Comparison of the retention of basic compounds in anionic and cationic microemulsion electrokinetic chromatographic systems

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Abstract

Retention of ionizable bases in microemulsion electrokinetic chromatography (MEEKC) has been studied using two different systems with anionic and cationic microemulsions. Microemulsion pseudostationary phase is composed of heptane (oil), 1-butanol (cosurfactant) and sodium dodecyl sulfate (SDS, anionic system) or tetradecyltrimethylammonium bromide (TTAB, cationic system) as surfactant.

In contrast with micellar electrokinetic chromatography (MEKC) where the retention of neutral compounds is very different in the two micellar pseudostationary phases (SDS and TTAB, respectively); in MEEKC, neutral compounds present very similar retention factor (*k)* values in SDS and TTAB microemulsion pseudostationary phases.

However, the *k vs.* p*H* profiles of protonable bases are very different in the two MEEKC systems. In TTAB system, retention increases with p*H* because of neutralization of the protonated base and partition of the unionized form into the microemulsion. However, a reversed trend is observed in SDS system. Retention decreases with p*H* because of the formation of an ionic pair between the protonated base and the anionic SDS, much more retained than the unionized base.

Thus, it is demonstrated that the two systems behave very similar in the retention of neutral bases, but completely different for retention of protonated bases.

Keywords: Retention mechanism, Bases, Microemulsion, MEEKC, Chromatography, Ion pair interaction.

1. Introduction

Capillary electrophoresis (CE) is a powerful separation technique able to separate compounds with different charge/size ratios. In order to separate both charged and neutral solutes new approaches of the technique, such as micellar and microemulsion electrokinetic chromatographies (MEKC and MEEKC, respectively) were developed [1–3]. In both cases a pseudostationary phase (e.g. a charged micelle or microemulsion (ME) with its own mobility) is added into the buffer solution. Therefore, the elution of the compounds not only depends on their charge to size ratio, but also on their affinity to the pseudostationary phase. In contrast with MEKC where the pseudostationary phase is simply a surfactant, in MEEKC the pseudostationary phase is a ME composed of small oil droplets which are stabilized by a surfactant and a cosurfactant [4]. Due to their properties, MEs have been used in different applications in both research and industry (for example in cosmetics and pharmacy) [5]. Moreover, MEEKC systems have been used as surrogates for the estimation of the lipophilicity of compounds. The octanol-water partition coefficient ($P_{o/w}$) has been estimated through the retention factor (k) of the compounds in similar MEEKC systems [6–10].

Whereas the retention processes in MEKC are well-known [2,3,11–13], retention in MEEKC has been studied scarcely and usually it is assumed to be similar to MEKC. Thus, the same equations developed for MEKC are used [6,8]. However, it is clear that micellar and microemulsion systems have different properties. For instance, we have demonstrated that the addition of the oil and cosurfactant, needed to form the microemulsion, change significantly the viscosity of the surfactant solution and the usual MEKC equation used to calculate the retention factor of partially ionized acids has to be corrected for this change of viscosity [14]. Also, the solvation properties of MEKC systems strongly depend on the surfactant used to form the micelle [15–18]. However, Ishihama *et al.* [19]

showed that in MEEKC, the nature of the surfactant does not affect the partition of neutral compounds between the aqueous buffer and the microemulsion, probably because the surfactants are shielded by the oil and the cosurfactant.

In a previous work [14], we studied the effect of the ionization of acids in a MEEKC system with a ME composed of heptane, 1-butanol and sodium dodecyl sulfate (SDS, an anionic surfactant), a system which showed to be a good surrogate for the determination of octanol-water partition coefficients [6–10]. However, the study of the retention of partially protonated bases was not intended because additional interactions, other than partition of the unionized form of the base into the ME, were expected. In the case of basic compounds, the retention mechanism into the SDS microemulsion can be more complex. The literature reports some studies based on micellar electrokinetic chromatography (MEKC) where an electrostatic interaction is observed when compounds and surfactant present opposite charges [11,13,20–22]. Indeed, Quang *et al.* obtained higher retention factors (*k*) for the ionized bases than for the neutral compounds, meaning that apart from hydrophobicity other equilibria, such as ion pairing, must exist, enhancing retention of cationic ionized bases [13]. Moreover, the presence of other ions in the media (such as buffer components) can also interfere and influence the ion pair interaction between opposite charged test compounds and charged surfactants [22].

