

Localization of EPR probes and labeled drugs in nanocarriers and their penetration and release in skin

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Introduction

The successful inclusion of active substances into nanocarriers, their penetration into the skin and the release of the drugs at the target sites are essential and important for effective local therapy approaches. The exterior layer of the skin (horny layer, *stratum corneum*) has a distinctive barrier function that provides protection against environmental influences [1, 2].

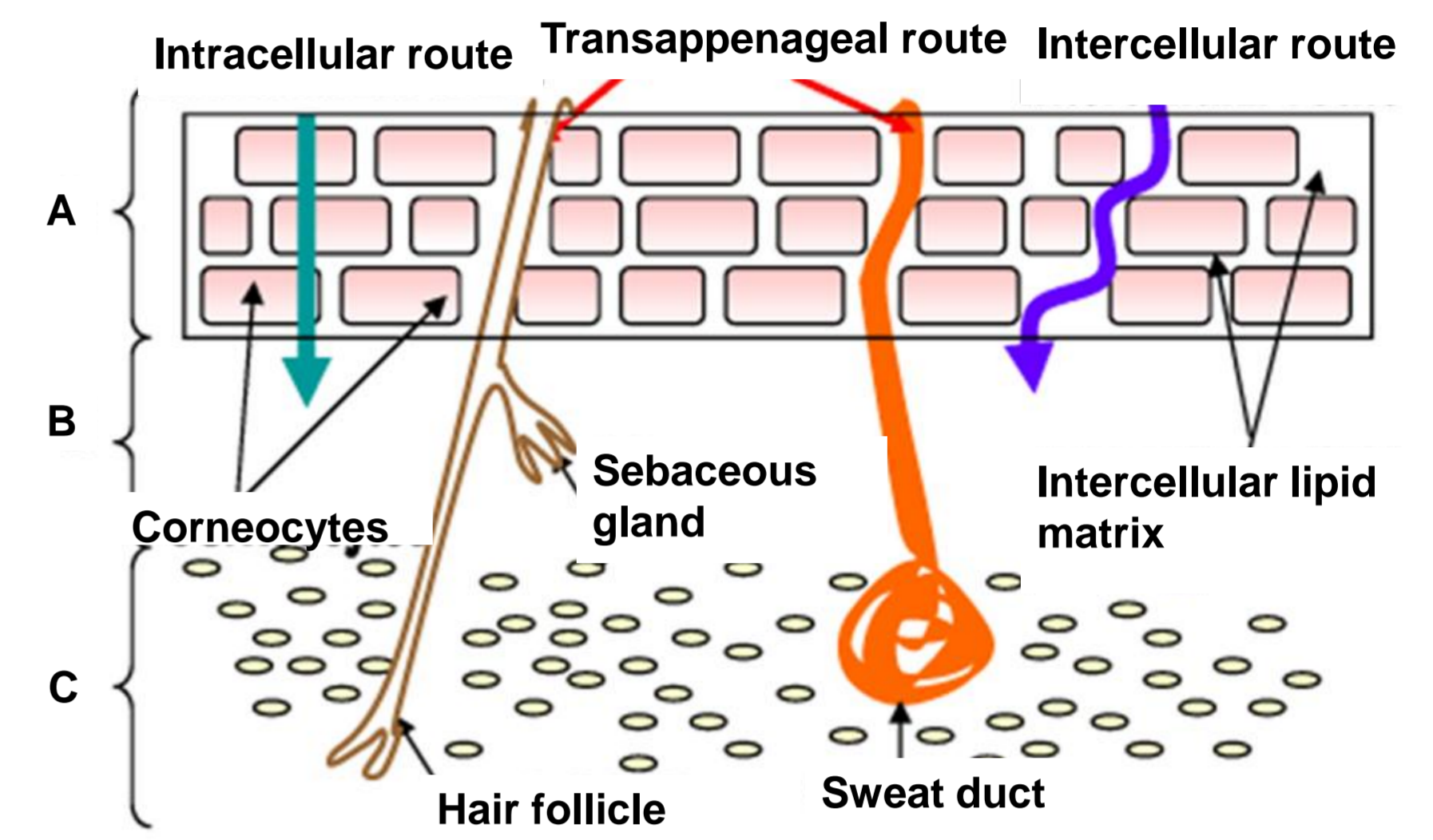
Aim of the study:

- Improvement of the penetration of active ingredients into the skin.
- Research is focused on promoting solvents and nano-transport systems (NTS) [1].

