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Short communication

Feasibility of the estimation of octanol-water distribution coefficients of acidic drugs by microemulsion electrokinetic chromatography

Alejandro Fernández-Pumarega[1], Susana Amézqueta[1,*], Elisabet Fuguet[1,2], Martí Rosés[1]

[1]*Departament de Química Analítica and Institute of Biomedicine of the University of Barcelona (IBUB), Universitat de Barcelona, Martí i Franquès 1-11, 08028, Barcelona, Spain*
[2]*Serra Húnter Programme, Generalitat de Catalunya*

*Corresponding Author: E-mail: samezqueta@ub.edu; Tel.:+34934021277

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Abstract

Previous studies have shown that a microemulsion electrokinetic chromatography (MEEKC) system can estimate the logarithm of the octanol-water partition coefficient (log $P_{o/w}$) of neutral solutes. In the present work, the applicability of the method to partially and fully ionized acids has been evaluated. Naproxen, a monoprotic acid, has been used as test solute. The retention factor (k) of this compound has been measured in MEEKC at several values of pH and the retention factor-pH profile has been established. As log $P_{o/w}$ correlates with log k_{MEEKC} for neutral compounds, this correlation has been used to estimate the logarithm of the octanol-water partition coefficient of the neutral (log $P_{o/w(HA)}$), and the fully ionized (log $P_{o/w(A^-)}$) forms of naproxen. Then, the logarithm of the octanol-water distribution coefficient (log $D_{o/w}$) of the partially ionized form of the acid has been estimated. The comparison of the estimated values with the ones obtained experimentally using the classical procedures, such as the shake-flask method, shows differences under 0.4 log $D_{o/w}$ units either if the acid is partially ionized or in its neutral form in the most part of the pH range. However, the method overestimates the log $D_{o/w}$ of the highly (>99.5 %) or fully ionized form of naproxen.

Keywords

Capillary electrophoresis; MEEKC; log $D_{o/w}$; lipophilicity

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 discovery. This property, among others, is related to the capacity of a substance to pass through different biological membranes, which are formed mainly by a lipid bilayer. Therefore, the viability of a compound as a drug candidate is clearly linked to its lipophilicity. 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 ($P_{o/w}$). However, the direct evaluation of this parameter (following the shake-flask method) is time-consuming and it is not fully automated. Because of these drawbacks, the development of faster high-throughput methods is of a great interest.

Ishihama and coworkers [1] introduced a method able to evaluate the lipophilicity of a substance through a system based on microemulsion electrokinetic chromatography (MEEKC). The MEEKC system used is composed of two main phases, an aqueous phase (buffer) and a pseudostationary phase (the microemulsion).

Further studies [2,3] followed the method and correlated satisfactorily the logarithm of the octanol-water partition coefficient (log $P_{o/w}$) to the logarithm of the retention factor in MEEKC (log k_{MEEKC}). Abraham *et al.* [2] correlated log $P_{o/w}$ to log k_{MEEKC} measured in a system with a microemulsion composed of 1.44 % (w/w) SDS, 6.49 % (w/w) 1-butanol, and 0.82 % (w/w) heptane, obtaining the following equation:

$$\log P_{o/w} = 1.542 + 1.276 \cdot \log k_{MEEKC} \quad , \qquad R^2=0.99;\ SD=0.096;\ n=53 \quad . \tag{1}$$

Subirats *et al.* [3] performed the same procedure using a microemulsion consisting of 1.3 % (w/v) SDS, 8.15 % (v/v) 1-butanol, 1.15 % (v/v) heptane, and 5 % (v/v) acetonitrile (at pH 7.4, in a 10 mM phosphate buffer). Acetonitrile was added to overcome the co-elution of the microemulsion marker with the most lipophilic compounds. The resulting equation of the correlation and the statistics are:

$$\log P_{o/w} = 1.48\ (\pm 0.05) + 1.48\ (\pm 0.05) \cdot \log k_{MEEKC} \quad , \qquad R^2=0.96;\ n=32 \quad . \tag{2}$$

Taking into account Equations 1 and 2 it can be concluded that this method is able to predict the octanol-water partition coefficient of new compounds in their neutral form through electrokinetic measurements.

Considering that most part of pharmaceutical drugs 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 test the applicability of MEEKC measurements to estimate the logarithm of the octanol-water distribution coefficient (log $D_{o/w}$) of acidic solutes at several degrees of ionization. Naproxen has been employed as test solute since it is an acid that presents a pK_a close to 4 (within the electrophoretic working pH range), its lipophilicity-pH profile is well-defined in the literature [4,5], and it contains chromophore groups (therefore detectable by UV-vis).

