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Separation of a group of *N*-phenylpyrazole derivatives by micellar electrokinetic chromatography: Application to the determination of solute-micelle association constants and estimation of the hydrophobicity

Micellar electrokinetic chromatography (MEKC) was applied to the separation of a group of *N*-phenylpyrazole derivatives. Sodium dodecyl sulfate (SDS) as micellar system and 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) as separation buffer (pH 10) were employed in the absence and presence of different percentages of medium chain alcohols (*n*-propanol or *n*-butanol). The separation of multicomponent mixtures of the solutes studied enabled the rapid determination of their retention factors which, in turn, allowed the study of the separation selectivity of compounds and the determination of their solute-micelle association constants (from the linear variation of the retention factors as a function of the total surfactant concentration in the separation buffer). Separation selectivity was studied according to the elution range and number of solutes separated in all the electrolyte solutions employed (45 micellar phases). The effect of the buffer concentration (0.05, 0.08 and 0.10 M), the alcohol nature (*n*-propanol or *n*-butanol) and the alcohol percentage (1, 3 or 5%) of the values obtained for the solute-micelle association constants was also studied. The best separation (12 solutes) was performed when a 0.08 M CHES buffer, pH 10, 0.02 M SDS modified by 5% *n*-butanol was used. The possibilities of using MEKC for evaluating the hydrophobicity of compounds was investigated through the study of the correlation between the logarithm of the retention factors of *N*-phenylpyrazole derivatives and their logarithm of the octanol-water distribution coefficients estimated by high performance liquid chromatography (HPLC).

Keywords: Micellar electrokinetic chromatography / *N*-phenylpyrazole derivatives / Separation selectivity / Solute-micelle association constants / Hydrophobicity estimation EL 3935

1 Introduction

In micellar electrokinetic chromatography (MEKC), a surfactant at a concentration above its critical micellar concentration (CMC) is added to the separation buffer of a capillary electrophoresis system [1–3]. With this technique, solutes are distributed between the micelle and the surrounding nonmicellar phase according to their solute-micelle association constants, and only one partition equilibrium (water-micelle) exists. For this reason, an accurate knowledge of solute-micelle association constants is essential for a better understanding of the separation mechanism and, consequently, predicting the retention behavior of compounds [4–6].

If the micellar concentration in the buffer is low (close to CMC) [2], the solute retention factor (k) in MEKC can be related to the total surfactant concentration in the buffer using the following equation [2, 5]:

$$k = (K_2 + \nu)\,(C - \mathrm{CMC}) \qquad (1)$$

where K_2 is the solute-micelle association constant per surfactant monomer, ν is the molar volume of the micelle and C is the total surfactant concentration in the buffer. Equation (1) allows the calculation of solute-micelle association constants for the solutes from the slope of the straight line obtained for the variation of the retention factor as a function of the total surfactant concentration in

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the separation buffer. Thus, a decrease in the error obtained in determining association constants can be expected with respect to other techniques such as micellar liquid chromatography (MLC) [7–11].

In MEKC, all neutral solutes migrate between the electroosmotic flow (t_o) and the micellar (t_m) migration times. From the parameters t_o and t_m the retention factor (k) of a neutral solute can be calculated as follows [1]:

$$k = (t_r - t_o)/t_o\,[1 - (t_r/t_m)] \quad (2)$$

where t_r is the migration time of the neutral solute. If the migration time of the solute is similar to t_o or t_m, the error in the determination of the retention factor increases exponentially [4]; then, the variation of k for very polar or very hydrophobic compounds as a function of the surfactant concentration (Eq. 1) could not be a straight line and it would not be possible to calculate solute-micelle association constants [4]. However, for other solutes (which are neither very polar nor very hydrophobic), MEKC can have a great potential in determining solute-micelle association constants due to the high efficiency and resolution of this technique. These characteristics make possible the rapid determination of the retention factors of groups of solutes by means of the control of the separation selectivity which can be modified by a great number of factors such as surfactant nature, buffer pH, temperature, and addition of organic solvents or other additives. Organic solvents are useful additives since they can extent the elution range (t_m/t_o) or improve the resolution of hydrophobic compounds [12, 13].

