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Journal of Chromatography A, 852 (1999) 559–571

JOURNAL OF CHROMATOGRAPHY A

pH effects on micelle–water partitioning determined by micellar electrokinetic chromatography

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Received 1 September 1998; received in revised form 19 April 1999; accepted 28 April 1999

Abstract

In many biological and environmental situations, the pH of aqueous media varies and differences in solute partitioning may result. However, the majority of biopartitioning and hydrophobicity studies conducted have been at pH 7. Using migration factors measured by micellar electrokinetic chromatography, we have determined pH effects on micelle–water partitioning for 19 compounds. We develop an improvement to the migration factor equation and the corrected migration factor for aniline shows a definite increase as pH decreases. The corrected migration factor was constant for the rest of the compounds over the pH ranged studied. We also investigated five micelle markers and determined that decanophenone is the best micelle marker to date. Decanophenone has a strong chromophore, detectable at all pH levels, and is easy to dissolve in the mobile phase.

Keywords: pH effects; Partitioning; Micelles; Decanophenone; Benzoic acids; Phenols; Amino acids; Anilines

1. Introduction

One of the major difficulties facing pharmaceutical and environmental scientists is the accurate measurement of biopartitioning data. These data are important in determining drug uptake, bioconcentration of compounds, and compounds' environmental fate and transport. Fent and Looser [1], and Wildi et al. [2] have studied the biopartitioning of selected solutes as a function of pH. Their results indicate that pH does influence biopartitioning. However, since the cost of determining biopartitioning values at a single pH level can range up to and exceed US$ 10 000 per compound [3], an alternative approach is needed to predict biopartitioning. Hydrophobicity is the most utilized physical property to predict biopartitioning. To determine hydrophobicity values, Hansch et al. [4–6] suggested measuring the partitioning of a solute between octanol and water. The octanol–water partition coefficient (K_{ow}) is defined as the concentration ratio of the solute in water-saturated octanol to octanol-saturated water. From these experiments, log K_{ow} values correlate with many biopartitioning values, but exceptions are common [7,8].

Since Hansch's work [5,6], several attempts to measure or estimate log K_{ow} values using different analytical techniques have been made. Reversed-phase liquid chromatography (RPLC) has been the alternate method of choice [9,10]. Examples of other analytical methods include: the slow stirring method [11], counter-current chromatography [12], computer

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00599-3

modeling [13], solubility values [14], micellar electrokinetic chromatography (MEKC) [15] and activity coefficients [16]. Except for MEKC, all other methods are either labor-, time- or equipment intensive.

To determine the relevance of the various methods used to predict biopartitioning values, the thermodynamics of octanol–water partitioning and biopartitioning have been studied. For example, Beezer et al. [17] investigated the octanol–water partitioning of resorcinol and Anderson et al. [18] studied the octanol–water partitioning of *p*-substituted phenols. Generally, octanol–water partitioning is enthalpically driven. However, Beezer et al. [17] indicated that, as the side chain of the resorcinol monoether increased in size, the partitioning became more entropically driven. In contrast, Matsuo [19] determined biopartitioning of polychlorinated biphenyls into fish to be entropically driven. Opperhuizen et al. [20] compared the thermodynamics of biopartitioning of five chlorobenzenes into fish lipids, which are entropically driven, to octanol–water partitioning, which is enthalpically driven. Unfortunately, log K_{ow} values are often not thermodynamically relevant to biopartitioning. Woodrow and Dorsey [21] have validated these results by measuring the thermodynamics of micelle–water partitioning of selected compounds and comparing these values to those for octanol–water partitioning.

Is RPLC a more relevant model? Sentell and Dorsey [22] investigated the thermodynamics of naphthalene partitioning into an octadecyl (C_{18}) chromatographic stationary phase with various surface coverages or bonding densities. They concluded that partitioning is enthalpically driven for a C_{18} bonding density less than about 3.0 $\mu mol/m^2$ and entropically driven for a C_{18} bonding density of greater than about 3.0 $\mu mol/m^2$. Thus, as the stationary phase becomes more ordered, entropy becomes the major force in the partitioning of the solute. Solute retention determined on a high C_{18} bonding density column is better correlated with biopartitioning values than retention measured on a low C_{18} bonding density column [23].

Solute retention determined by RPLC has often been used to correlate with log K_{ow}. Most RPLC mobile phases have a significant amount of organic modifier present to aid in the dissolution and elution of the solute. However, there is no one mobile phase composition that is useful for all solutes. Using several mobile phase compositions, solute retention is commonly plotted as a function of percent organic modifier and extrapolated to 100% water (k'_w). However, Hsieh and Dorsey [24] have shown that curvature in the k' versus percent composition of modifier is present at very low percent organic modifier. The error introduced by the curvature can be large and unpredictable.

MEKC may quickly become the method of choice for estimating hydrophobicity due to the minimal amount of hazardous waste produced, calculated migration factors (k'_m) are in 100% water, and shorter analysis times. Herbert and Dorsey [25] published the first comprehensive study of the use of k'_m values from MEKC to correlate with log K_{ow} values. Hanna et al. [26] have recently reinforced the ease and usability of MEKC to model octanol–water partitioning coefficients.

2. Theory

In capillary zone electrophoresis (CZE), charged species are separated due to differences in their electrophoretic mobilities (μ_s). Neutral compounds migrate with the electroosmotic flow (EOF) and are not separated by this technique. Therefore, the electrophoretic mobility of a neutral solute is defined as zero. To determine the electrophoretic mobility of a charged solute, the electroosmotic mobility (μ_{EOF}) is subtracted from the observed electrophoretic mobility (μ_{obs}), as shown in Eq. (1).

$$\mu_s = \mu_{obs} - \mu_{EOF} \quad (1)$$

In MEKC, as described by Terabe et al. [27,28], the buffer contains a surfactant above the critical micelle concentration. Thus, the solutes interact with micelles, causing separation of both the neutral and charged solutes. k'_m values are calculated using electrophoretic mobilities as shown below.

$$k'_m = \left(\frac{\mu_{sm}}{\mu_m - \mu_{sm}} \right) \quad (2)$$

where μ_m is the electrophoretic mobility of the micelle marker and μ_{sm} is the electrophoretic mobility of the solute determined in a MEKC system.

