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Determination of solute–micelle association constants for a group of benzene derivatives and polycyclic aromatic hydrocarbons with sodium dodecyl sulphate by micellar electrokinetic chromatography

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Abstract

Micellar electrokinetic chromatography was applied to determine solute–micelle association constants for a group of benzene derivatives and polycyclic aromatic hydrocarbons using sodium dodecyl sulphate as surfactant. Among the different buffers studied, only those of pH ≥ 9 [2-(N-cyclohexylamino)ethanesulphonic acid and ammonium acetate] gave an electroosmotic flow high enough to allow the elution of all compounds studied. Determination of association constants was achieved under different experimental conditions and the resulting errors were evaluated. The effect of the nature and concentration of the buffer and the alcohol (*n*-propanol and *n*-butanol) on the values obtained by this technique for solute–micelle association constants was studied. It was observed that these factors do not affect the association constants under the experimental conditions used. The values of the solute–micelle association constants and the errors in their determination were also compared with those obtained previously by micellar liquid chromatography.

Keywords: Association constants; Solute–micelle association constants; Polynuclear aromatic hydrocarbons; Benzenes

1. Introduction

The determination of solute–micelle association constants is of great interest in many areas of chemistry and other disciplines [1]. Several methods have been used to determine these constants. UV–Vis spectrophotometry and fluorimetry allow one to obtain these constants if a modification system is observed [2]. Micellar liquid chromatography (MLC) [3–5], in which a surfactant at a concentration above its critical micelle concentration (cmc) is introduced into the mobile phase, has also been employed in recent years. This technique can be applied to a great variety of compounds, and allows one to determine solute–micelle association constants

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for numerous compounds in aqueous and modified media [6–10]. However, this method is not suitable for very hydrophobic compounds experiencing great retention in the chromatographic system and furnishing large errors when determining association constants [7]. This is because association constants are obtained in MLC as the slope/intercept ratio of the straight line obtained for the variation of the inverse of the solute capacity factor with the surfactant concentration in the mobile phase [11]. For very hydrophobic compounds, the intercept of this straight line is close to zero, causing an increased error when determining the association constant.

Micellar electrokinetic chromatography (MEKC), a capillary electrophoresis method (CE) [12–14], is highly regarded as a technique for measuring solute–micelle association constants, although this possibility has largely remained unexplored [15]. In MEKC, solutes (ionized and non-ionized) are distributed between the micelle and the surrounding non-micellar phase according to their solute–micelle association constants. Since the micelles most often used in MEKC are negatively charged, their migration in an electric field is retarded with respect to the electroosmotic flow. Consequently, solutes elute from the separation capillary at a time somewhere between the migration time of the electroosmotic flow marker, t_0, and the migration time of the micelle, t_m. If the micellar concentration in the buffer is low, the solute capacity factor (k') in MEKC can be related to the total surfactant concentration in the buffer using the following equation [12,15]:

$$k' = (K_2 + v)(C - \text{cmc}) \quad (1)$$

where K_2 is the solute–micelle association constant per surfactant monomer, v is the molar volume of the micelle and C is the total surfactant concentration in the buffer. In MEKC, k' is defined as [12]

$$k' = (t_r - t_0)/t_0[1 - (t_r/t_m)] \quad (2)$$

where t_r is the migration time of the solute.

From Eq. 1, it can be observed that solute–micelle association constants can be calculated from the slope of the straight line obtained for the variation of the solute capacity factor with the total surfactant concentration in the separation buffer. Hence one would expect a decrease in the error obtained in determining association constants. However, this method is not appropriate for compounds that are very associated with the micelles, for which the term $[1 - (t_r/t_m)]$ in Eq. 2 can be close to zero.

Despite the interesting possibilities that MEKC offers in the determination of solute–micelle association constants, this technique has seldom been used to this end [15–17]. Therefore, the influence of the nature and concentration of the buffer and the additives present in the buffer on solute–micelle association constants values has not yet been studied. There has been little work on the comparison of association constants for organic solutes by MLC and MEKC [15] or on the errors obtained by both techniques when determining these constants.

In this work, MEKC was applied for determining solute–micelle association constants between a group of benzene derivatives and polycyclic aromatic hydrocarbons and sodium dodecyl sulphate (SDS). The influence of the nature and concentration of the buffer used and the nature and concentration of an organic modifier added to the separation buffer was studied. Also, values obtained for solute–micelle association constants by MEKC and those previously obtained by MLC were compared.

2. Experimental

2.1. Reagents

All reagents were of analytical-reagent grade. SDS was obtained from Schwarz/Mann Biotech (Cambridge, MA, USA), methanol and *n*-propanol from Scharlau (Barcelona, Spain), dimethylformamide, sodium hydroxide, sodium mono- and dihydrogenphosphate, sodium chloride, glacial acetic acid, ammonium acetate, *n*-butanol, benzo[*a*]pyrene and 2-(N-morpholino)-ethanesulphonic acid (MES) from Merck (Darmstadt, Germany), ammonia solution from Pan-

reac (Barcelona, Spain) and Sudan III and 2-(N-cyclohexylamino)ethanesulphonic acid (CHES) from Sigma (St. Louis, MO, USA).

The solutes studied, also of analytical-reagent grade, are listed in Table 1, which shows the logarithm of the octanol–water partition coefficient of each compound to indicate its hydrophobic character.

2.2. Apparatus

The capillary electrophoresis instrument employed was a P/ACE System 2050 (Beckman, CA, USA) connected to an AMC 486 computer. The signal was acquired by using a System Gold V711 hard- and software package (Beckman). All solutes were detected at 214 nm, except markers used to determine micelle migration times (Sudan III and benzo[*a*]pyrene), which were detected at 254 or 280 nm. The symmetry of the peaks was improved through the hydrodynamic injection of the solutes (pressure). The system was thermostated at 25°C.

Capillaries of 25 μm I.D. and various lengths (Polymicro Technologies, Phoenix, AZ, USA) were used. The electrolyte solutions were degassed in a ultrasonic system (Julavo, Seelbach, Germany). A pH meter (Metrohm, Herisau, Switzerland) was employed to adjust the pH of the separation buffers.

2.3. Procedure

Separation buffers were prepared by dissolving the appropriate amount of surfactant in the buffer. These solutions were filtered and degassed prior to their introduction into the capillary and the buffer reservoirs. The samples were prepared by dissolving an appropriate amount of each solute dissolved in methanol or dimethylformamide in the separation buffer. Under each measuring conditions, the peaks of the solutes in the mixture were identified by comparing their migration times with those of individual standard solutions injected under the same conditions. The symmetry of the peaks for the most hydrophobic compounds was improved when dimethylformamide was used in the sample solvent. The final concentration of the solutes ranged from 0.001 to 1 mg/ml, depending on the nature of the buffer and solute.

