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## Correlation between the logarithm of capacity factors for aromatic compounds in micellar electrokinetic chromatography and their octanol–water partition coefficients

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### Abstract

The correlation between the logarithm of the capacity factors ( $\log k'$ ) and the logarithm of the octanol–water partition coefficients ( $\log P_{ow}$ ) for a group of 20 organic compounds (ten benzene derivatives and ten polycyclic aromatic hydrocarbons) was studied in micellar electrokinetic chromatography. Sodium dodecyl sulphate micelles in different aqueous and hydro-organic (*n*-propanol and *n*-butanol) buffers were used as electrolyte solutions. A linear relationship between  $\log k'$  and  $\log P_{ow}$  was found. This correlation improved when the percentage of alcohol in the buffer system was increased and when the polarity of the alcohol was decreased.

**Keywords:** Octanol–water partition coefficients; Capacity factors; Buffer composition; Aromatic compounds; Benzenes; Polynuclear aromatic hydrocarbons

### 1. Introduction

Hydrophobicity is commonly understood as a measure of the relative tendency of a solute to prefer a nonaqueous rather than an aqueous environment and plays an important role in the biological and physicochemical behavior of numerous types of organic compounds [1–4]. Solute hydrophobicity can be expressed by the partition coefficient,  $P$ , between water and an immiscible, nonpolar solvent. It has been found that the use of *n*-octanol and water to determine  $P$  values (represented by  $\log P_{ow}$ ) is very useful in the study of the interactions between biological systems and organic compounds [4].

Traditionally, the logarithm of the octanol–water partition coefficient ( $\log P_{ow}$ ) was determined using the shake-flask technique. Long analysis times and certain problems encountered in the analysis of low solute concentrations in both phases at polarity extremes are some of the most significant disadvantages of this method [5]. The latter problem usually prevents application of this method to those solutes with  $\log P_{ow} > 4$ . Another direct method for  $\log P_{ow}$  measurement is counter-current chromatography (CCC) [6] which uses an octanol–water biphasic system solvent. The mobile phase is water saturated with octanol, and the stationary phase is octanol saturated with water. This technique is also time-consuming and unsuitable for high  $\log P_{ow}$  values.

Chromatographic methods for indirect  $\log P_{ow}$

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determination have also been explored. A relationship between the logarithm of the capacity factor ( $\log k'$ ) of a noncongeneric series of non-ionized solutes and their  $\log P_{ow}$  values was obtained in reversed-phase high-performance liquid chromatography (RP-HPLC) with hydro-organic mobile phases [7–9]. However, this method is also questionable because the scale of hydrophobicity obtained has a limited range and depends on the partition chromatographic system [7–10].

The use of a bulk solvent such as octanol as a model for complex systems such as biomembranes has been criticized. Biomembranes consisting of phospholipids are different from octanol–water systems in terms of rigid structures, anisotropic and amphiphilic properties, and limited sizes. In this case, some alternative models were proposed such as liposomes [11,12], cells [11,13] and micelles [14,15].

Micellar liquid chromatography (MLC), in which a surfactant at a concentration above its critical micellar concentration is used in the mobile phase, has proved to be a useful tool for evaluating  $\log P_{ow}$  values. Several studies on the correlation between MLC retention and  $\log P_{ow}$  have been published [16–23].

Micellar electrokinetic chromatography (MEKC), which is a mode of capillary electrophoresis (CE), is an interesting alternative to study the correlation between retention and  $\log P_{ow}$ , but it has been scarcely used in this type of study [24–26]. A linear correlation between the logarithm of the capacity factor obtained for a solute in MEKC and its  $\log P_{ow}$  can be expected. The separation mechanism in MEKC provides an ideal environment to generate hydrophobicity estimations without the complications that arise from HPLC stationary phase effects because there is no chemically bonded stationary phase present [25]. Elimination of the stationary phase decreases the error caused by intercolumn variability [25]. However, MEKC is a limited analytical technique for determination of capacity factor values for hydrophobic compounds [27]. For these compounds, the estimated error in capacity factors determination could be very high.

In this work, the correlation between  $\log k'$  for a group of 20 organic compounds obtained in a MEKC system and their  $\log P_{ow}$  has been studied in elec-

trolyte solutions of different nature. The experimental conditions to obtain good correlations between  $\log k'$  and  $\log P_{ow}$  have been established accordingly.

