# ESTIMATION OF BIOLOGICAL PROPERTIES OF PHARMACEUTICAL INTEREST USING LECITHIN LIPOSOMES AS THE PSEUDOSTATIONARY PHASE ON CAPILLARY ELECTROPHORESIS

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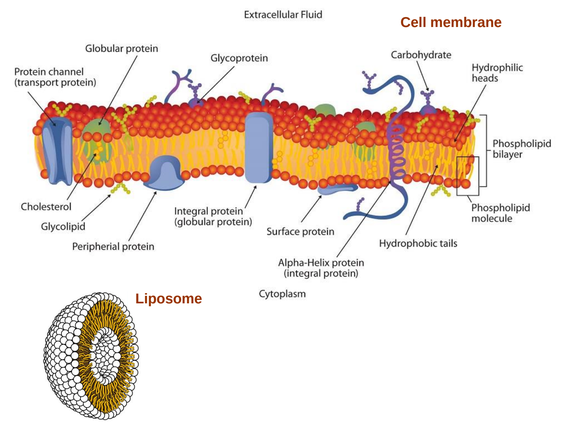
## INTRODUCTION

The cell membrane is selectively permeable to ions and organic molecules and regulates the concentration of the compounds in and out of the cell. There exist several biological functions that are regulated by cell membranes such as the intestinal absorption of nutrients or drugs, the skin absorption of drugs or the blood brain distribuiton of drugs. All of these processes depend on the partition of a solute (i.e. nutrient or drug) between two phases with different polarity (i.e. blood and a biological membrane). There are different methodologies to determine the nutrients/drugs partition in a biological system. While *in vivo* experiments provide reliable measurements, they often require expensive, long, and complex procedures. Furthermore, it is recommended to reduce the number of *in vivo* tests due to ethical reasons. As an alternative, predictive models allow to obtain information about biological properties using *in silico* or experimental procedures. The partition of solutes can be evaluated theoretically using QSAR (quantitative structure-activity relationships) models (*in silico*) or experimentally performing, for example, physicochemical measurements. The goal of this work is to develop a new physicochemical system able to surrogate biological properties of pharmaceutical interest.

Liposomes are microscopic hollow soft vesicles composed of one or more phospholipid bilayers surrounding an aqueous core which may mimic the cell membrane. Thus, we have measured the retention factor of several chemical substances in a LECK (liposome electrokinetic chromatography) system (using lecithin-based liposomes as pseudostationary phase); and next we have evaluated if this physicochemical property could be linearly related to a biological property of pharmaceutical interest (cell membrane mediated).




## EXPERIMENTAL

- Instrument: CE Agilent Technologies capillary electrophoresis device equipped with a DAD detector
- Capillary: fused silica TSP from Polymicro Technologies
- Separation solution: Liposome suspension, 48.5 cm total length, 40 cm effective length, 50 μm ID
- Separation conditions: 25 ºC, 15 kV
- Solutes: 200 mg/L in methanol:pbs 2:8, injection 3s 50 mbar
- Electroosmotic flow and liposome markers: methanol and dodecanophenone, respectively
- Pseudostationary phase preparation: Preliposome Pro-Lipo Neo™ from Lucas Meyer Cosmetics kindly provided by Comercial Química Jover (18-25% phospholipids, obtained from lecithin), 0.5% (w/w) of preliposome in phosphate buffer solution (PBS, 10 mM, pH 7.0). Magnetic stirring (1000 rpm, 45 min, <25ºC)

$$k = \frac{t_m - t_{eof}}{\left(1 - \frac{t_m}{t_{lp}}\right) t_{eof}}$$

k: retention factor
$t_m$: solute migration time
$t_{eof}$: methanol migration time
$t_{lp}$: dodecanophenone migration time

## RESULTS AND DISCUSSION

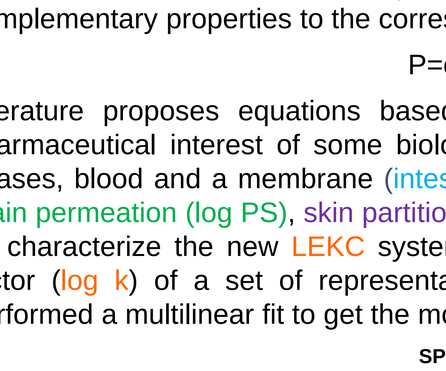
### SELECTION OF THE BIOLOGICAL SYSTEMS THAT MAY BE EMULATED WITH THE LEKC SYSTEM

The partition or a property related to the partition of a solute between two phases (P) can be modeled through quantitative structure-property relationship (QSPR) models, such as the solvation parameter model (SPM) (Eq 1) [1]. The model descriptors, those properties that define partition of a solute, are; E, that represents the excess molar refraction; S, the solute dipolarity/polarizability; A and B, the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively; and V, McGowan's solute volume. The coefficients of the equation (c, e, s, a, b, v) are characteristic of the system and reflect its complementary properties to the corresponding solute property.