The purposes of this work are, in a first instance, to compare the retention of compounds in equivalent (same surfactant) MEKC and MEEKC systems, in order to see how the nature of the pseudostationary phases affect the retention. In a second instance, the retention of ionizable bases in two different MEEKC systems (anionic and cationic), and the retention behaviour of the unionized and ionized forms of the bases in the two systems will be also compared. The anionic system will be the same used previously [14] with a ME composed of heptane, 1-butanol and SDS (SDS-MEEKC system). The cationic

system will have the same composition, but changing SDS by tetradecyltrimethylammonium bromide (TTAB; TTAB-MEEKC system). The two studies will provide a wide overview of the retention mechanisms in MEEKC.

2. Theory

2.1. Calculation of retention factors in MEKC and MEEKC

In MEKC, retention factors (*k*) are calculated from the well-known Eq. 1:

$$k = \frac{\mu - \mu_0}{\mu_{mc} - \mu} \qquad \text{Eq. 1}$$

where μ is the electrophoretic mobility of the compound in the MEKC system, μ_{mc} the electrophoretic mobility of the micellar pseudostationary phase (measured by the micellar marker) and μ_0 the electrophoretic mobility of the compound in capillary zone electrophoresis (CZE) mode where the electrophoretic mobility is measured using only the same aqueous buffer as for the MEKC system.

The formation of the ME implies the addition to the CZE buffer of the oil, surfactant and cosurfactant which may have viscosities very different from that of the aqueous buffer. Thus, the same type of equation can be applied to MEEKC with the introduction of a correction factor that accounts for this change of viscosity between the microemulsion MEEKC system and the CZE plain buffer (Eq. 2):

$$k = \frac{\mu - \left(\frac{\mu}{\mu_0}\right)_{unretained\ solute} \cdot \mu_0}{\mu_{ME} - \mu} \qquad \text{Eq. 2}$$

123 Where μ_{ME} is the electrophoretic mobility of the ME (measured by the ME marker) and
124 $\left(\frac{\mu}{\mu_0}\right)_{unretained\ solute}$ is the correction factor for the change of viscosity between the
125 water/surfactant/cosurfactant/oil MEEKC system and the water CZE system (which
126 cannot be reproduced with the same components as the microemulsion). The viscosity
127 correction factor is calculated measuring the ratio of mobilities, in MEEKC (μ) and CZE
128 (μ_0), of a compound that does not interact with the ME phase. In the case of the SDS-
129 MEEKC system, benzoate ion was used as compound for viscosity correction because it
130 is small and polar, and it can be easily detected [14]. The value of the correction for the
131 studied SDS system is $\left(\frac{\mu}{\mu_0}\right)_{benzoate\ ion}$ = 0.76.

132

133 *2.2 Influence of pH on mobility and retention factors*

134 Mobility (μ) and retention (k) of the compounds will change through the measured pH
135 range depending on their degree of ionization. Khaledi *et al.* [12] proposed a model to
136 relate k of acidic compounds in MEKC to buffer pH. A similar expression can be easily
137 derived to predict the behavior of basic compounds ($B+H^+ \leftrightarrow BH^+$) in MEKC or MEEKC.
138 k of a monoprotic basic compound can be defined as:

139

140 $$k = \alpha_B k_B + \alpha_{BH^+} k_{BH^+} \qquad \text{Eq. 3}$$

141

142 Where k_B and k_{BH^+} are the retention factor of the unionized and the fully ionized forms of
143 the base, respectively, and α_B and α_{BH^+} are their mole fractions. These can be calculated
144 using the apparent acidity constant (K_a') as follows:

145

146 $$\alpha_B = \frac{K_a'}{[H^+]+K_a'} \qquad \text{Eq. 4}$$

$$\alpha_{BH^+} = \frac{[H^+]}{[H^+]+K'_a} \quad \text{Eq. 5}$$

Finally, combining Eqs. 3-5 and organizing the terms Eq. 6 is obtained, which relates the retention factor of a monoprotic basic compound to p*H*.

$$k = \frac{k_{BH^+} + k_B \cdot 10^{pH-pK'_a}}{1+10^{pH-pK'_a}} \quad \text{Eq. 6}$$

