Skin Forum
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New Investigation Tools to Predict Percutaneous Penetration

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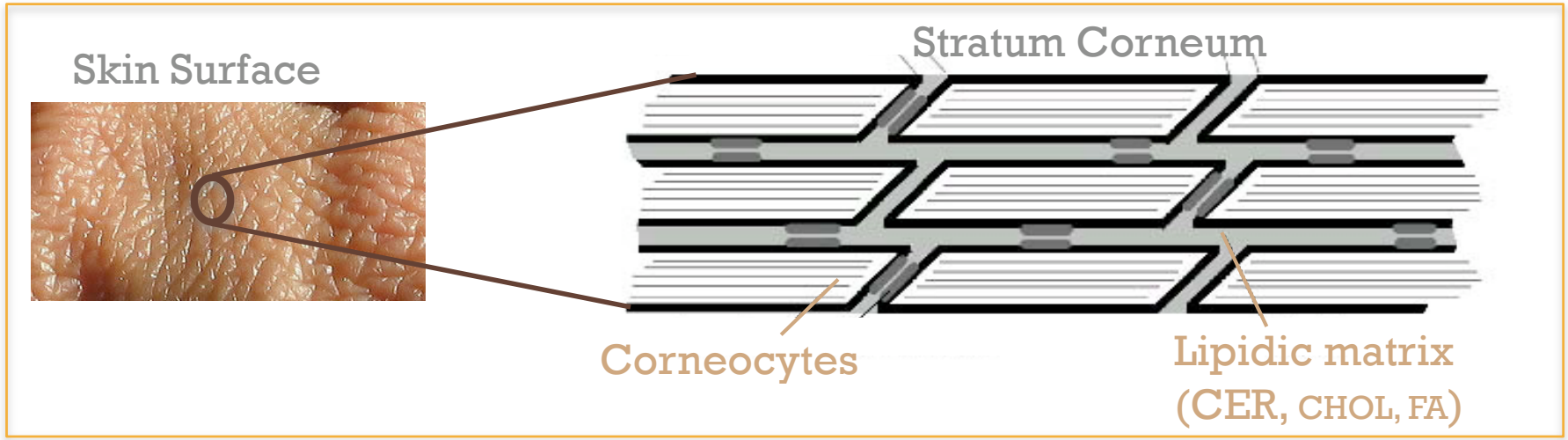
Introduction

- Developing a new parameter to predict percutaneous penetration
- Cosmetics risk analysis: cosmetic ingredients skin penetration and their systemic passage
- The stratum corneum (SC): barrier against penetration
- SC Lipids: crucial role against absorption by retaining molecules
- Trans and Intercellular pathway: correlated to SC lipids & molecules interactions

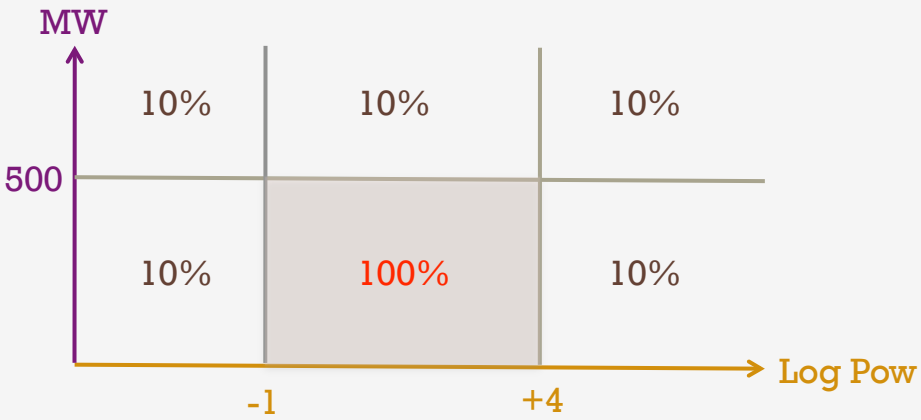


Introduction

Current approaches to study penetration:

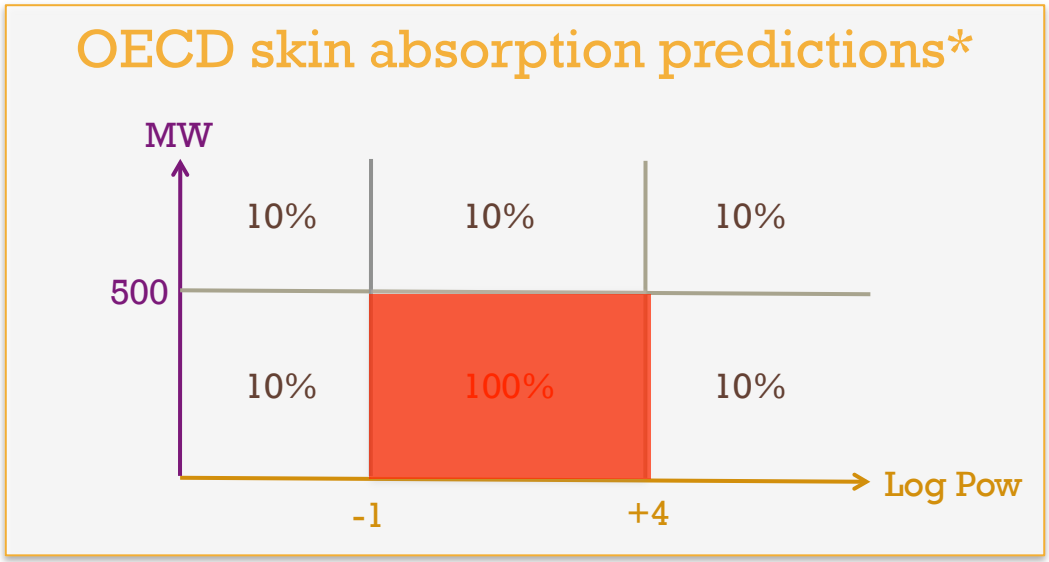
1. Ex vivo: Franz cell ➔ Methodological issues
2. In vivo: Non invasive approach ➔ Not validated yet
3. Mathematical Modelisation ➔ Depends on Log P, MW, Kp

OECD skin absorption predictions*



*OECD GUIDANCE NOTES ON DERMAL ABSORPTION DRAFT 22 OCTOBER 2010

Objective



➔ Need to clarify OECD values to have a more modulated prediction

- To predict more precisely skin absorption in the 100% area
- Addition of a **new parameter** focusing on **ceramide-molecule interaction**

*OECD Guidance Document for the Conduct of Skin Absorption Studies 1 Draft 26 May 08