Khaledi et al. [29] have performed some limited

experiments involving the migration of a solute at different pH values to understand the effect of pH on a hypophosphate buffer system. They determined that the migration of the solute by MEKC is a weighted average of the migration of the charged solute in the buffer, the migration of the solute interacting with micelles and the migration of the neutral molecule. Thus, if the migration of the solute is determined without the presence of surfactant, via CZE, the corrected k'_m value of the solute at the specific pH of the buffer can be calculated. A corrected k'_m value can be determined by subtracting the electrophoretic mobility of the solute determined by CZE from the electrophoretic mobility of the solute analyzed by MEKC. The difference is then divided by the electrophoretic mobility of the micelle subtracted by the electrophoretic mobility of the solute analyzed by MEKC. This is shown in Eq. (3) [29],

$$k'_m = \left(\frac{\mu_{sm} - \mu_s}{\mu_m - \mu_{sm}} \right) \quad (3)$$

where μ_s is the electrophoretic mobility of the solute analyzed under CZE conditions.

For our study, all calculations involving MEKC and CZE use electrophoretic mobilities because of variability of the EOF. EOF variability may be caused by ionic strength differences between the CZE and MEKC buffer, changes in column conditions beyond the control of the experimenters, changes in the dielectric constant, or changes in the zeta potential. We have assumed that the critical micellar concentration (CMC) and the aggregation number of the surfactant remain constant over the pH range of interest.

3. Experimental

3.1. Reagents

Reagents were obtained from various sources and were used as received. Water for buffer and sample preparation was obtained from a Barnstead Nanopure II water purification system (Barnstead, Boston, MA, USA), filtered with a 0.45-μm nylon filter. All buffers were filtered using Gelman acrodisk 0.45 μm nylon filters (Gelman Scientific, Ann Arbor, MI, USA).

3.2. Standard and sample preparation

To prepare stock standard solutions of the solutes listed in Table 1, each solute was weighed and added to 10 ml of methanol to make an approximately 0.5–0.25 *M* standard. To prepare an analytical sample, 15 μl of the standard were added to 100–250 μl of methanol, which is used as an EOF marker, and enough buffer to make 4 ml of sample.

Table 1
List of constituents and associated pK_a values used to determine pH effects on micelle–water partitioning

Compound	pK_a
Carboxylic acids	
Benzoic acid	(4.20)
p-Hydroxybenzoic acid	(4.58)
p-Chlorobenzoic acid	(3.99)
p-Nitrobenzoic acid	(3.44)
Phenols	
2,4-Dinitrophenol	(4.11)
p-Nitrophenol	(7.14)
Phenol	(9.95)
p-Bromophenol	(9.34)
Anilines	
Aniline	(4.64)
o-Nitroaniline	(−0.28)
p-Bromoaniline	(3.91)
2,4-Dinitroaniline	(−4.27)
Zwitterions	
Tryptophan	(2.38, 9.39)
Tyrosine	(2.20, 9.11, 10.07)
Phenylalanine	(1.83, 9.13)
m-Aminobenzoic acid	(3.07, 4.73)
Neutrals	
Nitrobenzene	(N/A)
Naphthalene	(N/A)
m-Dinitrobenzene	(N/A)
Micelle markers	
Decanophenone	(N/A)
Sudan IV	(N/A)
Sudan III	(N/A)
Orange OT	(N/A)
Yellow AB	(N/A)

(N/A), not applicable.
pK_a values are from Ref. [37].

Decanophenone was used as the micelle marker and was analyzed separately.

3.3. Buffer preparation

To prepare the phosphate buffer, a 20-m*M* Na_2HPO_4 solution was prepared and adjusted to the appropriate pH with either 20 m*M* H_3PO_4 or 20 m*M* Na_3PO_4 solution. To prepare the phosphate–sodium chloride buffer, a 20-m*M* Na_2HPO_4–50 m*M* NaCl solution was prepared and adjusted to the appropriate pH with either a 20-m*M* H_3PO_4–50 m*M* NaCl or 20 m*M* Na_3PO_4–50 m*M* NaCl solution. To prepare the MEKC buffer, a 20-m*M* Na_2HPO_4–50 m*M* sodium dodecyl sulfate (SDS) solution was prepared and adjusted to the appropriate pH using 20 m*M* H_3PO_4–50 m*M* SDS or 20 m*M* Na_3PO_4–50 m*M* SDS solution.

3.4. Instrumentation

The data were collected using a Waters Quanta 4000 capillary electrophoresis instrument (Waters, Milford, MA, USA), which was attached to a PE Nelson (Perkin-Elmer, Norwalk, CT, USA) data acquisition box and a 33-MHz 486 computer. The data were collected at a wavelength of 254 nm and processed and manipulated by Turbochrom v. 4.0 (Perkin-Elmer) data acquisition software. The temperature was controlled by a water jacket system made in-house using a design published by Lukkari et al. [30].

The column was constructed from a 50-μm I.D. fused-silica capillary with a total length of 70 cm. The detection window was placed 7.5 cm from the outlet end, yielding a separation length of 62.5 cm. For the analysis of solutes between the pH values of 8–6 inclusive, the applied voltage was 17.5 kV. Thus, the electric field gradient was 250 V/cm. The analysis times varied, depending on the migration of the compounds of interest. Injections were performed hydrostatically for 7–10 s.

4. Results and discussion

4.1. Capacity factors as a function of pH

One of the difficulties in determining a migration factor via MEKC is that there are several migration velocities involved in the overall observed migration. Khaledi et al. [29] have identified these velocities as the electroosmotic mobility of the uncharged solute, the electroosmotic mobility of the charged solute and the electroosmotic mobility of the solute interacting with the micelle. They also considered the possibility of the charged solute interacting with the micelle. This is so minute that we have assumed that the interaction of a like-charged solute with a micelle is inconsequential.