On the other hand, hydrophobicity estimation is of great importance in different disciplines, such as drug design and toxicology [14–16]. In this sense, MEKC has been utilized for the indirect estimation of the hydrophobicity of several groups of compounds [6, 18–22]. If solutes experience a similar partitioning in micelle-water and octanol-water systems, an approximately linear relationship between the logarithm of the retention factor (log k) and the logarithm of the octanol-water distribution coefficient (log P_{ow}) can be obtained [6, 17].

In this study, a group of *N*-phenylpyrazole derivatives with a pharmacological interest has been separated in a MEKC system using a 2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) buffer (pH 10) with sodium dodecyl sulfate (SDS) micelles with and without the addition of alcohols such as *n*-propanol or *n*-butanol. Pyrazoles and their derivatives have important applications in agricultural and medical fields [23]. In particular, they are of pharmacological interest owing to their capacity to act as antitumoral agents [24] or to be used for the treatment of leukemia, rheumatoid arthritis, psoriasis, or epilepsy [25, 26]. Determination of the retention factors of these compounds under different experimental conditions has enabled calculation of the solute-micelle association constants and the study of the possibility of MEKC in evaluating their hydrophobicity.

2 Materials and methods

2.1 Chemicals and samples

All reagents employed were of analytical grade. CHES was purchased from Sigma (St. Louis, MO, USA); SDS, dimethylformamide (DMF), benzo(a)pyrene (BaP), and *n*-butanol were from Merck (Darmstadt, Germany); *n*-propanol was obtained from Scharlau (Barcelona, Spain) and sodium hydroxide was from Panreac (Barcelona, Spain). All solutions were prepared with HPLC-grade water (Milli-Q system; Millipore, Bedford, MA, USA). The 26 *N*-phenylpyrazole derivatives were synthesized at the Department of Organic Chemistry of the University of Alcalá, Spain. Table 1 shows identification numbers, names, and structures of these compounds.

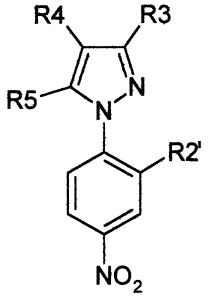
2.2 Apparatus

A capillary electrophoresis instrument 279A-HT model from Applied Biosystems (Norwalk, CT, USA), equipped with a UV detector, a temperature controlled capillary compartment, and an autosampler was used. Data treatment was performed with a Turbochrom acquisition system (Perkin Elmer, Norwalk, CT, USA). Capillary temperature was set at 30°C and UV detection at 238 nm was employed. A fused-silica capillary (25 μm ID; 375 μm OD) from Polymicro Technologies (Phoenix, AZ, USA) was employed. The total length was 75 cm and the effective length was 50 cm.

2.3 Procedure

Separation buffers were prepared by weighing and dissolving the appropriate amounts of CHES and surfactant (SDS) in HPLC-grade water. Next, the pH was adjusted to the selected value (pH 10) with a concentrated solution of sodium hydroxide. These solutions were filtered and degassed prior to their introduction into the capillary and the buffer reservoirs. Sample solutions were prepared by dissolving 5 μL of each *N*-phenylpyrazole derivative (previously dissolved in DMF at an approximate concentration of 10 mg/mL) in 0.75 mL of separation media (final concentration, approximately 0.07 mg/mL). All *N*-phenylpyrazole derivatives analyzed were injected as mixtures con-

Table 1. Identification numbers, names and structures of the *N*-phenylpyrazole derivatives studied