Experimental

A capillary electrophoresis system (CE 7100) equipped with a diode array from Agilent technologies (Santa Clara, CA, USA) was employed to do the electrophoretic measurements. The fused-silica capillary used was from Polymicro technologies (Lisle, IL, USA) with an effective length of 25 or 30 cm (depending on the pH value) and an internal diameter of 50 µm. The microemulsion used as the pseudostationary phase was prepared in a phosphate or acetate buffer at 0.05 M ionic strength. First, the surfactant (SDS) was dissolved in the buffer at 1.30 % (w/v), then the co-surfactant (1-butanol) was added at 8.15 % (v/v), followed by the addition of the oil (heptane) at 1.15 % (v/v). The solution was stirred and sonicated until the turbidity was no longer observed [3].

The analyses were performed using two different solutions (in the microemulsion and in a buffer without the pseudostationary phase) at 25 °C, applying a voltage ranging from 8 to 15 kV, and an internal pressure ranging from 0 to 50 mbar (depending on the buffer and the pH of each experiment). The solutes were injected with a pressure of 50 mbar during 5 s. Dodecanophenone was employed as the microemulsion marker and DMSO as the electroosmotic flow marker. The solute to be injected was disolved in microemulsion/methanol mixture at 9:1 ratio, in MEEKC measurements, and in water/methanol mixture at 9:1 ratio, in plain buffer measurements, with a final concentration of approximately 200 mg/L.

Theory

The migration behavior of an acidic compound when it is partially or totally ionized is a contribution of two main factors [6]: the hydrophobic interaction of the compound with the pseudostationary phase (the microemulsion), and the electrophoretic mobility of the anionic acid by its electrostatic attraction to the anode (with positive charge). Retention factor of ionized compounds can be calculated through the next equation:

$$k = \frac{\mu - \mu_0}{\mu_{me} - \mu} \quad , \tag{3}$$

where, k is the retention factor of the solute, μ the overall observed mobility of the compound, μ_{me} the mobility of the microemulsion phase (obtained by the mobility of the microemulsion marker), and μ_0 the mobility of the compound in the aqueous buffer without the microemulsion, i.e., in capillary zone electrophoresis (CZE).

In parallel studies of the research group, it has been observed that the viscosity of the buffer can affect the electrophoretic mobility of the compound. So, in order to determine the retention factor of ionizable acids, as solutions with different viscosities have been used, a viscosity correction factor has to be introduced [7].

Results and Discussion

In order to estimate the log $D_{o/w}$ values of naproxen at several degrees of ionization through electrophoretic measurements a calibration curve that correlates log $P_{o/w}$ *vs.* log k_{MEEKC} has been performed. 20 neutral compounds with known log $P_{o/w}$ values [8,9] have been used to establish the curve. The substances that have been chosen present log $P_{o/w}$ values uniformly distributed between approximately 0 and 4 units. The equation of the calibration curve obtained and their statistics are as follows:

$$\log P_{o/w} = 1.60\ (\pm 0.11) \cdot \log k_{MEEKC} + 1.51\ (\pm 0.08) \quad , \qquad R^2 = 0.92;\ SD = 0.33;\ n = 20 \quad , \tag{4}$$

as expected, Equation 4 shows a good correlation between log $P_{o/w}$ and log k_{MEEKC} for neutral compounds, and it is very similar to Equations 1 and 2.

As the retention factor of acidic compounds is the weighted average of the retention factor of the neutral ($k_{(HA)}$) and the fully ionized ($k_{(A-)}$) forms of the compound:

$$k = (1 - \alpha) \cdot k_{(HA)} + \alpha \cdot k_{(A-)} \quad , \tag{5}$$

and the ionization degree (α) can be calculated using the following formula:

$$\alpha = \frac{10^{pH - pKa}}{1 + 10^{pH - pKa}} \quad , \tag{6}$$

an equation that relates retention factor to pH can be easily obtained [6]:

$$k = \frac{k_{(HA)} + k_{(A-)} \cdot 10^{pH - pKa}}{1 + 10^{pH - pKa}} \quad . \tag{7}$$

To study the applicability of the method to ionizable acidic compounds, the retention factor of naproxen has been measured in the MEEKC system at 6 different pH values, ranging from 2 to 7 (covering all of the degrees of ionization of the acid). Then Equation 7 has been fitted to the measured retention factors. Figure 1 shows the experimental values (x) and the retention-pH profile obtained by fitting Equation 7 to the experimental data (solid line).

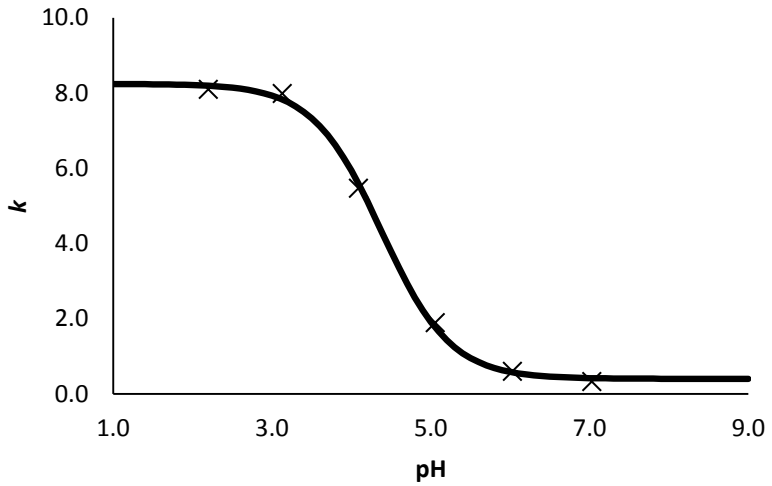

Figure 1. Retention factor-pH profile of naproxen.