Methodological Approach

Franz cells Experiment

Experimental approach

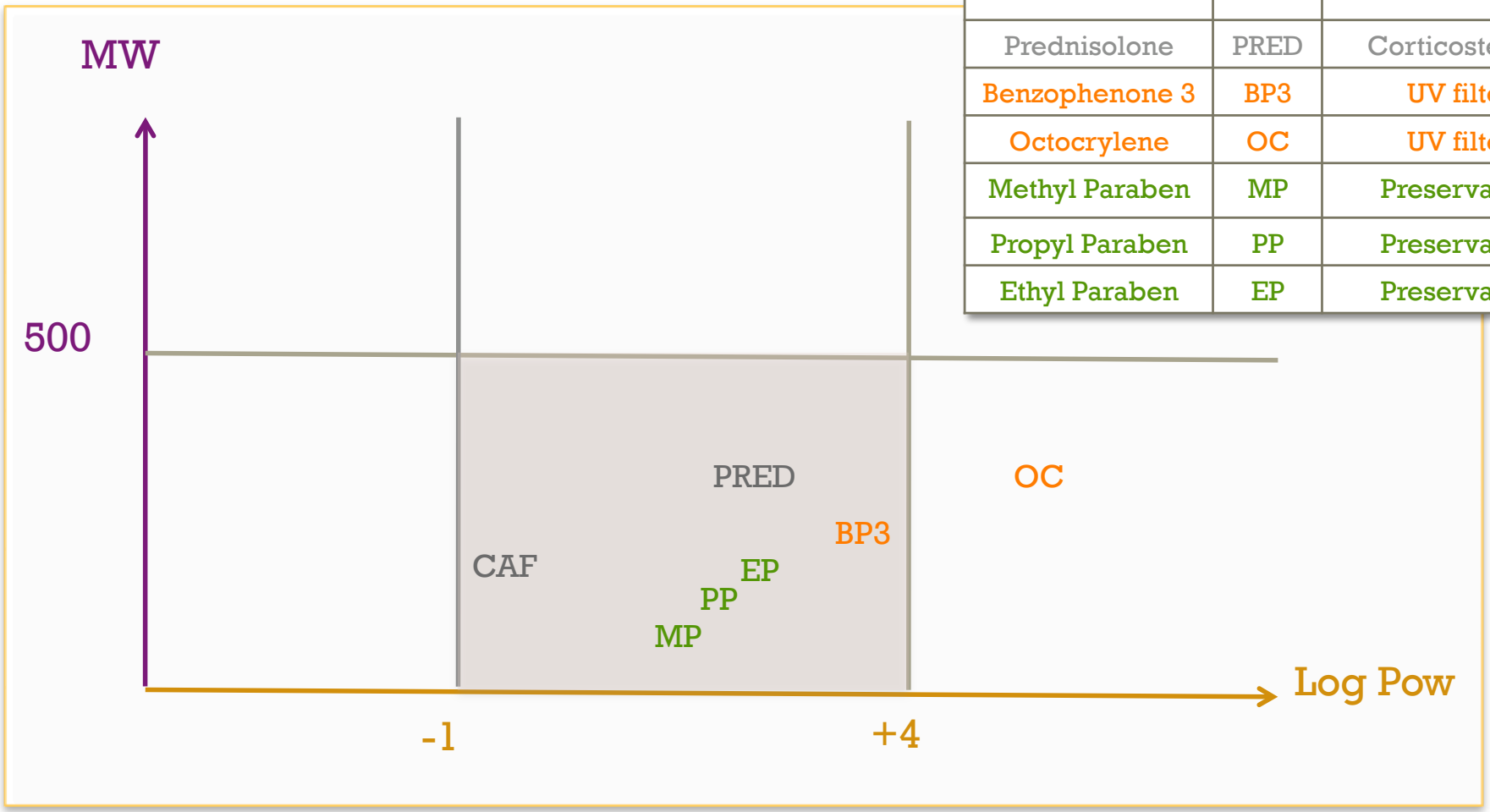
- **FTIR microspectroscopy**
  lipidic barrier status & molecule epidermal distribution

Predictive approach

- **Physico-Chemical Parameters**
  Log P and MW
- **Cer-Mol interaction**
  Affinity Chromatography

Methodological Approach

Name		Properties
Caffeine	CAF	Cellulite reduction
Prednisolone	PRED	Corticosteroid
Benzophenone 3	BP3	UV filter
Octocrylene	OC	UV filter
Methyl Paraben	MP	Preservative
Propyl Paraben	PP	Preservative
Ethyl Paraben	EP	Preservative



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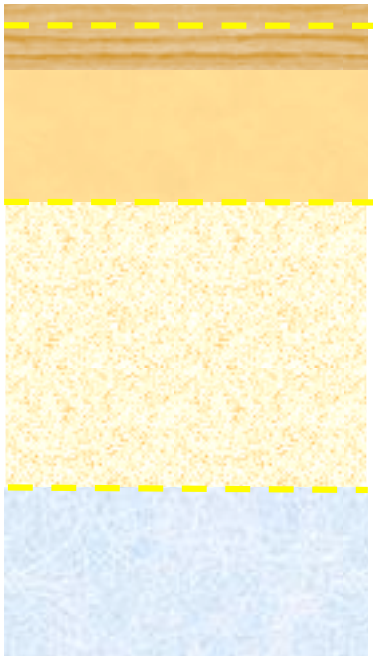
Franz Cells Experiments



Upper SC: 3 tapes stripping (**TS**)

Lower SC + Epidermis (**EP**)