Background information and experimental research:

- In the pharmaceutical industry stearic acid is used as an additive for e.g., skin creams and oils; their hydrophobic character (*log P* value 8.23) complicates the penetration into the skin extremely [3].
- Dendritic core-multi shell (CMS) nanocarriers belong to the multi-shell NTS. They allow the transport and storage of molecules with different chemical characters, consist of a polar core, a nonpolar inner shell and a hydrophilic outer shell.

In this study multi-frequency EPR spectroscopy (W, X band) was applied to investigate the localization of 5DSA within the carrier, the penetration properties of the carrier and the release of the drug.

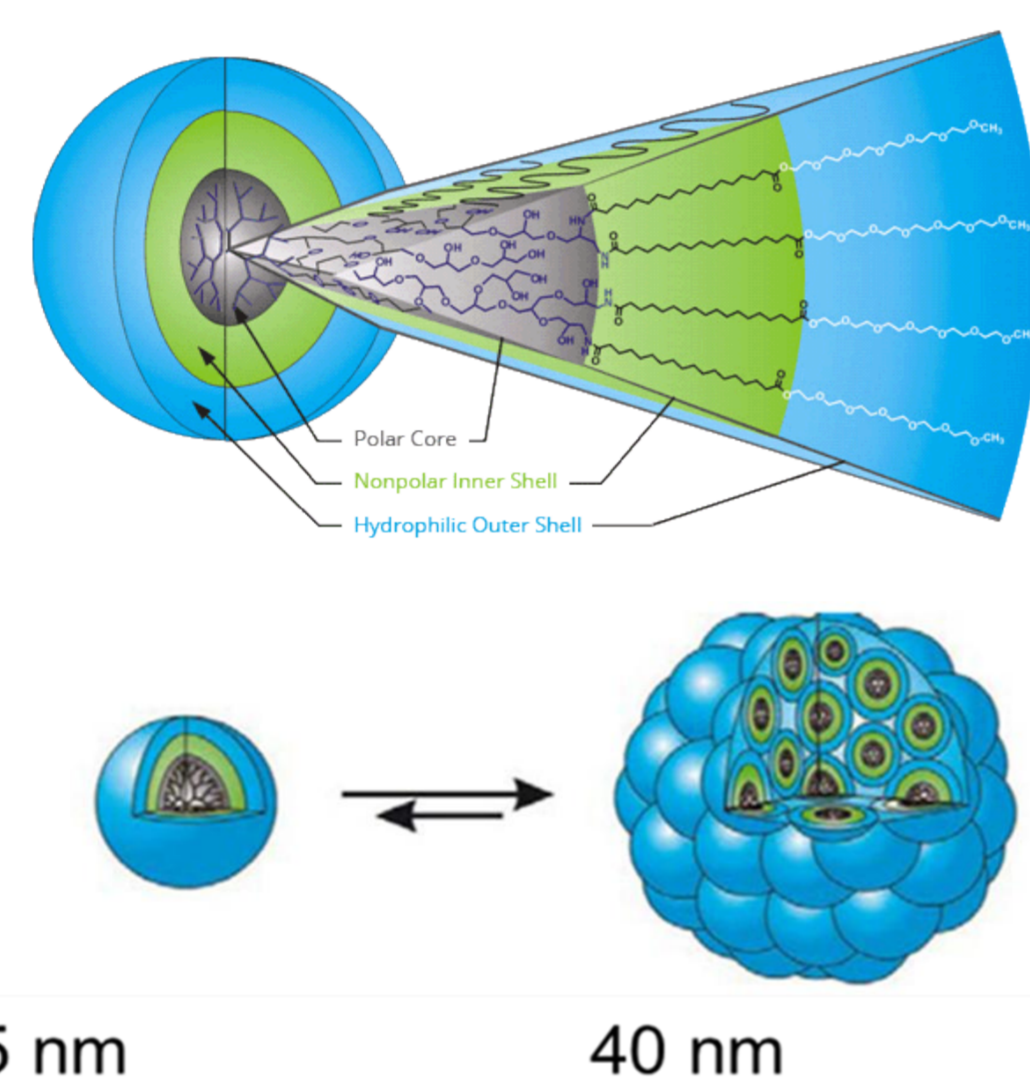


Pathways through the skin; A) Epidermis (S. corneum), B) Dermis, C) subcutaneous layer (http://www2.mst.dk/common/Udgivramme/Frame.asp?http://www2.mst.dk/udgiv/publications/2009/978-87-7052-980-8/html/kap05_eng.htm)