## 2. Theory

The capacity factor,  $k'$ , for neutral solutes in MEKC can be calculated as follows:

$$k' = (t_r - t_0)/t_0[1 - (t_r/t_m)] \quad (1)$$

where  $t_r$ ,  $t_0$  and  $t_m$  are the migration times of the solute, electro-osmotic flow marker and the micelle, respectively.

On the other hand, according to the well known equation in chromatography in which the capacity factor is related to the micelle–water partition coefficient ( $P_{mw}$ ), the capacity factor in MEKC can be expressed by:

$$k' = P_{mw}(V_m/V_w) \quad (2)$$

where  $V_m$  and  $V_w$  are the volumes of the micellar and the aqueous phases, respectively.

Despite the existence of certain differences in partitioning behavior in micelles compared to that in octanol, there is a correlation between micelle–water and octanol–water partition coefficients for a group of compounds with a similar partitioning behavior in these systems [14,16]. Hence, an approximately linear relationship between  $\log P_{mw}$  and  $\log P_{ow}$  is obtained and can be expressed by the Collander equation [28]:

$$\log P_{mw} = a_1 \log P_{ow} + a_2 \quad (3)$$

where  $P_{ow}$  is the octanol–water partition coefficient, and  $a_1$  and  $a_2$  are constants.

From Eqs. (2,3), an approximately linear relationship between  $\log k'$  and  $\log P_{ow}$  can also be obtained:

$$\log k' = b_1 \log P_{ow} + b_2 \quad (4)$$

where  $b_1$  and  $b_2$  are constants.

Table 1  
Log  $P_{ow}$  values for the 20 compounds studied

Solute	Log $P_{ow}$
1. Benzene	2.13 <sup>a</sup>
2. Benzylic alcohol	1.10 <sup>a</sup>
3. Benzamide	0.64 <sup>a</sup>
4. Toluene	2.69 <sup>a</sup>
5. Benzonitrile	1.56 <sup>a</sup>
6. Nitrobenzene	1.85 <sup>a</sup>
7. 2-Phenylethanol	1.36 <sup>a</sup>
8. Chlorobenzene	2.84 <sup>a</sup>
9. Phenylacetonitrile	1.56 <sup>a</sup>
10. 3,5-Dimethylphenol	2.35 <sup>a</sup>
11. Naphthalene	3.37 <sup>a</sup>
12. Naphthylamine	2.22 <sup>a</sup>
13. Pyrene	4.88 <sup>a</sup>
14. Phenanthrene	4.46 <sup>a</sup>
15. 2,3-Benzofluorene	5.03 <sup>b</sup>
16. Fluorene	4.18 <sup>a</sup>
17. Fluoranthene	4.50 <sup>b</sup>
18. Acenaphthylene	3.48 <sup>b</sup>
19. Acenaphthene	3.82 <sup>b</sup>
20. Anthracene	4.45 <sup>a</sup>

<sup>a</sup> Values from Ref. [4].

<sup>b</sup> Values from Ref. [29].

### 3. Experimental

Table 1 summarizes the benzene derivatives and the polycyclic aromatic hydrocarbons studied in this work together with their log  $P_{ow}$  values.

Experimental micellar electrokinetic chromatographic data used in this work were previously determined to obtain solute–micelle association constants of the compounds studied as indicated in Ref. [27]. Briefly: a P/ACE System 2050 capillary electrophoresis (Beckman, Fullerton, CA, USA) with UV detection (214 nm) and 25  $\mu$ m I.D. capillaries (Polymicro Technologies, Phoenix, AZ, USA) were used. Ammonium acetate (pH 9) and 2-(*N*-cyclohexylamino)-ethanesulphonic acid (CHES) (pH 10) buffers were used. Sudan III or benzo[*a*]pyrene and dimethylformamide were used to determine micelle migration time and electro-osmotic flow time respectively. The washing routine employed for the capillary prior to each injection in order to determine solutes capacity factors and solute–micelle association constants was the following: Milli-Q water for 3 min, 0.1 *M* sodium hydroxide for 3 min, Milli-Q

water for 2 min and separation buffer for 3 min. Working temperature was 25°C and the electrical field strength applied was 15 kV.