The pH range utilized for this study was limited because, at pH 5 and lower, the electrophoretic mobility of the micelle is higher than the EOF. This results in the micelle having a net migration in the opposite direction from the EOF. This phenomenon was first observed by Otsuka and Terabe [31]. The actual pH level where this phenomenon occurs is dependent on the buffer system used. As we increased pH, the ionic strength of the buffer also increased due to increased concentration of multiply charged ions. We kept the phosphate concentration constant at 20 m*M* rather than keeping the ionic strength constant. Since the majority of studied solutes are charged, the buffering capacity of the solution needed to be as strong as possible under the experimental conditions.

The pH range is also limited to pH 8 or lower due to the poor buffering capacity of the phosphate buffer at levels above pH 8 and the influence of carbon dioxide on the buffer system. Due to design of the buffer vials, the buffer solution could not be covered during analysis. As carbon dioxide is absorbed into the buffer system, it is quickly hydrated to form carbonic acid, thereby decreasing the pH of the buffer. The amount of carbon dioxide that can be absorbed into the buffer is dependent on the initial pH of the system. The higher the pH, the higher the level of carbon dioxide when equilibrium is reached [32]. Evidence of this effect can be observed by the change in EOF after a period of time.

In addition, we decided to keep the concentration of the sample as constant as possible to reduce the effect of conductivity differences between the sample and the buffer solution. This phenomenon was initially described by Hjerten [33]. If the concentration of the sample varies between analysis with each pH and buffer system, variability of the measurement of the migration factor is introduced.

4.2. Determination of migration factors

To determine the migration factor, each solute was analyzed four times and the average electrophoretic mobilities were determined. One migration factor was calculated using the average values. Electrophoretic mobilities were used to calculate the migration factors instead of time as Khaledi et al. [29] suggest. One assumption they made is that the EOF was identical for CZE and MEKC analyses at a given pH. The data collected by our system demonstrate that this assumption is not valid for this study under the analytical conditions described above.

As pH changes and solutes become less charged, the migration factors increase. Specifically, as pH decreases, migration factors should increase and level off for all acids and phenols. The migration factors for anilines should decrease as the pH decreases. Zwitterions may have a more complicated chemistry and no trend can be predicted.

Initially, we used Eq. (2) to calculate migration factors, which are reported in Table 2. *p*-Nitrophenol and *p*-hydroxybenzoic acid followed the expected trend, i.e., as pH decreased, the migration factor decreased. Migration factors for aniline and *p*-bromoaniline increased as the pH decreased. All other migration factors were random as pH decreased. The migration factor of aniline and *p*-bromoaniline increasing as pH decreased may be indicative of the positively charged solute electrostatically binding to the micelle. Also, since naphthalene is the last migrating compound, the error associated with its measurement is high. At first glance, the migration factor of naphthalene seems to decrease as pH decreases. However, the standard deviations of these measurements are large, and the values are equivalent within measurement error.

We used Eq. (3) to calculate corrected migration factors and the values are listed in Table 3. We observed three trends. The migration factors of *p*-nitrophenol, *p*-bromoaniline and aniline increased as the pH decreased to pH 7. k'_m values were relatively

Table 2
Summary of migration factors (k'_m) and standard deviations ($n=4$) determined using Eq. (2)

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	2.38±0.03	2.45±0.03	2.35±0.05	2.38±0.05	2.40±0.03
p-Hydroxybenzoic acid	1.94±0.02	1.82±0.02	1.67±0.02	1.67±0.02	1.59±0.01
p-Chlorobenzoic acid	1.95±0.02	2.00±0.03	1.92±0.03	1.93±0.03	2.07±0.02
p-Nitrobenzoic acid	2.02±0.02	2.01±0.02	1.99±0.03	2.00±0.03	2.01±0.02
Phenols					
2,4-Dinitrophenol	2.15±0.04	2.45±0.04	2.10±0.04	2.27±0.02	2.23±0.03
p-Nitrophenol	2.16±0.02	1.89±0.02	1.68±0.02	1.39±0.03	1.27±0.01
Phenol	0.663±0.005	0.591±0.005	0.644±0.006	0.629±0.007	0.553±0.009
p-Bromophenol	4.22±0.07	3.93±0.07	3.83±0.1	4.04±0.07	3.67±0.07
Anilines					
Aniline	0.534±0.004	0.492±0.006	0.628±0.008	0.684±0.005	1.14±0.01
o-Nitroaniline	2.22±0.1	2.13±0.03	2.49±0.06	2.39±0.1	2.10±0.2
p-Bromoaniline	3.50±0.05	3.21±0.04	3.29±0.08	3.55±0.08	3.84±0.06
2,4-Dinitroaniline	3.09±0.06	2.89±0.06	2.87±0.07	2.92±0.06	2.89±0.05
Zwitterions					
Tryptophan	0.701±0.004	0.607±0.006	0.704±0.01	0.652±0.01	0.655±0.01
Tyrosine	0.180±0.004	0.143±0.003	0.137±0.006	0.107±0.03	0.108±0.003
Phenylalanine	0.341±0.004	0.278±0.01	0.315±0.003	0.322±0.005	0.261±0.004
m-Aminobenzoic acid	1.95±0.02	1.96±0.3	1.88±0.03	1.92±0.02	1.77±0.02
Neutrals					
Nitrobenzene	1.63±0.02	1.47±0.01	1.46±0.05	1.43±0.01	1.38±0.01
Naphthalene	16.2±3	15.2±1	12.5±1	15.4±0.9	11.8±0.5
m-Dinitrobenzene	1.46±0.01	1.30±0.01	1.46±0.02	1.43±0.01	1.29±0.02

Table 3
Summary of migration factors (k'_m) and standard deviations ($n=4$) determined using Eq. (3)