Materials and Methods

Dendritic core-multishell (CMS) NTS

- Polar/ hydrophilic core of hyperbranched/dendritic polyglycerol
- Intermediate shell is hydrophobic and consists of linear C18 diacid building blocks which are covalently bound to the hyperbranched polar core and to the terminal hydrophilic outer shell
- Outer shell consists of monomethyl polyethylenglycol (mPEG)
- CMS particles are soluble in water and organic substances



Schematic representation of a dendritic core-multishell nanocarrier (size diameter: 5nm); cluster sizes range between 30 nm to 130 nm).

5-doxy-stearic acid (5DSA)

- Stearic acid is a member of the saturated carboxylic acids
- For cosmetics: emulsifying, emulsion stabilizing effect, moisturizing
- Low penetration (*logP* value 8.23)
- → Spin probe 5-doxy-stearic acid (5DSA)



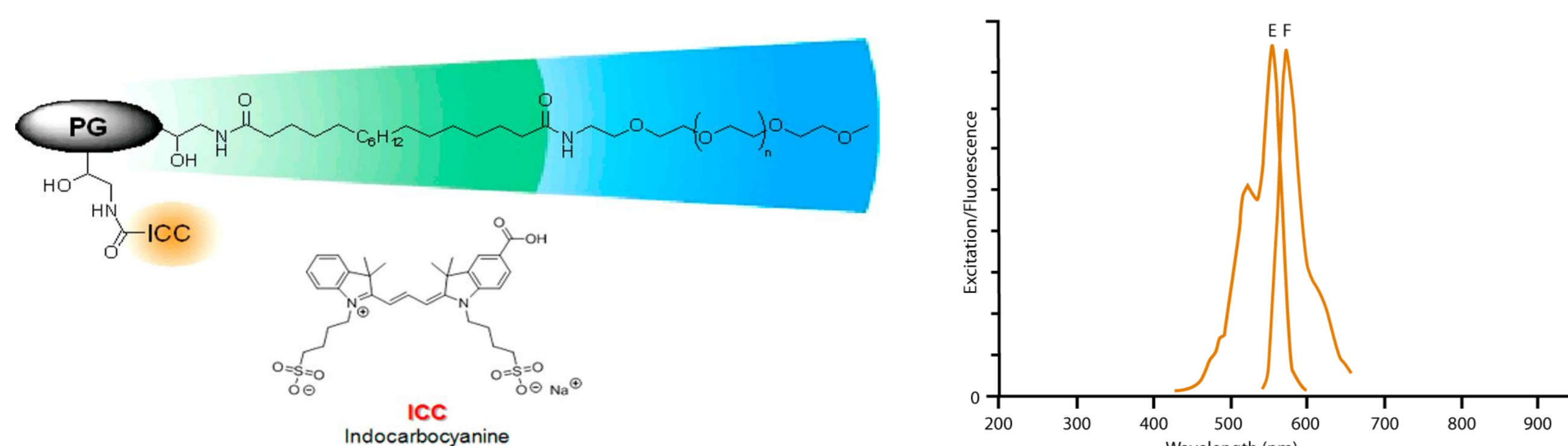
(Mukherjee et al (2010))

Electron Paramagnetic Resonance (EPR) Spectroscopy

- Analysis of the **localization**, **release** and **penetration** profile of an hydrophobic drug, here 5DSA
- Investigations in formulation and on porcine skin (*ex vivo*)
- Measurements were carried out with the use of an X- (9-10GHz; Miniscope, Magnettech, Berlin Germany), and W-band (94GHz; Bruker Biospin, Karlsruhe, Germany)

Fluorescence Microscopy (FM)

- Investigation of the **penetration** of CMS particle into porcine skin (*ex vivo*)
- Analysis of the **localization** of the CMS NTS within the skin
- Covalently bound fluorescent label indocarbocyanine (ICC) to the CMS-NTS



(Boreham et al., 2014)

