SCIENTIFIC PAPERS
OF THE UNIVERSITY OF PARDUBICE
Series A
Faculty of Chemical Technology
15 (2009)

POSSIBILITIES AND LIMITATIONS OF MICELLAR ELECTROKINETIC CHROMATOGRAPHY SEPARATION OF VERY HYDROPHOBIC ANTHRAQUINONE DYES USING SDS MICELLES

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Received September 30, 2009

The separation of five very hydrophobic anthraquinone dyes and five polar anthraquinone dye intermediates by micellar electrokinetic chromatography has been studied. Both types of analytes were separated using non-coated fused silica capillary in borate background electrolyte containing sodium dodecyl sulphate micellar pseudostationary phase. Acetonitrile was used for modification of polarity of the background electrolyte. The migration of anthraquinones was characterized by lipophilic and polar indices employing n-alkylbenzenes as calibration standards. Based on the results, the types of the interactions of anthraquinones with sodium dodecyl sulphate micelles and acetonitrile in aqueous phase are described. The limits of micellar electrokinetic chromatography for successful separation of very hydrophobic compounds are discussed.

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Introduction

Separation of two types of anthraquinone-based compounds with highly different polarity using capillary electrophoresis is described in this work. The first group, anthraquinone dye intermediates, represents moderately polar compounds with relatively good solubility in water and in common background electrolytes. In contrast to them, very hydrophobic anthraquinone dyes, almost insoluble in water belong to the second group being analyzed. The micellar electrokinetic chromatography separation of very hydrophobic analytes using conventional surfactants can be difficult, especially when the retention of analytes is so high, that they are presented almost only in the micellar phase. To reduce the retention factors, solubility of the analytes in background electrolyte has to be increased resulting in shift of the micellar/aqueous partitioning equilibrium towards the presence of anaytes in aqueous phase. The most frequently used approaches are based on addition of various modifiers into the background electrolyte, like organic solvents [1-3] and/or cyclodextrins [4,5]. Another possibility is to employ polymeric pseudostationary phase [6] or mixed micellar system, where the separation selectivity can be tuned *via* the molar fractions of the two surfactants [7,8]. In the present work, the addition of acetonitrile into the background electrolyte is used to improve the selectivity of separation of anthraquinone dyes and dye intermediates.

Compounds based on the anthraquinone skeleton are a wide group of chemical individuals both produced by chemical industry and naturally occurring in the plant kingdom. Some of the anthraquinones have biological activities and can be used for medical treatment; others can serve as natural colorants [9-11]. Hydrophobic anthraquinone-based dyes, produced by chemical industry, are applied mostly for coloring of textiles, preparation of color ink-jet inks and toners, lacquers and cosmetics. Dissolved in the miniemulsions, anthraquinone-based solvent dyes have recently been used for the preparation of nanocolorants, which exhibit excellent chromatic properties, migration and light fastness, thermal stability and good processibility [12].

Anthraquinones have been analyzed by many separation techniques, including high-performance liquid chromatography [9,13-18], capillary zone electrophoresis [10,15,17-21], micellar electrokinetic chromatography [10,11,22,23], capillary electrochromatography [24] and nonaqueous capillary electrophoresis [25]. Most of the publications are focused on the separation of anthraquinone derivatives containing polar and/or ionisable functional groups. Fast separation of such compounds can usually be achieved by capillary zone electrophoresis in phosphate or borate background electrolytes with addition of β-cyclodextrin [20,21] or acetonitrile [18,19], to improve selectivity of anthraquinone dyes separation. When the separation of both uncharged and charged derivatives of anthraquinones is needed, micellar electrokinetic

chromatography (MEKC) can be used, since micelles provide both ionic and hydrophobic interactions with the analyte. Using this method, in background electrolytes containing sodium dodecyl sulphate (SDS) and acetonitrile, separation and determination of a broad range of red organic anthraquinone-based pigments used in art work [11] or even 21 hydroxy- and dihydroxyanthraqinone positional isomers [22] were described. Determination of hydroxyanthraquinones in medicinal herb samples by MEKC using ternary mixed micellar system of SDS, sodium cholate (SC) and β-cyclodextrin was described by Shang *et al.* [23].