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	−0.0245±0.007	−0.0222±0.05	−0.0690±0.01	−0.0285±0.02	−0.0912±0.01
p-Hydroxybenzoic acid	−0.0191±0.004	−0.0663±0.03	−0.0559±0.005	−0.0139±0.01	−0.100±0.004
p-Chlorobenzoic acid	0.00361±0.01	0.0136±0.04	−0.0395±0.008	−0.0232±0.01	−0.0461±0.004
p-Nitrobenzoic acid	−0.00809±0.005	−0.0326±0.008	−0.0505±0.007	−0.0340±0.009	−0.0971±0.007
Phenols					
2,4-Dinitrophenol	−0.0142±0.02	−0.0127±0.01	−0.0512±0.006	−0.0408±0.009	−0.0731±0.009
p-Nitrophenol	0.0713±0.01	0.216±0.02	0.494±0.009	0.777±0.02	1.01±0.01
Phenol	0.617±0.005	0.591±0.005	0.644±0.006	0.629±0.007	0.553±0.009
p-Bromophenol	3.89±0.07	3.93±0.07	3.83±0.1	4.04±0.07	3.67±0.07
Anilines					
Aniline	0.534±0.004	0.491±0.006	0.628±0.008	0.684±0.006	1.16±0.01
o-Nitroaniline	2.22±0.1	2.13±0.03	2.49±0.06	2.39±0.1	2.10±0.2
p-Bromoaniline	3.50±0.05	3.21±0.04	3.29±0.08	3.55±0.08	3.84±0.06
2,4-Dinitroaniline	3.09±0.06	2.89±0.06	2.87±0.07	2.92±0.06	2.89±0.05
Zwitterions					
Tryptophan	0.626±0.004	0.607±0.006	0.704±0.01	0.652±0.01	0.655±0.01
Tyrosine	0.105±0.004	0.116±0.003	0.111±0.006	0.107±0.03	0.108±0.003
Phenylalanine	0.255±0.004	0.231±0.01	0.292±0.003	0.322±0.005	0.261±0.004
m-Aminobenzoic acid	−0.0224±0.007	−0.0280±0.009	−0.0750±0.008	−0.0348±0.006	−0.123±0.009

constant as pH decreased for nitrobenzene, naphthalene, *m*-dinitrobenzene, phenylalanine, *o*-nitroaniline, tyrosine, tryptophan, *p*-bromophenol, phenol and 2,4-dinitroaniline. The migration factors were negative for benzoic acid, *p*-hydroxybenzoic acid, *p*-chlorobenzoic acid, *p*-nitrobenzoic acid, 2,4-dinitrophenol and *m*-aminobenzoic acid.

A negative k'_m value results when the electroosmotic mobility of the solute is higher in the MEKC experiment than the CZE experiment. However, a negative value is an impossibility due to the definition of migration factors. Briefly, the migration factor describes the ratio of the amount of time the solute spends in the micellar phase to the aqueous phase. This ratio can be related to the concentration ratio of the solute in the micellar phase to the aqueous phase. Mathematically, to obtain a negative migration factor, the electrophoretic mobility of the solute in CZE must be higher than the electrophoretic mobility of the solute in MEKC. A reason that may cause the electrophoretic mobility of the solute in MEKC to be lower than the electrophoretic mobility in CZE is to increase the contribution of the neutral solute to the weighted average of the electrophoretic mobilities. This may be indicative of ion pairing. Work has been performed on the use of ion pairing in CZE and Shelton et al. [34] have recently provided a review of this.

4.3. Sodium chloride as a buffer modifier

Since the MEKC buffer has a higher concentration of sodium ions than the CZE buffer, sodium chloride was added to the 20 m*M* phosphate buffer to create a 20-m*M* phosphate–50 m*M* sodium chloride buffer. This buffer should mimic the concentration of sodium ions present in the MEKC buffer. Others have added sodium chloride and other alkali metal salts to the CZE buffer. Junppanen et al. [35] used alkali salts to aid in the separation of diuretics by CZE. Their results indicate reduced electroosmotic and electrophoretic mobility as ionic strength increased, in agreement with the data presented here.

As an adaptation of Eq. (3), we have replaced the electrophoretic mobility of the solute in CZE (μ_s) with the electrophoretic mobility of the solute in the presence of sodium chloride (μ_{ss}). The resulting equation is described below:

Table 4
Summary of migration factors (k'_m) and standard deviations ($n=4$) determined using Eq. (4)

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	0.0652±0.007	0.108±0.01	0.0593±0.009	0.0311±0.03	0.00952±0.01
p-Hydroxybenzoic acid	0.0562±0.006	0.107±0.009	0.0568±0.005	0.0288±0.009	−0.00462±0.003
p-Chlorobenzoic acid	0.0667±0.008	0.0995±0.01	0.0580±0.02	0.0278±0.01	0.0499±0.003
p-Nitrobenzoic acid	0.0567±0.004	0.0758±0.005	0.0618±0.007	0.0228±0.009	0.0000836±0.006
Phenols					
2,4-Dinitrophenol	0.0525±0.02	0.107±0.02	0.0339±0.005	0.0392±0.007	0.0196±0.01
p-Nitrophenol	0.258±0.01	0.377±0.01	1.68±0.02	0.810±0.02	1.03±0.01
Phenol	0.628±0.005	0.591±0.005	0.644±0.006	0.629±0.007	0.553±0.009
p-Bromophenol	3.89±0.07	3.75±0.07	3.71±0.1	4.04±0.07	3.67±0.07
Anilines					
Aniline	0.534±0.004	0.491±0.006	0.628±0.008	0.684±0.006	1.14±0.01
o-Nitroaniline	2.22±0.1	2.13±0.03	2.49±0.06	2.39±0.1	2.10±0.2
p-Bromoaniline	3.50±0.05	3.21±0.04	3.29±0.08	3.55±0.08	3.84±0.06
2,4-Dinitroaniline	3.09±0.06	2.89±0.06	2.87±0.07	2.92±0.06	2.89±0.05
Zwitterions					
Tryptophan	0.639±0.005	0.567±0.006	0.670±0.01	0.652±0.01	0.655±0.01
Tyrosine	0.126±0.004	0.101±0.003	0.103±0.006	0.107±0.03	0.108±0.003
Phenylalanine	0.281±0.005	0.237±0.01	0.283±0.003	0.322±0.005	0.261±0.004
m-Aminobenzoic acid	0.0473±0.003	0.0713±0.01	0.0312±0.01	0.0356±0.009	−0.0270±0.01

$$k'_m = \frac{(\mu_{sm} - \mu_{ss})}{(\mu_m - \mu_{sm})} \quad (4)$$

As shown in Table 4, the number of negative values calculated using Eq. (4) was sharply reduced. Only very small negative migration factors were calculated for *p*-hydroxybenzoic acid and *m*-aminobenzoic acid at pH 6.

