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Solute–solvent interactions in micellar electrokinetic chromatography: VII. Characterization of sodium cholate–sodium deoxycholate mixed-micellar systems

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ABSTRACT

Micellar electrokinetic chromatography (MEKC) systems with mixed pseudostationary phases of the bile surfactants sodium cholate (SC) and sodium deoxycholate (SDC) have been characterized by means of the solvation parameter model. The importance of characterizing systems with an appropriate set of solutes that embrace a wide range of descriptor values has been proven as they can significantly influence the value of the system constants. The fit of the solvation parameter model to the experimental log k data has been compared for each SC–SDC system when the Abraham descriptors and the Poole optimized descriptors, recently proposed, are used. In both cases, the variation in MEKC surfactant composition results in similar changes in the coefficients of the correlation equations, which in turn leads to similar information on solute–solvent and solute–micelle interactions. It is demonstrated that SDC is more hydrogen-bond acidic and hydrophobic but slightly less polarizable than SC. Systems with intermediate selectivity are obtained through mixtures of both surfactants.

1. Introduction

Micellar electrokinetic chromatography (MEKC) is a well-established separation technique based on electrophoretic and electroosmotic principles whose distinguishing feature is the addition of a surfactant above its critical micelle concentration to the separation buffer. As a consequence, solutes are separated not only by migration but also by distribution between the aqueous phase (bulk electrolyte) and the pseudostationary phase (charged micelles), which allows the separation of complex mixtures of both neutral and ionized solutes [1–3]. The uncharged solutes are separated according to their distribution constants between the aqueous and micellar phases, whereas the charged solutes are separated as a result of their distribution between phases and their electrophoretic mobility. The main advantage of MEKC is the possibility of modifying the migration behaviour and separation in a very easy and flexible way, only by changing the nature of the pseudostationary phase. This can be achieved by proper selection of the surfactant type or by the addition of complexing agents (e.g., cyclodextrins, urea, etc.) or organic solvents to the separation solution [3–8]. However, it is generally accepted that the choice of surfactant is the most important consideration for varying the chemical nature of the medium and optimizing selectivity [8,9].

The solvation parameter model is strongly recommended to characterize MEKC systems [10] because it allows a better understanding of the types and the relative strength of the chemical interactions that control retention. This model, developed by Abraham [11], provides information not only about how different the characterized systems are, but also about the magnitude of the different interactions between the phases (aqueous phase and micellar phase in MEKC) and neutral solutes. It is based on linear free energy relationships (LFERs) established with solute descriptors. The suitable form for MEKC can be written as:

$$\log k = c + eE + sS + aA + bB + vV \quad (1)$$

where k is the MEKC retention factor and E, S, A, B and V are the solute descriptors proposed by Abraham [11]. E is an excess molar refraction, S is the solute dipolarity/polarizability, A and B are the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively, and V is McGowan's solute characteristic volume. The correlation coefficients of Eq. (1) are characteristic of the system (micellar phase + aqueous buffer) and reflect the system properties that are complementary to the corresponding solute property, i.e. they are system constants.

Since the solute property correlated to the solvation descriptors (log k for MEKC) can be any property related to free energy,

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the solvation parameter model has also been applied to the characterization of many other physicochemical systems apart from MEKC systems [10,12,13], as well as it has been demonstrated to be extremely useful for the characterization of many biological processes [14–16].

As the MEKC retention factor is directly related to the partition of the solute between the micellar phase and the aqueous phase, *e* refers to the difference in capacity of each phase to interact with solute π- and n-bonding electrons; *s* is a measure of the difference in capacity of the micellar phase and the aqueous phase to take part in dipole–dipole and dipole-induced dipole interactions; *a* and *b* represent the differences in hydrogen-bond basicity and acidity, respectively, between the micellar and the aqueous phases; *v* is a measure of the relative ease of forming a cavity for the solute in the buffer and the micelles. The intercept of the correlation, *c*, is related to the pseudostationary/aqueous buffer phase ratio and its value influences the retention time but not the selectivity. For any MEKC system the coefficients of the correlation equation can be obtained by multiple linear regression analysis of the experimental log *k* values acquired for a group of varied solutes with known descriptor values. As the proper selection of an adequate collection of solutes is extremely important to accurately determine the coefficients of Eq. (1), the literature gives some recommendations [9,10,17]. In general terms, this set of solutes must have properties sufficiently varied to define all interactions in Eq. (1) and be of sufficient number to establish the statistical validity of the model. In order to obtain a good fit of the solvation parameter model, well-characterized descriptor values with the minimal uncertainty for the selected set of compounds are also required. Abraham et al. [18] constructed the available database of descriptor values, which collects 4000 solutes characterized by all or some of their descriptors. Recently, Poole et al. [19] have proposed a collection of new descriptor values which were optimized for chromatographic methods using well-characterized systems of gas chromatography, reversed-phase chromatography, micellar electrokinetic chromatography, and liquid–liquid partitioning. These Poole optimized descriptors are expected to afford a better fit to the experimental data and smaller standard deviations for the system constants than the Abraham descriptors.