The same type of equation can be derived for μ:

$$\mu = \frac{\mu_{BH^+} + \mu_B \cdot 10^{pH-pK'_a}}{1+10^{pH-pK'_a}} \quad \text{Eq. 7}$$

where μ_B and μ_{BH+} are the mobilities of the neutral and fully ionized species of the basic compound, respectively. Since the neutral base is uncharged, μ_B is equal to 0 and Eq. 7 can be simplified to Eq. 8.

$$\mu = \frac{\mu_{BH^+}}{1+10^{pH-pK'_a}} \quad \text{Eq. 8}$$

3. Experimental section

3.1 Equipment

A CE system equipped with a diode array from Agilent technologies (Santa Clara, CA, USA) was used to perform the electrophoretic measurements. The fused-silica capillary utilized was from Polymicro Technologies (Lisle, IL, USA) and presented an effective and a total length of 30 and 38.5 cm, respectively.

A p*H*-meter GLP 22 from Crison (Barcelona, Spain) was used to determine the p*H* of the solutions.

3.2 Reagents

Hydrochloric acid (1N Tritisol™), sodium hydroxide (0.5N Tritisol™), sodium dihydrogen phosphate monohydrate (≥99%), dimethyl sulfoxide (DMSO) (≥99.9%), and ammonium chloride (>99.8%) were from Merck (Darmstadt, Germany). Methanol (HPLC-grade) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Heptane (99%), dodecanophenone (98%), SDS (≥99%), TTAB (>99%), 1-butanol (≥99.7%), 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3diol (Bistris) (>99%), 2-amino-2-(hydroxymethyl)propane-1,3diol (Tris) (>99.8%), sodium phosphate dodecahydrate (>98%), and borax decahydrate (>99.5%) were from Sigma-Aldrich (St. Louis, MO, USA). Disodium hydrogen phosphate (99.5%) and sodium acetate anhydrous (99.6%) were from Baker (Center Valley, PA, US). Water was purified using a Milli-Q plus system from Millipore (Bedford, MA, US), up to a resistivity of 18.2 MΩ cm.
Ephedrine, alprenolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol and trimethoprim were supplied from Carlo Erba (Milan, Italy) and Sigma-Aldrich.

1.3 Analysis conditions

1.3.1 Buffer preparation

Two sets of buffers were prepared: in the first set, acidic compounds were used to prepare the buffers in the 4.0-12.0 p*H* range maintaining, in all the cases, the ionic strength (I) constant at 0.05 M. These solutions were prepared using 0.2 M stock solutions of the buffer salts and the p*H* was adjusted using hydrochloric acid 1.0 M or sodium hydroxide 0.5 M. Anhydrous sodium acetate was used to prepare the buffers at p*H* 4.0 and 5.0; the

buffers at p*H* 6.0, 7.0, and 8.0 were prepared using a mixture of sodium dihydrogen phosphate monohydrate and disodium hydrogen phosphate; borax decahydrate was utilized for the preparation of the acidic buffers at p*H* 9.0, and 10.0; for the rest of the p*H* values (p*H* 11.0, and 12.0) a mixture of disodium hydrogen phosphate and sodium phosphate dodecahydrate was used.

In the second set, basic compounds were used to prepare buffers in the 5.0-10.5 p*H* range, also maintaining I at 0.05 M. A Bistris solution previously protonated with HCl was used to prepare the buffers at p*H* 5.0, 6.0 and 7.0; a Tris solution previously protonated with HCl was used to prepare the buffer at p*H* 8.0; for the other two buffer solutions, p*H* 9.0 and 10.0, an ammonium chloride solution was used. p*H*s were adjusted to the desired value using NaOH.

1.3.2 ME preparation

Two different MEs were prepared in this study. In both cases the procedure followed was the same. First, the surfactant was dissolved in around 70 mL of the corresponding buffer solution (1.30 g of SDS, anionic ME, or 1.70 g of TTAB, cationic ME). Then, 8.15 mL of 1-butanol were added, finishing with the addition of 1.15 mL of heptane. The cosurfactant and the oil were added under continuous magnetic stirring, and if the solution remained turbid, it was sonicated until clarification [8]. Finally, buffer was added up to a total volume of 100 mL. The final concentration of each component was: 8.15% v/v of 1-butanol, 1.15% v/v of heptane, and 1.30% w/v of SDS or 1.70% w/v of TTAB for, the anionic and cationic ME, respectively.

