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Enhancement of the separation selectivity of a group of polycyclic aromatic hydrocarbons using mixed cyclodextrin-modified micellar electrokinetic chromatography

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Abstract

The separation selectivity of a group of twenty polycyclic aromatic hydrocarbons (PAHs) (including sixteen Environmental Protection Agency priority PAHs) was studied when mixtures of native β- and γ-cyclodextrins (CDs) are used as modifiers in the separation buffer in cyclodextrin-modified micellar electrokinetic chromatography. The ratio between the concentrations of β- and γ-CDs in the separation buffer as well as the total concentration was varied for various buffer concentrations. An enhancement in selectivity was obtained which enabled the separation of eighteen PAHs when using a 0.140-*M* borate buffer (pH 9) containing 0.100 *M* sodium dodecyl sulphate micelles, 0.042 *M* β-CD, 0.020 *M* γ-CD and 2.5 *M* urea. The correlation between the logarithm of the capacity factors of PAHs and the logarithm of their octanol–water distribution coefficients was also investigated. © 1997 Elsevier Science B.V.

Keywords: Selectivity; Polynuclear aromatic hydrocarbons; Cyclodextrins

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals of great environmental concern because many of them are suspected mutagens, teratogens and/or carcinogens [1–3]. Thus, the processes that affect the transport and fate of these contaminants must be understood in order to assess exposure risk to humans and the environment and to develop efficient and cost-effective remediation strategies. Their accurate analytical determination is of great interest. Fast, accurate and sensitive analytical methods are needed in order to unambiguously identify these compounds in polluted environments.

Different methods, using gas chromatography (GC) or high-performance liquid chromatography (HPLC) have been reported in the literature [4] to separate mixtures of PAHs in environmental samples. Capillary electrophoresis (CE) has recently emerged as one of the most efficient methods to achieve the separation of different components in mixtures. However, modified CE methods are necessary in order to make the analysis of neutral solutes by CE possible. Several approaches have been reported in order to separate PAHs by CE. One possibility is to use capillary electrochromatography (CEC) in which

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mobile phase transport through a capillary packed with stationary phase particles (as in HPLC) is performed by electroosmotic flow. The separation of PAHs by CEC has been shown under isocratic conditions [5] as well as with gradient elution [6]. Another possibility for the separation of PAHs is micellar electrokinetic chromatography (MEKC) [7,8] in which an ionic surfactant [usually sodium dodecyl sulphate (SDS)] is added to the separation buffer. MEKC has allowed the separation of PAHs and their determination in soil samples [9]. However, hydrophobic compounds tend to be totally incorporated into the micelle and then, the addition of cyclodextrins (CDs) to the buffer is used to enhance the separation power of MEKC. This method is named cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC). β- or γ-CDs are normally used as modifiers in CD-MEKC. CD systems are excellent candidates for analytes when conventional separation schemes based on polarity, molecular mass, etc., have been ineffective. Another approach used to separate PAHs was cyclodextrin-modified CE, in which solutes were separated on the basis of differential partitioning between two types of CD (one charged and one neutral CD) [10].

The separation of some PAHs has been demonstrated by CD-MEKC using γ-CD in the separation buffer [11–14]. Terabe et al. [11] separated a mixture of eight PAHs (naphthalene, four tricyclic PAHs and three tetracyclic PAHs) in less than 30 min using a borate buffer (pH 9) and a 0.030-*M* concentration of γ-CD and a mixture of sixteen PAHs, including pentacyclic and hexacyclic PAHs, using the same buffer and a 0.020-*M* concentration of γ-CD [12]. PAHs have also been separated using β-CD [12,15].

When β- or γ-CDs are used in MEKC, only the PAH molecules that have the right size and shape will fit into the cavity. However, molecules that are either too big or too small compared to the size of the cavity will either not be effected at all or will only be slightly effected by the presence of β- or γ-CD. This suggests that the use of a mixture of β- and γ-CD may provide better results than those obtained with the use of a single CD, although some authors reported that the use of a mixed solution of β- and γ-CD was explored but that the complete separation of sixteen PAHs was not successful [12].

The aim of this work was to study the possibilities of using mixtures of native β- and γ-CDs in order to increase the selectivity for the separation of a group of twenty PAHs, including sixteen Environmental Protection Agency (EPA) priority PAHs using mixed cyclodextrin-modified MEKC (MCD-MEKC). The correlation between the logarithm of the capacity factors of PAHs in MCD-MEKC and the logarithm of their octanol–water distribution coefficients was also investigated.