To improve the reproducibility of the solute capacity factor when CHES and acetate buffers were used, the following washing routine was employed before each sample injection: Milli-Q water for three min, 0.1 *M* sodium hydroxide for 3 min, Milli-Q water for 2 min and separation buffer for 3 min.

For the molar volume of SDS, a value of 0.246 l mol^{-1} was taken [1]. It was assumed that this value does not vary appreciably under the experimental conditions employed in this work [1].

2.4. Electrolyte solutions used in the determination of association constants

A buffer solution of 0.05 *M* ammonium acetate–0.1 *M* ammonia solution (pH 9) without organic modifiers was used. Also, buffers containing 0.1 and 0.05 *M* CHES without additives and 0.05 *M* CHES modified with 3% *n*-propanol and 1, 3 and 5% *n*-butanol were used. All CHES buffers were of pH 10, adjusted with 0.1 *M* NaOH. The SDS concentration in the buffers ranged from 0.01 to 0.07 *M* (five SDS concentration for each buffer).

2.5. Data manipulation

The error in determining solute–micelle association constants was ascertained from the statistical parameters of the least-squares fitting and from error propagation [20].

Non-parametric tests used in this work to compare association constant values were the Wilcoxon matched pairs test, the Mann–Whitney test and the Kolmogorov–Smirnov two-sample test. They were carried out by using the SOLO Statistical System [21].

3. Results and discussion

In order to calculate the solute–micelle association constants of the compounds studied, it is necessary to determine the solute capacity

factors at different SDS concentrations in the separation buffer (see Eq. 1). Owing to the hydrophobic character of some of the solutes studied, the experimental determination of the capacity factor was difficult owing to (a) the difficulty of solubilizing highly hydrophobic solutes in the separation buffer for injection into the capillary, (b) the need to achieve an electroosmotic flow strong enough as to drive SDS micelles pass the detector placed at the cathode and (c) the large errors obtained in determining the capacity factors of highly hydrophobic compounds owing to the similarity between the solute and micelle migration times (see Eq. 2). Some work was done prior to the determination of association constants to solve these problems.

3.1. Selection of elution conditions for benzene derivatives and polycyclic aromatic hydrocarbons in MEKC

The electroosmotic flow coefficient was measured for various buffers. Fig. 1 shows the results for the variation of this coefficient as a function of the buffer concentration [(a) phosphate, (b) MES, (c) acetate, (d) CHES] and methanol percentage in 0.1 *M* CHES buffer (e) for different capillary washing times with the separation buffer. For the sake of comparison, the micelle effective mobility, i.e., the electroosmotic mobility minus the apparent mobility of Sudan III, is also given in Fig. 1. As observed by other workers [14], Fig. 1 shows that the electroosmotic flow coefficient decreases when the buffer concentration and the percentage of the organic modifier in the buffer are increased. Micelle migration to the detection point occurs only for those buffers for which a high electroosmotic flow is obtained (electroosmotic flow coefficient higher than $6 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}). Only in these cases can the micelle migration time and the solute capacity factor be determined. In fact, when a phosphate buffer is used (Fig. 1a), the micelle does not migrate to the detection point at any buffer concentration. These results are different from those reported by other workers using a phosphate buffer [15]. As for the MES buffer, the micelle migrates to the detection point only at a 0.02 *M* concentration.

It is very important to establish appropriate washing routines for the capillary to obtain reproducible results [22]. The washing routines and the rinsing solution depend on the nature and concentration of the buffer, surfactant and sample. The capillary washing time with the separation buffer affects the reproducibility of the results only for phosphate and MES buffers. For the other buffers studied, good reproducibility is obtained regardless of the capillary washing time. This may be due to the low working pH of the buffers or to adsorption of the buffer on the capillary surface [23–25]. Consequently, CHES and acetate buffers seem to be the most suitable buffers to determine the capacity factors of compounds with adequate reproducibility to calculate solute–micelle association constants. The electrolyte solutions for which solute capacity factors were determined are indicated under Experimental. Low percentages of *n*-propanol and *n*-butanol were added to study the influence of organic modifiers on the separation buffer on the solute–micelle association constants.

The possibility of separating a large number of solutes in one injection would allow a considerable reduction in the time required to determine the association constants, since solutes should not be injected separately to measure capacity factors. The CHES and acetate buffers allowed good separations of approximately 20–22 compounds.

The influence of different factors on the separation of mixtures was investigated. An increase in the elution range can be obtained by increasing the buffer concentration and adding organic modifiers such as alcohols (*n*-propanol and *n*-butanol) to the separation buffer. Fig. 2a and b show the increase in the elution range when the CHES concentration is increased from 0.05 to 0.1 *M*, keeping the SDS concentration constant (0.05 *M*). Comparison of Fig. 2a and c shows that the addition of 3% *n*-propanol to 0.05 *M* CHES buffer 0.05 *M* in SDS also causes an increase in the elution range, as reported by other workers [26–28]. The addition of this organic modifier or 3% *n*-butanol allowed the separation of 2-