1.3.3 Instrumental parameters

Temperature was set at 25ºC for all the measurements. The analysis conditions varied depending on the ME used and the p*H* of work in order to obtain the appropriate electrophoretic window. For the SDS-MEEKC system the applied voltage varied between 8.5-15 kV and the separation pressure varied in the 0-50 mbar range. In the case of the TTAB-MEEKC system the voltage applied was negative, and it ranged between -11.5 to -14 kV, and the separation pressure was between 0 and 25 mbar. For the analysis performed in CZE the applied voltage varied between 8.5-15 kV and the separation pressure varied in the 0-50 mbar range.

To perform the MEEKC analysis the compounds were dissolved at 200 $mg \cdot L^{-1}$ in a 9:1 ME:methanol mixture, and in the CZE analysis, the solutes were dissolved in a 9:1 buffer:methanol mixture. The compounds were injected applying a pressure of 50 mbar during 5s, and they were detected at λ=200, 214 or 254 nm (depending on the absorbance profile of the solutes). The ME marker was dodecanophenone (at a concentration of 200 $mg \cdot L^{-1}$ and detected at λ = 254 nm). The electroosmotic flow marker was DMSO (at a concentration of 0.2% v/v and detected at λ = 214 nm) when the ME was based on SDS, and methanol (at a concentration of 10% v/v and detected at λ = 254 nm) when the ME was based on TTAB [23].

1.3.4 Data calculation

Electrophoretic mobilites have been calculated from the migration time using the well-known Eq. 9:

$$\mu = \left[\frac{1}{t_r} - \frac{1}{t_0}\right] \cdot \left[\frac{L_T L_D}{V}\right] \qquad \text{Eq. 9}$$

where, t_r is the migration time of the analyte, t_o the migration time of the electroosmotic flow marker, L_T and L_D are the total and the effective length of the capillary, respectively, and V the voltage applied.

TableCurve 2D v5.01 from Systat Software Inc. (San Jose, CA, USA) was used to fit the *k*-p*H* profiles. Excel from Microsoft (Redmond, WA, USA) was used to perform all the data calculations. Bio-Loom database v1.7 from BioByte Corporation (Claremont, CA, USA) was utilized to obtain the log $P_{o/w}$ values of the tested compounds.

4. Results and discussion

4.1 Microemulsion vs. micelle selectivity for neutral solutes

In a previous MEKC study [16,17], the solute solvent-interactions and the selectivity of the two surfactants studied, among others, were characterized. Results showed that TTAB is much more hydrogen bond acceptor, and less donor, than SDS. As a consequence, hydrogen bond donor compounds will be much more retained in TTAB than in SDS. Thus, the selectivity of the two systems might be different. To prove this, we have selected 56 neutral compounds that have a wide chemical diversity, and the logarithms of their retention factors in the two MEKC systems have been correlated. The correlation is presented in Eq. 10 and Figure 1A (data obtained from [16,17]), where it is seen that the it is not very good.

$$\log k_{(TTAB-MEKC)} = -0.01(\pm 0.05) + 0.80(\pm 0.06) \cdot \log k_{(SDS-MEKC)} \qquad \text{Eq. 10}$$

n= 56; R^2 = 0.793; SD = 0.34; F = 207

$k_{(TTAB\text{-}MEKC)}$ and $k_{(SDS\text{-}MEKC)}$ are the retention factor of the compounds determined in the MEKC systems composed of TTAB/aqueous buffer and SDS/aqueous buffer,