Although many methods for determination of anthraquinones by MEKC were published, to our best knowledge, separation of non-polar anthraquinones has not been reported so far. The aim of this paper is to develop the separation of very hydrophobic anthraquinone dyes and anthraquinone dye intermediates in micellar electrolytes containing different concentrations of SDS and acetonitrile. Simple two-indices calibration approach was used for description and prediction of their retention, introduced by Jandera *et al.* for prediction of retention in HPLC, micellar LC, normal and reduced-flow MEKC [26-31].

Experimental

Samples and Chemicals

Standards of anthraquinone dyes (C.I. Solvent Blue 36 (No. 1), C.I. Solvent Blue 104 (No. 2), C.I. Solvent Violet 13 (No. 3), C.I. Solvent Violet 14 (No. 4), C.I. Solvent Yellow 163 (No. 5)) and intermediates (anthraquinone (No. 6), 1-aminoanthraquinone (No. 7), 2-aminoanthraquinone (No. 8), 2,6-diaminoanthraquinone (No. 9), 1,2-dichloroanthraquinone (No. 10)) were obtained from Aliachem (Pardubice – Semtín, the Czech Republic) and from the reference collection of the Institute of Polymeric Materials, University of Pardubice. Their structures are shown in Fig. 1. The individual standards and mixtures of anthraquinone dyes were dissolved in isopropyl alcohol (Merck, Darmstadt, Germany), dye intermediates were dissolved in background electrolyte. Boric acid, sodium tetraborate, sodium dodecyl sulphate and *n*-alkylbenzene standards (from methyl- to *n*-hexylbenzenes) were obtained from Fluka (Buchs, Switzerland), acetonitrile from Sigma-Aldrich (Steinheim, Germany). Background electrolytes were prepared by dissolving the buffer components in water purified by means of Ultra CLEAR UV apparatus (SG, Hamburg, Germany). The pH was adjusted by mixing the buffer components in appropriate ratios and measured using Orion 3STAR pH meter (Orion, Boston, USA). All the buffers were filtered using a Milipore 0.45 μm filter and degassed by ultrasonication before use.

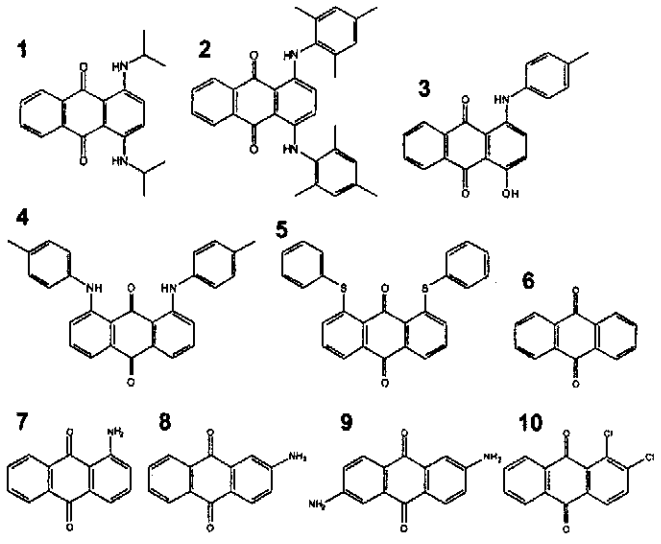


Fig. 1 Structures of anthraquinone dyes and anthraquinone dye intermediates. 1: C.I. Solvent Blue 36, 2: C.I. Solvent Blue 104, 3: C.I. Solvent Violet 13, 4: C.I. Solvent Violet 14, 5: C.I. Solvent Yellow 163, 6: anthraquinone, 7: 1-aminoanthraquinone, 8: 2-aminoanthraquinone, 9: 2,6-diaminoanthraquinone, 10: 1,2-dichloroanthraquinone

Equipment and Methods

All the MEKC experiments were performed in fused silica capillary, 47 cm × 50 mm I.D. (40 cm effective length to detector; Agilent, Palo Alto, USA) using Beckman P/ACE System 2100 capillary electrophoresis equipped with a UV detector. Mixtures of 25 mmol l^{-1} borate buffer (pH 8.5) with appropriate addition of SDS and acetonitrile were used as background electrolytes. New capillary was preconditioned before use by subsequent flushing with 0.2 mol l^{-1} sodium hydroxide (15 min), water (10 min) and with the background electrolyte (10 min). MEKC separations were performed at a potential of +15 kV applied across the capillary. The temperature of the capillary was 35 °C in all the MEKC experiments. The detector was operated at 254 nm. Thiourea (MERCK, Darmstadt, Germany) was used as the electoosmotic flow time, t_{EOF}, marker and Sudan III (Lachema, Brno, the Czech Republic) dissolved in isopropyl alcohol was used as the micelle migration time, t_{MC}, marker.

