Estimation of the octanol-water distribution coefficient of acidic compounds by microemulsion electrokinetic chromatography measurements

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Introduction

Lipophilicity, which is defined as the ability of a compound to be dissolved in lipids or non-polar solvents, is a key factor in drug development. This property is related to the capacity of a substance to go through different biological membranes, which are formed by a lipid bilayer. Therefore, the effectiveness of a compound as a drug candidate will be clearly linked to its lipophilicity, the higher the value of this property, the better, as well as the hydrophobic-hydrophilic balance is maintained.

In order to estimate the lipophilicity of a substance, different parameters can be evaluated. Among these, the most widely used is the octanol-water partition coefficient. However, the direct evaluation of this parameter (shake-flask method) is time-consuming and it is not automated. Because of these drawbacks, other alternatives have been studied. Recently, a method to estimate the octanol-water partition coefficient of neutral substances at different pH values using microemulsion electrokinetic chromatography (MEEKC) measurements has been developed [1]. However, considering that most part of drugs from the pharmaceutical industry are acids or bases, a fast determination of this parameter for ionic compounds is of great interest. Therefore, the aim of this work is to estimate the distribution coefficient of acidic solutes at several degrees of ionization through MEEKC measurements.

In order to estimate the $logP_{ow}$ values of new substances (neutral or ionic) a correlation between this parameter and the logarithm of their retention factor in the microemulsion system considered has been established.

Theory

In the MEEKC systems, the ionizable acids are separated due to two main separation processes [2]:

- The partition of the compound between the microemulsion and the aqueous phase.
- The electrostatic attraction of the deprotonated fraction of the analyte to the anode.

The work is performed in two solutions with different viscosities. So, a density correction factor is needed.

In order to calculate the retention factor (k) taking into account only the hydrophobic interaction between the microemulsion and the solutes tested, the following expression has been employed :

$$k = \frac{\mu - (\frac{\eta_{(CZE)}}{\eta_{(me)}})\mu_0}{\mu_{me} - \mu} \quad \text{Eq. 1}$$

In equation 1:

- k: Retention factor.
- μ: Electrophoretic mobility of the compound in microemulsion.
- $\eta_{(CZE)}/\eta_{(me)}$: Density correction factor.
- μ_0 : Electrophoretic mobility of the compounds in CZE conditions*.
- μ_{me} : Electrophoretic mobility of the microemulsion marker.

* Analysis performed with surfactant below the CMC (the microemulsion is not formed).

Experimental section

- Instrument: CE Agilent Technologies capillary electrophoresis device equipped with a DAD detector.
- Capillary: fused silica TSP from Polymicro Technologies, 38,5 cm total length, 30 cm effective length, 50 μm ID.
- Electroosmotic flow and microemulsion markers: DMSO and dodecanophenone, respectively.
- Solutes:
  - Analyses in microemulsion: 100 mg/L in methanol:microemulsion 1:9.
  - Analyses in CZE: 100 mg/L in methanol:CZE solution 1:9.