268 respectively. n is the number of data points, R^2 the determination coefficient, SD the
269 standard deviation, and F the Fisher's F parameter. Standard deviations of the fitting
270 parameters (slope and intercept) are in brackets.
271 In MEEKC, the retention of neutral compounds in the two equivalent systems considered
272 (indicated by SDS-MEEKC and TTAB-MEEKC subscripts) has been also compared
273 using data previously determined [24,25] and data measured in this work (Table 1). The
274 results are presented in Eq. 11 and Figure 1B.
275
276 $$\log k_{(TTAB-MEEKC)} = 0.11(\pm 0.03) + 0.99(\pm 0.04) \cdot \log k_{(SDS-MEEKC)} \quad \text{Eq. 11}$$
277 n= 22; R^2 = 0.973; SD = 0.11; F = 727
278
279 In Eq. 11, $k_{(TTAB\text{-}MEEKC)}$ and $k_{(SDS\text{-}MEEKC)}$ are the retention factor of the compounds
280 determined in the TTAB-MEEKC and SDS-MEEKC systems, respectively.
281 The correlation is much better than that of the equivalent MEKC systems and the slope is
282 not statistically different from 1 at 95% confidence level of Student's t-test. The intercept
283 is not zero, but its value would depend on the amounts of pseudostationary phases.
284 Consequently, it can be concluded that the selectivity of the two systems is practically the
285 same. These results support the theory of Ishihama *et al.* [19], that surfactants may be
286 shielded by the oil and the cosurfactant, which are responsible of the partition. So the
287 nature of the surfactant does not affect the partition of neutral compounds between the
288 aqueous buffer and the ME.
289
290 *4.2 Mobility and retention of protonated bases in MEEKC*
291 In order to evaluate the retention behavior of the ionized and the unionized forms of a
292 compound in the two MEEKC systems, the mobility-pH profiles of eight monoprotic

bases have been determined. These bases must have pK_a values between 5 and 10 so that the entire profile can be determined, and must be easily detectable by UV-vis. We have selected compounds of different lipophilicity to assure different levels of interaction with the microemulsions, since the retention factor of the unionized forms of the compounds presents a good correlation with the octanol-water partition coefficient (log $P_{o/w}$), commonly used to estimate lipophilicity [25]. The physicochemical properties (log $P_{o/w}$, McGowan volume, and pK_a) and chemical structures of the eight selected bases [26-30] are detailed in Table S-1 of the supplementary material.

Calculation of the retention of the bases totally or partially protonated according to Eq. 2 requires the determination of its mobility in the microemulsion (MEEKC mode) and also in plain buffer, i.e. without microemulsion (CZE mode). Thus, the variation of the mobility of the bases with p*H* of the buffer has been studied in three different systems: MEEKC with SDS, MEEKC with TTAB, and CZE in aqueous buffer. The obtained mobilities together with the p*H* and nature of the buffers studied are presented in Table S-2.

4.2.1 Mobility vs. pH profiles of bases in CZE mode

First of all, μ_0 of the selected bases has been measured at several p*H* values in the 4.0-12.0 p*H* range in CZE. Different types of buffers have been prepared using either acidic or basic electrolytes. Then, Eq. 8 has been fitted to the data obtained. The parameters and the statistics obtained from these fittings are presented in Table 2, and the μ_0 vs. p*H* obtained profiles are shown in Figure S-1. Good μ_0-p*H* profiles have been obtained in all the cases and, as expected in CZE for cationic solutes, it is observed that all the bases behave in a similar way regardless of the buffer type used: mobility decreases from positive values down to zero when p*H* increases, according to the decrease in the

ionization degree of the bases. Since there are no differences between the mobilities determined in buffers of different nature, the formation of ion pairs between the protonated base and the counter-ions present in the media has been considered negligible. Furthermore, the pK_a' values obtained in these fittings (I=0.05 M) are of the same order as the ones presented in the literature determined by potentiometric methods (Table S-1). Slight differences between both set of data can be seen as different conditions, such as buffer and ionic strength (I), have been utilized when measuring the pK_a.

4.2.2 Effect of buffer pH in mobility of bases in the SDS and TTAB MEEKC systems

Next, the mobilities of the bases have been measured in the MEEKC systems with SDS and TTAB at the same p*H* values than in CZE. The measured μ values are plotted in Figure 2 against the aqueous buffer p*H*. It can be observed that the variation with p*H* is small in both cases, but the values are completely different. Mobilities in TTAB microemulsions are positive, which correspond to cations as expected in protonated bases. Mobilities also vary when p*H* increases from the mobility of the fully protonated base, scarcely partitioned into the microemulsion, to the mobility of the neutral base partially partitioned into the microemulsion. However, mobilities in SDS microemulsions are negative, which indicates that an anionic species is formed, regardless of the nature of the buffer used (anionic or cationic), and thus the anionic species has to be aggregates of the cationic protonated base with the anionic microemulsions. In this case, the variation observed is between the mobility of the anionic aggregate and that of the neutral base partitioned into the SDS microemulsion.