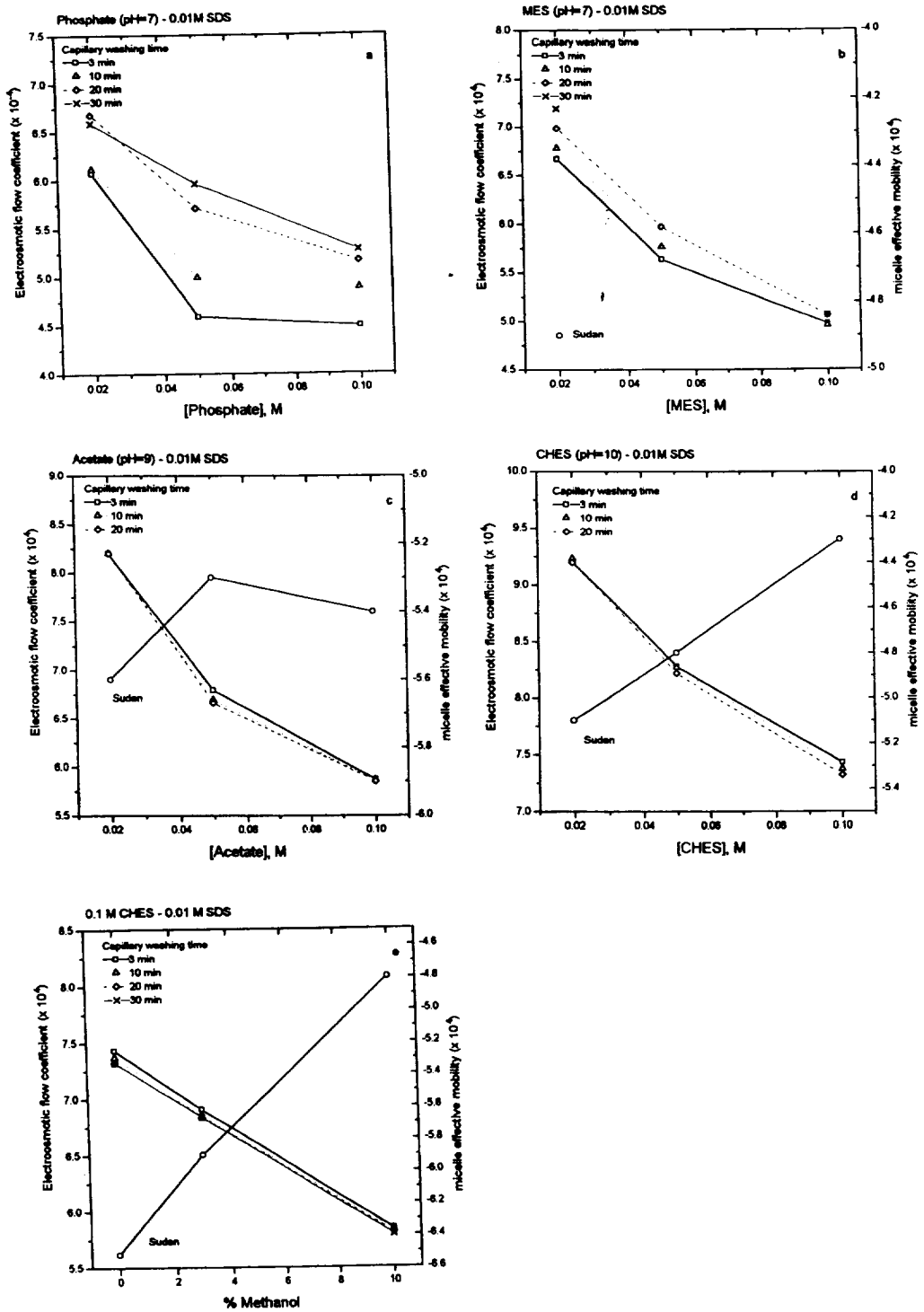

Fig. 1. Variation of the electroosmotic flow coefficient as a function of the buffer concentration, the methanol percentage and the washing time of the capillary with the electrolyte solution. Temperature, 45°C. Total capillary length, 37 cm; length to detector, 30 cm. Concentration of SDS, 0.01 *M*. (a) Phosphate (pH 7); (b) MES (pH 7); (c) acetate (pH 9); (d) CHES (pH 10); (e) 0.1 *M* CHES (pH 10) at different percentages of methanol.

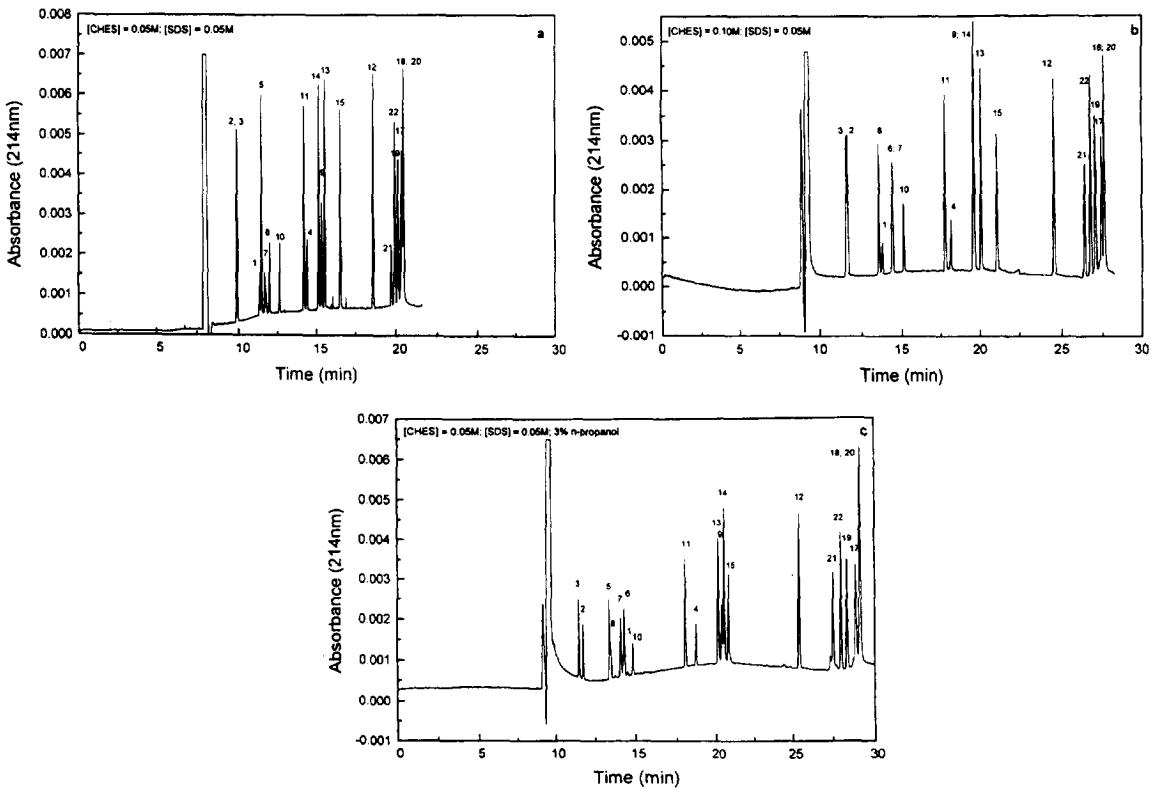

Fig. 2. Electrophoregrams corresponding to the separation of mixtures of the compounds studied with CHES buffer (pH 10) and 0.05 *M* SDS. Applied voltage, 15 kV. Injection by pressure, 10 s. Temperature, 25°C. Total capillary length, 66 cm; length to detector, 59 cm. (a) 0.05 *M* CHES ($t_m/t_0 = 2.83$); (b) 0.1 *M* CHES ($t_m/t_0 = 3.22$); (c) 0.05 *M* CHES–3% *n*-propanol ($t_m/t_0 = 3.17$). For peak identification, see Table 1.

phenylethanol and benzonitrile, which could not be separated under any of the other conditions studied. An increase in the SDS concentration both increases the elution range and enhances the separation of moderately hydrophobic compounds. However, the separation of highly hydrophobic solutes is enhanced by lowering the micelle concentration. A better separation of polycyclic aromatic hydrocarbons is obtained on decreasing the SDS concentration while the lowest hydrophobic compounds are closer to t_0.