With the wide range of surfactants commercially available, several individual MEKC surfactants have been characterized through the solvation parameter model [3,4,8,9,13,17,20]. Such model has also been applied to the characterization of mixed-micellar systems [5,21–23]. The attention that has been paid to the use of mixed micelles is due to the fact that the properties of the pseudostationary phase, and therefore the coefficients of Eq. (1) for the MEKC system, can be continuously varied by changing the proportion of the surfactants in the mixture. This is especially interesting as it can facilitate the fine-tuning of selectivity, as has been reported in some studies carried out to classify the chemical selectivity of electrokinetic chromatography systems [13,23].

In this work, we characterize mixed-micellar systems of sodium cholate (SC) and sodium deoxycholate (SDC) (Fig. 1). The manuscript is part of a series devoted to the characterization and selectivity of different MEKC systems, composed of individual or mixed micelles, and to the study of several factors that influence the results. Some individual surfactant systems included in this series are the anionic sodium dodecyl sulfate (SDS), lithium dodecyl sulfate (LDS), lithium perfluorooctanesulfonate (LPFOS), SC, SDC and the cationic tetradecyltrimethylammonium bromide (TTAB) and hexadecyltrimethylammonium bromide (HTAB). Regarding mixed-micellar systems, this series has only included the characterization of the following binary mixtures: SDS with the neutral surfactant Brij 35, and two surfactants with very different properties, LDS and LPFOS. In the present work, we characterize another mixture composed of two individual bile salt surfactants that are very similar, SC and SDC. We study how their composition in the mixtures affects the coefficients of the solvation parameter model. The effect of the set of solutes and descriptors used and the pH of the buffer on the characterization coefficients are also discussed.

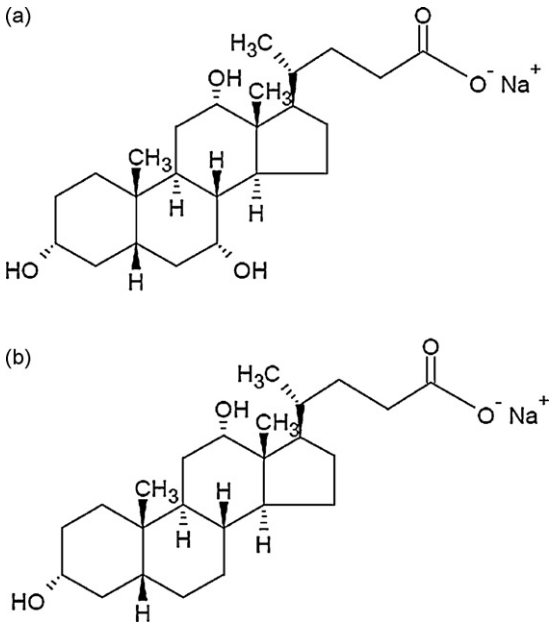

Fig. 1. Chemical structure of the monomers of the bile surfactants: (a) sodium cholate (SC) and (b) sodium deoxycholate (SDC).

2. Experimental

2.1. Apparatus and conditions

All separations were performed with a Beckman P/ACE System 5500 Capillary Electrophoresis with a UV diode array detector. The fused-silica separation capillaries were 40 cm effective length × 50 μm I.D. and were obtained from Composite Metal Services Ltd (Shipley, West Yorkshire, UK). The capillary was activated by the following washing sequence: water (10 min), 1 *M* NaOH (10 min), water (5 min), 0.1 *M* NaOH (10 min), water (5 min) and separation solution (20 min). This sequence was also used for conditioning the capillary when the pseudostationary phase was changed. As daily conditioning when using the same pseudostationary phase, the capillary was flushed with water for 5 min, followed by 0.1 *M* NaOH for 5 min, water for 5 min and separation solution for 20 min. Prior to each separation when using the same pseudostationary phase the capillary was flushed with separation solution for 3 min. Retention measurements were made at 25 °C and +15 kV. Detection was at 214 nm.

All systems (separation solutions) were 20 m*M* in aqueous buffer. The systems at pH 8 were prepared by dissolving the surfactants in NaH_2PO_4:$Na_2B_4O_7$ (65:35) buffer. For 80 m*M* SC at pH 7 the system was prepared by dissolving the surfactant in NaH_2PO_4:Na_2HPO_4 (50:50) buffer. For 80 m*M* SC at pH 6 the system was prepared by dissolving the surfactant in NaH_2PO_4:Na_2HPO_4 (50:50) buffer and neutralizing with HCl. Surfactant concentrations were similar to the ones used by other authors [4,8,17], and they were chosen to be well above the critical micelle concentration (CMC) in order to obtain a reasonable volume of pseudostationary phase and an acceptable elution window. The proportion of each surfactant in the mixtures was chosen in such a way that the total surfactant concentration ranged from 40 m*M* to 80 m*M*, with the total surfactant concentration varied in increments of 10 m*M*.

Solutes were dissolved in methanol (used as electroosmotic flow marker) and contained 2 mg mL^{-1} of dodecanophenone as micellar marker [24]. The injection of methanol produces a local disruption of the micellar phase. For low micellar concentrations, the disruption causes peak splitting [25], which can be avoided by working at higher concentrations such as those used in this work. The concentration of the solutes was 2 mg mL^{-1}, except for the alcohols which were 40% (v/v) in order to obtain measurable absorbance. All solutions were filtered through 0.45-μm nylon syringe filters obtained from Albet (Dassel, Germany). Samples were introduced into the capillary by applying a pressure of 0.5 p.s.i. for 1 s (1 p.s.i. = 6894.76 Pa). All measurements were taken in triplicate.

