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APPLICATION OF SKIN PAMPA TO COSMETIC SOLVENT SCREENING

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PURPOSE

Transdermal drug delivery has become one of the most popular alternative drug administration routes due to its numerous advantages. Also, topical route is preferred for skin improvement action **in cosmetics**. The main barrier and regulator of transport through the skin is the **stratum corneum** (SC), the outermost layer of epidermis. Estimation of skin penetration of compounds designed for dermal usage or transdermal delivery is needed at early stage of development, when the *in vitro* or *in vivo* human skin studies are not feasible to use. Recently, an **artificial membrane based microtiter-plate method** Skin PAMPA was developed to mimic the permeability through SC and introduced as relevant alternative for **skin absorption[1]**. The **Skin PAMPA** has already been applied for **formulations** as well, both **semi-solid ones[2]** and **transdermal patches[3]** . The purpose of the project was to validate the applicability of Skin PAMPA assay for permeability study of two model compounds: **Symwhite** and (2) **Aminexi**l dissolved in **23 different solvents**.

MATERIALS and METHODS

To run the assay with saturated solutions, preliminary solubility classification test has been performed. In a stepwise procedure, increasing volumes of medium pre-warmed to 32°C was added to approximately 0.1 g of the sample in a 10 mL glass-stoppered measuring cylinder. After each addition of pre-warmed (32°C) medium, the mixture was shaked for 10 minutes and was visually checked 10 minutes later for any undissolved parts. This procedure was repeated till the compound was fully dissolved, or till we reached 1 mg/mL solubility limit, where compounds were reported as >1mg/mL. Visual observations had been done after 10 minutes or at the end after 1 hour. Sample is kept at 32°C with stirring or shaking for 24 hours to see long term stability. The approximate solubility data are presented in Figure 1.

For permeability study commercially available Skin PAMPA plates (Skin PAMPA™ , Pion Inc., USA) were used. The membrane was hydrated during overnight with standard hydration solution (Pion Inc., USA). The donor phase solutions of **Symwhite** and **Aminexi**l in different solvents were prepared freshly according to the approximate solubility data and 70 µl was applied to the donor (upper) plate. The acceptor (lower) plate contained 180 µl of Prisma buffer pH 7.4 in each wells and a magnetic stirrer. The PAMPA sandwich was incubated at **32 °C** for 6 h. 150 µl sample was taken out from the acceptor phase at 2, 4, 6 h. The concentration of the samples was measured by UV spectroscopy, by **HPLC-UV or LC-MS.**

RESULTS

Symwhite: The **transdermal permeability** of Symwhite was investigated by **Skin PAMPA** method using the 23 solvent. The preliminary semi-quantitative solubility study define the starting concentrations in the permeability test, as **saturated solutions** were applied in the tests. The **thermodynamic equilibrium intrinsic solubility (So)** of Symwhite in the acceptor media at 32°C was also determined to monitor the saturation in the acceptor and avoid any limitation effect. So: 3.45 ± 0.01 mg/ml (n = 8).

The **flux** (J) was obtained as the slope of the permeability profile (total permeated amount vs time) and the logP_m was derived using $J = P_m C_D$ equation (**Eq.1**), where C_D is the initial donor concentration. Data for 20 solvents were possible to be determined as acceptor concentration was below the detection limit for the rest of samples.

The different solvents have **significant impact on the permeability** of the model compound. The logP_m values span more than three orders of magnitude and divide the solvents into three main types in accordance with their chemical structures. The first group of solvents providing **low permeability** (logP_m < -3) includes three small molecular size, **polar organic solvents** (DMSO, dimethylisosorbide, ethanol). Solvents providing **medium permeability** (logP_m: (-3) - (-2)) cover two chemical types, (a) the **highly apolar solvents** like long chain fatty acid esters, long chain alcohol and corn oil, and (b) the "**glycol type" solvents**. **The highest permeability** (logP_m > (-2)) was provided by **water and water containing mixtures.**