- Injection: 5s 50 mbar.
- Separation conditions: 25 ºC, 8-15 kV.
- Separation solution in CZE:
  - Surfactant: SDS at 0.06% w/v (under CMC).
  - Phosphate or acetate buffer at 50 mM ionic strength.
- Separation solution in microemulsion [1] (see Figure 1):
  - Surfactant: SDS 1.3% w/v.
  - Cosurfactant: 1-butanol 8.15% v/v.
  - Oil: Heptane 1.15% v/v.
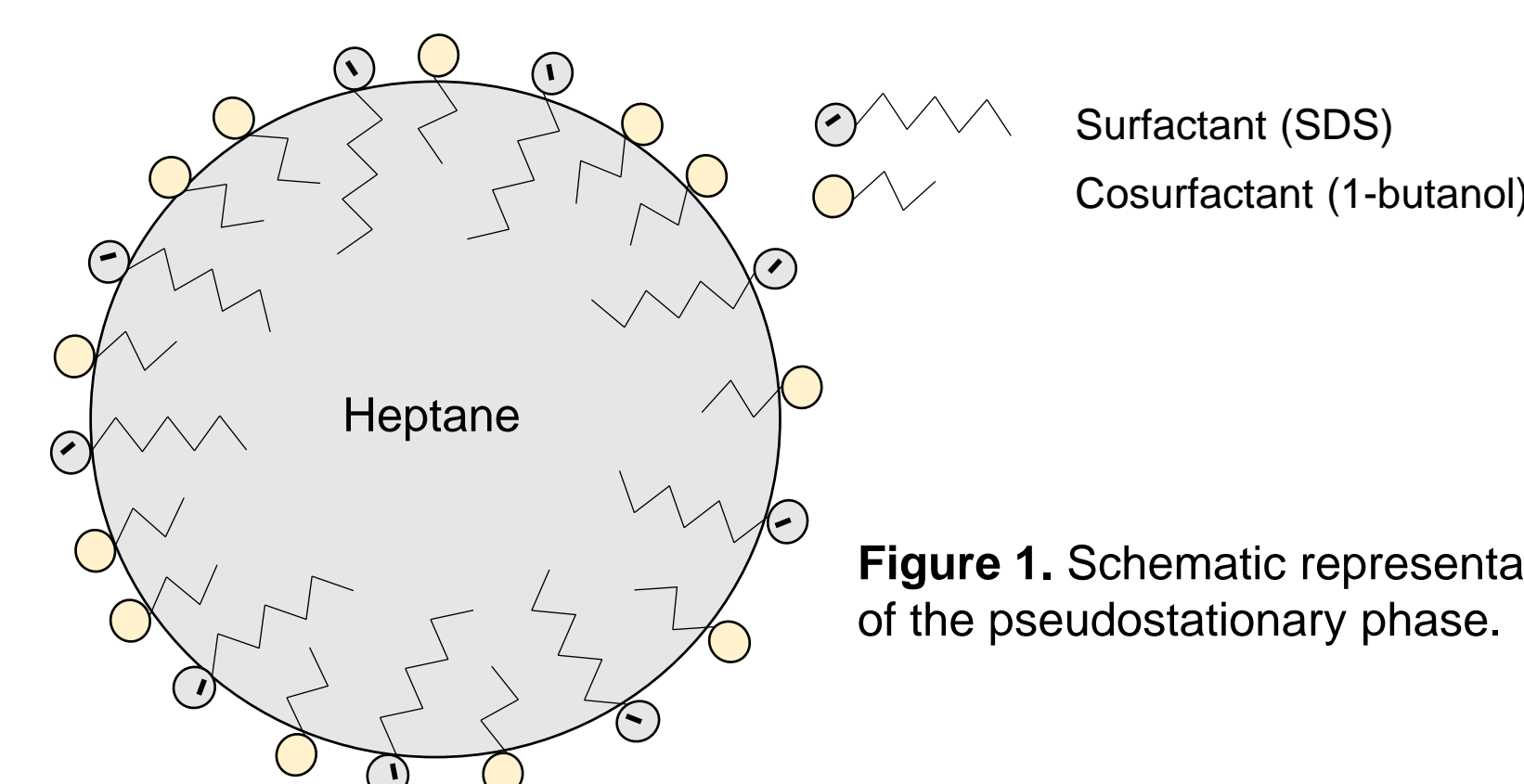 - Phosphate or acetate buffer at 50 mM ionic strength.

Figure 1. Schematic representation of the pseudostationary phase.