No.	Name	R_3	R_4	R_5	R'_2	**Structure**
1	DNPP	H	H	H	NO_2	
2	3-Methyl DNPP	Me	H	H	NO_2	
3	4-Methyl DNPP	H	Me	H	NO_2	
4	4,5-Dimethyl DNPP	H	Me	Me	NO_2	
5	3-Ethyl DNPP	Et	H	H	NO_2	
6	4,5-Dimethyl pNPP	H	Me	Me	H	
7	3,4,5-Trimethyl DNPP	Me	Me	Me	NO_2	
8	4-Methyl pNPP	H	Me	H	H	
9	3-Methyl-4-nitro-5-chloro DNPP	Me	NO_2	Cl	NO_2	
10	3,5-Dimethyl pNPP	Me	H	Me	H	
11	4-Bromo pNPP	H	Br	H	H	
12	3-Bromo-4-methyl DNPP	Br	Me	H	NO_2	
13	3,5-Dimethyl-4-bromo DNPP	Me	Br	Me	NO_2	
14	5-Methyl-4-bromo pNPP	H	Br	Me	H	
15	3-*tert*-Butyl pNPP	t-Bu	H	H	H	
16	3,4-Dibromo DNPP	Br	Br	H	NO_2	
17	3-Ethyl-4-bromo DNPP	Et	Br	H	NO_2	
18	4-Methyl-5-phenyl DNPP	H	Me	Ph	NO_2	
19	3-Phenyl-4-bromo DNPP	Ph	Br	H	NO_2	
20	3,5-Di-*tert*-butyl-4-methyl DNPP	t-Bu	Me	t-Bu	NO_2	
21	3,4-Dimethyl-5-phenyl DNPP	Me	Me	Ph	NO_2	
22	3,5-Dimethyl-4-*tert*-butyl DNPP	Me	t-Bu	Me	NO_2	
23	4-Bromo-5-methyl DNPP	H	Br	Me	NO_2	
24	3-Phenyl-4-bromo-5-methyl DNPP	Ph	Br	Me	NO_2	
25	3-Methyl-4-bromo-5-phenyl DNPP	Me	Br	Ph	NO_2	
26	3,5-Diphenyl DNPP	Ph	H	Ph	NO_2	

DNPP: dinitro phenylpyrazole

pNPP: paranitro phenylpyrazole

Me: Methyl
Et: Ethyl
t-Bu: *tert*-Butyl
Ph: Phenyl

taining the maximum number of solutes that could be separated in each of the measuring conditions. Peaks of solutes in the mixtures were identified by comparing their migration times with those of individual standards injected under the same conditions. DMF and benzo(a)pyrene were used as electroosmotic flow and micelle migration markers, respectively. Buffers containing 0.05, 0.08, and 0.10 M CHES without alcohols and 0.08 M CHES modified by 1, 3, or 5% *n*-propanol or *n*-butanol were used. All CHES buffers, pH 10, were adjusted with 0.1 M sodium hydroxide. SDS concentration in the buffers ranged from 0.01 to 0.05 M (five SDS concentrations for each buffer, 0.01, 0.02, 0.03, 0.04, and 0.05 M). At the beginning of the day, the capillary was rinsed with HPLC-grade water (5 min), followed by 0.1 M sodium hydroxide (5 min), and finally with HPLC-grade water (5 min). Typically, analyses were performed automatically using a run sequence that included the following steps: (i) 2 min rinse with HPLC-grade water; (ii) 2 min rinse with 0.1 M sodium hydroxide; (iii) 2 min rinse with HPLC-grade water; (iv) 4 min rinse with the separation buffer, in a separate inlet vial; (v) hydrodynamic sample injection (67.73 kPa for 1 s) from the sample vial; and (vi) sample separation run during the necessary time (voltage applied was 15 kV).

2.4 Data treatment

A value of 0.246 $Lmol^{-1}$ was taken as molar volume for SDS. It was assumed that this value did not vary appreciably under the experimental conditions used in this study [27]. The error in determining solute-micelle association constants was ascertained from the statistical parameters of least-squares fitting and from error propagation [28]. Nonparametric tests used in this work to compare association constant values obtained under different experimental conditions were the Wilcoxon matched pair test and the Kolmogorov-Smirnov two-samples test, for a significant level of 95%. They were carried out by using the Statgraphics plus program [29].

