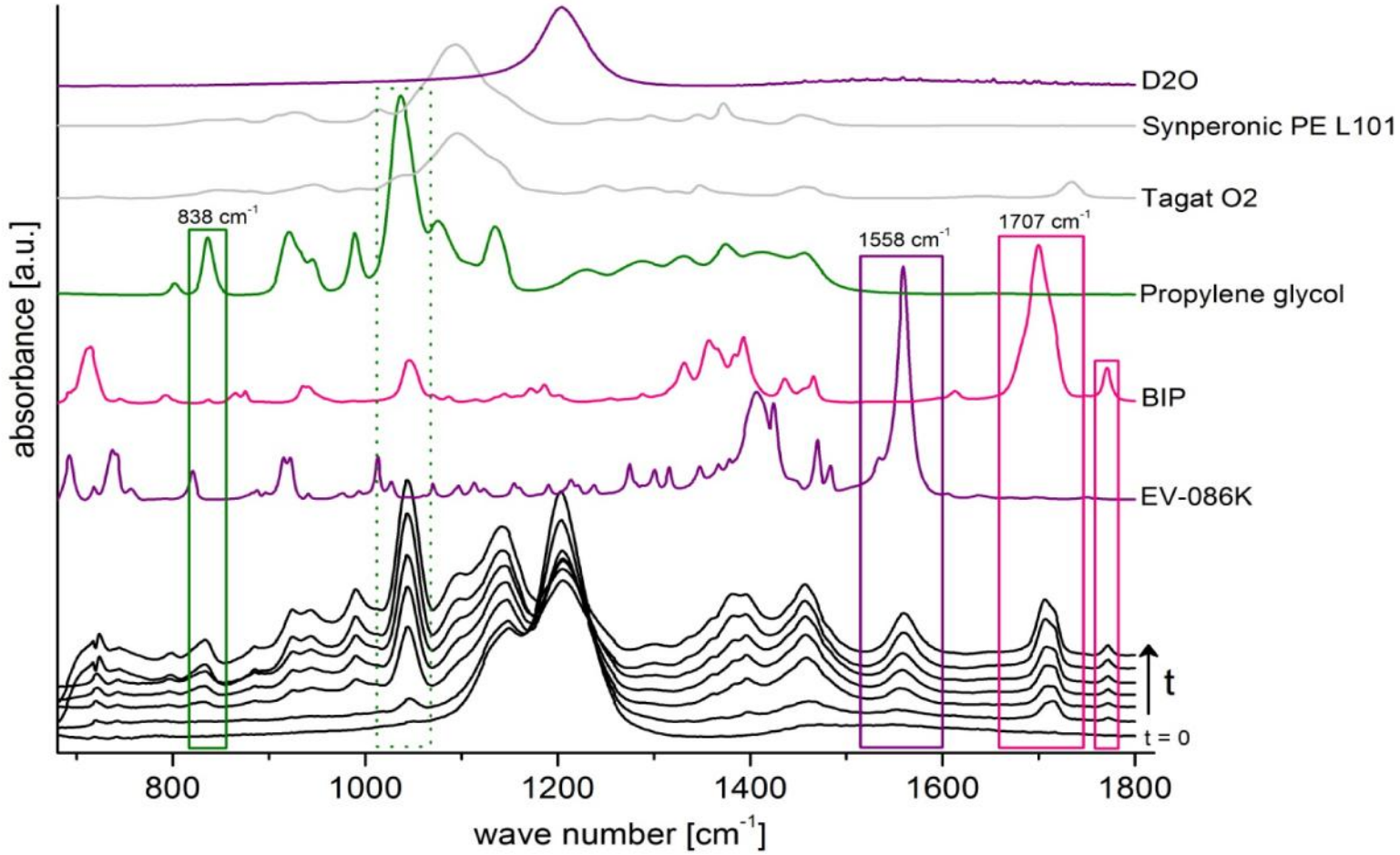
Physicochemical properties of EV-086K, M_w = 322.44 g/mol

Solubilities:

Medium	*c_s [mg/ml]*	*Classification according to Ph. Eur. 7.8*
Water	327.9 ± 5.2	freely soluble
Propylene glycol	607.9 ± 67.4	freely soluble
Pentylene glycol	554.9 ± 63.9	freely soluble
Methanol	2152.4 ± 65.2	very soluble
Ethanol	47.3 ± 2.2	soluble
Transcutol® P	1634.2 ± 138.2	very soluble
Soluphor® P	720.2 ± 67.6	freely soluble
Glycerol	0.4 ± 0.1	very slightly soluble
Isopropanol	3.6 ± 0.1	slightly soluble

CMC = 18 µg/ml; log P = 4.54 ± 1.39

FTIR-ATR spectra



FTIR-ATR spectra (colored) of the single components of the CCS and FTIR-ATR spectra (black) of the uptake of EV-086K and the other ingredients of the CCS into the **acceptor of the diffusion cell** during the penetration experiment (24 hours) through **equine hoof** membranes.