2. Experimental

2.1. Reagents

All reagents employed were of analytical grade. Urea, β- and γ-CDs were obtained from Fluka (Buchs, Switzerland). Sodium borate and boric acid were from Sigma (St. Louis, MO, USA). Sodium hydroxide and sodium dodecyl sulphate (SDS) were from Merck (Darmstadt, Germany). Dimethylformamide (DMF) was from Scharlau (Barcelona, Spain). Water was purified using a Milli-Q Water System (Millipore). PAH standards, including sixteen EPA priority PAHs, were provided individually by Dr. D.G. Patterson (CDC, Atlanta, GA, USA), as 1 ml solutions at a concentration of 1 mg/ml in toluene, and are listed in Table 1. This Table also includes the values of the octanol–water distribution coefficients (log P_{ow}) used throughout this work for the compounds studied. Individual standards were concentrated and a mixture containing all of these compounds was made using DMF as the solvent.

2.2. Apparatus

The CE system consisted of a programmable injector, model Prince, a Lambda 1000 UV-detector and a high voltage power supply, all purchased from Lauer Laboratories (Netherlands). The integrator employed was a HP3394 from Hewlett Packard (Avondale, PA, USA). The temperature was kept constant at 35°C and detection was carried out at 230 nm. The injection of solutes was performed by pressure for 0.02 min at 20 mbar. The applied voltage was 15 kV. A fused-silica capillary tube (50 μm I.D. and 375 μm O.D.) from Polymicro Tech-

Table 1
PAHs used in this study, their identification numbers and log P_{ow} values

Code	Name	log P_{ow}	Number of rings
1	Acenaphthene	3.82[a]	3
2	Acenaphthylene	3.48[a]	3
3	Naphthalene	3.37[b]	2
4	Benzo[*g,h,i*]perylene	6.85[a]	6
5	Fluorene	4.18[b]	3
6	Phenanthrene	4.46[b]	3
7	Pyrene	4.88[b]	4
8	Chrysene	5.91[a]	4
9	Perylene	6.58[a]	5
10	Anthracene	4.95[b]	3
11	Benzo[*e*]pyrene	6.50[a]	5
12	Benzo[*a*]pyrene	6.50[a]	5
13	Benzo[*a*]anthracene	5.92[a]	4
14	Fluoranthene	5.85[b]	4
15	Dibenzo[*a,h*]anthracene	6.85[a]	5
16	Benzo[*k*]fluoranthene	6.40[c]	5
17	Triphenylene	6.30[b]	4
18	Benzo[*j*]fluoranthene	6.40[c]	5
19	Benzo[*b*]fluoranthene	6.62[a]	5
20	Indeno[1,2,3-*cd*]pyrene	–	6

[a] Values taken from [16].
[b] Values taken from [17].
[c] Values taken from [18].

nologies (Phoenix, AZ, USA) was employed. The total length was 65 cm and the effective length was 50.5 cm. The capillary was rinsed prior to use for 5 min with 1 *M* sodium hydroxide followed by 5 min with the separation buffer. This separation buffer was degassed in an ultrasonic system Transsonic 460 from Elma (Germany). A 654 pH-meter (Metrohm, Herisau, Switzerland) was employed to measure the pH.

2.3. Procedure

Micellar solutions were prepared by dissolving the appropriate amounts of SDS, urea and CDs in borate buffer solutions (pH 9) of the selected concentration. Solutions were passed through a membrane filter with a pore size of 0.45 μm. Analytes were dissolved in DMF at an appropriate concentration (usually 0.1–0.5 mg/ml).

Electropherograms were evaluated by determining the peak-to-peak resolution and the analysis time.

The capacity factor (k') for each solute was calculated as follows [12]:

$$k' = (t_m - t_0)/[t_0(1 - t_m/t_{mic})]$$

where t_m, t_0 and t_{mic} are the migration times of the solute, the electroosmotic flow marker and the micelle, respectively.

The value of the migration time for the most hydrophobic compound (indeno[1,2,3-*cd*]pyrene) was taken as being the t_{mic}.

3. Results and discussion

3.1. Study of the separation selectivity of PAHs using β- and γ-CDs mixtures

In order to separate the individual components of a complex mixture containing twenty PAHs, CD-MEKC was used with SDS as the micellar system and β- and γ-CDs were added as modifiers. Borate buffer was used at a pH of 9 to obtain a high electroosmotic flow. Some preliminary experiments were performed in order to select the optimal concentration of SDS micelles in the separation buffer. Among the tested concentrations, a SDS concentration of 0.100 *M* was selected, since it appeared to provide the best performance. Urea, the concentration of which was kept constant and equal to 2.5 *M*, was also added to the separation buffer in order to increase the solubility of CDs and lipophilic compounds in the aqueous media [15,19].