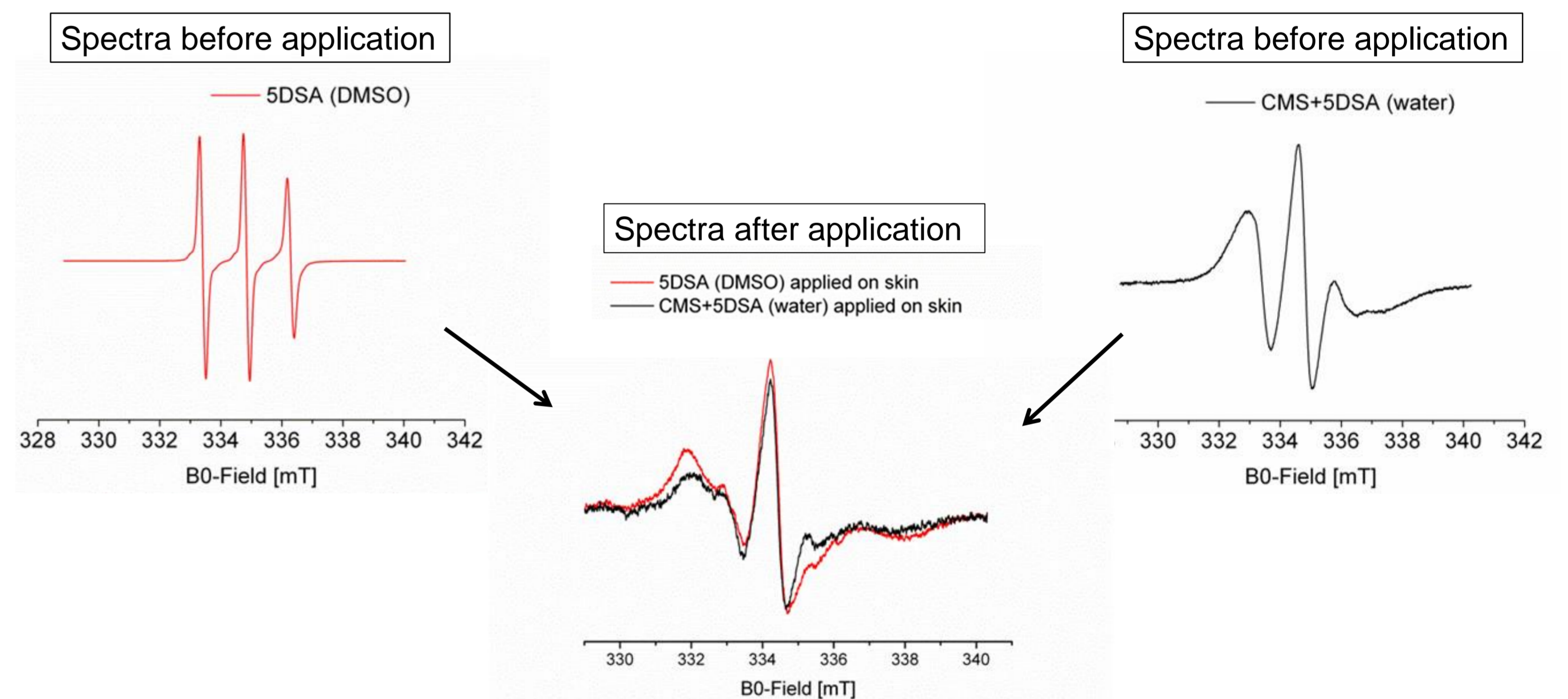
(<https://ireurope.com/technical/products/conjugate-selection/cyanine/cy3>)

References

- [1] Haag SF, Fleige E, Chen M, Fahr A, Teutloff C, Bittl R, Lademann J, Schafer-Korting M, Haag R, Meinke MC: Skin penetration enhancement of core-multishell nanotransporters and invasomes measured by electron paramagnetic resonance spectroscopy. *Int J Pharm* 2011;416:223-228
- [2] Haag SF, Chen M, Peters D, Keck CM, Taskoparan B, Fahr A, Teutloff C, Bittl R, Lademann J, Schafer-Korting M, Meinke MC: Nanostructured lipid carriers as nitroxide depot system measured by electron paramagnetic resonance spectroscopy. *Int J Pharm* 2011;421:364-369.
- [3] S. Mukherjee, M. Edmunds, X. Lei, M.F. Ottaviani, K.P. Ananthapadmanabhan, N.J. Turro, Stearic acid delivery to corneum from a mild and moisturizing cleanser, *J Cosmet Dermatol*, 9 (2010) 202-210.
- [4] A. Boreham, M. Pfaff, E. Fleige, R. Haag, U. Alexiev, Nanodynamics of dendritic core-multishell nanocarriers, *Langmuir*, 30 (2014) 1686-1695.