Reinhard Neubert

18/07/2014

Formulations

How to influence penetration of EV-086K into and through the human nails?

Application of the following formulations:

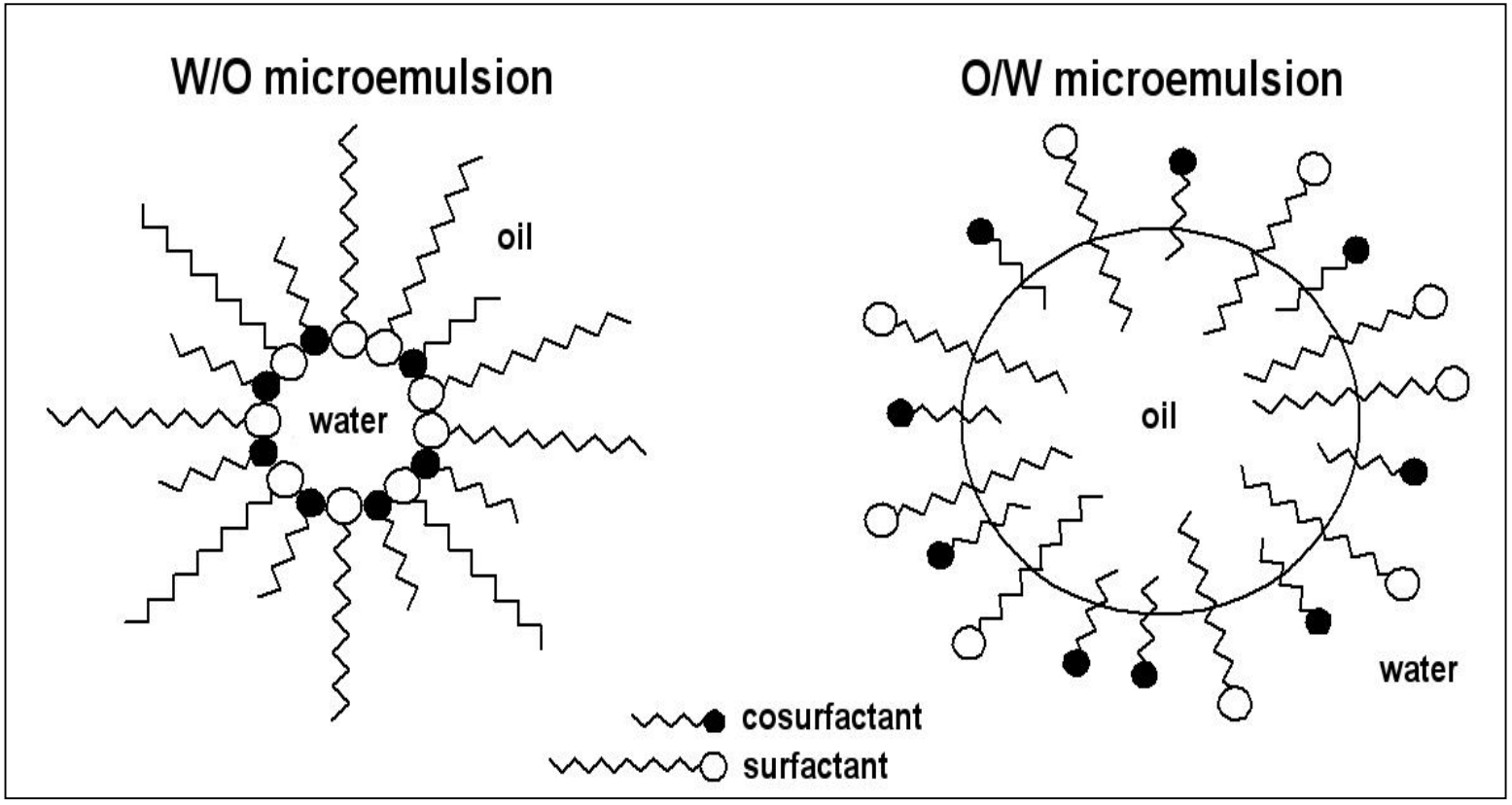
1. **Nail laquer (NL):** Based on dimethylaminoethyl methacrylate, butyl and methyl methacrylate