#### 3.1. Data manipulation

Wilcoxon matched-pairs test and box plots were carried out using the SOLO Statistical System [30]. Box plot is used when a sample summary display of the distribution is desired. This is defined in terms of percentiles and shows the median and spread of the

Table 2  
Values for the squared correlation coefficients for the variations log  $k'$ –log  $P_{ow}$  for different SDS electrolyte solutions and for all compounds studied

Buffer	[SDS] ( <i>M</i> )	log $k'$ (20)
0.10 <i>M</i> CHES (pH 10)	0.010	0.9318
	0.020	0.9510
	0.030	0.9516
	0.040	0.9524
	0.050	0.9500
0.05 <i>M</i> Acetate (pH 9)	0.010	0.9392
	0.015	0.9460
	0.020	0.9521
	0.025	0.9507
	0.030	0.9514
0.05 <i>M</i> CHES (pH 10)	0.030	0.9523
	0.040	0.9497
	0.050	0.9454
	0.060	0.9399
	0.070	0.9448
0.05 <i>M</i> CHES–3% <i>n</i> -propanol (pH 10)	0.010	0.9561
	0.020	0.9669
	0.030	0.9665
	0.040	0.9681
	0.050	0.9663
0.05 <i>M</i> CHES–1% <i>n</i> -butanol (pH 10)	0.010	0.9590
	0.015	0.9604
	0.020	0.9586
	0.025	0.9642
	0.030	0.9657
0.05 <i>M</i> CHES–3% <i>n</i> -butanol (pH 10)	0.010	0.9682
	0.015	0.9703
	0.020	0.9716
	0.025	0.9737
	0.030	0.9721
0.05 <i>M</i> CHES–5% <i>n</i> -butanol (pH 10)	0.010	0.9755
	0.015	0.9800
	0.020	0.9810
	0.025	0.9740
	0.030	0.9760

data, as well as the mean, minimum and maximum values for the variable studied. The length of the upper and lower relative to the box show how stretched the tails of the distribution are. The plot allows a partial assessment of the symmetry of the values.

#### 4. Results and discussion

Capacity factors obtained for the 20 compounds studied in a MEKC system in which 35 different electrolyte solutions of SDS with and without *n*-propanol and *n*-butanol were used. These values were obtained in a previous paper [27] and they have been used in this work to study the correlation

between  $\log k'$  and  $\log P_{ow}$ . Acetate (pH 9) and CHES (pH 10) buffers were used. High pH values were necessary to obtain an electro-osmotic flow strong enough to bring the micelle to the detection point placed at the cathode.

The squared linear correlation coefficients ( $r^2$ ) obtained for the variation of  $\log k'$  as a function of  $\log P_{ow}$  for all compounds studied in the 35 electrolyte solutions are given in Table 2. Good correlations were obtained for  $\log k'$  and  $\log P_{ow}$  in all electrolyte solutions studied Table 2.

The box plots corresponding to the squared correlation coefficients for the variation  $\log k'$ - $\log P_{ow}$  in each buffer employed (each one includes five SDS concentrations) and for all compounds studied are shown in Fig. 1. For the buffers not containing an