4.2.3 Effect of buffer pH in retention of bases in the SDS and TTAB MEEKC systems

From the measured mobilities, *k* has been calculated through Eq. 2, and Eq. 6 has been fitted to the experimental data. For the analysis in the TTAB system, ephedrine ion was selected to correct the mobilities in CZE due to the different viscosity of the two media. When ephedrine is fully ionized it has a very low lipophilicity (log $P_{o/w(BH+)}$ = -1.36; determined at p*H* = 4.5 by the reference shake-flask method [31]) and is a small and polar compound. Therefore, it is not supposed to interact with the ME. With this aim, the ratio between the mobilities of ephedrine in the TTAB-MEEKC system and CZE at p*H* 5.0 was calculated, which provided a mobility correction value of 0.84. In this case, benzoate ion has not been used as for the SDS-based ME, since it could interact electrostatically with the cationic surfactant. The parameters and statistics resulting from these fittings are shown in Table 3. In addition, these profiles are plotted in Figure 3.

Good fittings have been obtained for the *k*-p*H* profiles obtained using the TTAB-MEEKC system (Table 3 and solid lines in Figure 3). All eight compounds show low retention at the lowest p*H* values, where the solutes are in their cationic form, and *k* increases with p*H*. This fact means that the unionized species of the compounds interact much more with the pseudostationary phase than the ionized species of the bases do. Note that k_{BH+} values are very low, almost zero for most of the compounds, and in case of showing some retention, it is in all cases less than 10% the retention of the unionized form. This tendency is the same observed when *k*-p*H* profiles of acidic compounds were measured using a ME formed by SDS, 1-butanol, and heptane [14].

However, a reversed trend is observed for the *k*-p*H* profiles determined in the SDS system (Table 3 and dotted lines in Figure 3). High retention is observed at low p*H* values, and it decreases when p*H* increases. In all the profiles k_{BH+} is always higher than k_B, when we would expect the contrary if only hydrophobic partition would take place. The neutral species of the ionizable compounds (B) are more lipophilic than the ionized species (BH^+)

and thus, we expect them to partition better into the ME. The higher *k* of ionic species points out that, as it has been seen before from the μ-p*H* profiles, there is an electrostatic interaction between the cationic bases and SDS microemulsions leading to ion aggregation, which increases retention even to a larger value than *k* of the unionized form. Analyzing pK_a from Table 3 it can be seen that generally similar values have been obtained in both approaches except for nadolol and penbutolol. Note that for these two compounds the difference between k_{BH+} and k_B is very small, which increases uncertainty in the pK_a determination. The rest of obtained pK_a' values are consistent with the literature ones reported in Table S-1, and the slight differences observed between both sets of pK_a' values can be due to the experimental conditions selected (different medium and/or I).

The degree of retention of the neutral species depend on the own structure of the compounds. For this reason the logarithm of the retention factor of the compounds in each of the two ME systems has been correlated to two structural descriptors that may have an important influence on their retention: the McGowan volume and the log $P_{o/w}$. On one hand, it has been demonstrated that the McGowan volume has a very important contribution when the retention of neutral compounds is evaluated in MEEKC systems. On the other hand, good correlations have been observed between log $P_{o/w}$ and the retention of neutral compounds in these two MEEKC systems [6,25]. Correlations are shown in Figures S2a and S2b of the supplementary information. As expected, a good correlation between the lipohilicity parameter, log $P_{o/w}$, and retention is observed. On the contrary, McGowan volume itself does not directly correlate to the retention of neutral species.

Due to the important retention of the protonated forms of the bases in the SDS MEEKC system, correlation with these two structural descriptors has also been performed (Figure S2c of the supplementary information). Similarly to what occur for neutral compounds,

the retention of the ion-pairs is not explained by the volume of the compounds, whereas it is closely related to the lipophilicity of the neutral base.

4.2.4 Conjoint comparison of the retention of unionized and ionic species in MEEKC.

To compare the retention of neutral and ionic species in the two systems, the log *k* determined in the TTAB-MEEKC system (log $k_{(TTAB\text{-}MEEKC)}$) have been correlated against log *k* values determined in the SDS-MEEKC system (log $k_{(SDS\text{-}MEEKC)}$) for both unionized (log k_B) and fully protonated (log k_{BH+}) forms. The equations of the resulting correlations, which are plotted in Figure S-~~2~~3, are:

$$\log k_{B(SDS\text{-}MEEKC)} = -0.04\ (\pm 0.05) + 0.94\ (\pm 0.05) \log k_{B(TTAB\text{-}MEEKC)} \quad \text{Eq. 12}$$

n = 8; R^2 = 0.981; SD = 0.10; F = 309

$$\log k_{BH+(SDS\text{-}MEEKC)} = 1.28\ (\pm 0.07) + 0.69\ (\pm 0.08) \log k_{BH+(TTAB\text{-}MEEKC)} \quad \text{Eq. 13}$$

n = 6; R^2 = 0.952; SD = 0.15; F = 79.