The parameters obtained from the retention profile in Figure 1, with their respective statistics are: $k_{(HA)}$ = 8.24 (±0.13); $k_{(A-)}$ = 0.40 (±0.11); pK_a = 4.38 (±0.04); (R^2 = 0.999; F = 1223; SD = 0.16).

Using Equation 4, and $k_{(HA)}$ and $k_{(A-)}$ obtained from the fit of Equation 7 (Figure 1), the logarithm of the octanol-water partition coefficient of the neutral and fully ionized forms of the acid can be calculated: log $P_{o/w(HA)}$=2.97 and log $P_{o/w(A-)}$=0.87, respectively.

Similarly to the retention factor, the octanol-water distribution coefficient ($D_{o/w}$) is also related to the ionization degree of the compound through the following equation:

$$D_{\mathrm{o/w}} = (1-\alpha)\cdot P_{\mathrm{o/w(HA)}} + \alpha \cdot P_{\mathrm{o/w(A-)}} \cdot \qquad (8)$$

So, using Equation 8 and considering the pK_a value of the compound (pK_a=4.38, obtained from Figure 1 data) it is possible to estimate the log $D_{o/w}$ of naproxen at any degree of ionization. Table 1 presents the experimental log $D_{o/w}$ values reported in the literature determined by the shake-flask [4] or the pH-metric methods [5], and the log $D_{o/w}$ estimated values which have been calculated using Equation 8.

Table 1. Differences between literature and estimated log $D_{o/W}$ values of naproxen at several pH values.

pH	log $D_{o/w}$ (literature)	α	(1-α)·$D_{o/w(HA)}$	α·$D_{o/w(A-)}$	log $D_{o/w}$ (estimated)	log $D_{lit.}$-log $D_{est.}$
1.00[a]	3.35	0.000	942.36	0.00	2.97	0.38
2.00[a]	3.34	0.004	938.84	0.03	2.97	0.37
3.35[a]	3.05	0.085	862.28	0.63	2.94	0.11
4.50[a]	2.68	0.569	406.67	4.22	2.61	0.07
5.50[a]	2.07	0.929	66.47	6.90	1.87	0.20
5.5[b]	2.1	0.929	66.47	6.90	1.9	0.2
6.5[b]	1.1	0.992	7.10	7.37	1.2	-0.1
6.70[a]	0.79	0.995	4.49	7.39	1.07	-0.28
7.40[a]	0.33	0.999	0.90	7.42	0.92	-0.59
7.4[b]	0.3	0.999	0.90	7.42	0.9	-0.6
8.20[a]	0.27	1.000	0.14	7.43	0.88	-0.61
9.20[a]	0.25	1.000	0.01	7.43	0.87	-0.62

[a] Data obtained from ref. [4].
[b] Data obtained from ref. [5].

The differences between estimated (obtained by MEEKC measurements) and literature log $D_{o/W}$ data are in most cases under 0.4. Higher differences have been observed only at those values of pH where the acid is highly or fully ionized (>99.5 %). This higher error could be explained due to the possible formation of different ion-pairs, depending on the experimental conditions, or due to the low retention of fully ionized acid species in the MEEKC system (meaning higher experimental errors as the magnitude of *k* is smaller than that of the neutral species). Moreover, these differences could also be attributed to a surface between the aqueous and the lipid phase bigger in the microemulsion than in the octanol-water system. In this way, the retention of the compounds in MEEKC will be larger even if they are charged as they will be attached to the hydrophobic phase even if they do not partition into it. Further research is needed to confirm these differences, clarify them, and propose additional correction for the ionized forms of the acidic drugs. The direct applicability of the method to ionizable basic compounds is doubtful because of the possible ion-pair formation between the protonated base and the anionic surfactant. Work is in progress to clarify this point.

Conclusions

Accurate predictions of log $D_{o/w}$ have been obtained for naproxen at different degrees of ionization using the MEEKC system presented in this work. At those pH values where the compound is in its neutral form or only partially ionized, the error obtained is in the order of the error obtained for the estimation of the log $P_{o/w}$ of neutral compounds and the one of experimental determinations. Only at very high ionization degrees (>99.5 %) an overestimation of the log $D_{o/w}$ is observed. This work shows that the method based in MEEKC is an alternative to the classical shake-flask procedure to determine log $D_{o/w}$ values for ionized species of this acid. A deeper study is necessary to check if this behavior can be extrapolated to other compounds.

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