2.2. Reagents and materials

Hydrochloric acid (25% in water), sodium hydroxide (>99%), sodium dihydrogenphosphate monohydrate (>99%), disodium hydrogenphosphate (>99%) and methanol (HPLC grade) were from Merck (Darmstadt, Germany). Disodium tetraborate decahydrate (>99.5%) was from Sigma (Steinheim, Germany). SC (>98%) was from Fluka (Steinheim, Germany). SDC (98%) and dodecanophenone (98%) were from Aldrich (Steinheim, Germany). Water was purified by a Milli-Q plus system from Millipore (Bedford, MA, USA), with a resistivity of 18.2 MΩ cm. The test solutes employed were reagent grade or better and obtained from several manufacturers (Merck (Darmstadt, Germany), Sigma (Steinheim, Germany), Fluka (Steinheim, Germany), Aldrich (Steinheim, Germany), Carlo Erba (Milano, Italy), Baker (Deventer, Netherlands)).

2.3. Calculation

The MEKC retention factor, k, was calculated according to Eq. (2) with the migration time of methanol used to determine the electroosmotic flow (t_0), and the migration time of dodecanophenone used to determine the migration time of the micelles (t_m). t_R is the solute migration time:

$$k = \frac{t_R - t_0}{(1 - (t_R/t_m))t_0} \quad (2)$$

Microsoft Excel XP was used to perform data calculations and multiple linear regression analysis.

3. Results and discussion

3.1. Influence of the set of solutes on the characterization of systems

Although most MEKC systems are usually characterized at pH 7, it was decided to characterize all the systems studied here at pH 8 because SDC presents low solubility at pH 7.

The 80 m*M* SC pure surfactant system at pH 8 was the first one to be characterized by means of the solvation parameter model through Eq. (1) by analysis of the log k data of a series of 69 solutes with known E, S, A, B and V descriptor values. The studied solutes and their descriptors are given in Table 1, where both the Abraham descriptor values [18] and the optimized descriptor values recently proposed by Poole et al. [19] for some of them are presented. In this section, only the Abraham descriptors have been employed for the system characterizations. The collection of solutes has been selected according to a study carried out in a previous work [17] in which they were considered an appropriate group of compounds for MEKC characterizations covering a wide range of solute descriptor values. Initially, the compounds 4-chlorophenol, catechol, resorcinol, hydroquinone, 2-naphthol, and 1,2,3-trihydroxybenzene were not included in the characterization set because they have aqueous pK_a values between 9 and 10 and therefore they are susceptible to be partially ionized at the working pH (pH 8). The k values obtained were calculated by Eq. (2). The system constants and the statistics for the fit of the solvation parameter model to the experimental log k data for the 80 m*M* SC system at pH 8 are summarized in Table 2 (correlation a). The compounds of the set that were not included in the final correlation (because they coeluted with the electroosmotic flow marker or were outliers) are also detailed in the table.

Noticeable differences were observed between the coefficients in Eq. (1) (system constants) for the 80 m*M* SC system characterized at pH 8 in this work, and a 80 m*M* SC system characterized at pH 7 in a previous work [17]. Since literature reports that pH does not have effect on the system constants [8], we decided to characterize 80 m*M* SC at pH 7, as well as, at pH 6 in order to prove if the change of pH was or not responsible for the differences found between the coefficients.

The characterizations of the 80 m*M* SC system at pH 7 and 6, unlike the 80 m*M* SC system at pH 8, were carried out including the substances 4-chlorophenol, catechol, resorcinol, hydroquinone, 2-naphthol, and 1,2,3-trihydroxybenzene because these phenols are totally neutral at these values of working pH. The system constants and the statistics for the fit of the solvation parameter model to the experimental log k data for each correlation are also shown in Table 2 (correlations e and f).

Comparing the coefficients of the correlations a, e, and f detailed in Table 2, it is observed that they are nearly identical for 80 m*M* SC at pH 7 and 6, whereas those of the system at pH 8 are different. Since the only difference between the characterization at pH 8 and at pH 7 and 6 is the fact that the substances 4-chlorophenol, catechol, resorcinol, hydroquinone, 2-naphthol, and 1,2,3-trihydroxybenzene were not included at pH 8, it was decided to calculate again the correlations at pH 7 and 6 without these phenols in order to investigate if they were responsible for the discrepancies between the coefficients. The results obtained are also summarized in Table 2 (correlations b and c).

After removing these six phenols, coefficients became very similar, independently of the pH of the separation solution. According to this fact, it is confirmed that changing the pH (after excluding ionized solutes) has little influence on the selectivity, as it is reported in the literature [8]. Taking everything into account, what is highly remarkable is the role that 4-chlorophenol, catechol, resorcinol, hydroquinone, 2-naphthol, and 1,2,3-trihydroxybenzene play in these characterizations. These phenolic compounds are especially important because they are of the few ones of the set of solutes with a high value for the A descriptor. As a consequence, if 4-chlorophenol, catechol, resorcinol, hydroquinone, 2-naphthol, and 1,2,3-trihydroxybenzene are not included in the characterizations, a very narrow range of values for the A descriptor is used and the accuracy in the determination of system constants diminishes. In fact, it can be observed that when these phenolic compounds are not included in the correlations, the a coefficient is approximately 50% higher than the value obtained when the more varied set of solutes that includes them is used. Therefore, this reveals how important is to employ a varied set of solutes that span a wide range of descriptor values when systems are characterized by means of the solvation parameter model.