All k'_m values remained constant over the pH range, with the exception of aniline, where k'_m increased as pH decreased. The increase in migration factor for aniline can be explained by increased electrostatic attraction between the solute and the micelle as the solute becomes positively charged. The low values of k'_m, between zero and one, make sense since most of these compounds are charged or very hydrophilic. A disturbing trend of k'_m decreasing as pH decreases seems to be present for the acidic compounds. However, the points are all within the rather high standard deviations, so, within experimental error, the partitioning for acidic solutes remains constant over the pH range of interest.

4.4. Comparison of different micelle markers

We evaluated five micelle markers described in the

Table 5
Electrophoretic mobilities (cm^2/min V) and standard deviations ($n=4$) of micelle markers as a function of pH

	pH of buffer				
	8	7.5	7	6.5	6
Decanophenone	−0.0263±0.00007	−0.0263±0.00008	−0.0261±0.0001	−0.0261±0.00007	−0.0257±0.00007
Sudan IV	N/A	N/A	N/A	N/A	N/A
Sudan III	N/A	N/A	N/A	N/A	N/A
Orange OT	−0.0263±0.00007	−0.0251±0.0007	−0.0260±0.0001	−0.0260±0.00004	−0.0255±0.0004
Yellow AB	−0.0263±0.0001	−0.0265±0.00005	−0.0258±0.00003	−0.0259±0.0001	−0.0250±0.00005

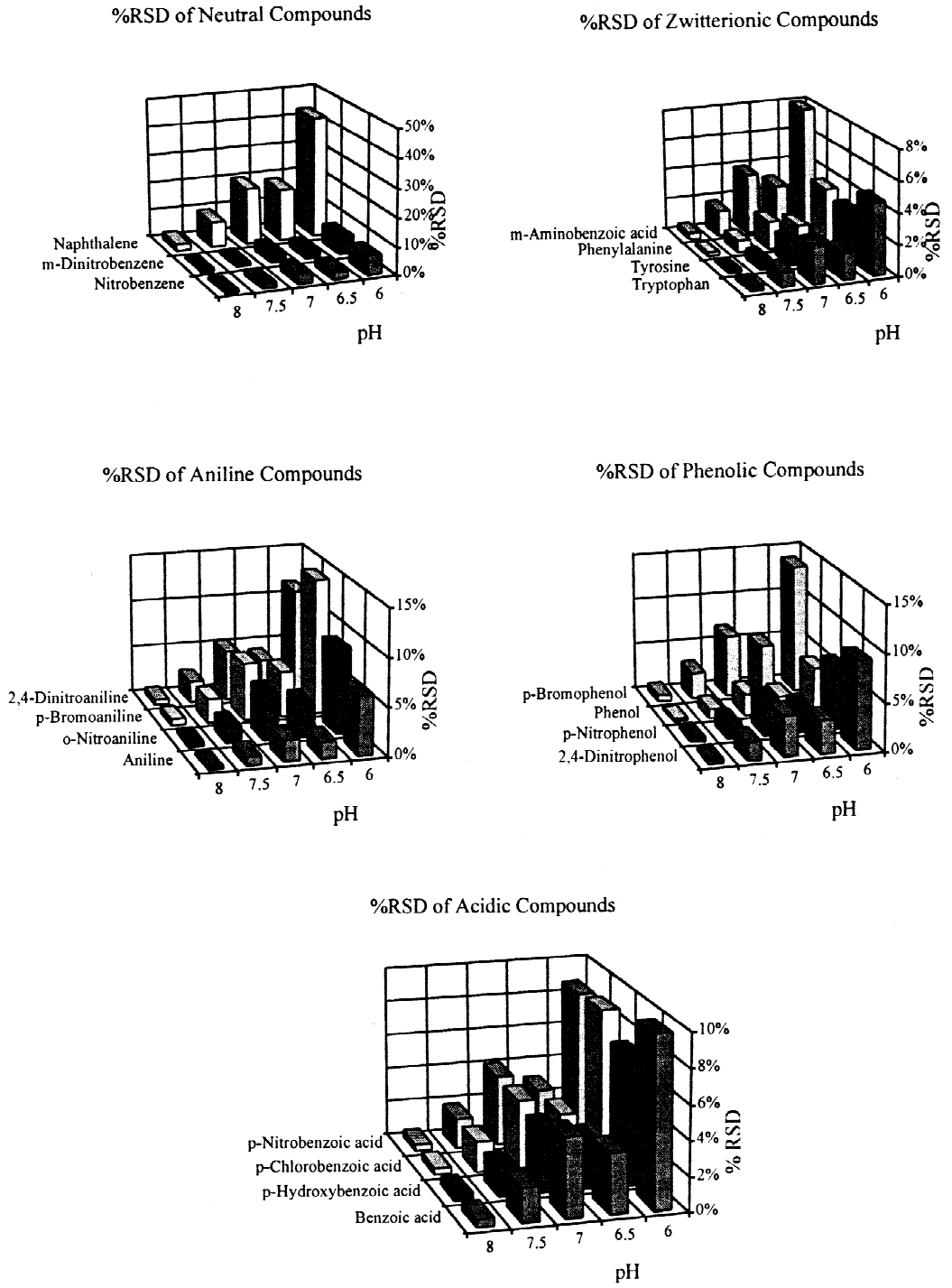

Fig. 1. Set of plots showing the absolute value of the % RSD of each solute at each pH level.

literature to determine which compound is most convenient [36]. The five compounds are Sudan III, Sudan IV, Orange OT, Yellow AB and decanophenone, and these were chosen due to their availability. All of these compounds are assumed to be neutral in the pH range of interest. Using a standard concentration, only three solutes were detected, Yellow AB, Orange OT and decanophenone. We tried to detect both Sudan III and Sudan IV by making the sample according to sample preparatory method described in the Experimental section and by making a saturated solution of Sudan III in ethanol and Sudan IV in methanol. We chose these solvents for their ability to solubilize the micelle marker. Then, we analyzed up to 400 μl of each standard. We were not able to detect Sudan III or IV.