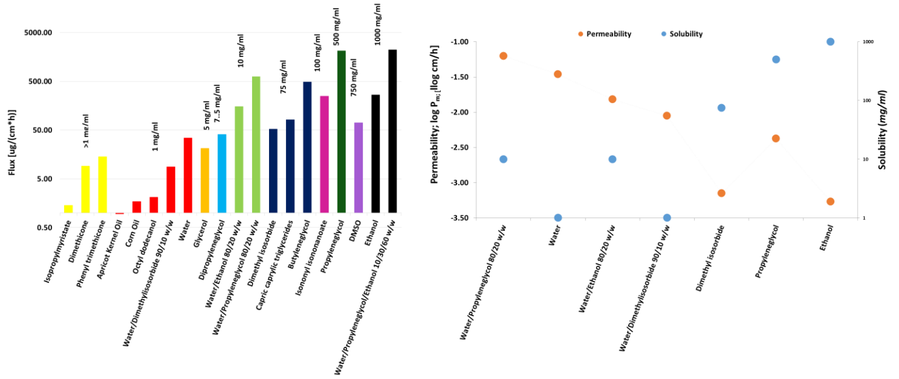

Figure 1. Flux of Symwhite as a function of solvents, grouped by the solubility (on the left) and comparison of permeability and solubility for selected solvents (on the right)

Flux of Symwhite is shown on **Figure 1 (left)** as a function of solvents and data are grouped based on the solubility. We have found that **different solvents provide significantly different solubility and permeability**. Since flux is given as the product of permeability and the donor concentration (**Eq.1**), the **higher solubility** at donor side in a given solvent does not mean proportional **increase in the flux**, but it depends on the **permeability in that solvent** as well. The expected relationship between solubility and permeability is supported by **Figure 2 (right)** i.e. **the higher donor phase solubility** induces **a decrease in permeability**, so flux do not change proportionally with donor solubility, which can be explained by the **solubility-permeability interplay** described by many publications[4].

RESULTS

Aminexil: Aminexil provided far **lower solubility** in most of the 23 solvents. 65 % of the solvents (15/23) belong to the c < 1 mg/ml category, since the polar AMX is **very slightly soluble in apolar solvents**. Despite the **good sensitivity of LC-MS** technique (LoQ = 0.04 µg/ml) the detected amount was below LoQ for 12 solvents therefore these results are not shown.

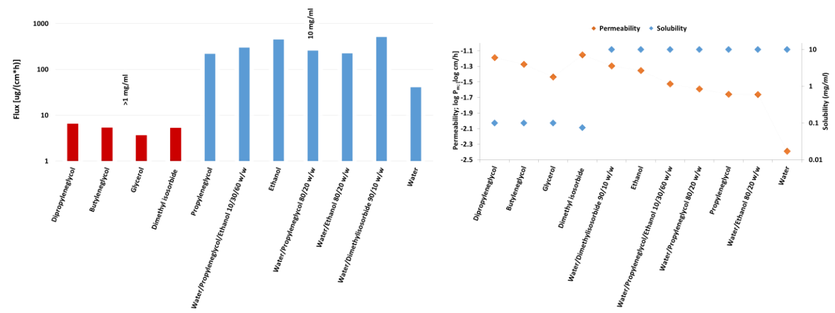

Figure 2. Flux of Aminexil as a function of solvents, grouped by the solubility (on the left) and comparison of permeability and solubility for selected solvents (on the right)

Permeability classification has been done similarly to Symwhite, but only **medium** or **high permeability** group could be identified, and solubility varied in a narrow range as well. **Figure 2 (left)** shows that one fold increase in donor concentration result in similar change in flux, so permeability of aminexil is less affected by the solvents. **Figure 2 (right)** demonstrates that the **expected trend** in solubility and permeability behavior have been found, but the differences are smaller. The **highest flux** was provided by **water/dimethylisosorbide 90/10 w/w**, which may be **possible solvents for product development. Dimethylisosorbide provided the highest permeability**, but solubility was too low to provide suitable flux, while water provides a good enough solubility with llow permeability, therefore the mixture of water and dimethylisosorbide is a beneficial combination, as demonstrated by the data. For AMX, permeability coefficient seems to be **relatively insensible to the nature of the solvent** except for **water** which shown **significantly lower permeability.**

CONCLUSION

Our results have **proved the applicability of Skin PAMPA** for the purpose. The system **distinguished the effect on permeability** of model compounds dissolved in **solvents with different polarity**. Based on the **logP_m values three categories** of solvents were defined: low, medium and high permeability providing ones. The Skin PAMPA can **help the solvents selection** at **early stage of product development**. **Study on *ex vivo* human skin** will be done on selected solvents for PER and AMX to compare ranking with **Skin PAMPA.**

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