2. **Colloidal carrier system (CCS)**: Radii = 20 - 40 nm, based on the surfactants Tagat® O2 V/ Synperonic™ PE/L and Pelemol® BIP

3. **Solution (S):** Based on water, ethanol and Transcutol® P

Colloidal carrier systems (CCS)

Application of **colloidal carrier systems** (radii = 20 - 40 nm):



Properties of the CCS

Application of colloidal drug carrier systems (CCS):

- Radii = 20 - 40 nm
- Content of surfactants < 20 %
- Physiologically well accepted (nontoxic) surfactants
- Variable over a wide range (O/W- and W/O- systems)
- Thermodynamically stable (very low interface tension)

Penetration of EV-086K: Bovine hoof

Comparison of the parameters of the diffusion experiment using bovine hoof membranes

(mean ± SD, $p < 0.05$ vs. superscript formulation)

Formulation $c_{EV\text{-}086K}$ [%]	***n***	D_{bovine} [x10^{-9} cm²/s]	$t_{lag\text{-}bovine}$ [min]	***linear range*** [min] $R^2 \geq 0.993$	J_{bovine} [µg/cm^{-2} min^{-1}]	P_{bovine} [x10^{-4} cm/min]
NL 12	3	3.0 ± 1.0^{S}	60 ± 18^{CCS}	60 – 156	43.9 ± 10.0	3.7 ± 0.8^{S}
				163 – 694	15.4 ± 2.3	1.3 ± 0.2
S 5.7	3	1.2 ± 0.5^{NL}	96 ± 21^{CCS}	96 – 171	33.8 ± 6.4	5.9 ± 1.1^{NL}
				171 – 1440	11.7 ± 0.4	2.1 ± 0.1
CCS 10	6	11 ± 10.4	29 ± 9^{NL,S}	19 – 79	76.8 ± 35.9	7.7 ± 3.6
				79 – 324	22.9 ± 8.8	2.3 ± 0.9

Penetration of EV-086K: Equine hoof

Comparison of the parameters of the diffusion experiment using equine hoof membranes

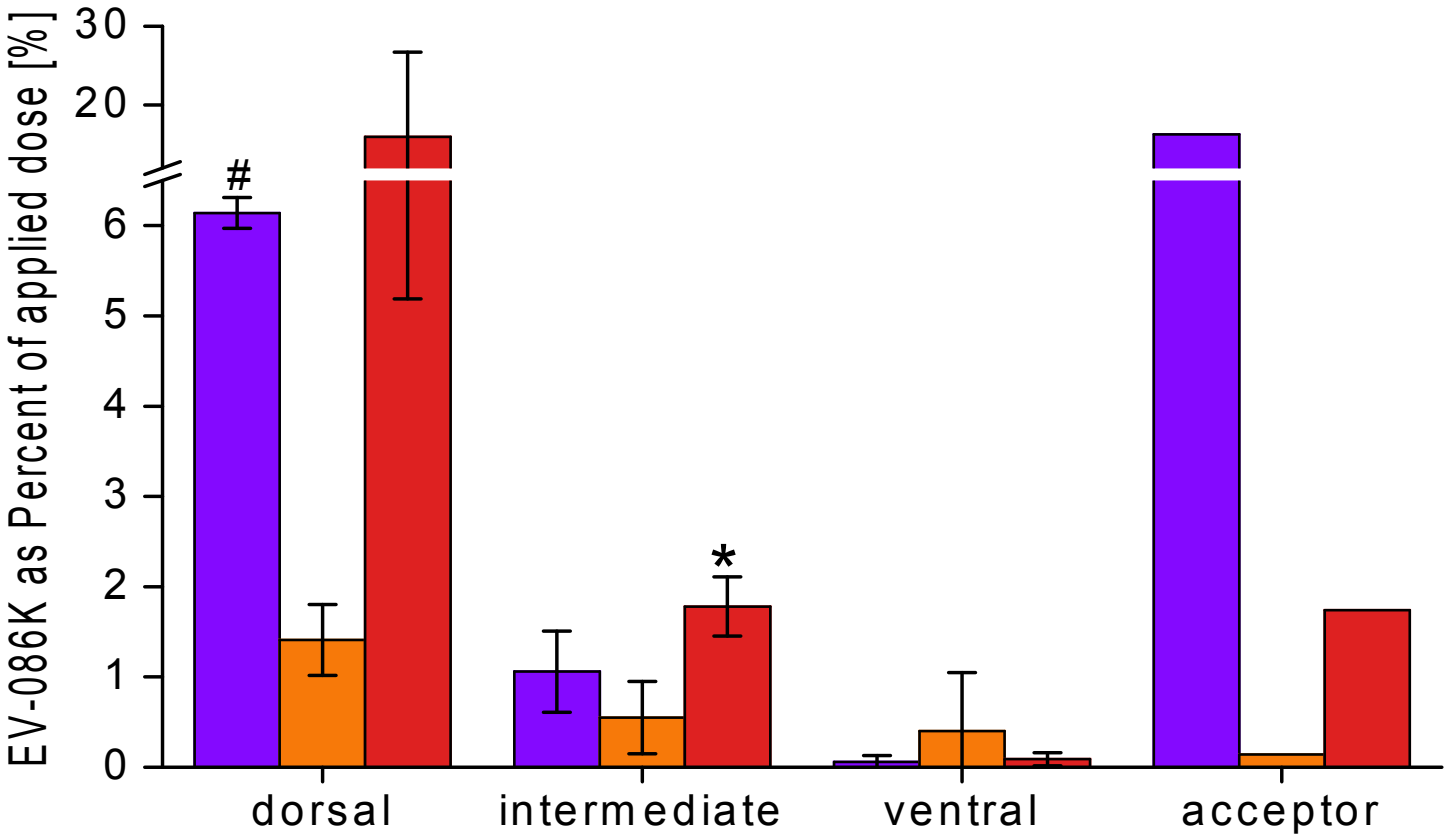
(mean ± SD, $p < 0.05$ vs. superscript formulation)