Results

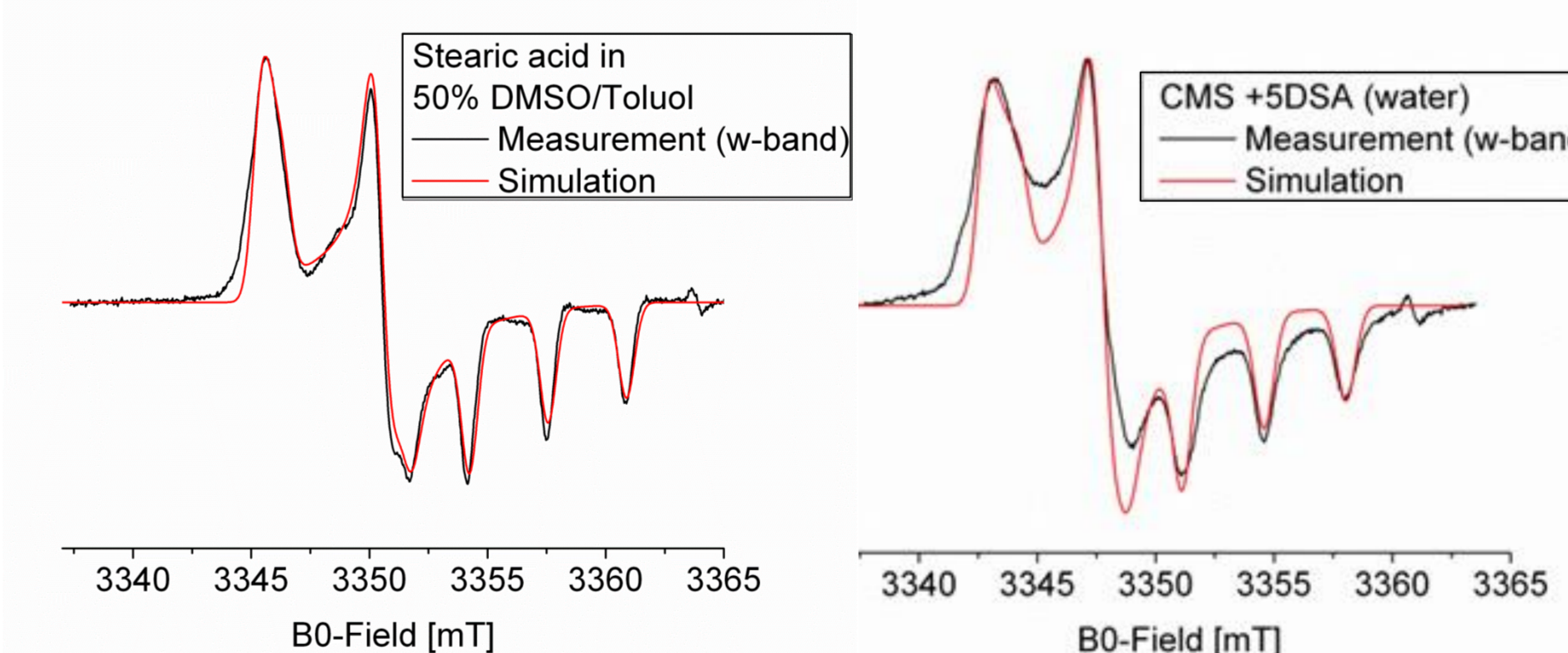
Analysis of the localization of the EPR probe 5DSA in solution (DMSO) respectively within CMS NTS (water) via X-band EPR spectra, before and after penetration into porcine skin (*ex vivo*)



- In DMSO 5DSA shows a higher mobility than if loaded to CMS NTS
- *Ex vivo* studies demonstrate nearly the same properties for 5DSA loaded to CMS NTS and in solution

Multi-frequency analysis

- Multi-frequency analysis for determination of magnetic parameters of the EPR label