As a first step in our study on the modification of separation selectivity through the use of β- and γ-CD mixtures, the separation of PAHs was carried out using either β- or γ-CDs as modifiers in the separation buffer. Firstly, experiments were done using γ-CD alone as the modifier. Several concentrations ranging from 0.020 to 0.080 *M* were investigated. The best selectivity achieved with a 0.100 *M* borate buffer was obtained for a 0.050-*M* concentration of γ-CD. Under these conditions, the separation of fourteen peaks from the twenty PAHs was achieved in 30 min. Five groups of PAHs, fluoranthene/benzo[*a*]anthracene, benzo[*k*]fluoranthene/triphenylene/dibenzo[*a,h*]anthracene, anthracene/perylene, benzo[*a*]pyrene/phenanthrene, and pyrene/benzo[*e*]pyrene were not resolved. When β-CD was used on its own as the modifier in the separation buffer, concentrations ranging from 0.020 to 0.080 *M* were

also tested. The best results were obtained with a β-CD concentration of 0.040 *M* and, under these conditions, the separation of twelve peaks from the twenty PAHs was achieved in 33 min. According with Terabe et al. [12], the use of β-CD gave poor resolution as a whole, although a change in the selectivity with respect to γ-CD was obtained. In this work, the authors used a mixed solution of β- and γ-CDs, but the separation of a mixture of sixteen PAHs was not successful.

A set of preliminary experiments was designed in order to determine the effect of using mixtures of β- and γ-CDs. In spite of the fact that the complete separation of the twenty PAHs was not successful, better results were obtained compared to those achieved when using β- or γ-CDs alone in the separation buffer. Table 2 shows the results regarding analysis times and the numbers of peaks for the separations carried out using different mixtures of β- and γ-CDs at a constant concentration of borate buffer (0.100 *M*). First, β-CD was added to a fixed concentration of 0.050 *M* γ-CD, which gave good results when this CD was used alone in the separation buffer. The migration order was altered by the addition of β-CD. When 0.030 or 0.040 *M* β-CD was added to a 0.050-*M* γ-CD separation buffer, fifteen peaks were obtained for the mixture containing twenty PAHs. These preliminary results suggested that the use of a mixture of β- and γ-CDs may provide better results than those obtained using a single CD. In fact, one more compound was resolved from the twenty PAHs than when γ-CD was used alone, in the same analysis time. Then, the ratio between the concentrations of β- and γ-CDs in the separation buffer (β/γ) as well as the total concentration of both CDs in the mixture were varied. The addition of two different concentrations of β-CD (0.040 and 0.070 *M*) to a constant concentration of γ-CD (0.020 *M*), which was less than that employed in the first experiments, gave separations that were worse than those obtained when the β/γ ratio was less than unity, with the number of peaks separated being twelve and fourteen, respectively. When the concentration of β-CD was kept constant at 0.070 *M* and different concentrations of γ-CD were used (ranging from 0.015 to 0.040 *M*), worse selectivity was obtained, except for a 0.070-*M* β-CD–0.015-*M* γ-CD mixture, which gave rise to fifteen peaks. Table 2 shows that when the total concentration of CDs is kept constant but the β/γ ratio is varied, the numbers of peaks obtained differ, indicating an influence of the β/γ ratio on the selectivity.

A new set of experiments was conducted using a 0.120-*M* buffer concentration in order to increase the elution window. In fact, an increase in the borate buffer concentration could improve the separations. The cost of this resolution enhancement is an increase in the analysis time due to a decrease in the electroosmotic flow. Table 3 summarizes the results obtained in terms of analysis time and the number of peaks for all experiments carried out with mixtures of β- and γ-CDs at different ratios and total concentrations. Table 3 shows that, in all experiments carried out with values of the β/γ ratio higher than two, separation could not be improved to more than fourteen peaks, indicating again the dependence of the separation on the ratio of both CDs, as suggested by the differential partitioning model [20]. In addi-

Table 2
Number of peaks and analysis times obtained using different mixtures of β- and γ-CDs in a 0.100-*M* borate separation buffer containing 2.5 *M* urea and 0.100 *M* SDS