3.2. Determination of solute–micelle association constants

To exclude the existence of possible temperature variations during the determination of solute–micelle association constants, the variation of the capacity factor (k') for all compounds as a function of the working voltage was determined for 0.05 *M* CHES buffer 0.05 *M* in SDS. The results for benzene derivatives are shown in Fig. 3, where the straight line obtained for the above-mentioned variation can be observed. The spread of the points obtained for the most hydrophobic compounds (results not shown) is due to the errors in the capacity factor determination, as will be demonstrated later. This indicates that the possible thermal effect that could exist is negligible and that the capillary temperature was the same as that of the cooling liquid (25°C). Also, the coefficient of electroosmotic flow was constant at different voltages.

From Eq. 1, the variation of a solute capacity factor as a function of the total SDS concentration in the buffer should allow a straight line

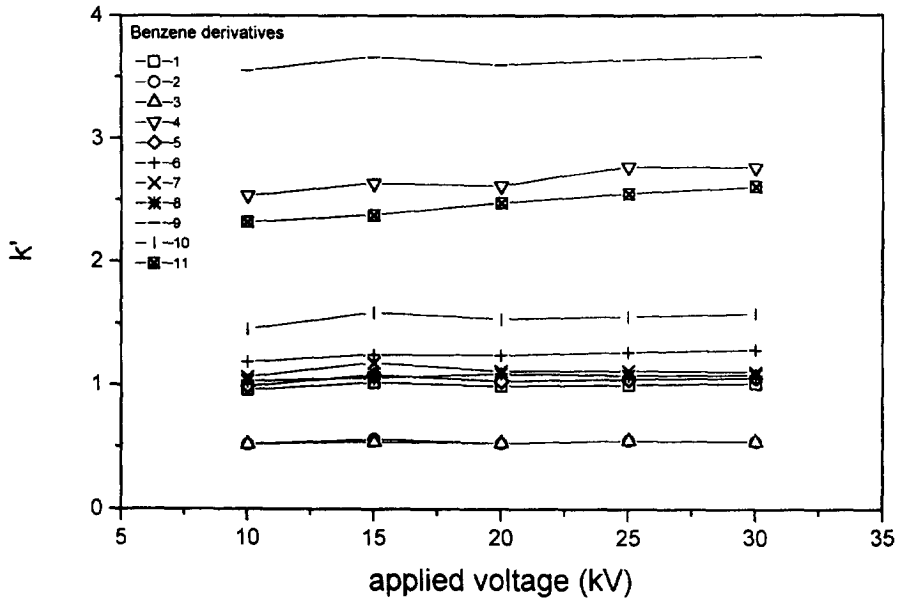

Fig. 3. Variation of the capacity factor (k') for benzene derivatives as a function of the applied voltage. Buffer: 0.05 *M* CHES, 0.05 *M* SDS. For solute identification, see Table 1.

to be obtained. From the slope of this straight line, the solute–micelle association constant can be obtained if the molar volume of the surfactant is known. As an example, Fig. 4 shows the linear variation of the capacity factor as a function of the SDS concentration for the benzene deriva-

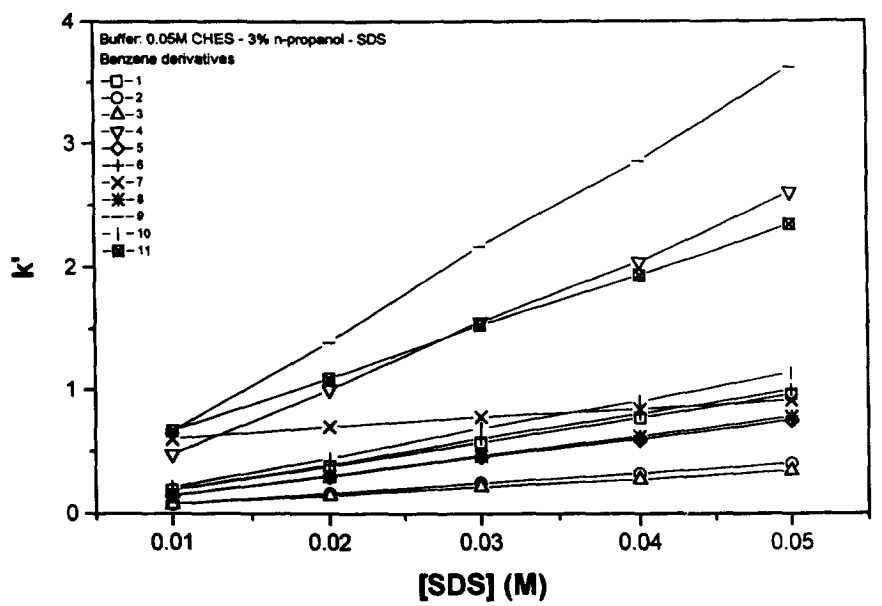

Fig. 4. Variation of the capacity factor (k') for benzene derivatives as a function of the SDS concentration in 0.05 *M* CHES buffer modified with 3% *n*-propanol. Other conditions as in Fig. 2. For solute identification, see Table 1.

tives in 0.05 *M* CHES buffer modified with 3% *n*-propanol. However, it has been observed (results not shown) that for the most hydrophobic compounds (see Table 1) this variation is not a straight line, a larger scatter of the points being observed. Similar plots were obtained for the other separation buffers, but the number of solutes for which a good fit to a straight line is obtained depends on the experimental conditions (nature and concentration of the organic modifier and nature of the buffer employed). This is why association constants for highly hydrophobic compounds could not be obtained under some experimental conditions.

The fact that the variation of the capacity factor as a function of the surfactant concentration is not a straight line for the most hydrophobic compounds may be due to the large error in the determination of the capacity factor as a result of the solute migration times, which were similar to the micelle migration time.

To show that the error in the determination of the capacity factor is the cause of the non-linearity for highly hydrophobic compounds, the error variation in determining the capacity factor was calculated as a function of the t_r/t_m ratio. For each t_r value, the error in the capacity factor was calculated as the relative error between the capacity factor values obtained when values 1% higher and 1% lower than t_r is assigned to this parameter. The results are shown in Fig. 5. It can be observed that when the solute migration time is similar to t_0 or t_m, the error in the determination of the capacity factor increases exponentially. This indicates that there exists a range of values for the ratio t_r/t_m for which it is possible to attain an acceptable error in determining the capacity factor. Under the conditions detailed in Fig. 5, achieving capacity factors with errors lower than 5% implies that solute migration times should be 14% greater than t_0 and 25% lower than t_m.