$$P = c + eE + sS + aA + bB + vV \qquad \text{(Eq 1)}$$

Literature proposes equations based on the solvation parameter model to estimate properties of pharmaceutical interest of some biological systems that depend on the solutes partition between two phases, blood and a membrane (intestinal absorption (log HIA), blood-brain distribution (log BB), blood-brain permeation (log PS), skin partition (log $K_{sc}$), skin permeation (log $K_p$)) [2-5] (shown in the next table). To characterize the new LEKC system through the SPM, we have determined the logarithm retention factor (log k) of a set of representative substances with known SPM descriptors [6] and we have performed a multilinear fit to get the model coefficients (also shown in the next table).

| | | SPM Coeficients | | | | | | Statistics | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ***c*** **($SD_c$)** | ***e*** **($SD_e$)** | ***s*** **($SD_s$)** | ***a*** **($SD_a$)** | ***b*** **($SD_b$)** | ***v*** **($SD_v$)** | ***n*** | ***r*** | **SD** | ***F*** |
| Literature | log HA | 0.544 (0.092) | -0.025 (0.075) | 0.141 (0,062) | -0.409 (0.068) | -0.514 (0.042) | 0.204 (0.059) | 127 | 0.894 | 0.290 | 94 |
| | log BB | 0.044 (-) | 0.511 (-) | -0.886 (-) | -0.724 (-) | -0.666 (-) | 0.861 (-) | 148 | 0.843 | 0.367 | 71 |
| | log PS | -0.639 (0,498) | 0.312 (0.515) | -1.009 (0.158) | -1.895 (0.385) | -1.636 (0.410) | 1.709 (0.392) | 30 | 0.933 | 0.520 | 32 |
| | log $K_{sc}$ | 0.341 (0,093) | 0.341 (0.133) | -0.206 (0.096) | -0.024 (0.137) | -2.178 (0.158) | 1.850 (0.106) | 45 | 0.962 | 0.216 | 97 |
| | log $K_p$ | -5.426 (0,101) | -0.106 (0.129) | -0.473 (0.095) | -0.473 (0,148) | -3.000 (0.152) | 2.296 (0.137) | 119 | 0.912 | 0.461 | 112 |
| This work | log k | -1.869 (0.083) | 0.885 (0.118) | -0.809 (0.078) | 0.339 (0.065) | -2.865 (0.112) | 2.809 (0.092) | 46 | 0.968 | 0.125 | 240 |




Comparison of coefficients through PCA

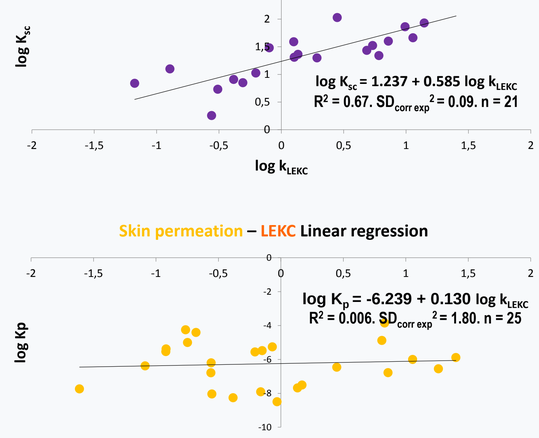
The closest systems to the **LECK** one are **Skin partition** and **Skin permeation**.

The main PCs plot (scores plot) distributes the different systems in a chemical space, so that systems with similar characteristics are close in the scores plot.

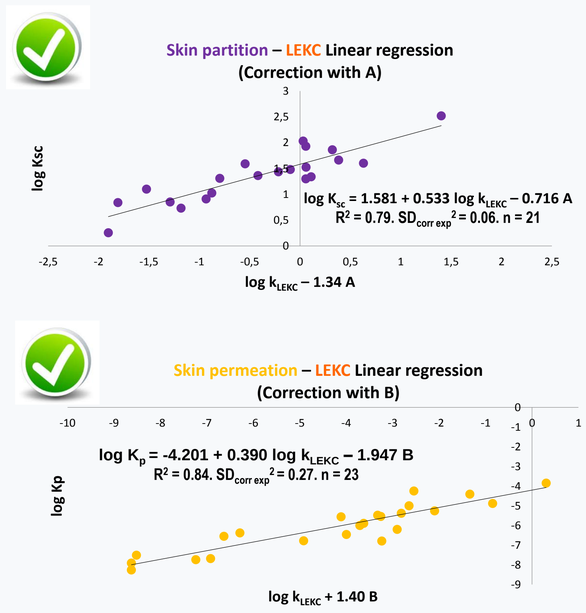
Emulation?

### BIOLOGICAL – PHYSICOCHEMICAL CORRELATIONS

Next graphics show the correlation parameters and statistics of the biological - physicochemical system experimental correlations found for Skin partition and Skin permeation. First, a direct correlation has been evaluated.




To improve the prediction ability the correlations have been corrected with one of the SPM descriptors (acidic descriptor A in the case of skin partition and basic descriptor B in the case of skin permeation).




## CONCLUSIONS

- The partition of solutes in a new LEKC system based on lecithin liposomes has been characterized. Those with a high hydrophobicity are highly retained while those with high hydrogen bond basicity are low retained.
- The new LEKC system is able to emulate the skin partition. The surrogation of skin partition improves when corrected by the acidity of the compound.
- A correction of the basicity of the compounds also allows to emulate the skin permeation.

## REFERENCES

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