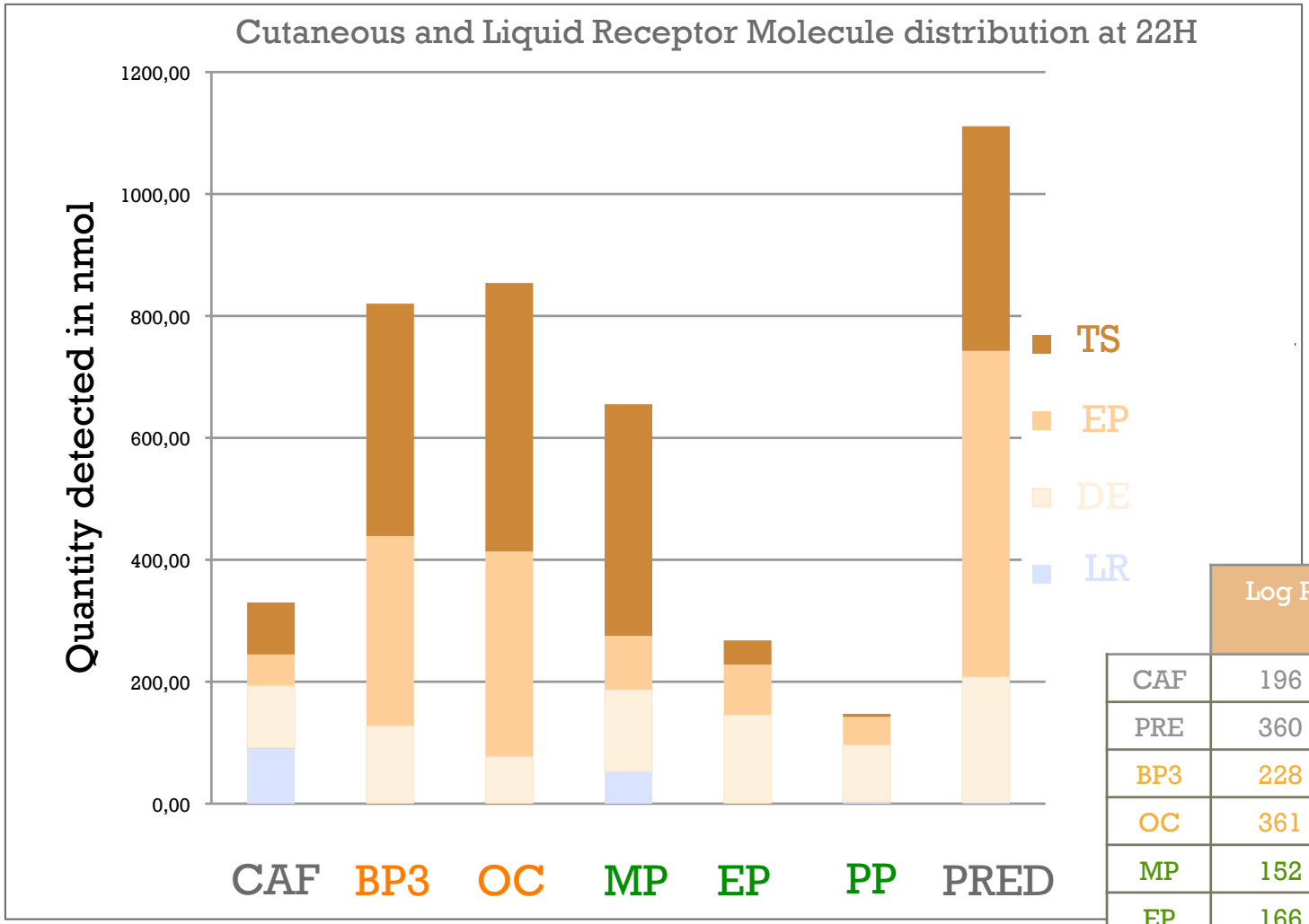
Dermis (**DE**)

Liquid Receptor (**LR**)

HPLC analysis

- Abdominal human skin from plastic surgery
- n = 3
- 2 μmol molecule dissolved in 100μl ethyl acetate and dropped on skin
- Experiment length: 22h

Franz Cells Results



	Log P	MW	Rate nmol.cm^{-2}.h^{-1}
CAF	196	-0,07	1,32 ± 0,090
PRE	360	1,62	0,02 ± 0,02
BP3	228	3,79	0,00 ± 0,00
OC	361	6,88	0,00± 0,00
MP	152	1,93	0,76 ± 0,11
EP	166	2,27	0,00 ± 0,00
PP	180	2,81	0,03 ± 0,05