As the compounds 4-chlorophenol, catechol, resorcinol, hydroquinone, 2-naphthol, and 1,2,3-trihydroxybenzene have a great effect on the system constants, it was studied if their ionisation degree was significant enough to include them in the correlation at pH 8. These phenolic compounds were injected in 80 m*M* SC at pH 8. Afterwards, the log k values obtained for the 80 m*M* SC system at pH 8 were plotted against those obtained before at pH 6 and 7 for all the solutes of the set (Fig. 2a). According to the proved little effect of the pH on the selectivity, good correlations should be obtained

Table 1
Solute descriptors used in the solvation parameter model.

Solute	*V*	Abraham descriptors [18]				Poole optimized descriptors [19]			
		E	*S*	*A*	*B*	*E*	*S*	*A*	*B*
Propan-1-ol	0.5900	0.236	0.42	0.37	0.48				
Propan-2-ol	0.5900	0.212	0.36	0.33	0.56				
Butan-1-ol	0.7309	0.224	0.42	0.37	0.48	0.224	0.44	0.34	0.52
Pentan-1-ol	0.8718	0.219	0.42	0.37	0.48	0.219	0.44	0.34	0.52
Pentan-3-ol	0.8718	0.218	0.36	0.33	0.56	0.218	0.40	0.27	0.57
Propan-1,3-diol	0.6487	0.397	0.91	0.77	0.85				
Butan-1,4-diol	0.7860	0.395	0.93	0.72	0.90				
Pentan-1,5-diol	0.9305	0.388	0.95	0.72	0.91				
Thiourea	0.5696	0.840	0.82	0.77	0.87				
Benzene	0.7164	0.610	0.52	0.00	0.14	0.608	0.51	0.00	0.14
Toluene	0.8573	0.601	0.52	0.00	0.14	0.606	0.50	0.00	0.14
Ethylbenzene	0.9982	0.613	0.51	0.00	0.15	0.613	0.50	0.00	0.14
Propylbenzene	1.1391	0.604	0.50	0.00	0.15	0.610	0.50	0.00	0.14
Butylbenzene	1.2800	0.600	0.51	0.00	0.15	0.595	0.50	0.00	0.14
p-Xylene	0.9982	0.613	0.52	0.00	0.16	0.615	0.49	0.00	0.16
Naphthalene	1.0854	1.340	0.92	0.00	0.20	1.240	0.91	0.00	0.19
Chlorobenzene	0.8388	0.718	0.65	0.00	0.07	0.718	0.66	0.00	0.06
Bromobenzene	0.8914	0.882	0.73	0.00	0.09	0.882	0.72	0.00	0.09
Anisole	0.9160	0.708	0.75	0.00	0.29	0.712	0.77	0.00	0.31
Benzaldehyde	0.8730	0.820	1.00	0.00	0.39	0.813	1.03	0.00	0.39
Acetophenone	1.0139	0.818	1.01	0.00	0.48	0.806	1.03	0.00	0.50
Propiophenone	1.1548	0.804	0.95	0.00	0.51	0.804	1.03	0.00	0.50
Butyrophenone	1.2957	0.797	0.95	0.00	0.51	0.798	1.03	0.00	0.50
Valerophenone	1.4366	0.795	0.95	0.00	0.50	0.795	1.03	0.00	0.50
Heptanophenone	1.7184	0.720	0.95	0.00	0.50				
Benzophenone	1.4808	1.447	1.50	0.00	0.50	1.224	1.33	0.00	0.58
Methyl benzoate	1.0726	0.733	0.85	0.00	0.46	0.738	0.92	0.00	0.44
Benzyl benzoate	1.6804	1.264	1.42	0.00	0.51	1.264	1.30	0.00	0.59
Benzonitrile	0.8711	0.742	1.11	0.00	0.33	0.742	1.14	0.00	0.33
Aniline	0.8162	0.955	0.96	0.26	0.50	0.955	1.00	0.25	0.43
o-Toluidine	0.9751	0.970	0.90	0.23	0.59	0.966	1.05	0.19	0.49
3-Chloroaniline	0.9390	1.050	1.10	0.30	0.36				
4-Chloroaniline	0.9390	1.060	1.10	0.30	0.35	1.017	1.13	0.37	0.31
2-Nitroaniline	0.9904	1.180	1.37	0.30	0.36	1.182	1.44	0.39	0.35
3-Nitroaniline	0.9904	1.200	1.71	0.40	0.35	1.248	1.60	0.47	0.42
4-Nitroaniline	0.9904	1.220	1.91	0.42	0.38	1.236	1.83	0.60	0.34
Nitrobenzene	0.8906	0.871	1.11	0.00	0.28	0.846	1.14	0.00	0.27
2-Nitroanisole	1.0902	0.965	1.34	0.00	0.38				
Benzamide	0.9728	0.990	1.50	0.49	0.67	1.258	1.34	0.65	0.66
4-Aminobenzamide	1.0726	1.340	1.94	0.80	0.94				
Acetanilide	1.1137	0.870	1.36	0.46	0.69	0.960	1.12	0.53	0.71
4-Chloroacetanilide	1.2357	0.980	1.50	0.64	0.51				
Phenol	0.7751	0.805	0.89	0.60	0.30	0.769	0.76	0.72	0.32
3-Methylphenol	0.9160	0.822	0.88	0.57	0.34	0.810	0.78	0.67	0.35
2,3-Dimethylphenol	1.0569	0.850	0.90	0.52	0.36	0.866	0.77	0.59	0.40
2,4-Dimethylphenol	1.0569	0.840	0.80	0.53	0.39				
Thymol	1.3387	0.822	0.79	0.52	0.44				
Furan	0.5363	0.369	0.53	0.00	0.13				
2,3-Benzofuran	0.9053	0.888	0.83	0.00	0.15	0.921	0.77	0.00	0.19
Quinoline	1.0443	1.268	0.97	0.00	0.51	1.268	1.09	0.00	0.60
Pyrrole	0.5774	0.613	0.73	0.41	0.29				
Pyrimidine	0.6342	0.606	1.00	0.00	0.65				
Antipyrine	1.5502	1.320	1.50	0.00	1.48				
Caffeine	1.3632	1.500	1.60	0.00	1.33	1.557	1.62	0.00	1.27
Corticosterone	2.7389	1.860	3.43	0.40	1.63				
Cortisone	2.7546	1.960	3.50	0.36	1.87				
Hydrocortisone	2.7975	2.030	3.49	0.71	1.90				
Estradiol	2.1988	1.800	3.30	0.88	0.95				
Estriol	2.2575	2.000	3.36	1.40	1.22				
Monuron	1.4768	1.140	1.50	0.47	0.78				
Myrcene	1.3886	0.483	0.29	0.00	0.21				
α-Pinene	1.2574	0.446	0.14	0.00	0.12				
Geraniol	1.4903	0.513	0.63	0.39	0.66				
4-Chlorophenol[a]	0.8975	0.915	1.08	0.67	0.20	1.016	0.79	0.89	0.21
Catechol[a]	0.8338	0.970	1.10	0.88	0.47				
Resorcinol[a]	0.8338	0.980	1.00	1.10	0.58	0.968	0.91	1.37	0.51
Hydroquinone[a]	0.8338	1.000	1.00	1.16	0.60				
2-Naphthol[a]	1.1441	1.520	1.08	0.61	0.40	1.457	1.18	0.81	0.35
1,2,3-Trihydroxybenzene[a]	0.8925	1.165	1.35	1.35	0.62				