Total CD concentration (*M*)	[β] (*M*):[γ] (*M*)	β/γ ratio	Analysis time (min)	Number of peaks
0.060	0.040:0.020	2.00	31	12
0.080	0.030:0.050	0.60	31	15
0.085	0.070:0.015	4.67	33	15
0.090	0.040:0.050	0.80	32	15
0.090	0.070:0.020	3.50	34	14
0.095	0.070:0.025	2.80	32	14
0.100	0.070:0.030	2.33	33	14
0.110	0.070:0.040	1.75	25	6

Table 3
Number of peaks and the analysis times obtained using different mixtures of β- and γ-CDs in a 0.120-*M* borate separation buffer containing 2.5 *M* urea and 0.100 *M* SDS

Total CD concentration (*M*)	[β] (*M*):[γ] (*M*)	β/γ ratio	Analysis time (min)	Number of peaks
0.059	0.021:0.038	0.55	36	16
0.062	0.042:0.030	1.40	41	16
0.068	0.038:0.030	1.27	42	17
0.068	0.040:0.028	1.43	43	17
0.070	0.020:0.050	0.40	39	17
0.070	0.035:0.035	1.00	38	15
0.070	0.040:0.030	1.33	39	17
0.070	0.050:0.020	2.50	41	13
0.072	0.040:0.032	1.25	41	17
0.074	0.021:0.053	0.40	38	16
0.074	0.042:0.032	1.31	40	17
0.075	0.025:0.050	0.50	41	17
0.075	0.040:0.035	1.14	39	15
0.075	0.045:0.030	1.50	40	15
0.080	0.025:0.055	0.45	37	17
0.080	0.030:0.050	0.60	38	15
0.080	0.035:0.045	0.78	38	17
0.080	0.050:0.030	1.67	41	16
0.085	0.030:0.055	0.55	40	15
0.085	0.035:0.050	0.70	40	16
0.085	0.050:0.035	1.43	43	15
0.090	0.035:0.055	0.64	38	16
0.090	0.070:0.020	3.50	42	14
0.095	0.070:0.025	2.80	41	14

tion, when comparing similar situations that differ only in the borate buffer concentration (0.100 and 0.120 *M*), differences were not observed in the number of peaks obtained, just in the better resolution between peaks. Table 3 shows the influence that both the ratio between the concentrations of β- and γ-CDs and the total concentration of CDs have on the separation selectivity. However, it is difficult to establish a general behaviour. As a result of these experiments, the number of peaks obtained in the separation of the mixture of twenty PAHs could be increased from fifteen to seventeen.

Some of the conditions summarized in Table 3 that gave the best separations were selected and investigated with a new borate concentration of 0.140 *M*. The results are presented in Table 4. In this case, all values for the β/γ ratio were in the range of 0.45 to 1.61 and the total concentration of CDs in the mixture was in the range from 0.062 to 0.084 *M*. It can be seen that, of all of the mixtures investigated in this work, a mixture containing 0.042 *M* β-CD and 0.026 *M* γ-CD was found to be the most effective in the separation of the compounds studied, allowing eighteen peaks to be determined out of the twenty PAHs studied. Fig. 1 shows the separation of all of the PAHs contained in the mixture studied, except for two pairs, perylene/anthracene and benzo[*a*]pyrene/benzo[*a*]anthracene. These results are better than those obtained when γ-CD was used alone in the separation buffer. In fact, more compounds are separated (selectivity is improved) and the cost is reduced also because β-CD is used at higher concentrations than those of γ-CD, which is much more expensive.

3.2. Study of the correlation between the logarithm of the capacity factors of PAHs and the logarithm of their octanol–water partition coefficients

Fig. 1 shows that no rigorous relationship was observed between the size of the PAHs and their migration order. In fact, one of the studied hexa-

Table 4
Number of peaks and the analysis times obtained using different mixtures of β- and γ-CDs in a 0.140-*M* borate separation buffer containing 2.5 *M* urea and 0.100 *M* SDS

Total CD concentration (*M*)	[β] (*M*):[γ] (*M*)	β/γ ratio	Analysis time (min)	Number of peaks
0.068	0.042:0.026	1.61	54	18
0.069	0.040:0.029	1.38	48	14
0.071	0.040:0.031	1.29	53	17
0.072	0.042:0.030	1.40	49	16
0.074	0.023:0.051	0.45	48	15
0.074	0.043:0.031	1.39	56	16
0.075	0.025:0.050	0.50	52	14
0.080	0.025:0.055	0.45	46	15
0.080	0.044:0.035	1.25	51	15
0.084	0.026:0.058	0.45	46	14
0.085	0.049:0.035	1.39	44	15