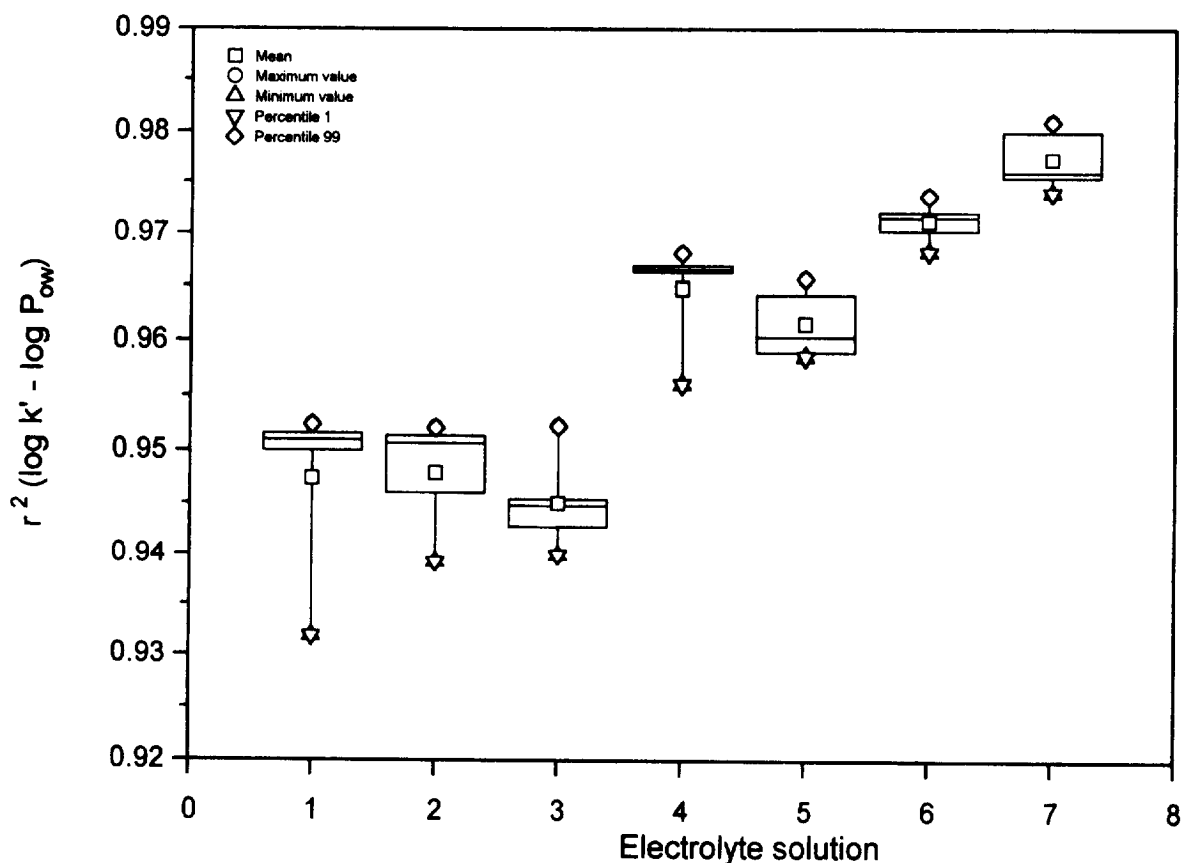


Fig. 1. Box plots for  $r^2$  values for the group of 20 compounds and for  $\log k' - \log P_{ow}$  correlations in the seven buffer systems used (five SDS concentrations have been included in each buffer). (1) 0.10 M CHES, (2) 0.05 M Acetate, (3) 0.05 M CHES, (4) 0.05 M CHES-3% *n*-propanol, (5) 0.05 M CHES-1% *n*-butanol, (6) 0.05 M CHES-3% *n*-butanol, (7) 0.05 M CHES-5% *n*-butanol.

organic modifier, a similar value for the  $r^2$  average is obtained for the three buffers. The application of a non-parametric test (Wilcoxon matched-pair test) indicated that the three groups of  $r^2$  values are not statistically different. Fig. 1 also shows the effect of the alcohols in the electrolyte solution on the  $\log k'$ - $\log P_{ow}$  correlation in MEKC. The average of  $r^2$  values increases in presence of alcohols, with the Wilcoxon test indicating that the  $r^2$  values obtained for buffers 4, 5, 6 and 7 are statistically different from those corresponding to aqueous buffers (1, 2 and 3). This result is in agreement with those obtained by other authors, who state that the addition of an alcohol to a MLC system can provide an environment more closely related to that of octanol-water than a purely aqueous micellar system [16].

To study the influence of the nature of the alcohol

added to the separation buffer, the values obtained for  $r^2$  have been compared for buffers 4 and 6 (0.05 M CHES–3% *n*-propanol and 0.05 M CHES–3% *n*-butanol). The average of  $r^2$  values is higher for 3% *n*-butanol, and the Wilcoxon test indicated that the two groups of values of  $r^2$  of both buffers are statistically different. The influence of the percentage of organic modifier present in the separation buffer can be studied for a 0.05 M CHES buffer in presence of 1, 3 or 5% *n*-butanol. Fig. 1 shows that the mean and median values of  $r^2$  are improved as the percentage of *n*-butanol increases (buffers 5, 6 and 7), the three groups of values obtained for  $r^2$  for the three percentages of *n*-butanol being statistically different as indicated by the Wilcoxon test.

As an example, Fig. 2 shows the variation obtained for  $\log k'$  with  $\log P_{ow}$  for a 0.02 M sodium