[a] Phenols with pK_a values between 9 and 10 and therefore susceptible to be partially ionized at pH 8.

Table 2
Coefficients in Eq. (1) for the 80 m*M* SC micellar separation system at pH 8, 7 and 6.

System	Coefficients						Statistics				Solutes excluded from the correlations
	c	*e*	*s*	*a*	*b*	*v*	*n*	*r*	SD	*F*	
Phenols with pK_a 9–10 not included											
(a) 80 m*M* SC at pH 8	−1.544 (0.110)	1.022 (0.185)	−0.849 (0.138)	0.152 (0.134)	−1.955 (0.132)	2.266 (0.116)	60	0.958	0.241	120	Propan-1,3-diol[a], Butan-1,4-diol[a] Furan[a]
(b) 80 m*M* SC at pH 7	−1.543 (0.110)	1.022 (0.185)	−0.819 (0.140)	0.144 (0.135)	−1.959 (0.133)	2.245 (0.122)	59	0.956	0.242	112	Propan-1,3-diol[a], Butan-1,4-diol[a] Furan[a], α-Pinene[b]
(c) 80 m*M* SC at pH 6	−1.570 (0.110)	1.018 (0.184)	−0.819 (0.140)	0.141 (0.134)	−1.954 (0.132)	2.246 (0.121)	59	0.956	0.241	113	Propan-1,3-diol[a], Butan-1,4-diol[a] Furan[a], α-Pinene[b]
Phenols with pK_a 9–10 included											
(d) 80 m*M* SC at pH 8	−1.501 (0.101)	0.910 (0.152)	−0.776 (0.116)	0.109 (0.096)	−1.961 (0.126)	2.255 (0.112)	66	0.958	0.234	133	Propan-1,3-diol[a], Butan-1,4-diol[a] Furan[a]
(e) 80 m*M* SC at pH 7	−1.502 (0.101)	0.915 (0.152)	−0.748 (0.118)	0.095 (0.096)	−1.968 (0.127)	2.235 (0.117)	65	0.956	0.234	126	Propan-1,3-diol[a], Butan-1,4-diol[a] Furan[a], α-Pinene[b]
(f) 80 m*M* SC at pH 6	−1.531 (0.100)	0.916 (0.151)	−0.751 (0.117)	0.097 (0.095)	−1.963 (0.126)	2.237 (0.116)	65	0.957	0.232	127	Propan-1,3-diol[a], Butan-1,4-diol[a] Furan[a], α-Pinene[b]

Standard deviations are in parentheses.
[a] Solutes that coelute with methanol.
[b] Solute considered outlier (with a standard residual higher than |2.5|).