3 Results and discussion

The *N*-phenylpyrazole derivatives studied were injected in a MEKC system using as separation media CHES buffers at pH 10 containing SDS micelles. CHES buffer at pH 10 originated an electroosmotic flow strong enough to allow the elution of hydrophobic compounds [4]. Concentrations of CHES and SDS in the separation buffer were varied from 0.05 to 0.10 M (three different concentrations, 0.05, 0.08, and 0.10 M) and from 0.01 to 0.05 M (five different

concentrations, 0.01, 0.02, 0.03, 0.04, and 0.05 M), respectively. *n*-Propanol and *n*-butanol were added to 0.08 M CHES buffers at percentages ranging from 1 to 5%. Retention factors were determined from migration times of solutes and migration times of electroosmotic flow and micelle markers. Multicomponent mixtures of the compounds studied were employed in order to decrease the time required to determine retention factors. From the results obtained, separation selectivity could be investigated, solute-micelle association constants could be determined, and the possibilities of MEKC techniques for hydrophobicity estimation were evaluated.

3.1 Variation of the separation selectivity with buffer, SDS, and alcohol concentrations in the electrolytic solution

Separation selectivity was studied on the basis of the elution range (t_m/t_o) obtained for each buffer employed and the number of solutes which could be separated under each of the experimental conditions tested in this work. Table 2 groups the values obtained for t_m/t_o in each buffer employed at the five SDS concentrations indicated above. An increase in t_m/t_o could be observed when increasing CHES concentration due to the decrease in the electroosmotic flow. When the CHES concentration was kept constant (0.08 M), an increase in the elution range was observed by adding alcohols (*n*-propanol or *n*-butanol). According to these results, an improvement in the separation of *N*-phenylpyrazole derivatives by MEKC was observed when adding alcohols to the separation buffer.

A statistical study of the results showed that the values of t_m/t_o obtained for *n*-propanol and *n*-butanol at percentages of 1 or 5% were not significantly different (for a 95% significance level) while for a percentage of 3% the differences between the results obtained for the two alcohols were statistically significant (*n*-propanol enabling a greater elution range than *n*-butanol). Percentages of alcohol higher than 5% were not used in order to maintain the integrity of SDS micelles in the electrolytic solution. The effect of SDS concentration in the separation buffer on the elution range (for each SDS concentration, all t_m/t_o values obtained in different buffers were considered) was also studied. As expected [4, 6], it could be observed that when the SDS concentration was increased, an increase in the elution window (see Table 2) and in solute retention was obtained, resulting in a loss in resolution for the later migrating compounds. In fact, when a mixture of 15 *N*-phenylpyrazole derivatives was injected in a MEKC system using SDS concentrations from 0.01 to 0.05 M (results not shown), it could be observed that for the lowest SDS concentration (0.01 M) a poor separation selectivity was obtained for the earlier migrating compounds whose resolution increased with the SDS concentration. When the SDS concentration was increased, a loss in efficiency and then in resolution for the most hydrophobic compounds was observed, together with a significant increase in the analysis time. Thus, intermediate SDS concentrations (0.02, 0.03 M) enabled better separation selectivity for the group of compounds considered than high micellar concentrations (0.04 M, 0.05 M). Figure 1 shows the electropherograms corresponding to the injection of two mixtures of the *N*-phenylpyrazole derivatives studied in this work (a mixture of 15 *N*-phenylpyrazole derivatives in Fig. 1a and a mixture of the remaining 11 *N*-phenylpyrazole derivatives in Fig. 1b) using a 0.08 M CHES buffer, pH 10, 0.02 M in SDS, with 5% *n*-butanol. The comparison of these separations with those obtained at lower or higher SDS concentrations illustrates that the intermediate concentration of SDS employed (0.02 M) can be a good compromise between resolution of all compounds and analysis time.

Table 2. Elution range (t_m/t_o) obtained in all separation media employed at different SDS concentrations

	SDS concentration (M)				
	0.01	0.02	0.03	0.04	0.05
0.05 M CHES	3.31	3.14	3.31	3.09	3.14
0.08 M CHES	3.51	3.55	3.69	4.15	4.24
0.10 M CHES	4.36	4.43	4.69	5.02	5.59
0.08 M CHES 1% *n*-Propanol	3.86	4.41	4.44	4.35	4.76
0.08 M CHES 3% *n*-Propanol	5.19	5.39	6.51	6.28	7.35
0.08 M CHES 5% *n*-Propanol	5.97	5.17	5.83	6.56	6.55
0.08 M CHES 1% *n*-Butanol	4.00	3.88	4.16	4.44	4.73
0.08 M CHES 3% *n*-Butanol	4.40	4.81	4.61	4.99	5.22
0.08 M CHES 5% *n*-Butanol	3.91	4.21	5.35	6.03	7.27