Of the three micelle markers detected, Yellow AB had the highest absorbance, then decanophenone and Orange OT. As shown in Table 5, the electrophoretic mobilities of the micelle markers are independent of pH, with decanophenone having the highest electrophoretic mobility. However, there are slight differences in the electrophoretic mobility between the three micelle markers, which can affect the calculation of the capacity factors. For calculating migration factors in our study, we used decanophenone as the micelle marker.

4.5. Recalculated capacity factors using the appropriate micellar electrophoretic mobility

We would like to stress the importance of choosing the most appropriate solute to use as a micelle marker. As shown in Table 5, the different micelle markers each had an individual electrophoretic mobility. We recalculated the migration factors using Eq. (2) and Yellow AB as the micelle marker. Fig. 1 shows the absolute value of the percent relative standard deviation (RSD) as a function of pH for each compound. As pH decreases, the RSD in-

Table 6
Summary of migration factors ($k_{m'}$) and standard deviations ($n=4$) determined using Eq. (2) with yellow AB as the micelle marker for pH values of 8–6

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	2.37±0.03	2.40±0.009	2.460±0.01	2.47±0.04	2.65±0.04
p-Hydroxybenzoic acid	1.93±0.02	1.79±0.01	1.73±0.008	1.71±0.05	1.71±0.05
p-Chlorobenzoic acid	1.94±0.03	1.97±0.02	2.00±0.01	1.98±0.02	2.26±0.03
p-Nitrobenzoic acid	2.01±0.02	1.98±0.009	2.07±0.01	2.06±0.02	2.19±0.03
Phenols					
2,4-Dinitrophenol	2.14±0.05	2.21±0.02	2.19±0.007	2.34±0.02	2.44±0.03
p-Nitrophenol	2.15±0.03	1.86±0.008	1.74±0.007	1.43±0.03	1.35±0.02
Phenol	0.662±0.005	0.586±0.005	0.658±0.002	0.640±0.01	0.577±0.02
p-Bromophenol	4.19±0.09	3.82±0.02	4.09±0.04	4.26±0.03	4.21±0.06
Anilines					
Aniline	0.533±0.005	0.487±0.008	0.642±0.008	0.696±0.01	1.21±0.008
o-Nitroaniline	2.21±0.1	2.09±0.01	2.61±0.02	2.47±0.07	2.29±0.03
p-Bromoaniline	3.48±0.07	3.13±0.009	3.49±0.01	3.72±0.04	4.43±0.06
2,4-Dinitroaniline	3.07±0.07	2.82±0.03	3.02±0.02	3.04±0.04	3.24±0.05
Zwitterions					
Tryptophan	0.700±0.005	0.602±0.006	0.720±0.01	0.663±0.02	0.686±0.02
Tyrosine	0.179±0.004	0.142±0.004	0.139±0.008	0.108±0.04	0.111±0.003
Phenylalanine	0.340±0.005	0.276±0.01	0.321±0.003	0.326±0.01	0.271±0.01
m-Aminobenzoic acid	1.94±0.02	1.93±0.01	1.96±0.01	1.97±0.02	1.92±0.02
Neutrals					
Nitrobenzene	1.63±0.02	1.45±0.006	1.51±0.04	1.46±0.02	1.48±0.02
Naphthalene	15.9±3	13.9±0.1	15.3± 0.08	18.4±0.1	18.2±1
m-Dinitrobenzene	1.46±0.01	1.28±0.008	1.51± 0.007	1.47±0.02	1.38±0.02

Table 7
Summary of migration factors (k'_m) and standard deviations ($n=4$) determined using Eq. (3) with yellow AB as the micelle marker for pH values of 8–6

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	−0.0244±0.07	−0.0217±0.05	−0.0722±0.01	−0.0295±0.02	−0.101±0.01
p-Hydroxybenzoic acid	−0.0189±0.004	−0.0652±0.03	−0.0580±0.005	−0.0143±0.01	−0.108±0.004
p-Chlorobenzoic acid	0.00359±0.01	0.0134±0.04	−0.0411±0.008	−0.0239±0.01	−0.0503±0.004
p-Nitrobenzoic acid	−0.00806±0.05	−0.0321±0.008	−0.0526±0.008	−0.0351±0.01	−0.106±0.007
Phenols					
2,4-Dinitrophenol	−0.0142±0.02	−0.0125±0.01	−0.0534±0.006	−0.0422±0.009	−0.0802±0.01
p-Nitrophenol	0.0710±0.01	0.212±0.02	0.512±0.007	0.796±0.02	1.08±0.01
Phenol	0.616±0.005	0.586±0.005	0.658±0.002	0.640±0.01	0.577±0.01
p-Bromophenol	3.86±0.08	3.82±0.02	4.09±0.04	4.26±0.03	4.21±0.02
Anilines					
Aniline	0.533±0.005	0.487±0.008	0.642±0.008	0.696±0.01	1.24±0.01
o-Nitroaniline	2.21±0.1	2.09±0.01	2.61±0.02	2.47±0.07	2.29±0.1
p-Bromoaniline	3.48±0.06	3.13±0.009	3.49±0.01	3.72±0.04	4.43±0.02
2,4-Dinitroaniline	3.07±0.07	2.82±0.02	3.02±0.02	3.04±0.03	3.24±0.02
Zwitterions					
Tryptophan	0.625±0.005	0.602±0.007	0.720±0.01	0.663±0.02	0.686±0.02
Tyrosine	0.105±0.004	0.115±0.004	0.113±0.008	0.108±0.04	0.111±0.005
Phenylalanine	0.254±0.005	0.230±0.01	0.297±0.003	0.326±0.01	0.271±0.006
m-Aminobenzoic acid	−0.0223±0.007	−0.0275±0.009	−0.0780±0.008	−0.0359±0.006	−0.133±0.01

Table 8
Summary of migration factors (k'_m) and standard deviations ($n=4$) determined using Eq. (4) with yellow AB as the micelle marker for pH values of 8–6