Table 1 summarizes the values of the solute–micelle association constants for all the experimental conditions detailed under Experimental and for those solutes for which the capacity factor variation as a function of the surfactant concentration is a straight line in such a medium (squared correlation coefficient $r^2 > 0.99$). Also in Table 1, it can be observed that the solute–

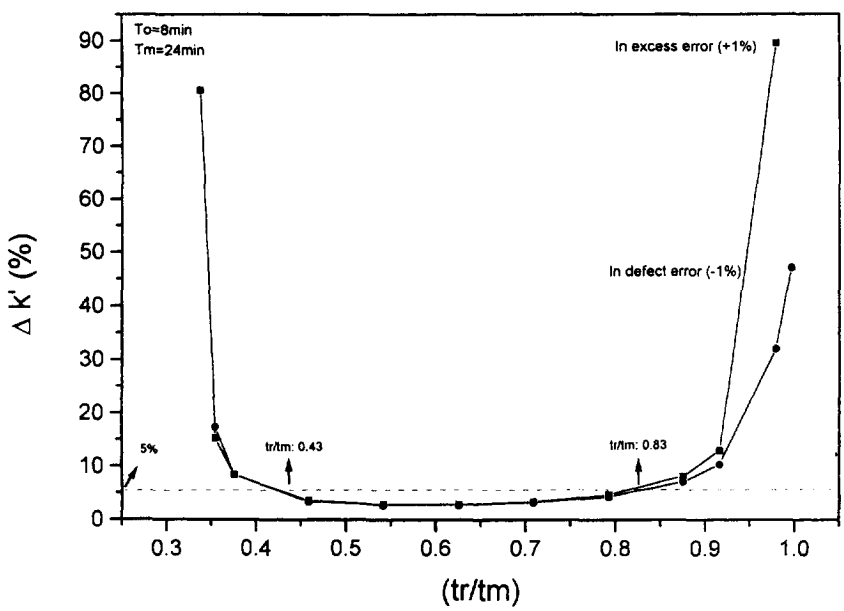

Fig. 5. Variation of the estimated error in the determination of the capacity factor calculated as a function of the t_r/t_m ratio in a hypothetical case in which $t_0 = 8$ min and $t_m = 24$ min. (■) With excess error (+1%); (●) with defect error (−1%).

Table 1
Association constants (and relative errors, %) for SDS solutions in different buffers

No.	Solute	Log P_{ow}	0.05 *M* CHES	0.10 *M* CHES	0.05 *M* acetate	0.05 *M* CHES–3% PrOH	0.05 *M* CHES–1% BuOH	0.05 *M* CHES–3% BUOH	0.05 *M* CHES–5% BuOH
1	Benzene	2.13[a]	23.01 (3.08)	21.35 (1.69)	20.05 (2.46)	19.07 (0.37)	20.83 (5.11)	21.51 (1.25)	24.35 (1.57)
2	Benzyl alcohol	1.10[a]	11.40 (2.12)	10.10 (1.42)	9.65 (2.73)	7.88 (0.37)	8.37 (2.35)	6.83 (2.84)	7.25 (1.12)
3	Benzamide	0.64[a]	11.56 (3.74)	9.23 (1.25)	9.75 (2.70)	6.34 (1.30)	7.49 (3.60)	5.87 (2.03)	5.81 (1.25)
4	Toluene	2.69[a]	60.50 (3.47)	57.35 (1.74)	59.27 (1.09)	52.80 (0.93)	58.17 (2.72)	57.11 (1.97)	67.11 (2.63)
5	Benzonitrile	1.56[a]	22.61 (2.95)	19.69 (1.27)	21.89 (3.52)	14.79 (0.80)	16.19 (2.11)	12.79 (0.77)	13.37 (0.85)
6	Nitrobenzene	1.85[a]	27.45 (2.54)	24.69 (0.89)	26.83 (1.37)	19.97 (0.59)	21.47 (2.45)	18.03 (1.21)	18.89 (2.50)
7	Phenol	1.46[a]	8.13 (6.78)	10.87 (1.06)	9.13 (7.15)	7.49 (4.76)	8.29 (13.36)	6.64 (6.36)	10.49 (30.84)
8	2-Phenylethanol	1.36[a]	21.86 (2.98)	19.20 (2.14)	21.64 (2.97)	15.55 (0.47)	16.51 (2.58)	13.93 (1.41)	13.29 (1.23)
9	Chlorobenzene	2.84[a]	78.40 (2.35)	77.61 (2.15)	77.82 (1.45)	73.60 (0.65)	79.53 (2.87)	77.73 (2.14)	103.69 (3.77)
10	Phenylacetonitrile	1.56[a]	34.12 (2.94)	30.06 (0.87)	33.04 (2.73)	22.85 (0.21)	25.10 (2.49)	19.29 (1.55)	18.85 (2.54)
11	3,5-Dimethylphenol	2.35[a]	41.07 (2.51)	43.99 (2.19)	62.47 (2.44)	41.81 (0.43)	40.41 (2.04)	38.31 (1.95)	37.85 (5.12)
12	Naphthalene	3.37[a]	233.38 (4.39)	225.61 (3.34)	289.97 (2.19)	229.66 (2.35)	254.59 (1.87)	227.46 (3.14)	259.19 (5.21)
13	1-Naphthol	2.98[a]	35.05 (6.23)	56.46 (5.49)	132.15 (3.77)	47.63 (2.58)	47.73 (4.74)	47.93 (1.70)	48.35 (6.61)
14	2-Naphthol	2.84[a]	46.59 (3.81)	51.46 (6.17)	141.55 (3.44)	62.06 (1.28)	56.05 (2.14)	54.45 (3.33)	55.25 (6.44)
15	1-Naphthylamine	2.22[a]	111.28 (4.62)	94.35 (7.23)	115.67 (4.32)	80.10 (1.30)	86.03 (2.03)	69.43 (0.65)	54.57 (11.01)
16	Pyrene	4.88[a]							
17	Phenanthrene	4.46[a]					1089.99 (21.99)	1492.85 (4.37)	1814.97 (17.99)
18	2,3-Benzofluorene	5.03[b]							
19	Fluorene	4.18[a]		563.90 (5.93)	1286.51 (4.95)	754.93 (8.49)	898.03 (8.49)	860.73 (5.50)	1018.85 (11.94)
20	Fluoranthene	4.50[b]							
21	Acenaphthylene	3.48[b]		430.46 (11.53)	713.17 (1.62)	477.46 (4.56)	561.23 (3.62)	485.51 (4.42)	556.53 (7.56)
22	Acenaphthene	3.82[b]		495.45 (8.41)	950.67 (3.20)	603.13 (5.94)	725.77 (5.26)	630.61 (5.87)	813.48 (9.69)
23	Anthracene	4.45[a]						3195.51 (2.31)	

[a] Values taken from Ref. [18].
[b] Values taken from Ref. [19].

micelle association constants generally increase when the solute hydrophobicity increases and the same can be stated for the errors in determining such constants. In fact, the error increase is due to the solute migration times obtained, which are similar to the micelle migration time. The influence of the nature and concentration of the buffer and the nature and percentage of the alcohol on the association constants will be discussed.