Critical micelle concentration (CMC) of SDS in background electrolyte was determined by measuring of refractive index using differential refractive detector M 401 (Waters, Milford, USA). A consecutive series of 12 solutions of SDS covering both submicelar and micellar regions (1-50 mmol l^{-1}) was prepared and the relative change of refractive index in comparison with SDS free solution was measured at 35 °C.

Results and Discussion

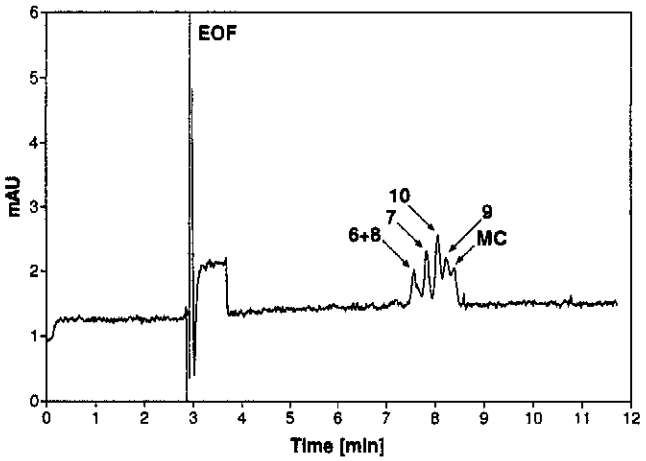
Effect of Background Electrolyte Composition on Separation of Anthraquinones

Anthraquinone dyes and anthraquinone dye intermediates used in this study (Fig. 1) are more or less hydrophobic compounds that do not contain functional groups, which could be ionized in background electrolytes commonly used for CZE separations. Moreover, the solubility of the anthraquinone dyes in water is very low. To obtain successful separation of these compounds by capillary electrophoresis, the presence of pseudostationary phase in background electrolyte is needed. Sodium dodecyl sulphate (SDS) micelles were tested as the pseudostationary phase. SDS was added to the background electrolyte in concentrations, c_{SDS}, of 30-70 mmol l^{-1}. Preliminary experiments were carried out in 25 mmol l^{-1} phosphate background electrolyte pH 8.5 with addition of 50 mmol l^{-1} SDS. In this electrolyte, migration of negatively charged micelles towards the injection end of the capillary was faster than the electroosmotic flow (EOF) and hydrophobic anthraquinones did not reach detector in reasonable time. Therefore, the background electrolyte was changed to 25 mmol l^{-1} borate pH 8.5, which has lower ionic strength and faster electroosmotic flow velocity than migration velocity of micelles. Separation of anthraquinone dye intermediates in borate background electrolyte with addition of 50 mmol l^{-1} SDS is shown in Fig. 2A. Hydrophobic anthraquinones are strongly retained in SDS micelles and only partial separation of compounds from micellar marker was accomplished. Anthraquinone (No. 6) and 2-aminoanthraquinone (No. 8) were not separated at all. Anthraquinone dyes (compounds No. 1-5) were completely retained in SDS micelles and migrate as one zone (picture not shown). Increased baseline absorbance after the migration of EOF marker can be attributed to the isopropyl alcohol zone, present in the sample as solvent of the micelle marker Sudan III.