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	0.0649±0.007	0.106±0.01	0.0621±0.009	0.0322±0.03	0.0105±0.01
p-Hydroxybenzoic acid	0.0560±0.006	0.106±0.009	0.0589±0.04	0.0296±0.009	−0.00498±0.004
p-Chlorobenzoic acid	0.0665±0.008	0.0978±0.01	0.0603±0.02	0.0287±0.01	0.0545±0.004
p-Nitrobenzoic acid	0.0565±0.004	0.0745±0.005	0.0644±0.009	0.0235±0.009	0.0000912±0.006
Phenols					
2,4-Dinitrophenol	0.0523±0.02	0.105±0.02	0.0353±0.006	0.0405±0.007	0.0215±0.01
p-Nitrophenol	0.257±0.01	0.371±0.01	1.74±0.005	0.830±0.02	1.10±0.01
Phenol	0.626±0.005	0.586±0.005	0.658±0.06	0.640±0.01	0.577±0.01
p-Bromophenol	3.86±0.08	3.65±0.02	3.96±0.03	4.26±0.03	4.21±0.02
Anilines					
Aniline	0.533±0.005	0.487±0.008	0.642±0.003	0.696±0.01	1.23±0.01
o-Nitroaniline	2.21±0.1	2.09±0.01	2.61±0.03	2.47±0.07	2.29±0.1
p-Bromoaniline	3.48±0.06	3.13±0.009	3.49±0.04	3.72±0.04	4.43±0.02
2,4-Dinitroaniline	3.07±0.07	2.82±0.02	3.02±0.04	3.04±0.03	3.24±0.02
Zwitterions					
Tryptophan	0.637±0.005	0.562±0.007	0.686±0.01	0.663±0.02	0.686±0.02
Tyrosine	0.125±0.004	0.100±0.004	0.104±0.04	0.108±0.04	0.111±0.004
Phenylalanine	0.281±0.005	0.236±0.01	0.288±0.03	0.326±0.01	0.271±0.006
m-Aminobenzoic acid	0.0471±0.005	0.0701±0.01	0.0324±0.006	0.0367±0.01	−0.0292±0.01

Table 9
Summary of electrophoretic mobilities (cm^2/min V) and standard deviations ($n=4$) in 20 m*M* phosphate buffer

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	−0.0187±0.00003	−0.0189±0.0004	−0.0189±0.00003	−0.0186±0.00004	−0.0188±0.00002
p-Hydroxybenzoic acid	−0.0175±0.00003	−0.0176±0.0003	−0.0169±0.00001	−0.0165±0.00007	−0.0168±0.00002
p-Chlorobenzoic acid	−0.0173±0.00008	−0.0175±0.0003	−0.0175±0.00002	−0.0174±0.00003	−0.0177±0.00001
p-Nitrobenzoic acid	−0.0176±0.00004	−0.0179±0.00006	−0.0178±0.00002	−0.0177±0.00003	−0.0180±0.00003
Phenols					
2,4-Dinitrophenol	−0.0181±0.00004	−0.0183±0.00003	−0.0181±0.00003	−0.0185±0.00006	−0.0183±0.00002
p-Nitrophenol	−0.0174±0.00008	−0.0153±0.0002	−0.0116±0.00004	−0.00671±0.00003	−0.00288±0.00003
Phenol	−0.000723±0.00002	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.00000
p-Bromophenol	−0.00167±0.00004	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.00000
Anilines					
Aniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.000348±0.00003
o-Nitroaniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.000000±0.0000
p-Bromoaniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.000000±0.0000
2,4-Dinitroaniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.000000±0.0000
Zwitterions					
Tryptophan	−0.00116±0.00002	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
Tyrosine	−0.00167±0.00006	−0.000616±0.00002	−0.000581±0.00003	0.0000±0.0000	0.0000±0.0000
Phenylalanine	−0.00169±0.00002	−0.000958±0.00002	−0.000460±0.000001	0.0000±0.0000	0.0000±0.0000
m-Aminobenzoic acid	−0.0176±0.00005	−0.0177±0.00002	−0.0177±0.000003	−0.0175±0.000008	−0.0176±0.00002

Table 10
Summary of electrophoretic mobilities (cm^2/V min) and standard deviations ($n=4$) in 20 m*M* phosphate–50 m*M* sodium chloride buffer

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	−0.0180±0.00003	−0.0179±0.00007	−0.0179±0.00001	−0.0182±0.0001	−0.0181±0.00002
p-Hydroxybenzoic acid	−0.0168±0.00005	−0.0160±0.00002	−0.0158±0.00002	−0.0161±0.00003	−0.0158±0.00002
p-Chlorobenzoic acid	−0.0168±0.00003	−0.0167±0.00003	−0.0167±0.00021	−0.0170±0.00001	−0.0169±0.00001
p-Nitrobenzoic acid	−0.0171±0.00001	−0.0169±0.00002	−0.0168±0.00001	−0.0172±0.00002	−0.0172±0.00002
Phenols					
2,4-Dinitrophenol	−0.0175±0.00006	−0.0174±0.00007	−0.0174±0.00002	−0.0178±0.00004	−0.0176±0.00005
p-Nitrophenol	−0.0158±0.00010	−0.0138±0.0001	0.0000±0.00000	−0.00635±0.00001	−0.00264±0.00001
Phenol	−0.000559±0.00002	−0.0000±0.0000	0.0000±0.00000	0.0000±0.0000	0.0000±0.0000
p-Bromophenol	−0.00166±0.00005	−0.000950±0.00004	−0.000654±0.00002	0.0000±0.0000	0.0000±0.0000
Anilines					
Aniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.000311±0.00001
o-Nitroaniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
p-Bromoaniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
2,4-Dinitroaniline	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
Zwitterions					
Tryptophan	−0.000964±0.00003	−0.000652±0.00003	−0.000518±0.00001	0.0000±0.0000	0.0000±0.0000
Tyrosine	−0.00121±0.00004	−0.000952±0.00003	−0.000780±0.00002	0.0000±0.0000	0.0000±0.0000
Phenylalanine	−0.00118±0.00003	−0.000831±0.00004	−0.000640±0.00003	0.0000±0.0000	0.0000±0.0000
m-Aminobenzoic acid	−0.0169±0.00002	−0.0168±0.00005	−0.0168±0.0001	−0.0169±0.0000	−0.0167±0.00004

creases, with the highest error being associated with the compounds that migrate more slowly. This trend is also apparent when the migration factors are calculated using Eq. (3) or Eq. (4). These results can be seen in the supplementary material supplied with this paper.