3.2.1. Influence of nature of the buffer

To study the effect of the nature of the buffer on the solute–micelle association constants, their values for CHES (pH 10) and acetate (pH 9) buffers at the same concentration (0.05 *M*) were compared. This comparison was made only for the fifteen benzene and naphthalene derivatives since for the polycyclic aromatic hydrocarbons the association constants were not calculated in CHES for the reasons stated before. Table 1 shows that for most compounds the association constants have similar values in both buffers except for 1-naphthol and 2-naphthol, for which the largest differences were obtained. Considering the pK_a values for 1-naphthol and 2-naphthol (9.34 and 9.51, respectively, measured in water), these compounds may be more negatively charged in CHES buffer (pH 10) than in acetate buffer (pH 9). Therefore, their interaction with the negatively charged SDS micelles could be smaller in CHES buffer than in acetate buffer, as shown in Table 1.

Although in this work Eq. 2 was used to calculate k' for all compounds, it should be noted that if the ionization of 1-naphthol and 2-naphthol occurs under these conditions, this equation is no longer valid [29,30]. If non-parametric tests are employed to compare the groups of constants obtained in CHES and acetate, the results would indicate that the association constants in these two buffers are not statistically different. Under these conditions (0.05 *M*), the relative error in determining association constants is always smaller than 5%, except for phenol and 1-naphthol. For these compounds, the errors obtained are close to 7% and 6%, respectively. Phenol is the only compound for which the variation of the capacity factor as a function of the surfactant concentration in solution is a straight line with a slope close to zero. This could be due to the high working pH. In fact, if phenol ($pK_a = 9.89$) is dissociated at this pH, its negative charge may cause a repulsion with respect to the SDS negative micelles and, consequently, a non-binding behaviour is observed. Further, hydrophobic phenol and SDS micelle interactions are smaller than for 1-naphthol and 2-naphthol with a higher hydrophobic character. If this is the case, Eq. 2 should be modified to calculate k' of the ionized compound in MEKC as indicated before.

3.2.2. Influence of buffer concentration

The influence of the buffer concentration on association constants will be discussed for the CHES buffer employed at two concentrations (0.05 and 0.1 *M*). Table 1 shows that the values are very similar regardless of the buffer concentration. In fact, if non-parametric tests are used to compare the values obtained, they show that the values are not statistically different. With regard to the errors obtained, these are generally lower for the highest concentration. Only for two compounds, 2-naphthol and naphthylamine, is the error in the determination of the association constant smaller at a concentration of 0.05 *M*. In 0.1 *M* CHES it was possible to calculate the association constants for fluorene, acenaphthylene and acenaphthene with SDS, that is, in 0.1 *M* CHES the association constants can be calculated for compounds that have log P_{ow} up to 4.18 whereas for 0.5 *M* CHES the log P_{ow} of the most hydrophobic compound for which the association constant can be calculated is 3.37, corresponding to naphthalene. This is due to the fact that an increase in the buffer concentration causes the elution range to increase.

3.2.3. Influence of addition of an alcohol

The effect of adding an alcohol was studied by comparing the association constants obtained in 0.05 *M* CHES, 0.05 *M* CHES modified with 3% *n*-propanol and 0.05 *M* CHES modified with 3% *n*-butanol. Table 1 shows that the association constants for the fifteen compounds for which

the comparison is possible are generally slightly higher in the absence than in the presence of either 3% *n*-propanol or *n*-butanol.

Fig. 6 shows the box plot for the association constants in the three buffers. This plot is used when a sample summary display of the distribution is desired. The box plot is defined in terms of percentiles and gives a quick overview of the median and spread of the data, and also the mean, minimum and maximum values for the variable studied. The length of the upper and lower lines pertaining to the box shows how stretched the tails of the distribution are. The plot allows a partial assessment of the symmetry of the values. Fig. 6 shows that the addition of an alcohol decreases the association constants and decreases the mean and the median, as would be expected since the alcohol can compete with the solute to associate with the micelle. To determine whether the differences between the association constant in the absence and presence of each alcohol are statistically different, the non-parametric tests described under Experimental were applied. When the constants for the fifteen benzene and naphthalene derivatives in the absence of alcohol are compared with those in the presence of 3% *n*-propanol or *n*-butanol, the tests indicated that the difference observed in Fig. 6 was not significant in statistical terms. Fig. 6 also shows that the nature of the alcohol does not affect the association constants appreciably, as was statistically tested.

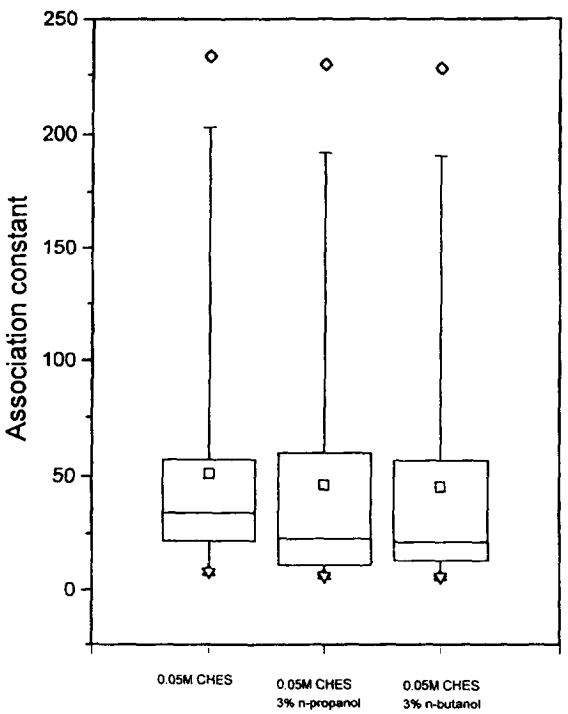

Fig. 6. Box plot for the association constants values for the fifteen benzene and naphthalene derivatives in 0.05 *M* CHES in the absence of alcohols and in the presence of 3% *n*-propanol and 3% *n*-butanol. □ = Mean; ○ = maximum value; △ = minimum value; ▽ = percentile 1; ◊ = percentile 99. The line across the middle of the box is the median.

The effect of the percentage of alcohol in the buffer was studied using 1, 3 and 5% *n*-butanol. Fig. 7 shows the three association constants obtained for each compound and the maximum error bars obtained for the three percentages. In general terms, it can be observed that the alcohol percentage does not affect the values of the association constants appreciably in the range studied. This result was statistically tested. Further, these tests also indicated that the association constants in 0.05 *M* CHES and 0.05 *M* CHES modified with 1% *n*-butanol and 5% *n*-butanol are statistically similar. The small variation of the association constants measured by MEKC with the alcohol percentage will be discussed in the next section by comparing these results with those obtained by MLC.