To decrease the partition coefficients and retention factors of anthraquinones in SDS micelles and to improve the separation, the polarity of aqueous phase was decreased by addition of acetonitrile (AcN) in concentrations of 0, 5, 10 and 20 % (v/v) to the background electrolyte. Figure 2B shows separation of anthraquinone dye intermediates using 25 mmol l^{-1} borate background electrolyte pH 8.5 with addition of 50 mmol l^{-1} SDS and 20 % acetonitrile. The retention window limited by migration of EOF and SDS micelles is significantly broadened and baseline separation of all five compounds was achieved under these conditions. Other peaks eluting close to the peaks of 2,6-diaminoanthraquinone (No. 9) and 1,2-dichloranthraquinone (No. 10) can be most likely attributed to the positional isomers (as byproducts of amination or chlorination reaction) in production of anthraquinone dye intermediates.

In comparison to the anthraquinone dye intermediates, anthraquinone dyes are more hydrophobic and thus cannot be separated using SDS micelles in back-

A)



B)

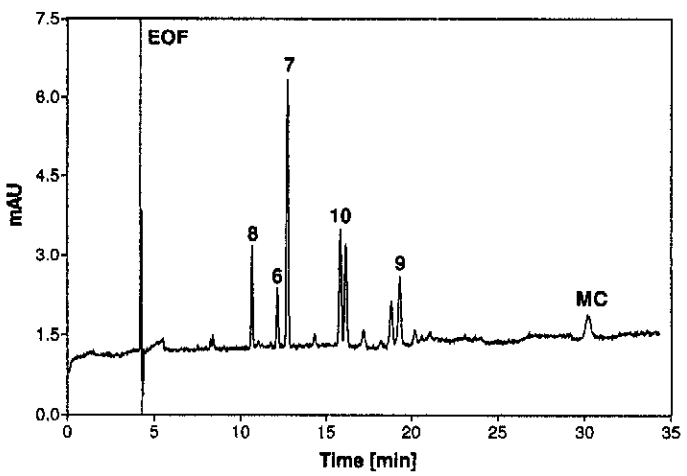
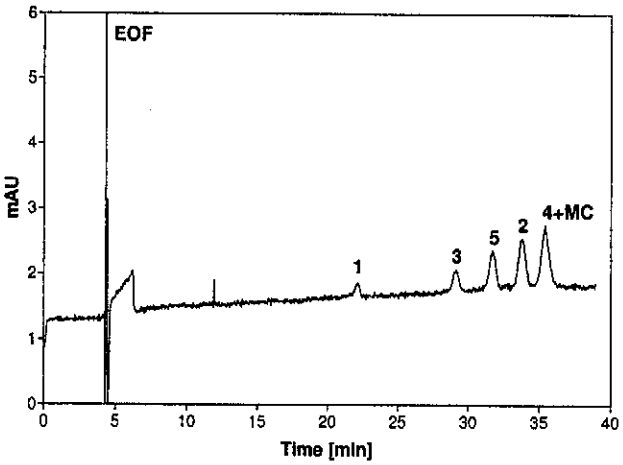


Fig. 2 Separation of anthraquinone dye intermediates in background electrolyte without acetonitrile (A) and with addition of 20 % acetonitrile (B). Compound numbers as in Fig. 1. Background electrolyte 25 mmol l^{-1} borate pH 8.5 with addition of 50 mmol l^{-1} SDS. Non-coated fused silica capillary 47 cm (40 cm effective length) × 50 mm I.D., applied voltage 15 kV, temperature 35 °C, detection UV at 254 nm. EOF - thiourea (electroosmotic flow marker), MC - Sudan III (SDS micelles marker)

ground electrolytes without organic solvent. Using 25 mmol l^{-1} borate background electrolyte pH 8.5 with addition of SDS in the range of 40-70 mmol l^{-1} and acetonitrile content lower than 20 %, the anthraquinone dyes were completely retained in micelles and did not separate from micellar marker. The best separation

of four anthraquinone dyes (No. 1,2,3,5) was achieved in background electrolyte with 50 mmol l^{-1} SDS and 20 % acetonitrile (Fig. 3A). Increasing concentration of SDS up to 70 mmol l^{-1} decreases resolution of anthraquinone dyes (Fig. 3B). The separation of Solvent Violet 14 (No. 4) from micelle marker Sudan III was not achieved in any of the experiments. The addition of AcN into the background electrolyte allows separation of both moderately polar anthraquinone dye intermediates and very hydrophobic anthraquinone dyes in a single run. The separation improves with addition of AcN; however, extremely hydrophobic compounds are retained in SDS micelles even at high AcN concentrations. The concentration of AcN in the background electrolyte cannot be increased above the certain limit given by disaggregation point of SDS micelles.