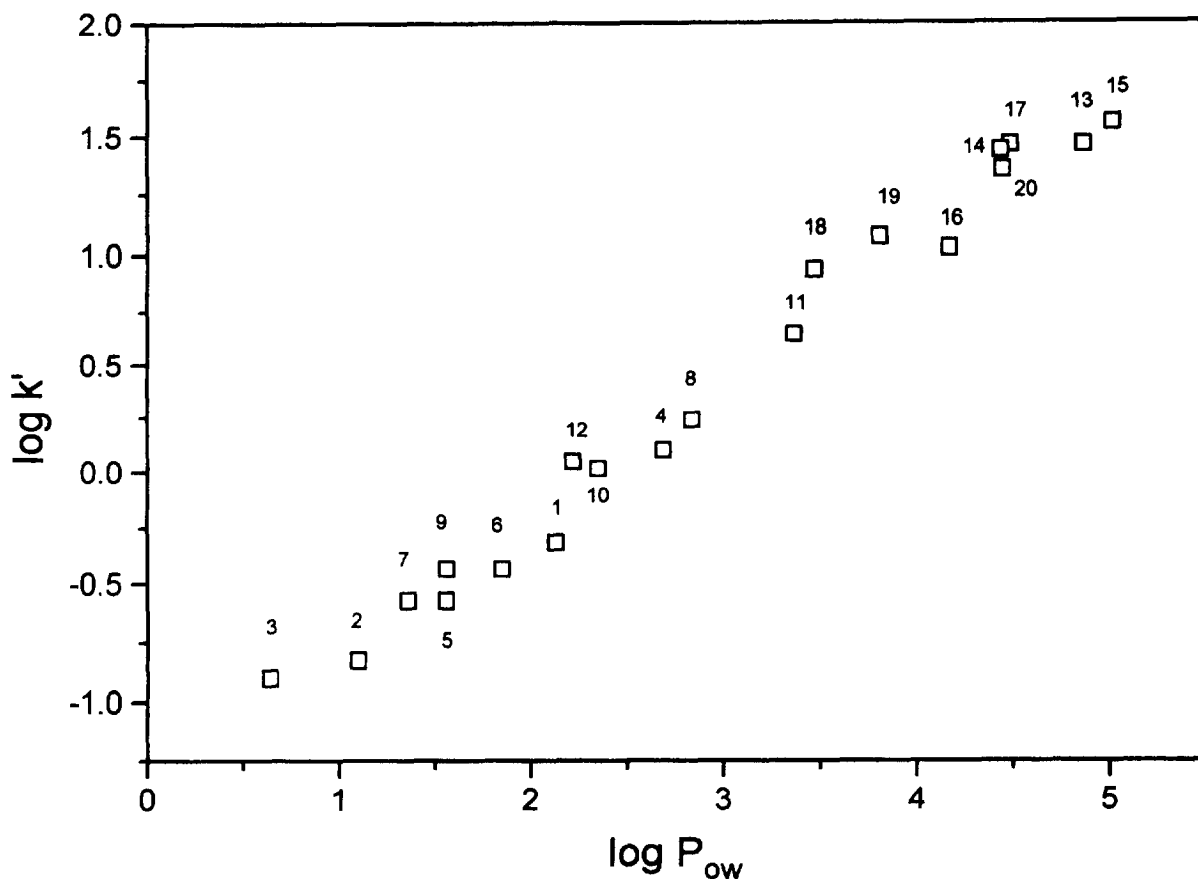


Fig. 2. Variation of  $\log k'$  with  $\log P_{ow}$  for a 0.02 M SDS–0.05 M CHES–5% *n*-butanol buffer. The number corresponding to each solute is specified in Table 1.

dodecyl sulphate (SDS) 0.05 M CHES–5% *n*-butanol buffer. To evaluate the error obtained for the  $\log P_{ow}$  calculated from the straight line of variation of  $\log k'$  with  $\log P_{ow}$  in these experimental conditions, the equation for the variation of this parameter with  $\log P_{ow}$  has been obtained. The equation of the straight line obtained is:

$$\log k' = -1.4517 + 0.6217 \log P_{ow} \quad (5)$$

with  $r^2 = 0.9810$ ,  $N = 20$ . From Eq. (5), a  $\log P_{ow}$  value can be obtained for each solute and the relative error between experimental and calculated  $\log P_{ow}$  can be obtained. The average of the relative errors obtained from Eq. (5) between calculated and experimental  $\log P_{ow}$  values for all solutes was 7.48%. The results obtained in this work on the  $\log k'$ – $\log P_{ow}$  correlation agree with those obtained by other authors who have found a good linear correlation  $\log P_{ow}$ – $\log k'$  in MEKC for a group of 100 solutes using an aqueous phosphate–borate buffer (pH 7) 0.05 M in SDS [25].

## 5. Conclusions

It is concluded that, for the group of compounds studied in this work, good correlations can be obtained in MEKC between  $\log k'$  and  $\log P_{ow}$ . These correlations improve when organic modifiers are added to the buffer, the polarity of the alcohol decreases, and the alcohol percentage in the buffer increases. Results show that MEKC could be considered a valuable technique in quantitative structure–migration relationships research.

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