Formulation $c_{EV\text{-}086K}$ [%]	***n***	D_{equine} [x10^{-9} cm²/s]	$t_{lag\text{-}equine}$ [min]	***linear range*** [min] $R^2 \geq 0.993$	J_{equine} [µg/cm^{-2} min^{-1}]	P_{equine} [x10^{-4} cm/min]
NL 12	2	4.6 ± 0.8^{CCS}	83 ± 0.1^{CCS}	83 – 190 222 – 1440	36.4 ± 0.7^{S} 23.2 ± 0.7^{S}	3.0 ± 0.1 1.9 ± 0.1^{S,CCS}
S 5.7	3	11.3 ± 5.9^{CCS}	57 ± 18	59 – 200 279 – 1440	25.9 ± 4.1^{NL} 4.3 ± 0.9^{NL,CCS}	4.5 ± 0.7 0.8 ± 0.2^{NL,CCS}
CCS 10	2	11.3 ± 1.2^{NL,S}	25 ± 6^{NL}	29 – 200	33.3 ± 4.0^{S}	3.3 ± 0.4^{NL,S}

Penetration of EV-086K: Human nail

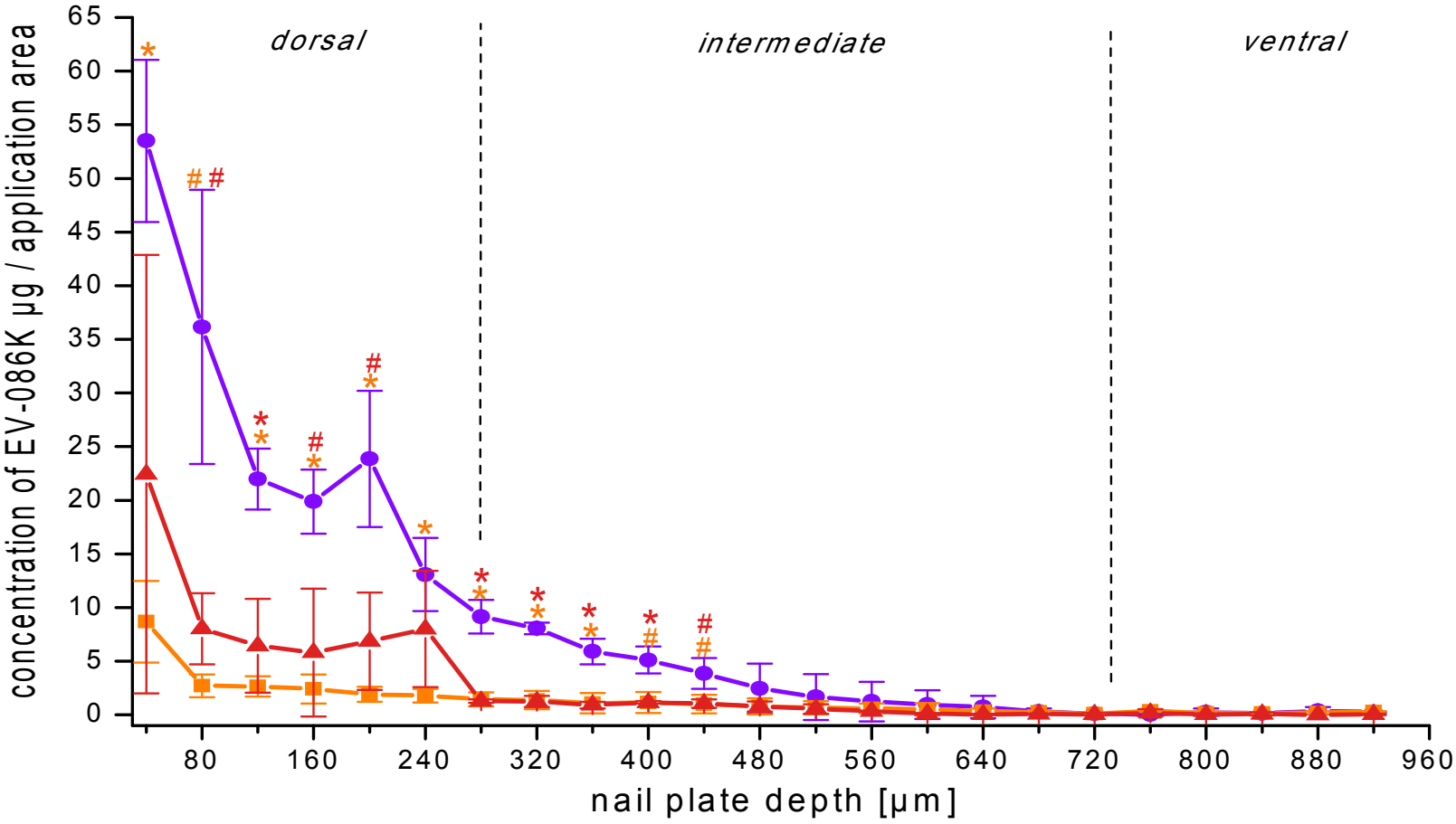
Comparison of the amounts of EV-086K in % after 24 hours after administration of:

CCS●, NL● and S●, (mean ± SD, *n* = 3, acceptor: n=1; **p* < 0.05 vs. S; #*p* < 0.01 vs. S).



Penetration of EV-086K: Human nail

Comparison of the penetration profiles of EV-086K in µg/area after 24 hours after administration of: CCS●, NL▲ and S■



(mean ± SD, n = 3, *p < 0.01, #p < 0.05 CCS vs. S/NL)

SN1 In diesem Diagramm sind nur die absoluten Konz. dargestellt und nicht die zur applizierten Dosis.
Sandy Naumann; 26.03.2014

Comparison of EV-086K fluxes

Comparison of the flux values of EV-086K using the different model system:

(Diffusion area of FTIR-ATR diffusion cell: 0.0962 cm²; penetration area of the human nail plates: 2.01 cm², time: 24 hours).

Formulation	$J_{bovine/20}$ [% /cm⁻²h⁻¹]	$J_{equine/20}$ [% /cm⁻²h⁻¹]	J_{human} [% /cm⁻²h⁻¹]	$\frac{J_{bovine}}{J_{human}}$	$\frac{J_{equine}}{J_{human}}$
NL	0.75 ± 0.05	1.25 ± 0.13	0.41 ± 0.22	**1.8**	**3.1**
S	1.4 ± 0.07	0.75 ± 0.18	0.05 ± 0.01	**28**	**15**
CCS	0.9 ± 0.20	1.05 ± 0.13	0.49 ± 0.01	**1.8**	**2.1**

(see Naumann et al. J Control Rel 180 (2014) 60-70)

Conclusions I

1. **Human nails can be defined as hydrogel concerning drug diffusion.**

2. **Onychomycosis is one of the most relevant nail disease.**

3. **Effective in vitro models are missing for optimizing nail diffusion/penetration from relevant formulations.**

4. **Diffusion/penetration of the highly lipophilic drug E-086K from different formulations was studied using the methods presented in this lecture.**

Conclusion II

5. It could be shown that the equine hoof slices have are simulating the diffusion/penetration conditions into human nails in a better way than the bovine hoof slices.

6. Colloidal carrier systems are the most effective formulation concerning nail delivery of a highly lipophilic drug such as EV-086K.

Thank you very much

for your attention!