Fig. 2. (a) Log *k* values for the 80 m*M* SC system at pH 6 (■) and pH 7 (○) against the log *k* values at pH 8. (b) Log *k* values for the 80 m*M* SC system at pH 6 (■) and pH 7 (○) against the log *k* values at pH 8, with the values for catechol and 1,2,3-trihydroxybenzene determined at pH 8 in phosphate buffer.

if these compounds are not significantly ionized. The correlations illustrated in Fig. 2a show a good fit for all the compounds except for the phenols catechol and 1,2,3-trihydroxybenzene, which deviate from both correlations. However, the most probably reason for the deviations of these two compounds is the fact that they have vicinal diol groups in their chemical structure and the borate buffer, which was used mixed with phosphate buffer, has a well-known ability to complex vicinal diol groups [26,27]. In order to prove this, catechol and 1,2,3-trihydroxybenzene were injected again in 80 m*M* SC at pH 8 but in this case the aqueous buffer was prepared only with phosphate. Plots of the log *k* values of the 80 m*M* SC system at different pHs were represented again including these new log *k* values acquired for catechol and 1,2,3-trihydroxybenzene at pH 8. Plots are shown in Fig. 2b. The good correlations observed in this figure for all the compounds of the set confirm that the previously noticed deviations of catechol and 1,2,3-trihydroxybenzene were due to the modification of the effective mobility that both compounds experiment when borate buffer interacts with their consecutive diols. The good correlations shown in Fig. 2b also prove that 4-chlorophenol, catechol, resorcinol, hydroquinone, 2-naphthol, and 1,2,3-trihydroxybenzene are not significantly ionized at pH 8, since none deviates from the values acquired at pH 7 and 6 (pH in which they are clearly neutral). In fact, a simple calculation shows that the ionisation degree of these compounds in water at pH 8 is less than 5% (except for the most acidic compound 1,2,3-trihydroxybenzene). This degree is decreased by partition of the neutral forms of the compounds to the pseudostationary phase. Thus, it is possible to include them in any characterization at pH 8. For the 80 m*M* SC at pH 8, system constants were calculated again including these six phenols and the results are shown in Table 2 (correlation d).

3.2. Characterization of SC–SDC mixtures

Separation systems composed by mixtures of SC–SDC at different concentrations were also characterized by means of the solvation parameter model through Eq. (1) by analysis of the log *k* data of the 69 solutes selected (Table 1). Correlation equations for each system were calculated employing both the Abraham descriptors and the Poole optimized descriptors, whose values are detailed in Table 1, in order to check if the use of these optimized descriptors improves the correlation equations of the SC–SDC systems studied in this work.

The coefficients obtained for each system and the statistics for the fit of the solvation parameter model to the experimental log *k* data when the Abraham descriptors were employed in the regressions are summarized in Table 3. The model provides a good statistical fit and its interpretation is chemically reasonable. The solutes that were not included in the correlations are detailed in the table (all them coeluted with methanol, except for heptanophenone that coeluted with dodecanophenone in the 40 m*M* SDC system). The coefficients obtained for the 80 m*M* SC and 40 m*M* SDC systems (only ones that are reported in the literature) are similar to the ones obtained for other authors in similar conditions [8,17]. The small differences observed in some constants may come from the different solutes employed in the characterizations.

The constants for each system were also calculated employing the Poole optimized descriptors that are available for some solutes of the characterization set (for the rest of solutes of the set, the Abraham descriptors were used). The results are summarized in Table 4.

As the correlation equation of each system was calculated with the same group of solutes in Tables 3 and 4, the coefficients and statistics presented in these tables can be directly compared. For all the characterized systems the fit of the experimental data to the solvation parameter model was only slightly better when the proposed optimized descriptors were used. Regarding the coefficients, it is observed that for all the systems, the values of *e*, *b*, and *v* are a bit higher when the Poole optimized descriptors are used in the correlation equations instead of the Abraham descriptors. On the contrary, it is observed that the *s* and *a* coefficients are a bit lower if the characterizations are performed using the Poole optimized descriptors. Anyway, the coefficients summarized in both tables lead to the same chemical interpretation.

In order to illustrate more clearly that the experimental data better fit the model when the Poole optimized descriptors are used, we also calculated the system constants for all the studied SC–SDC systems just including the solutes with available Poole optimized descriptors. The *F* statistic ranged from 71 to 83 when the Abraham descriptors were used, whereas it ranged from 144 to 197 with the Poole optimized descriptors. Regarding the standard deviation, it ranged from 0.190 to 0.212 using the Abraham descriptors, whereas it ranged from 0.127 to 0.151 using the Poole optimized descriptors. Taking into account these statistics, a greater improvement in the fit is observed when the Poole optimized descriptors are used instead of the Abraham descriptors. However, we consider that interpreting the system constants according to these regressions is not rigorous enough, since the solutes with available Poole optimized descriptors are not sufficiently representative to define all interactions (40 solutes that do not include large polar hydrogen-bonding solutes such as corticosteroids). Therefore, the chemical interpretation of the characterizations will be discussed according to the regressions calculated with the 69 solutes, i.e. using Abraham descriptors for the 69 solutes in one hand (Table 3) and the available Poole optimized descriptors for 40 solutes and Abraham descriptors for the remaining 29 solutes on the other hand (Table 4).