➔ Results served as reference for the next experiments

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- **FTIR microspectroscopy**
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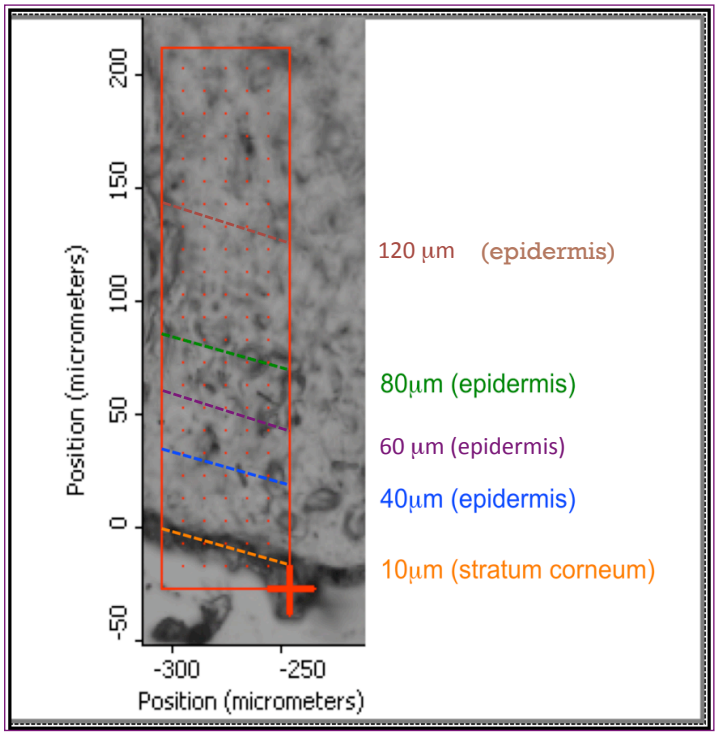
FTIR Microspectroscopy

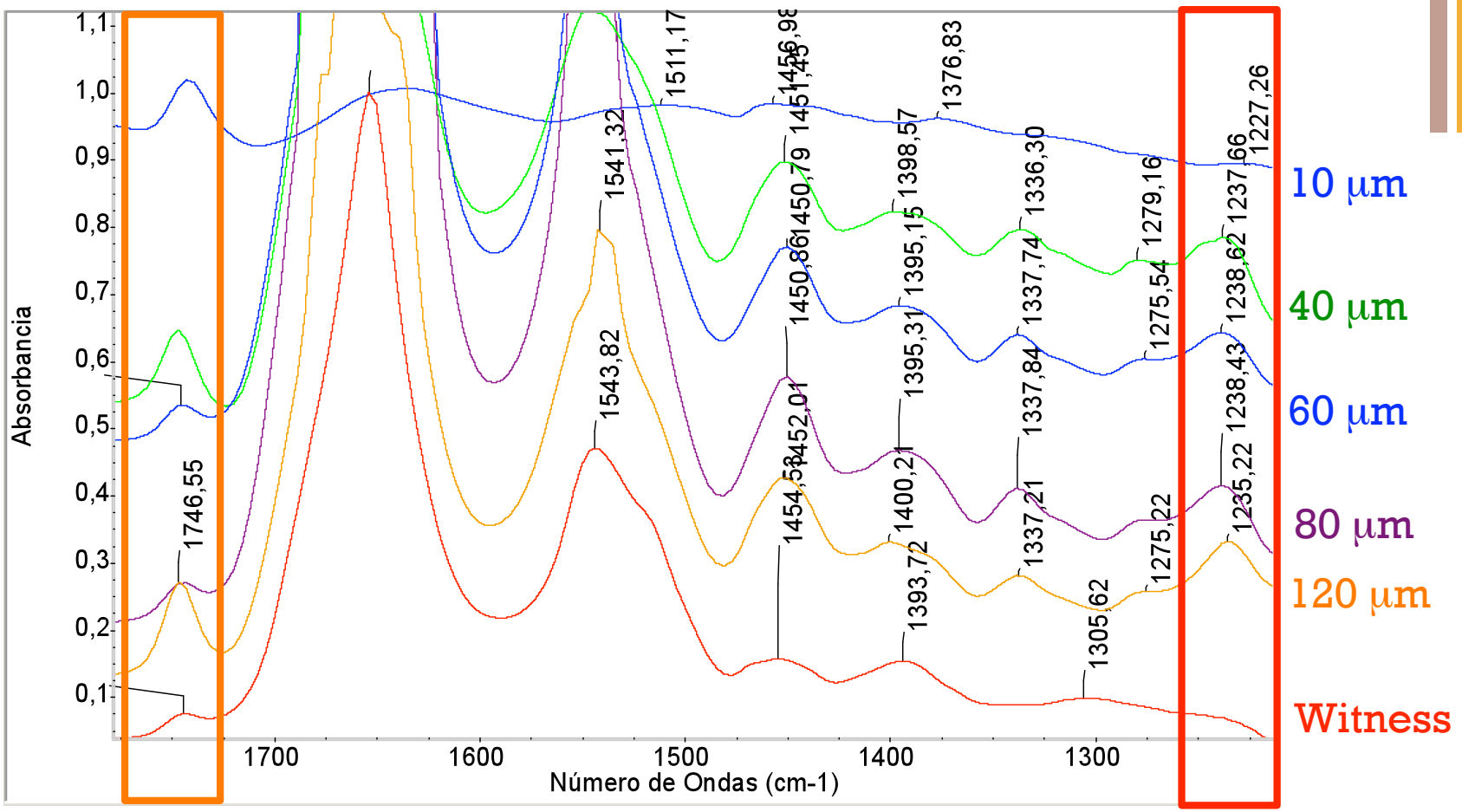
- Removed biopsies at 22h from franz cells, frozen at -20°C and -80°C
- Cryotomed at -20°C and mounted on CaF_2 lame
- FTIR microspectroscopy performed on Synchrotron FTIR microspectroscopy (SMIS beam-line at *SOLEIL*)
- Spectra recorded at a resolution of 4 cm^{-1} between 4000 cm^{-1} and 800 cm^{-1} or 650 cm^{-1} with 32 accumulations and a pixel size of 6 µm x 6 µm