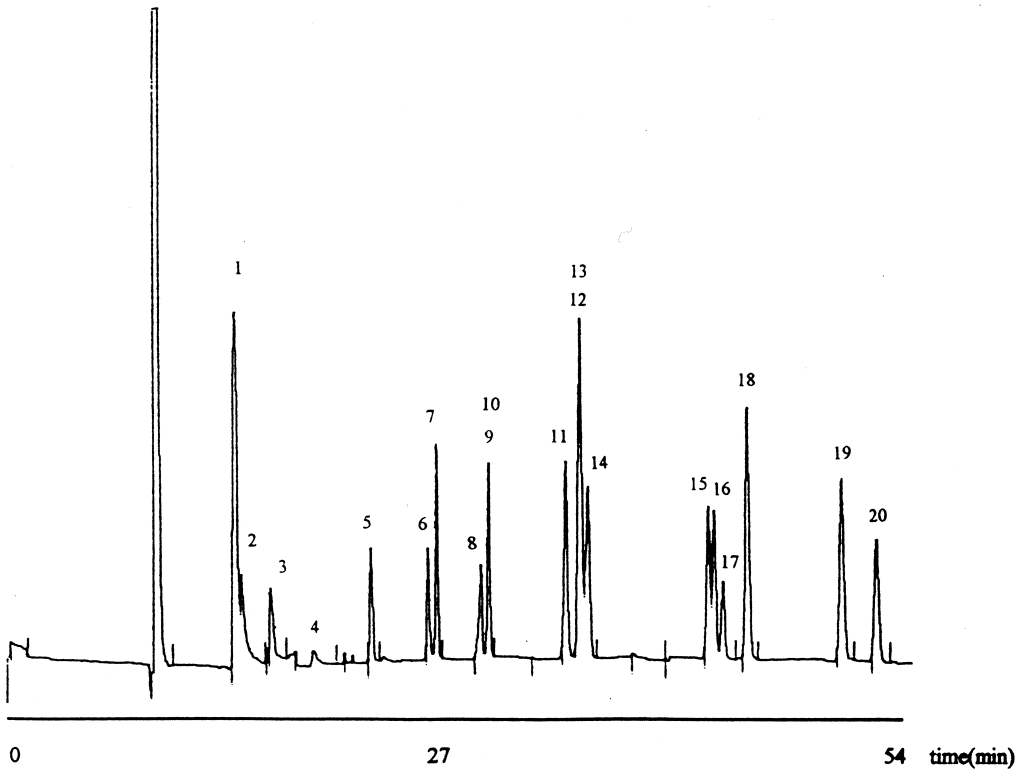

Fig. 1. Separation of twenty PAHs (including sixteen EPA priority PAHs) by MCD-MEKC. Conditions: Capillary, 65 cm×50 μm I.D. (50.5 cm length to the detector); injection by pressure, 0.02 min at 20 mbar, the concentration of each PAH in the mixture was 0.1 μg/μl; UV detection at 230 nm; separation solution, 0.042 *M* β-CD, 0.026 *M* γ-CD, 2.5 *M* urea and 0.100 *M* SDS in 0.140 *M* borate buffer (pH 9); applied voltage, 15 kV. For solute identification, see Table 1.

cyclic PAHs (benzo[*g,h,i*]perylene, number 4) eluted among PAHs one to eight, which are naphthalene, four tricyclic PAHs and two tetracyclic PAHs. However, the other hexacyclic PAH studied, indeno[1,2,3-*c,d*]pyrene (number 20), eluted last. A similar behaviour was reported by Terabe et al. [12], who showed that benzo[*g,h,i*]perylene was eluted first and indeno[1,2,3-*c,d*]pyrene eluted last in the separation of a mixture of sixteen PAHs using γ-CD in the separation buffer. In fact, determination of the migration order in MCD-MEKC can be a rather complex exercise involving differential partitioning between the two types of hydrophobic domains (micelles and CDs) as a function of partition coefficients, inclusion complex formation, hydrophobicities and molecular geometries [21]. This is why it was of interest to study the correlation between the logarithm of the capacity factors of compounds and the logarithm of the octanol–water distribution coefficients (P_{ow}), which account for the hydrophobicity of a solute. The studies achieved for PAHs when β-CD or γ-CD are used alone in the separation buffer in CD-MEKC show that a linear correlation can be found for the log k'–log P_{ow} correlation [22,23]. This may indicate that hydrophobic interactions play an important role in the separation mechanism of PAHs by CD-MEKC, especially when C_1 and C_{18} surface-treated capillaries are used [23].