As shown in Tables 3 and 4, the largest coefficients in absolute value are *v* and *b* independently of the composition of SC and

Table 3
Coefficients in Eq. (1) for the SC–SDC mixed-micellar systems at 25 °C and pH 8 calculated employing the Abraham descriptors.

System	x_{SDC}	Coefficients						Statistics				Solutes excluded from the correlations
		c	*e*	*s*	*a*	*b*	*v*	*n*	*r*	SD	*F*	
80 m*M* SC	0.000	−1.501 (0.101)	0.910 (0.152)	−0.776 (0.116)	0.109 (0.096)	−1.961 (0.126)	2.255 (0.112)	66	0.958	0.234	133	Propan-1,3-diol, Butan-1,4-diol Furan
60 m*M* SC–10 m*M* SDC	0.143	−1.578 (0.095)	0.897 (0.144)	−0.768 (0.110)	0.125 (0.090)	−1.903 (0.119)	2.241 (0.106)	66	0.961	0.221	145	Propan-1,3-diol, Butan-1,4-diol Furan
40 m*M* SC–20 m*M* SDC	0.333	−1.658 (0.091)	0.858 (0.142)	−0.795 (0.108)	0.145 (0.090)	−1.847 (0.119)	2.300 (0.105)	67	0.961	0.221	147	Propan-1,3-diol Butan-1,4-diol
20 m*M* SC–30 m*M* SDC	0.600	−1.753 (0.102)	0.865 (0.155)	−0.809 (0.116)	0.117 (0.097)	−1.827 (0.130)	2.354 (0.114)	66	0.955	0.239	125	Propan-2-ol, Propan-1,3-diol Butan-1,4-diol
40 m*M* SDC	1.000	−1.786 (0.112)	0.813 (0.170)	−0.803 (0.125)	0.061 (0.103)	−1.771 (0.140)	2.400 (0.127)	64	0.947	0.255	102	Propan-1-ol, Propan-2-ol, Propan-1,3-diol Butan-1,4-diol, Heptanophenone

Standard deviations are in parentheses.

Table 4
Coefficients in Eq. (1) for the SC–SDC mixed-micellar systems at 25 °C and pH 8 calculated employing the Poole optimized descriptors (Abraham descriptors were used for solutes with no available optimized descriptors).

System	x_{SDC}	Coefficients						Statistics				Solutes excluded from the correlations
		c	*e*	*s*	*a*	*b*	*v*	*n*	*r*	SD	*F*	
80 m*M* SC	0.000	−1.536 (0.094)	0.956 (0.146)	−0.825 (0.110)	0.029 (0.081)	−1.944 (0.119)	2.303 (0.104)	66	0.964	0.216	158	Propan-1,3-diol, Butan-1,4-diol Furan
60 m*M* SC–10 m*M* SDC	0.143	−1.610 (0.090)	0.936 (0.138)	−0.814 (0.105)	0.045 (0.077)	−1.883 (0.113)	2.287 (0.099)	66	0.966	0.205	169	Propan-1,3-diol, Butan-1,4-diol Furan
40 m*M* SC–20 m*M* SDC	0.333	−1.686 (0.088)	0.892 (0.138)	−0.836 (0.104)	0.059 (0.078)	−1.821 (0.115)	2.340 (0.099)	67	0.965	0.209	166	Propan-1,3-diol, Butan-1,4-diol
20 m*M* SC–30 m*M* SDC	0.600	−1.785 (0.098)	0.909 (0.152)	−0.854 (0.113)	0.033 (0.084)	−1.804 (0.126)	2.396 (0.108)	66	0.960	0.226	141	Propan-2-ol, Propan-1,3-diol Butan-1,4-diol
40 m*M* SDC	1.000	−1.822 (0.109)	0.865 (0.169)	−0.852 (0.123)	−0.022 (0.091)	−1.750 (0.137)	2.444 (0.122)	64	0.952	0.244	112	Propan-1-ol, Propan-2-ol, Propan-1,3-diol Butan-1,4-diol, Heptanophenone

Standard deviations are in parentheses.