3.2 Determination of solute-micelle association constants

Calculation of solute-micelle association constants by MEKC was performed according to Eq. (1). Different electrolytic solutions (0.05, 0.08 and 0.10 M CHES in absence of modifiers and 0.08 M CHES in the presence of 1, 3, or 5% *n*-propanol or *n*-butanol) were employed in order to determine the variation of the retention factor of each solute as a function of the SDS concentration (five SDS concentrations for each electrolytic solution). However, the calculation of the solute-micelle association constants per

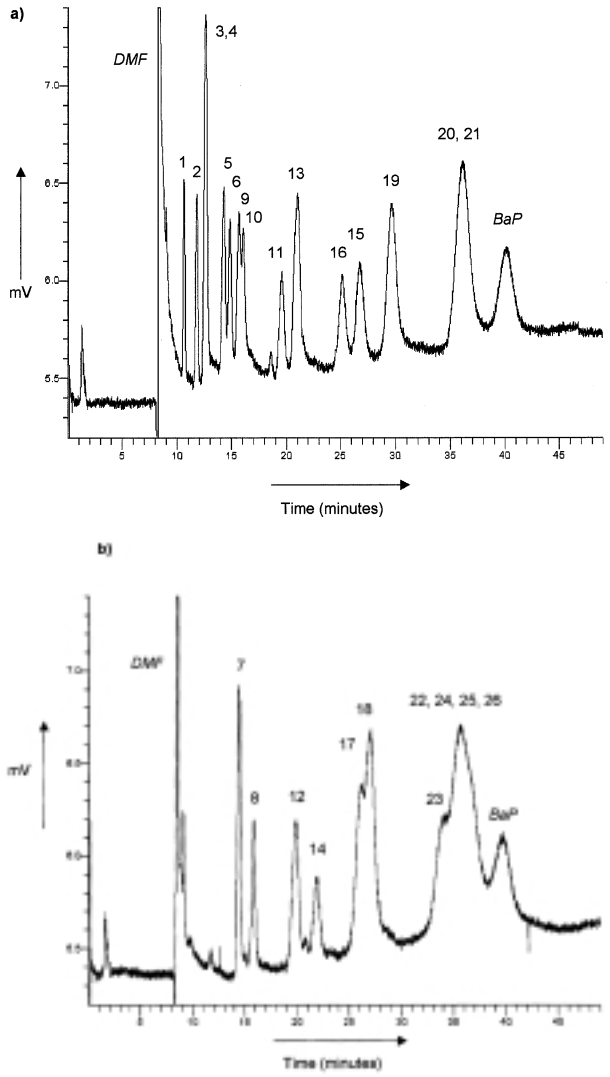


Figure 1. Electropherogram corresponding to the injection of two mixtures of the N-phenylpyrazole derivatives. (a) Mixture of 15 compounds; (b) mixture of the 11 remaining *N*-phenylpyrazole derivatives. Experimental conditions: 0.08 M CHES buffer (pH 10), 0.02 M SDS, 5% *n*-butanol; detection wavelength, 238 nm; applied voltage, 15 kV; injection by vacuum, 67.73 kPa during 1 s; temperature, 30°C; capillary, 75 cm (50 cm to the detector) × 25 μm ID (375 μm OD). See Table 1 for peak identification.

monomer was possible only for those compounds for which a linear variation of the retention factor was obtained as a function of the SDS concentration (when the retention factor could be determined in the medium considered). Table 3 groups the values obtained for the solute-micelle association constants for *N*-phenylpyrazole derivatives with SDS and the relative error associated with this determination. It can be observed that solute-micelle association constants could not be obtained for all compounds in all electrolytic solutions employed. A 0.08 M CHES buffer (pH 10) modified with 5% *n*-butanol enabled the calculation of the highest number of solute-micelle association constants.