Spectra Analysis

- Molecules distribution at 10, 40, 60, 80, 120µm
- Lipidic barrier status (νCH_2): 2848-2854 cm^{-1}

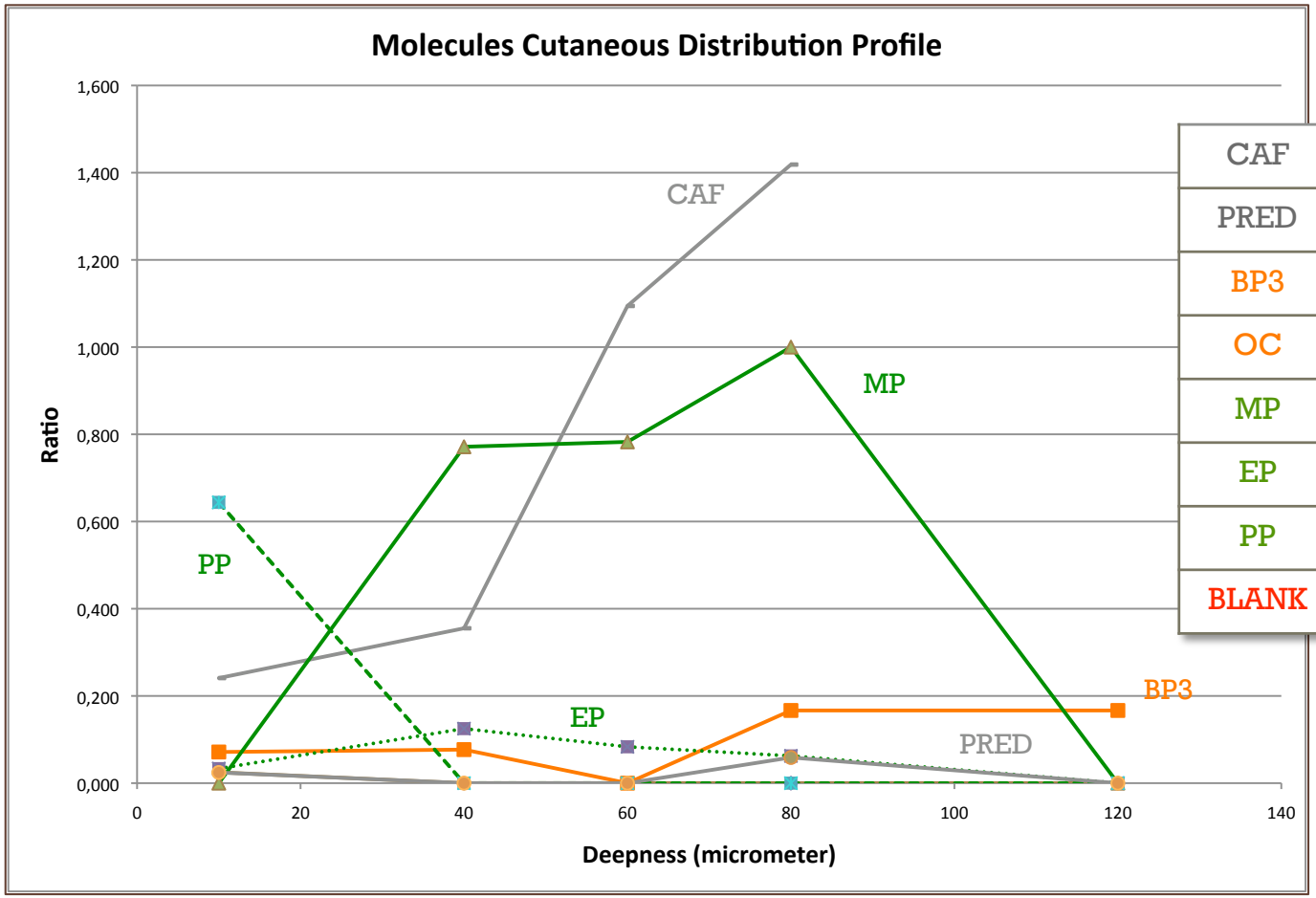


Results



- Average spectra of 10 spectra for each deepness
- Reference band recorded at 1745 cm^{-1}
- Ratio *specific band intensity / reference band intensity*

Results



	νCH_2
CAF	2854
PRED	2851
BP3	2854
OC	2851
MP	2851
EP	2855
PP	2854
BLANK	2848

➔Illustration of the molecule distribution
➔Follow stratum corneum barrier lipidic status
➔Qualitative information

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Ceramide-Molecule Interaction

PGC

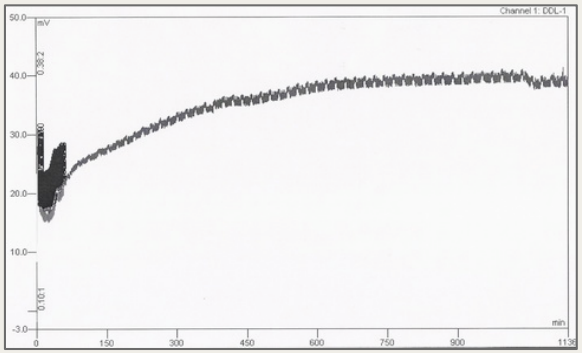
Molecules injections
(UV detection)

➔ Retention time (tr)

➔ Retention factor k_{PGC}

$$k_{PGC} = (tr-t_0) / t_0$$

CER----**PGC**---→



Picture of the PGC loading with Ceramides-like mobile phase by DEDL

PGC-CER

Molecules injections
(UV detection)

➔ Retention time

➔ Retention factor $k_{PGC-CER}$

➔ α ratio calculated

$$\alpha = k_{PGC} / k_{PGC-CER}$$

Results

➔ Molecules chromatographic behavior involves the interaction between ceramide and molecule