Eq. 12 is not significantly different from Eq. 11 at a 95% confidence interval, confirming that both ME systems are equivalent for the unionized species of the bases. However, a different trend is observed when the bases are completely ionized (Eq. 13), where the charge of the surfactant modifies completely the retention behavior of ionic forms leading to profiles in SDS totally different than in TTAB. The intercept of Eq. 13 is higher than 0 as expected from the additional retention by ion aggregation in SDS. However, the slope is lower than 1 showing that the hydrophobicity of the compound has a lower effect in the aggregation than in the partition.

The effect of the additional retention by aggregation of cations with SDS can be also observed in Figure ~~S-3~~S-4, where the retention of the ionic species ($k_{ionized}$) has been plotted vs. the retention of the unionized forms of bases ($k_{neutral}$). The plot also includes retention of acids in SDS-MEEKC obtained in a previous work [14]. As expected from the similarity of both systems, retention of acids in SDS-MEEKC and bases in TTAB-MEEKC can be assembled in the same straight line, described in Eq. 14.

$$\log k_{ionized} = -1.27\ (\pm 0.12) + 1.01\ (\pm 0.11) \log k_{neutral} \qquad \text{Eq. 14}$$

n = 12; R^2 = 0.876; SD = 0.23; F = 79.

However, retention of protonated bases in SDS-MEEKC is higher because of the extra-retention by aggregation, described by a straight line (Eq. 15) with a higher intercept and a lower slope than Eq. 14.

$$\log k_{ionized} = 0.38\ (\pm 0.06) + 0.81\ (\pm 0.07) \log k_{neutral} \qquad \text{Eq. 15}$$

n = 8; R^2 = 0.953; SD = 0.13; F = 142.

Concluding remarks

The direct comparison of the retention of a set of 56 neutral compounds with a wide chemical diversity in a MEKC and a MEEKC system, both using SDS as surfactant, has revealed that the selectivity of the systems is quite different. This fact points out that the retention of neutral compounds in MEEKC is strongly influenced not only by the surfactant, but by the whole ME components. Indeed, the retention of neutral compounds in two MEEKC systems with different surfactant (SDS or TTAB) and the same oil

(heptane) and cosurfactant (butanol) is very similar, indicating that the surfactant used does not alter significantly the selectivity of the microemulsions.

However, this behavior is completely different when the compounds are charged. Protonated bases show higher retention in the SDS-MEEKC system than in the TTAB-MEEKC system because of the aggregation between the cationic protonated base and the anionic surfactant. The use of basic or acidic compounds to prepare the buffers does not affect the mobilities obtained, indicating that aggregation of protonated bases is mainly caused by the SDS surfactant.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

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572 **FIGURE CAPTIONS**

573 **Figure 1.** Effect of surfactant change in: A) MEKC, B) MEEKC.

574

575 **Figure 2.** μ-p*H* profiles in MEEKC mode: μ in TTAB-MEEKC (○), μ_{ME} in TTAB-
576 MEEKC (●), μ in SDS-MEEKC (□), μ_{ME} in SDS-MEEKC (■). a) Alprenolol, b)
577 ephedrine, c) nadolol, d) oxprenolol, e) penbutolol, f) pindolol, g) propranolol, and h)
578 trimethoprim.

579

580 **Figure 3.** *k*-p*H* profiles in MEEKC. Symbols and compounds as in Figure 2. Dotted and
581 solid lines correspond to *k*-p*H* profiles determined in the SDS-MEEKC and TTAB-
582 MEEKC systems, respectively.