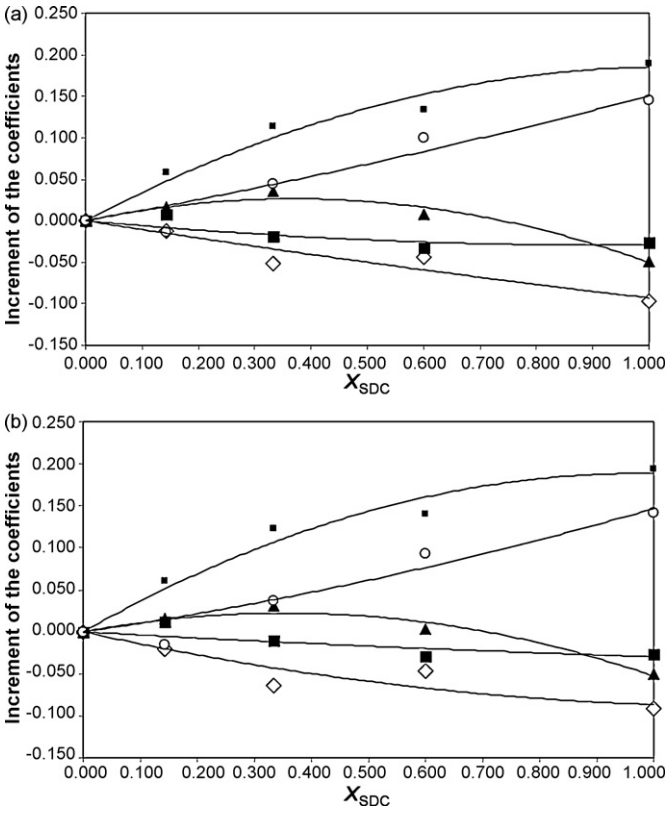

Fig. 3. Plot of the increment of each coefficient depending on the molar fraction of SDC in the mixed-micellar systems when the coefficients in Eq. (1) have been calculated with: (a) the Abraham descriptors, and (b) the Poole optimized descriptors: (◊) Δ*e*; (■) Δ*s*; (▲) Δ*a*; (■) Δ*b*; (○) Δ*v*.

SDC. This means that the McGowan's characteristic volume (*V*) and the hydrogen-bond basicity (*B*) are the solute descriptors that contribute most to retention. On the other hand, the *a* coefficient is nearly zero in all the systems, which indicates that the hydrogen-bond acceptor ability of micelles is similar to that of water and the retention of solutes is then scarcely influenced by its hydrogen-bond acidity (*A* descriptor plays a minor role). Regarding the sign of the coefficients, in all the SC–SDC systems the *e*, *a* and *v* coefficients are positive whereas the *s* and *b* coefficients are negative. According to the positive sign, the partition of the solutes towards the micellar phase is favoured by their excess molar refraction (*E*), their hydrogen-bond acidity (*A*) and their molar volume (*V*). On the contrary, since the *s* and *b* coefficients are negative, the more dipolar and hydrogen-bond basic is a solute (high values of *S* and *B*, respectively), the more favoured is its partition into the aqueous phase and it becomes less retained.

In order to study the variation of the coefficients depending on the composition of SC–SDC in the separation buffer, the increment of each coefficient has been plotted against the composition of SDC in the mixtures, using the coefficients of the 80 m*M* SC system as a zero point. Fig. 3a shows the plot corresponding to the variation of the system constants calculated with the Abraham descriptors whereas Fig. 3b represents the variation of the coefficients that were obtained employing the Poole optimized descriptors. It can be observed that Fig. 3a and b is nearly identical, which means that both collection of descriptors lead to the same conclusions about the variation of the coefficients. The hydrogen-bond acidity is the property that makes the two surfactants more different since the *b* coefficient has the greatest increment, followed by the *v* coefficient, which represents the hydrophobicity of the system. The increment of the rest of coefficients is modest according to the similar nature

of SC and SDC bile salts. From Fig. 3a and b we come to the conclusion that SDC is more hydrogen-bond acidic than SC ($b_{SDC} > b_{SC}$) and it is also a bit more hydrophobic ($v_{SDC} > v_{SC}$). However, SDC is moderately less polarizable than SC since it is observed a smooth decrease of the *e* coefficient ($e_{SDC} < e_{SC}$). On the other hand, there is hardly any difference in dipolarity and hydrogen-bond basicity between both surfactants, since a meaningful variation of its coefficients is not observed ($s_{SDC} \approx s_{SC}$, $a_{SDC} \approx a_{SC}$). This means that at any concentration of SC and SDC, the micellar pseudophase has the same capacity to take part in dipole–dipole interactions with the solute, as well as the same hydrogen-bond basicity.

4. Conclusions

It has been shown how significant is employing a suitable set of solutes for characterizing systems by means of the solvation parameter model in order to obtain accurate coefficients, as well as it has been proved that the pH of the aqueous phase does not have effect on the studied MEKC system characterizations. Initial comparison of the results obtained here for 80 m*M* SC at pH 8 and literature results for 80 m*M* SC at pH 7 showed significant differences, but we have demonstrated that these differences are caused by differences in the set of solutes used, not by differences in pH. No significant differences were found when the same set of non-ionized solutes were used for the two characterizations.

Both the Abraham descriptor values and the optimized descriptor values recently proposed by Poole et al. have been employed to calculate the correlation equations of all the studied SC–SDC mixtures. It has been proved that both group of descriptors lead to the same variation of coefficients depending on the composition of SC–SDC in the systems. Although the statistics obtained are a bit better when the optimized descriptors are used, there are not optimized descriptor values for all the solutes of the set. For this reason, enlarging the optimized descriptors database would be very interesting.

The characterizations of SC–SDC mixed-micellar phases demonstrate that the addition of SDC to SC micelles increases the hydrogen-bond acidity of the micellar phase and its hydrophobicity whereas the rest of interactions are only slightly changed. Therefore, the more rich in SDC is the micellar phase, the more appropriate the system is to separate mixtures of compounds of different hydrogen-bond basicity and size.

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