Results and discussion

The retention factor of six ionizable acids, with a well-known lipophilicity behavior has been measured in the MEEKC system at several pH values ranging from 2 to 8.
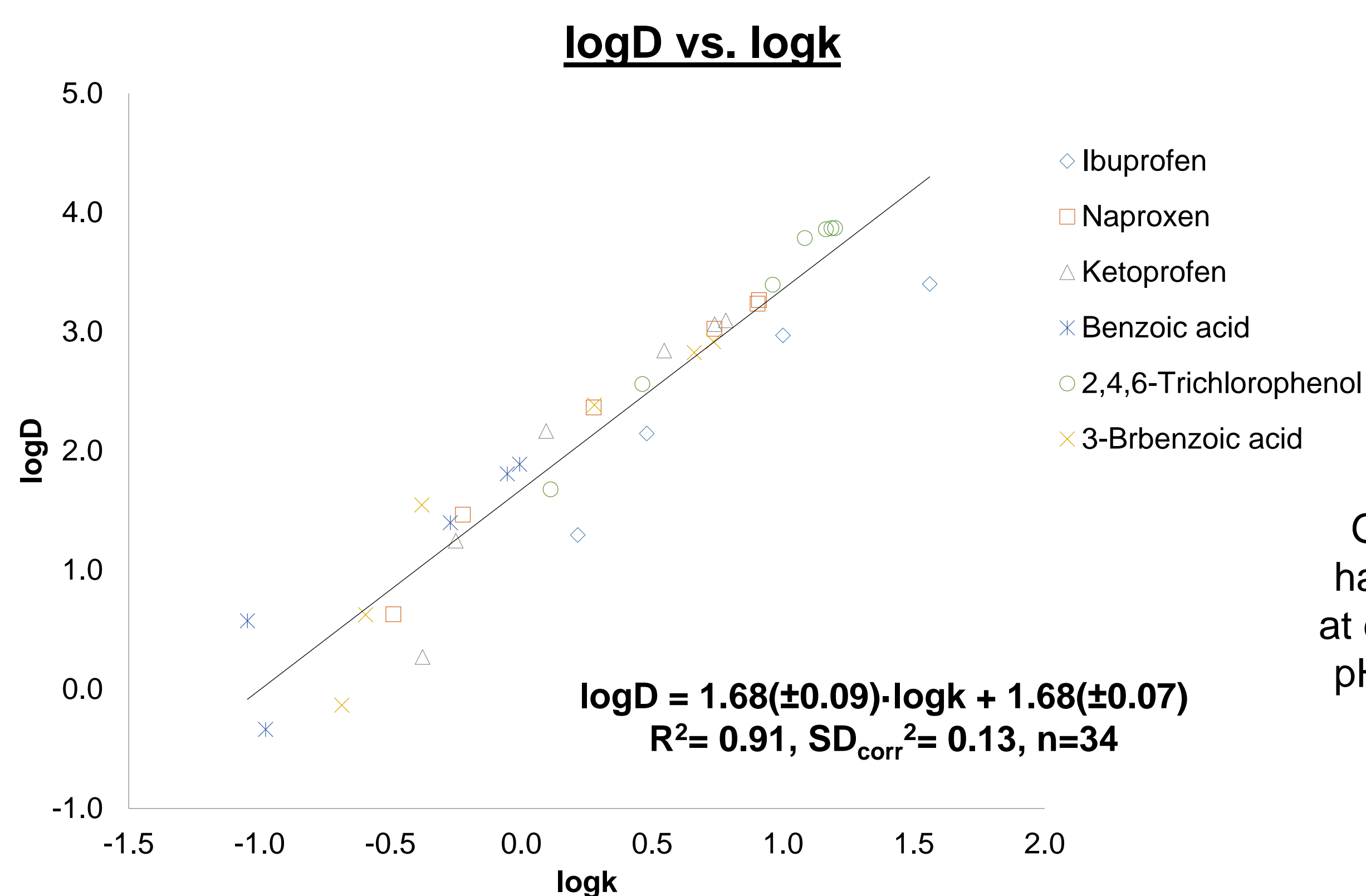
The acids that have been selected are:

- Benzoic acid
- 3-Bromobenzoic acid
- 2,4,6-trichlorophenol
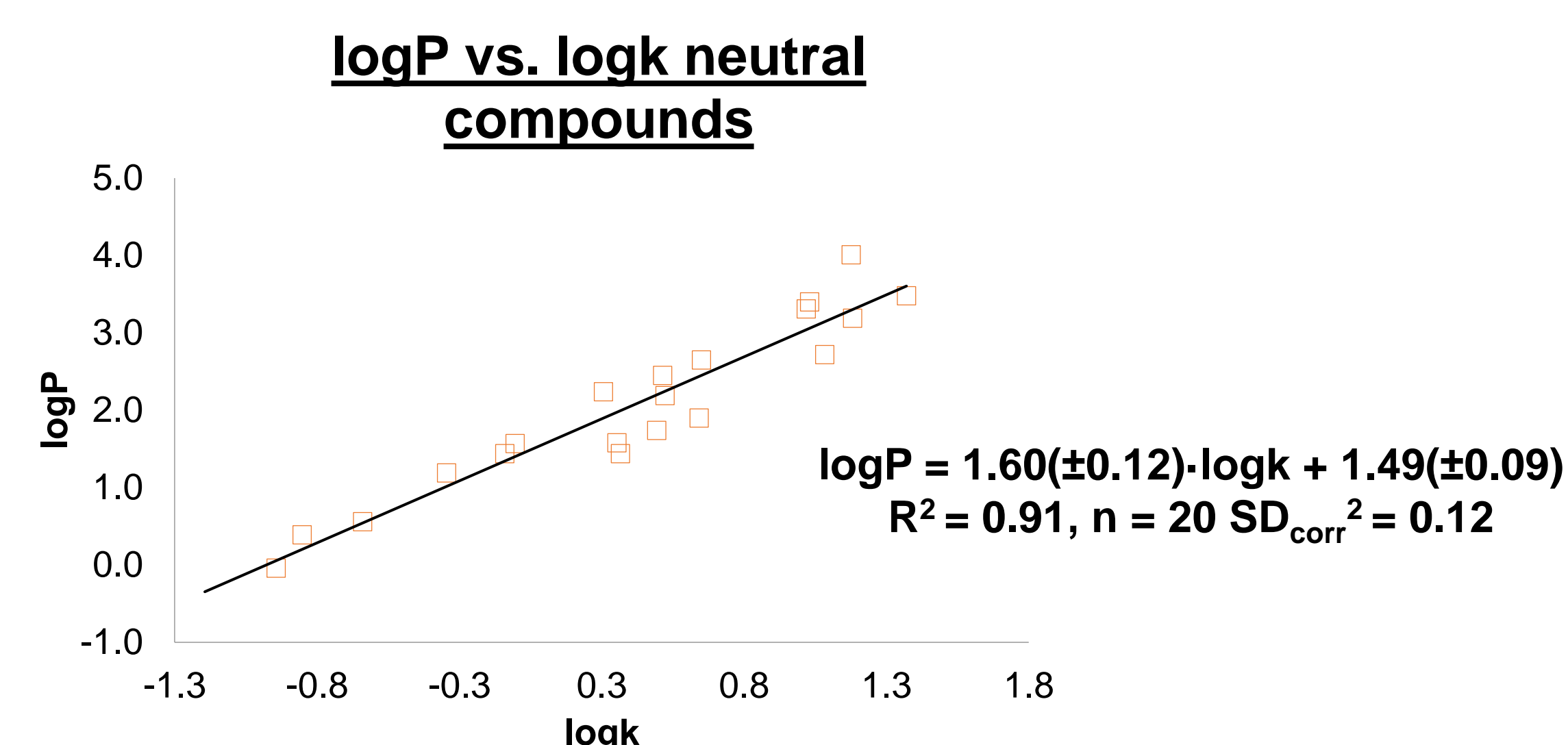- Ibuprofen
- Naproxen
- Ketoprofen

They have:

- pK_a values between 3.5 – 6.5.
- A well-defined lipophilicity profile [3-6].
- Chromophore groups.

The logD of the compounds has been calculated at specific pH values by extrapolation of the logD-pH profile. Then, it has been plotted the logD vs. the logk of the six acids at different degrees of ionization. The results obtained are as follows:

logD vs. logk

Legend:
- ◇ Ibuprofen
- □ Naproxen
- △ Ketoprofen
- ✕ Benzoic acid
- ○ 2,4,6-Trichlorophenol
- ✕ 3-Brbenzoic acid