Since the log k'–log P_{ow} correlation for PAHs has not been studied when β- and γ-CD mixtures were used in the separation buffer, we have thought that it would be interesting to investigate this correlation in MCD-MEKC. Fig. 2 shows the variation of log k' as a function of log P_{ow} for PAHs 1–19 in Table 1. In accordance with other works in which β- and γ-CDs were used alone in CD-MEKC, a linear correlation can be found ($r^2 = 0.846$) if some of the compounds studied are excluded from the linear regression. In this case, the compounds that deviated from the linear relationship were benzo[*g,h,i*]perylene, perylene and benzo[*b*]fluoranthene. These deviations were attributed in other works on CD-MEKC to different interactions of these compounds with the CDs' cavities [22]. This could be the case for compounds 4 and 9, whose capacity factors are lower than expected from the log k'–log P_{ow} correlation. It can also be noted that the error that affects the determination of the capacity factor in MEKC

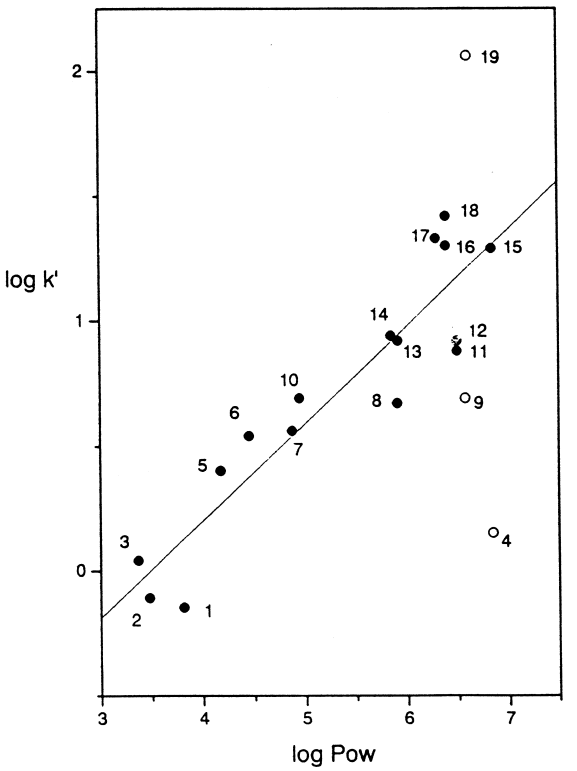

Fig. 2. Variation of log k' for PAHs as a function of their log P_{ow} values. Values of k' were determined using the experimental conditions given in Fig. 1. For solute identification and log P_{ow} values, see Table 1.

increases when the migration time of the solute is close to the migration time of the micelle [24] and this may be the case for compound 19.

Multiple regression analyses of data were performed, taking log P_{ow} and n (number of rings of the PAH) or V (Van der Waals volume) or L/B (the ratio of the maximum length-to-breadth of the rectangle enclosing the molecules) as the regression parameters [25,26]. Values for r^2 alone are higher than those obtained for the linear regression of log k'–log P_{ow} when compounds numbered 4, 9 and 19 were excluded from the multiple regression analysis. In this case, r^2 values were 0.850, taking log P_{ow} and n as being the regression parameters, 0.861, taking log P_{ow} and V as being the regression parameters, and 0.898, taking log P_{ow} and L/B as being the regression parameters.

4. Conclusions

From the results presented in this work, it can be concluded that selectivity in CD-MEKC can be improved by using mixtures of β- and γ-CDs in the separation buffer. A β-CD concentration of 0.042 *M* and a γ-CD concentration of 0.026 *M* (β/γ ratio equal to 1.6) enabled the separation of eighteen PAHs from a mixture of twenty, when a 0.140-*M* borate buffer (pH 9) containing 0.100 *M* SDS and 2.5 *M* urea was used. The selectivity that can be achieved in MCD-MEKC made this technique an interesting alternative for the separation of PAHs. The use of detection techniques such as laser-induced fluorescence detection will enable the sensitive determination of PAHs in real samples as performed by MEKC techniques [27].

With some exceptions, a linear correlation was found between log k' and log P_{ow} for the compounds studied, which showed the importance of hydrophobic interactions in the separation mechanism by MCD-MEKC. A higher correlation was found by multiple regression analysis taking log P_{ow} and the length-to-breadth ratio of molecules as the regression parameters.

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