On the other hand, Table 3 also shows that solute-micelle association constants decreased generally with increasing CHES concentration (0.05, 0.08, and 0.10 M) although the use of nonparametric tests to compare these values indicated that differences were not statistically significant for a significance level of 95%. Also, when nonparametric tests were used to compare the values of solute-micelle association constants obtained with a 0.08 M CHES buffer in the absence and presence of an alcohol, differences statistically significant were not found, except when the values obtained in a 0.08 M CHES buffer in absence and presence of 5% *n*-butanol were compared. This decrease in the solute-micelle association constants in presence of an alcohol can be justified by considering that, when the alcohol is absorbed into the surface of the micelle, it displaces some of the water molecules lying in the palisade layer. Since alcohols are weaker, hydrogen bond acceptor/donors do not interact as strongly with solutes partitioning in the surface of the micelle [30].

3.3 Study of the possibilities of MEKC in evaluating hydrophobicity

Estimation of hydrophobicity of compounds with pharmacological activity such as *N*-phenylpyrazole derivatives is important due to the correlation of this property with biological activity. The logarithm of the octanol-water distribution coefficient (log P_{ow}) determined by the shake-flask technique was traditionally used for hydrophobicity quantitation [31]. However, due to the disadvantages of this method, other techniques (such as chromatographic) were employed for the indirect estimation of hydrophobicity. Usually, the investigation of the possibilities of a chromatographic technique in evaluating hydrophobicity was performed through the study of the correlation between the retention factors of the group of compounds under study or their logarithm and the logarithm of their octanol-water distribution coefficients [32]. In the case of the group of *N*-phenylpyrazole derivatives studied in this work, the log P_{ow} value determined by the shake-flask method was known only for two of these compounds (numbers 12 and 22) while for the others the parameter known was the log P_{ow} estimated by HPLC [33]. Next we studied the correlation between the logarithm of the retention factors obtained for *N*-phenylpyrazole derivatives in the different micellar phases in which the retention factors could be determined for the 26 compounds, and the log P_{ow} values taken from literature. Best correlations were obtained when a concentration of 0.01 M in SDS was used

Table 3. Solute-micelle association constants per monomer (K_2) and relative errors (RE, %) for the *N*-phenylpyrazole derivatives studied with SDS as surfactant (five concentrations) in different media