3.3. Comparison between MEKC and MLC association constants and errors

Where possible, association constants and the errors obtained in their determination by MEKC were compared with the values obtained previously by our using MLC [31]. As stated in the Introduction, the error in the determination of the association constants by MEKC should be smaller than that obtained by MLC. As an example, Fig. 8 shows the error obtained in the determination of the association constants for fifteen benzene and naphthalene derivatives with SDS by MLC (without buffer in the mobile phase) and by MEKC (0.05 *M* CHES buffer). Similar results were obtained when comparing

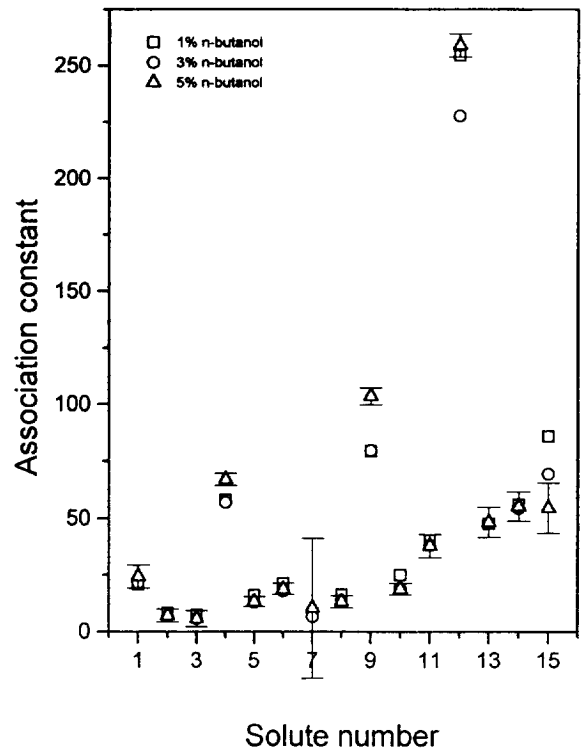

Fig. 7. Association constants obtained by MEKC in 0.05 *M* CHES buffer modified with 1, 3 and 5% *n*-butanol. For each compound the error bars correspond to the percentage of alcohol for which the error obtained is maximum. For solute identification, see Table 1.

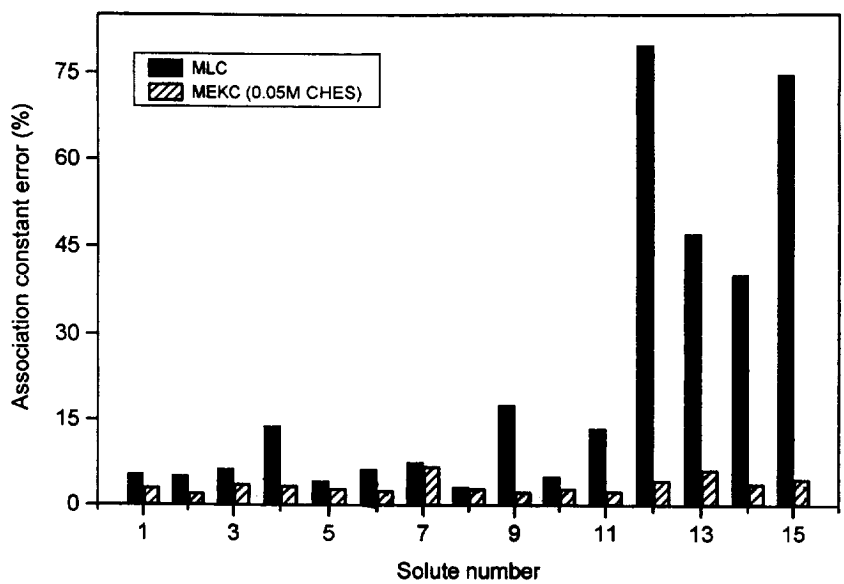

Fig. 8. Comparison of the errors obtained in the determination of the solute–micelle association constants for the fifteen benzene and naphthalene derivatives with SDS by MEKC and by MLC. For solute identification, see Table 1.

MLC and MEKC in the absence of alcohol by using other buffers in MEKC and when comparing MLC and MEKC in the presence of alcohols. The error decreases in MEKC especially for highly hydrophobic compounds (for naphthalene and naphthylamine a decrease of nearly 95% can be obtained) and in absence of alcohols. The largest difference in the errors obtained by MLC and MEKC is obtained in the absence of modifiers because in the presence of alcohols the error in determining the association constants by MLC decreases considerably. For other compounds the decrease in the error ranged from 5% to 90%.

To compare the association constants obtained by MLC and MEKC, the values measured for each solute by both techniques are presented in Fig. 9 together with the error bars corresponding to MLC. Fig. 9a compares the values obtained in micellar aqueous solutions (MLC without additives and MEKC with 0.05 *M* CHES, 0.1 *M* CHES and 0.05 *M* acetate buffers). Fig. 9b, c and d show the same data for micellar solutions modified with 3% *n*-propanol, 3% *n*-butanol and 5% *n*-butanol, respectively.

In aqueous micellar solutions (Fig. 9a), all values of the association constants by MEKC are included in the error bars corresponding to MLC, except for 1-naphthol, 2-naphthol and naphthylamine. This behaviour may be due to the different degrees of ionization of these compounds in the MLC mobile phase (pH close to 7) and in the MEKC buffer (pH 10 for CHES and pH 9 for acetate). Although Fig. 9a shows that for 1-naphthol, 2-naphthol and naphthylamine