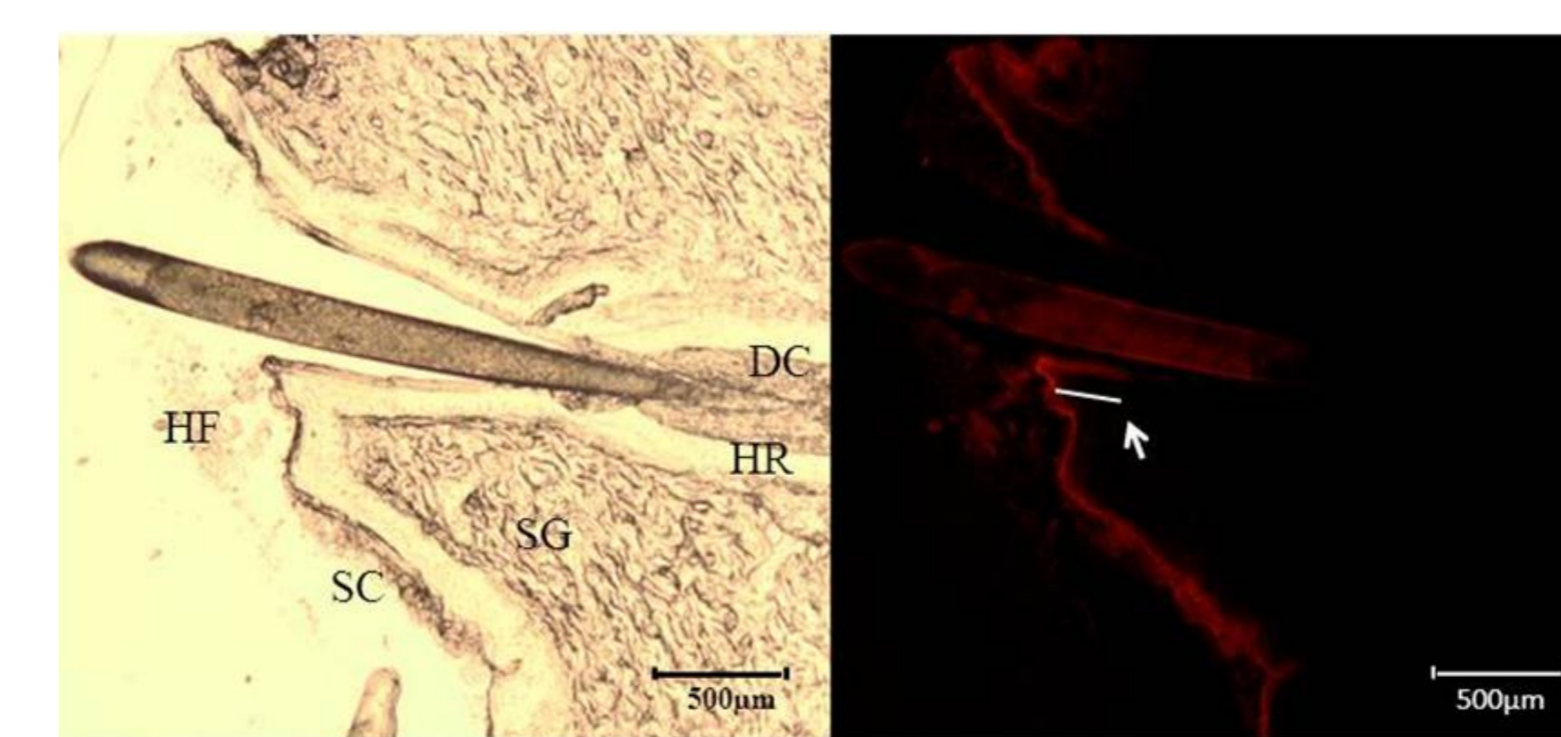


- Linear relation between *Azz* and *gxx* shows polarity changes in different environments

	<i>Azz</i> / MHz	<i>gxx</i>
5DSA [DMSO/Toluol]	93	2.009
5DSA+CMS NTS [water]	97	2.008

- 5DSA in DMSO and 5DSA in CMS indicates similar polarity

Investigation of the penetration efficiency for 5DSA-CMS NTS (ICC labeled)



Hair follicle with transmitted light (a) and a fluorescence filter (b). Abbreviations: hair follicle (HF), stratum corneum (SC), stratum granulosum (SG), hair root (HR), dermis (corium; DC).

- Average penetration depth into hair follicle: 340µm +/- 82µm
- A penetration into the viable epidermis was not observed
- CMS NTS which had not penetrated into the hair follicles, remain localized on the stratum corneum (SC)

Summary

- 5DSA is localized in the nonpolar shell
- Polarity within the nonpolar shell is similar to DMSO
- Penetration depth of the CMS into the hair follicles: 340µm +/- 82µm

5DSA
Fluorescence dye (ICC)