Compound	0.05 M CHES K_2 (RE, %)	0.08 M CHES K_2 (RE, %)	0.10 M CHES K_2 (RE, %)	0.08 M CHES 1% *n*-Propanol K_2 (RE, %)	0.08 M CHES 3% *n*-Propanol K_2 (RE, %)	0.08 M CHES 5% *n*-Propanol K_2 (RE, %)	0.08 M CHES 1% *n*-Butanol K_2 (RE, %)	0.08 M CHES 3% *n*-Butanol K_2 (RE, %)	0.08 M CHES 5% *n*-Butanol K_2 (RE, %)
1	37.85 (1.51)	37.85 (2.82)	39.65[a] (1.30)	31.85 (3.11)	23.55 (6.12)	22.95 (1.74)	28.85 (1.78)	19.05 (3.57)	14.15 (5.99)
2	67.75 (0.61)	66.25 (2.13)	66.75[a] (2.58)	54.35 (3.68)	41.75[a] (2.06)	37.85 (3.82)	48.75 (1.55)	32.65 (2.29)	22.35 (6.61)
3	93.05 (0.74)	95.45 (2.06)	91.45[a] (2.93)	73.45 (2.82)	52.85[a] (2.95)	53.95 (4.95)	71.95 (8.58)	44.45 (5.54)	28.95 (7.26)
4	112.55 (1.85)	108.65 (2.18)	108.55[a] (3.79)	83.05 (3.44)	59.95 (7.31)	53.95 (4.95)	73.98[a] (1.63)	44.45 (5.54)	28.95 (7.26)
5	158.55 (1.08)	161.05 (1.99)	152.45[a] (1.83)	132.75 (1.61)	93.65[a] (4.11)	81.35 (6.00)	114.85 (1.58)	67.45[a] (2.10)	42.25[a] (3.28)
6	195.85 (2.70)	188.05 (1.28)	172.25[a] (3.02)	129.05 (4.87)	93.65[a] (4.11)	84.92[a] (5.52)	114.85 (1.58)	67.45[a] (2.10)	48.95 (8.12)
7	203.45 (2.84)	195.85 (2.45)	160.89[a] (10.63)	148.25 (2.65)	103.75[a] (8.52)	102.05 (5.89)	132.95 (0.78)	84.05 (1.57)	58.95 (4.19)
8	225.35[a] (2.02)	323.15 (2.96)	218.45[a] (2.98)	182.05 (2.35)	130.05[a] (8.76)	129.45 (6.12)	164.25 (0.67)	107.75 (1.23)	77.85 (3.88)
9	274.05[a] (2.29)	235.55 (3.71)	228.05[a] (1.69)	185.25 (3.23)	132.25[a] (5.35)	110.21 (7.87)	164.65 (1.69)	98.55 (3.18)	54.25[a] (3.74)
10	256.45 (1.66)	266.85 (0.99)	243.05[a] (4.01)	313.05 (1.86)	137.65[a] (4.38)	127.85 (5.01)	164.65 (1.69)	98.55 (3.18)	60.75[a] (2.96)
11	560.55[a] (8.19)	358.55[a] (1.50)	348.15[a] (2.02)	362.85 (0.58)	222.45[a] (3.35)	203.65 (6.36)	265.55 (2.51)	175.65 (5.01)	101.25[a] (2.64)
12	487.15 (4.15)	402.65 (3.53)	386.05[a] (1.26)	399.05 (2.50)	271.75[a] (6.57)	268.65 (5.63)	329.05 (1.25)	222.85 (1.96)	152.85 (5.52)
13	456.35[a] (4.03)	475.15 (5.07)	463.05[a] (2.63)	324.45 (3.09)	287.15[a] (4.07)	261.45 (3.93)	343.15 (2.56)	221.05 (6.41)	117.35[a] (2.77)
14	–	699.05 (5.38)	827.65[a] (1.02)	732.65 (0.99)	530.65[a] (7.97)	431.15[a] (1.77)	511.25 (3.21)	331.45 (2.59)	209.45 (6.07)
15	858.45[a] (3.83)	783.75 (6.93)	603.75[a] (1.37)	749.85 (0.85)	595.55 (8.47)	583.45[a] (6.63)	644.35 (2.26)	341.45[a] (4.57)	249.65[a] (2.76)
16	904.95[a] (3.78)	733.35 (3.81)	728.45[a] (2.14)	516.15 (4.68)	621.95[a] (8.67)	603.55 (7.88)	656.15 (2.75)	356.15[a] (2.81)	195.95[a] (2.51)
17	–	1553.65 (8.68)	1515.65[a] (7.77)	1466.25 (8.03)	1186.55[a] (7.03)	1000.85[a] (7.03)	1349.25 (7.73)	769.65 (3.30)	410.15[a] (7.04)
18	–	–	2133.92[a] (4.79)	1281.55 (11.69)	1096.35[a] (9.32)	952.15[a] (6.14)	1244.65 (7.61)	756.65 (4.04)	391.15[a] (5.74)
19	–	–	1393.05[a] (5.98)	–	2119.75[a] (12.88)	–	2527.25[a] (4.76)	746.15[a] (2.76)	299.65[a] (4.65)
20	–	–	–	–	–	–	–	2714.55[a] (7.43)	1742.35[a] (6.28)
21	–	–	2905.25[a] (8.29)	–	–	–	–	2714.55[a] (7.43)	1742.35[a] (6.28)
22	–	–	–	–	–	–	–	–	2409.55[a] (10.26)
23	–	–	–	–	–	–	–	–	2409.55[a] (10.26)
24	–	–	–	–	–	–	–	–	2409.55[a] (10.26)
25	–	–	–	–	–	–	–	–	2594.55[a] (11.21)
26	–	–	–	–	–	–	–	–	2358.35[a] (11.50)

a) Values calculated with four SDS concentrations

in buffers 0.08 M CHES (pH 10) modified by 3 or 5% *n*-butanol. Data of retention factors obtained when using these two last separation buffers for 10 *N*-phenylpyrazole derivatives (numbers 1, 2, 3, 9, 10, 12, 13, 14, 17, and 19) covering a wide range of known log P_{ow} [33] were chosen to obtain a straight line log k–log P_{ow} which was employed to calculate log P_{ow} for the remaining *N*-phenylpyrazole derivatives from their retention factor values. The least-squares equation with the best correlation coefficient was obtained for a 0.08 M CHES buffer (pH 10), 0.01 M in SDS modified by 5% *n*-butanol:

$$\log k = -4.708 + 1.969 \log P_{ow} \quad (3)$$

($r = 0.983$, $n = 10$)