$logD = 1.68(\pm 0.09)\cdot logk + 1.68(\pm 0.07)$
$R^2 = 0.91$, $SD_{corr}^2 = 0.13$, n=34
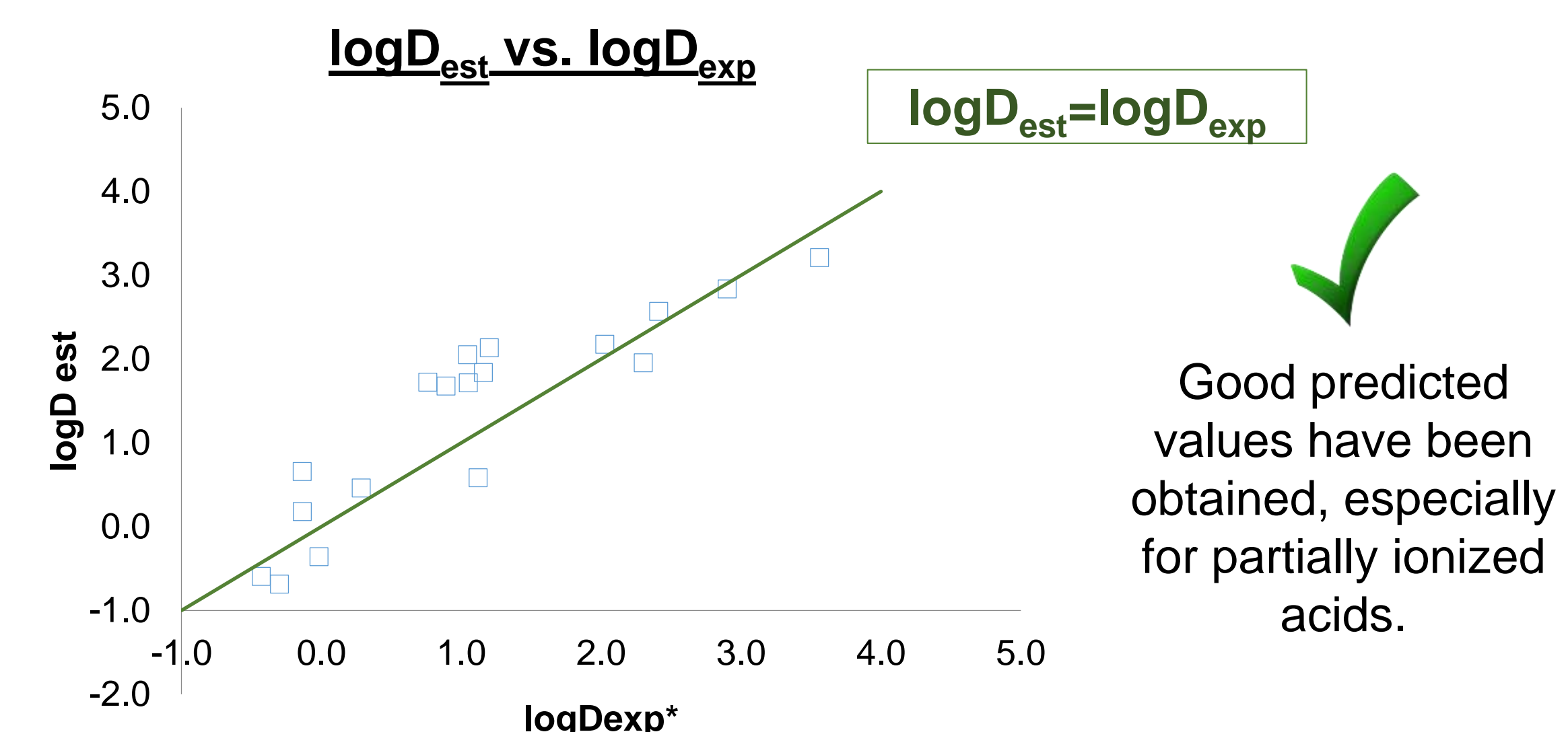
Good correlation has been obtained at different values of pH and degrees of ionization.

For a concrete pH value (7.4, blood's pH), the logP vs. logk of neutral compounds have been plotted:

logP vs. logk neutral compounds

$logP = 1.60(\pm 0.12)\cdot logk + 1.49(\pm 0.09)$
$R^2 = 0.91$, n = 20 $SD_{corr}^2 = 0.12$

This equation for neutral compounds agrees with the one obtained for the six acids considered in the present study analyzed at different degrees of ionization. Therefore, using the equation obtained with the neutral solutes at a pH of 7.4 as calibration, the logD values for 18 acids at different degrees of dissociation can be estimated.

logD_est vs. logD_exp

$logD_{est}=logD_{exp}$

Good predicted values have been obtained, especially for partially ionized acids.

* Experimental logD values at pH 7.4 have been obtained from Bioloom database [7].

Conclusions

- Good correlations between the logD and logk in MEEKC at different pH values and degrees of ionization for several compounds have been observed.
- For a specific pH (7.4, blood's pH) the logD values for partially and fully ionized acids can be predicted using a calibration performed with neutral compounds.

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