583

584 **TABLES**

585 **Table 1**. log *k* values of neutral compounds determined in the SDS-MEEKC and TTAB-

586 MEEKC systems.

Compound	log $k_{(SDS\text{-}MEEKC)}$ a)	log $k_{(TTAB\text{-}MEEKC)}$ b)
Acetaminophen	-0.80	-0.56 c)
Acetanilide	-0.30	-0.17
Acetophenone	-0.05	-0.03
Antipyrine	-0.59	-0.67
Butyrophenone	0.60	0.68
Caffeine	-0.89	-0.77
Carbamazepine	0.46	0.48 c)
Corticosterone	0.59	0.65
Coumarin	-0.09	0.00 c)
Dexamethasone	0.44	0.78 c)
Estradiol	1.13	1.35
Naphthalene	1.13	1.21
Hydrocortisone	0.30	0.39
Hydrocortisone-21-acetate	0.47	0.65 c)
Lormetazepam	1.03	0.90 c)
Prednisolone	0.32	0.49 c)
Progesterone	1.32	1.44 c)
Propiophenone	0.26	0.35
Testosterone	0.97	1.07 c)
Valerophenone	0.98	1.05
3-Nitroaniline	-0.15	0.07
Thymol	0.96	1.20

587 a) Data from [24]; b) Data from [25]; c) Measured in this work.

588

589 **Table 2.** Values of pK_a', μ_{BH+} ($\cdot 10^5$ cm^2 s^{-1} V^{-1}), and statistics for the fit of Eq. 8 to

590 electrophoretic mobilities determined in CZE (μ_0).

Compounds	**pK_a' (SD)**	**μ_{BH+} (SD)**	**R^2**	**SD**	**F**
Alprenolol	9.73 (0.03)	17.34 (0.18)	0.992	0.62	2385
Ephedrine	9.85 (0.04)	21.68 (0.26)	0.987	0.95	1524
Nadolol	9.84 (0.07)	14.78 (0.32)	0.960	1.15	479
Oxprenolol	9.76 (0.04)	16.97 (0.19)	0.990	0.66	1983
Penbutolol	9.89 (0.05)	15.36 (0.22)	0.981	0.80	1055
Pindolol	9.79 (0.04)	17.74 (0.19)	0.990	0.69	2005
Propranolol	9.69 (0.04)	17.05 (0.19)	0.990	0.67	1993
Trimethoprim	7.10 (0.06)	17.21 (0.43)	0.984	0.98	1196

591

Table 3. pK_a', k_B, k_{BH+}, and statistics obtained from the fit of Eq. 6 to retention factor (k) determined at different pH values using the SDS-MEEKC and TTAB-MEEKC systems.

Compounds	SDS-MEEKC						TTAB-MEEKC					
	pK_a' (SD)	**k_{BH+} (SD)**	**k_B (SD)**	**F**	**SD**	**R^2**	**pK_a' (SD)**	**k_{BH+} (SD)**	**k_B (SD)**	**F**	**SD**	**R^2**
Alprenolol	9.61 (0.21)	17.89 (0.35)	10.40 (0.73)	58	1.03	0.906	9.66 (0.10)	0.69 (0.29)	11.67 (0.57)	188	0.57	0.989
Ephedrine	9.75 (0.10)	1.99 (0.03)	0.58 (0.07)	238	0.09	0.975	9.74 (0.20)	-0.06 (0.04)	0.63 (0.07)	49	0.07	0.970
Nadolol	9.24 (0.23)	1.56 (0.03)	0.99 (0.05)	55	0.09	0.901	9.96 (0.11)	0.02 (0.02)	0.92 (0.05)	156	0.05	0.987
Oxprenolol	9.70 (0.11)	6.32 (0.08)	3.05 (0.17)	215	0.23	0.973	9.78 (0.05)	0.16 (0.03)	2.91 (0.07)	841	0.07	0.998
Penbutolol	9.37 (1.36)	47.53 (1.65)	43.67 (2.13)	1.47	3.36	0.269	9.78 (0.06)	4.13 (0.86)	62.54 (1.76)	578	1.68	0.997
Pindolol	9.55 (0.16)	2.16 (0.03)	1.31 (0.06)	107	0.09	0.947	9.84 (0.07)	0.10 (0.04)	2.53 (0.09)	420	0.08	0.995
Propranolol	9.47 (0.31)	23.14 (0.68)	13.63 (1.29)	27	1.96	0.820	9.80 (0.05)	1.27 (0.19)	16.78 (0.39)	854	0.36	0.998
Trimethoprim	7.07 (0.08)	2.18 (0.04)	0.59 (0.03)	490	0.08	0.988	7.35 (0.11)	-0.08 (0.03)	0.60 (0.02)	218	0.04	0.991