The variation of log P_{ow} values predicted by this equation and log P_{ow} values estimated by HPLC (or determined by the shake-flask method for compounds 12 and 22) [33] is shown in Fig. 2. A linear correlation between the two groups of values was observed. Relative error obtained in the prediction of each log P_{ow} by MEKC was calculated with respect to the corresponding value estimated by HPLC. Although for solute 23 the error was close to 24% (this compound was not included in Fig. 2), the errors obtained for the other 25 compounds ranged from 0.3 to 14.1% (average error 3.3%).

4 Concluding remarks

MEKC enabled the separation of mixtures of a group of *N*-phenylpyrazole derivatives with pharmacological activity. The separation of multicomponent mixtures using CHES buffers (pH 10) with SDS as micellar system in the absence and in the presence of medium chain alcohols allowed the rapid determination of retention factors for these compounds. Separation selectivity studied in terms of elution range (t_m/t_o) and number of compounds separated showed that an increase in the CHES concentration or the addition of low percentages of *n*-propanol or *n*-butanol increased the elution range enhancing the separation. However, although an increase in the elution range was also observed when increasing the SDS concentration in the separation buffer, better separations were obtained for intermediate SDS concentrations due to the increase in the retention of the solutes for high SDS concentrations, which provoked a loss in resolution for highly hydrophobic compounds together with a significant increase in analysis time. Mixtures of up to 12 *N*-phenylpyrazole derivatives were separated using a 0.08 M CHES buffer (pH 10), 0.02 M in SDS modified by 5% *n*-butanol.

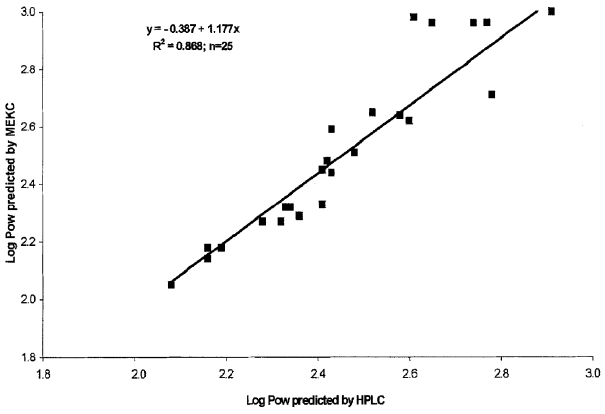


Figure 2. Variation of log P_{ow} values estimated by MEKC for 25 *N*-phenylpyrazole derivatives in a 0.08 M CHES buffer (pH 10), 0.01 M SDS, 5% *n*-butanol as a function of their log P_{ow} values estimated by HPLC or determined by the shake-flask method (compounds 12 and 22).

Solute-micelle association constants for *N*-phenylpyrazole derivatives with SDS were determined in different media. Differences statistically significant for the values obtained for these association constants were not observed when varying the CHES concentration or when adding alcohols to the separation buffer, except in the case of the addition of 5% *n*-butanol. A higher number of solute-micelle association constants could be calculated in media modified by *n*-butanol, especially for a percentage of this alcohol equal to 5%.

Finally, the study of the correlation between the logarithm of the retention factors obtained by MEKC for *N*-phenylpyrazole derivatives as a function of their octanol-water distribution coefficients (log P_{ow}) estimated by HPLC showed a linear variation which enabled the calculation of log P_{ow} values by MEKC for the compounds studied by using calibration with data corresponding to 10 *N*-phenylpyrazole derivatives. Errors obtained for log P_{ow} predicted by MEKC for 25 solutes with respect to those predicted by HPLC ranged from 0.3 to 14.1% with an average error of 3.3%.

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