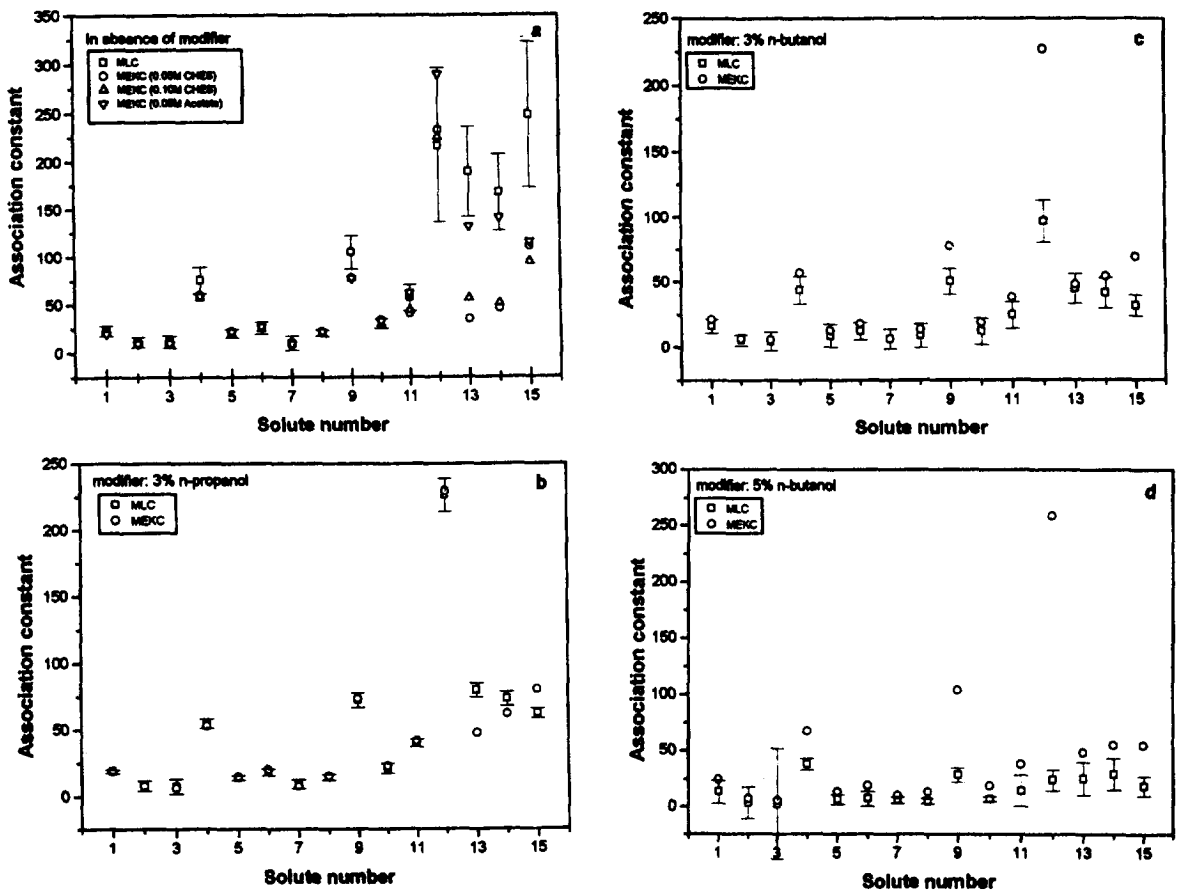

Fig. 9. Comparison of the association constants for the fifteen benzene and naphthalene derivatives obtained by MEKC and by MLC. (a) In the absence of alcohol and for different buffers in MEKC; (b) in the presence of 3% *n*-propanol; (c) in the presence of 3% *n*-butanol; (d) in the presence of 5% *n*-butanol. For solute identification, see Table 1.

the association constants obtained by MEKC are smaller than those obtained by MLC, application of non-parametric tests indicated that this difference was not statistically significant. Similar results were obtained on comparing MLC and MEKC in 3% *n*-propanol (Fig. 9b), where the association constants were not statistically different. Fig. 9c and d show that the association constants obtained by MEKC are generally higher than those obtained by MLC in solutions modified with 3% and 5% *n*-butanol. However, the results obtained when non-parametric tests for this group of fifteen benzene and naphthalene derivatives were applied are in the limit between the equality and the difference for 3% *n*-butanol, showing for 5% *n*-butanol that the association constants obtained by the two techniques are statistically different.

The difference between the MEKC and MLC values increases when the hydrophobicity of the solute increases. Table 2 groups the values for some of the polycyclic aromatic hydrocarbons for which it was possible to determine the solute–micelle association constants by MLC and to compare them with MEKC. It is observed that the association constants obtained by MEKC are much higher than those obtained by MLC and the opposite applies to the errors. This difference between MLC and MEKC may be due to (1) the absence of a stationary phase in MEKC, which in MLC causes a distribution of the solute between the stationary and micellar phases, especially for highly hydrophobic compounds showing a high affinity for non-polar chemically bonded phases, and (2) the high polarity of the separation buffer in MEKC with respect to the unbuffered micellar mobile phases used in MLC to determine solute–micelle association constants of the compounds studied here [31], which would lead to a higher solute affinity for the micelle in MEKC.

The decrease in the polarity of the aqueous micellar phase in MLC due to the addition of alcohols such as *n*-propanol and *n*-butanol can also explain the decrease in the solute–micelle association constants obtained by this technique for hydrophobic compounds in the presence of these alcohols. In fact, a decrease in these constants is observed in MLC with increasing alcohol chain length and percentage of alcohol in the mobile phase [7,31] (association constants statistically different for various alcohol percentages and different alcohols). This result conflicts with that obtained in this work, in which the values of the solute–micelle association constants for the fifteen benzene and naphthalene derivatives are not appreciably influenced by the nature and percentage of the alcohol. The fact that in MEKC the percentage of alcohol does not affect the association constants whereas in MLC a decrease in these constants is observed with increasing alcohol content in the mobile phase explains why the values of the association con-

Table 2
Association constants (and relative errors, %) for SDS solutions by obtained MLC and MEKC

Solute	3% *n*-Propanol		3% *n*-Butanol		5% *n*-Butanol	
	MLC	MEKC	MLC	MEKC	MLC	MEKC
Phenanthrene			173.98 (40.26)	1492.85 (4.37)	75.23 (25.23)	1814.97 (17.99)
Fluorene	1326.44 (320.23)	754.93 (8.49)	179.27 (34.25)	860.73 (5.50)	61.76 (23.63)	1018.85 (11.94)
Acenaphthylene	496.08 (26.44)	477.46 (4.56)	129.28 (21.14)	485.51 (4.42)	56.36 (16.26)	556.53 (7.56)
Acenaphthene	380.90 (100.28)	603.13 (5.94)	249.30 (46.33)	630.61 (5.87)	71.40 (13.70)	813.48 (9.69)
Anthracene			229.37 (38.51)	3195.51 (2.31)		

stants obtained by MLC and MEKC are statistically different only for 5% *n*-butanol. The slight influence of the percentage of alcohol on the association constants in MEKC may be explained by the smaller decrease in the polarity of the separation buffer in MEKC on adding a nonpolar alcohol with respect to the decrease in polarity of an unbuffered mobile phase in MLC.

These results suggest that for hydrophobic solutes, MEKC allows one to obtain the association constant of a solute with the micelle but if the solute and micelle migration times are similar, the association constant cannot be calculated. On the other hand, there is no limitation on the retention time in MLC when a peak is obtained for the solute (it is not irreversibly retained in the stationary phase). However, in this technique the solute would experience partitioning between the stationary and the micellar phases, which can alter the association constant. Also, the error in the determination of these constants can be appreciably higher in MLC than in MEKC if very elevated capacity factors are obtained. Regarding pH values, MLC and MEKC can be considered as complementary techniques since in MLC there is a limit close to pH 7 for determining solute–micelle association constants whereas these constants can be obtained at higher pH values by MEKC.

Acknowledgements

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