The difference in the electrophoretic mobility between decanophenone and Yellow AB ranges up to $-7.0 \cdot 10^{-4}$ cm^2/V min (1.2%) at pH 6. This small difference can manifest in a large RSD, especially for naphthalene at pH 6, which has a RSD of over 40% between the migration factors calculated using Yellow AB and decanophenone. Thus, accurate determination of the micelle marker is very crucial to the accuracy of migration factors. This error will manifest itself in hydrophobic solutes that partition strongly into micelles. A study to accurately determine the electrophoretic mobility of the micelle may be warranted.

4.6. Statistical review of the results

In Tables 6–11, we list the electrophoretic mobilities of the solutes in each buffer system and the corresponding standard deviation. Since the standard deviation of the electrophoretic mobilities of the solutes are small, the calculated standard deviation for the migration factors is relatively small. The error for the corrected migration factors is higher because of the increased manipulation of the data. Since the migration factors are dependent on the difference between electrophoretic mobilities, the error propagation of the measurements is compounded by the small number of the difference. Thus, the migration

Table 11
Summary of electrophoretic mobilities (cm^2/min V) and standard deviations ($n=4$) in 20 m*M* phosphate–50 m*M* SDS buffer

	pH of buffer				
	8	7.5	7	6.5	6
Carboxylic acids					
Benzoic acid	−0.0185±0.00005	−0.0187±0.00006	−0.0183±0.00007	−0.0184±0.0001	−0.0181±0.00007
p-Hydroxybenzoic acid	−0.0173±0.00002	−0.0170±0.00008	−0.0163±0.00005	−0.0163±0.00008	−0.0158±0.00003
p-Chlorobenzoic acid	−0.0174±0.00007	−0.0176±0.00010	−0.0172±0.00007	−0.0172±0.00009	−0.0173±0.00003
p-Nitrobenzoic acid	−0.0176±0.00003	−0.0176±0.00004	−0.0174±0.00006	−0.0174±0.00007	−0.0172±0.00005
Phenols					
2,4-Dinitrophenol	−0.0179±0.00014	−0.0182±0.0001	−0.0177±0.00004	−0.0181±0.00004	−0.0177±0.00007
p-Nitrophenol	−0.0180±0.00003	−0.0172±0.00004	−0.0164±0.00004	−0.0152±0.0002	−0.0144±0.00008
Phenol	−0.0105±0.00005	−0.00979±0.00005	−0.0102±0.00002	−0.0101±0.00009	−0.00915±0.00013
p-Bromophenol	−0.0212±0.00005	−0.0210±0.00005	−0.0207±0.00012	−0.0210±0.00006	−0.0202±0.00007
Anilines					
Aniline	−0.00915±0.00006	−0.00868±0.00009	−0.0101±0.00008	−0.0106±0.00006	−0.0137±0.00007
o-Nitroaniline	−0.0181±0.00032	−0.0179±0.00007	−0.0186±0.00009	−0.0184±0.0004	−0.0174±0.00063
p-Bromoaniline	−0.0204±0.00005	−0.0201±0.00002	−0.0200±0.00004	−0.0204±0.0001	−0.0204±0.00004
2,4-Dinitroaniline	−0.0199±0.00010	−0.0196±0.0001	−0.0194±0.00008	−0.0195±0.0001	−0.0191±0.00008
Zwitterions					
Tryptophan	−0.0108±0.00004	−0.00995±0.00007	−0.0108±0.00013	−0.0103±0.0001	−0.0102±0.00017
Tyrosine	−0.00401±0.00008	−0.00329±0.00006	−0.00314±0.00013	−0.00253±0.0006	−0.00250±0.00006
Phenylalanine	−0.00668±0.00008	−0.00573±0.0002	−0.00626±0.00003	−0.00637±0.00009	−0.00533±0.00008
m-Aminobenzoic acid	−0.0174±0.00004	−0.0174±0.00007	−0.0171±0.00007	−0.0172±0.00005	−0.0164±0.00008
Neutrals					
Nitrobenzene	−0.0163±0.00007	−0.0157±0.00003	−0.0155±0.00031	−0.0154±0.00005	−0.0149±0.00006
Naphthalene	−0.0246±0.00029	−0.0247±0.0001	−0.0242±0.00009	−0.0245±0.00007	−0.0237±0.00005
m-Dinitrobenzene	−0.0156±0.00003	−0.0149±0.00006	−0.0155±0.00005	−0.0154±0.00007	−0.0145±0.00010
Micelle marker					
Decanophenone	−0.0263±0.00007	−0.0263±0.00008	−0.0261±0.00013	−0.0261±0.00007	−0.0257±0.00007
Orange OT	−0.0263±0.00007	−0.0251±0.0007	−0.0260±0.0001	−0.0260±0.00004	−0.0255±0.0004
Yellow AB	−0.0263±0.00010	−0.0265±0.00005	−0.0258±0.00003	−0.0259±0.0001	−0.0250±0.00005

factors determined using Eq. (2) are more precise, but the migration factors determined by Eq. (4) may be more accurate.

5. Conclusion

We have confirmed Khaledi's assumption that the observed electrophoretic mobility of the sample as a weighted average of the EOF, electrophoretic mobility of the micelle and the electrophoretic mobility of the charged species is valid. The negative values obtained using Eq. (3) can be corrected by factoring in the ion-pairing effects. Thus, a corrected migration factor that compensates for ion pairing is introduced as Eq. (4). This ion-pairing phenomenon may have an effect on calculated micelle–water partitioning. Also, small errors in determining the electrophoretic mobility of the micelle marker and the proper choice of marker to use can lead to drastic differences in the determination of migration factors. Thus, a study to develop an easier method to determine the electrophoretic mobility of micelles is needed. As shown, current methods to determine micelle markers may not be accurate or precise.

Since the micelle–water partitioning of the solutes is constant over the pH range of this study, biopartitioning should be constant over this pH range and the changes in biopartitioning observed by Fent and Looser [1] and Wildi et al. [2] are due to physiological changes in the test species.

Acknowledgements

The authors are grateful for support of this work by NIH GM-48561. J.G.D. also thanks Merck Research Laboratories for continued support of our work and Waters, Inc. for the donation of the Quanta 4000 instrument.

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