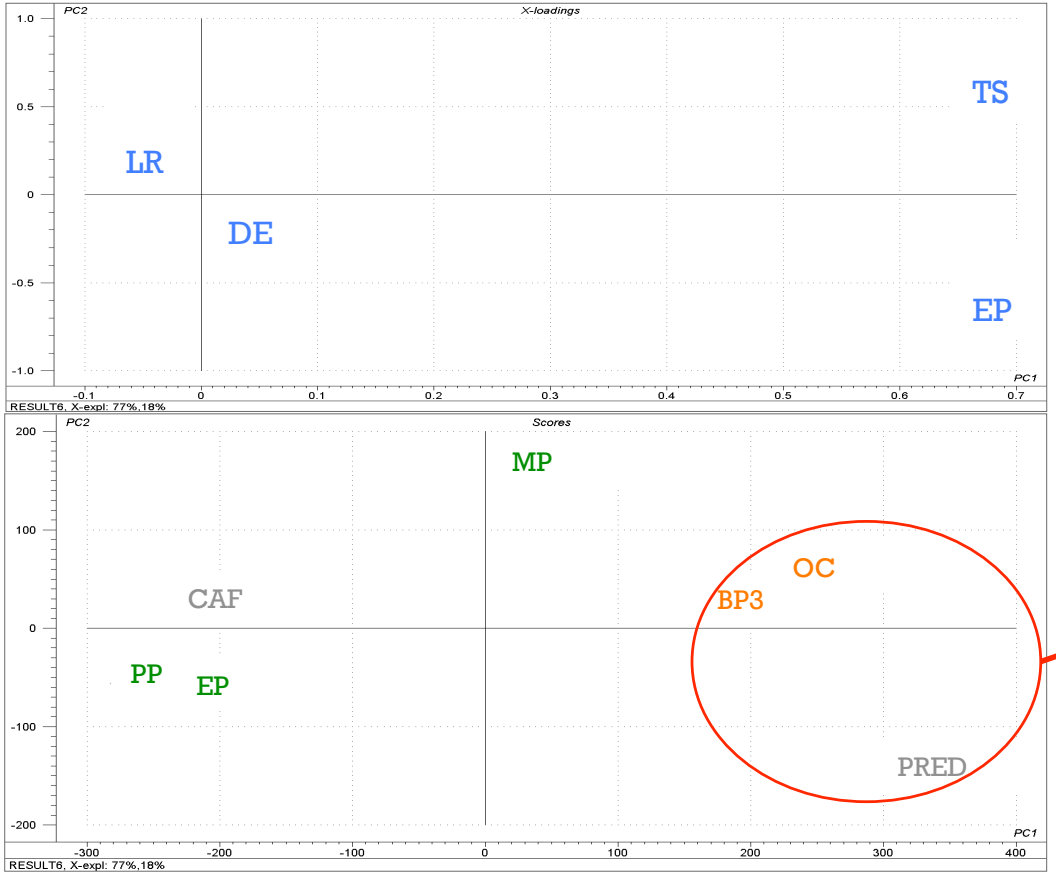
➔ The α ratio is a complementary parameter to Log P and MW.

	Log P	MW	k_{PGC}	$k_{PGC-CER}$	α
CAF	196	-0,07	26,5	19,9	1,3
PRED	360	1,62	7,7	5,4	1,4
BP3	228	3,79	26,3	18,3	1,4
OC	361	6,88	4,2	3,1	1,4
MP	152	1,93	6,1	3,4	1,8
EP	166	2,27	6,3	3,7	1,7
PP	180	2,81	8,4	5,0	1,7

Chemometrics

- Linear Multiple Regression Analysis (LMR) : not enough molecules
- Principal Component Analysis (PCA) : Source of variability of molecules penetration profiles in the Franz cell experiments

➔ PCA : PC1+PC2 = 77% + 18% = 95%



	TS	EP	DE	LR
CAF	85±6	51±14	102±32	91±62
PRED	369±148	534±99	207±91	1±2
BP3	381±30	311±74	128±52	0±0
OC	440±173	336±36	77±27	0±0
MP	380±40	88±12	135±35	52±8
EP	40±40	82±43	146±70	0±0
PP	5±3	46±29	94±94	2±3

Molecules quantity (nmol) in the skin different compartments and liquid receptor

	α	MW	Log P
CAF	1,3	196	-0,07
PRED	1,4	360	1,62
BP3	1,4	228	3,79
OC	1,4	361	6,88
MP	1,8	152	1,93
EP	1,7	166	2,27
PP	1,7	180	2,81

Conclusion

- To predict percutaneous penetration Log P and MW are not sufficient parameters
- Additive parameter: ceramide – molecule interaction
- Feasibility study that provides new trends
  - Future experiments with a higher molecules number → PLS
  - New spectroscopic fluorescence method to deepen the ceramide-molecule interaction study
- FTIR microspectroscopy data highlight vibrational methods to develop in vivo investigation (ex: Raman)

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Thank you for your attention