A)



B)

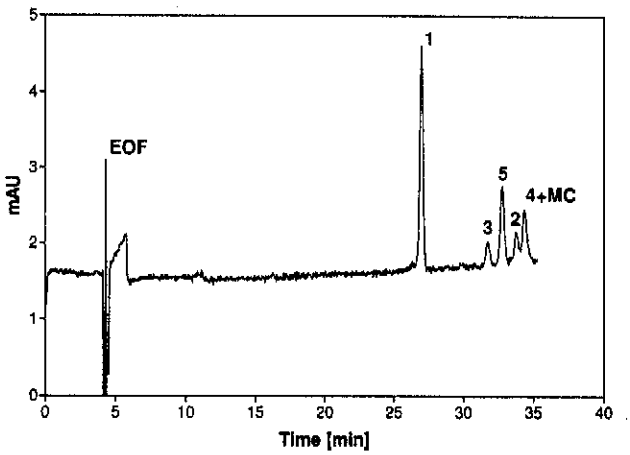


Fig. 3 Separation of anthraquinone dyes in background electrolyte containing 50 mmol l^{-1} SDS (A) and 70 mmol l^{-1} SDS (B). Background electrolyte 25 mmol l^{-1} borate pH 8.5 with addition of 20 % acetonitrile. For compounds and other separation conditions, see Fig. 2

The retention of anthraquinone dyes and intermediates in SDS micelles was characterized by mean of lipophilic and polar indices. The two-indices calibration approach, originally used for characterization of retention in reversed-phase chromatography [27-29] and adapted for application in MEKC [26,30,31], is based on the lipophilicity and polarity indices of solutes. The lipophilicity index, n_{ce}, is the hypothetical equivalent carbon atoms number of the homologous calibration series, while polar index, q_i, describes polarity of solute-micelle interactions. The lipophilicity index, usually less dependent on tested system, characterizes hydrophobicity of the solute and can be used for prediction of migration in background electrolytes with different composition and for the transfer of the separation methods between various techniques, i.e. when changing MEKC method to MEKC with reduced flow (RF-MEKC) [26]. The polar index is a measure of the polarity of solute-micelle interactions; it depends strongly on the composition of the background electrolyte. Using n_{ce} and q_i, the retention of analytes in MEKC can be described by the following equation

$$\log k = (a_0 + a_1 n_{ce})(1 + p \log c_{MC}) + q_i \log c_{MC} \quad (1)$$

where k is retention factor, c_{MC} is concentration of micelles in background electrolyte ($c_{MC} = c_{SDS} - CMC$), a_0, a_1 and p are constants of homologous calibration series compounds.

For calculation of the indices n_{ce} and q_i of the non-homologous analytes, these constants a_0, a_1 and p and constants a and m of linear dependencies of log $k = a + m.\log c_{MC}$ of analytes are used

$$n_{ce} = \frac{a - a_0}{a_1} \quad (2)$$

$$q_i = m - p(a_0 - a_1 n_{ce}) \quad (3)$$

Theoretically, the values of $q_i = 1$ and $p = 0$ are expected in MEKC if the distribution between the aqueous and micellar phases is controlled only by the hydrophobic mechanism and Eq. (1) can be simplified [26] as follows

$$\log k = a_0 + a_1 n_{ce} + q_i \log c_{MC} \quad (4)$$

This approach was applied to the characterization of retention of analysed samples using calibration homologous series of *n*-alkylbenzenes.

The method based on the measurement of refractive index of solution with different concentration of SDS was used for the exact concentration of micelles

in background electrolyte. The critical micelle concentration was determined as the crossing point of the two linear sections — below and above CMC — of the dependence of refractive index *vs.* concentration of SDS in mobile phase (Fig. 4). Measured values of CMC are shown in Table I. Parameters a and m of tested anthraquinones were determined by linear regression of dependencies log k *vs.* log c_{MC}. For illustration, retention of analytes in micellar mobile phases containing 20 % AcN and 30 to 70 mmol l^{-1} SDS are shown in Figs. 5A,B.

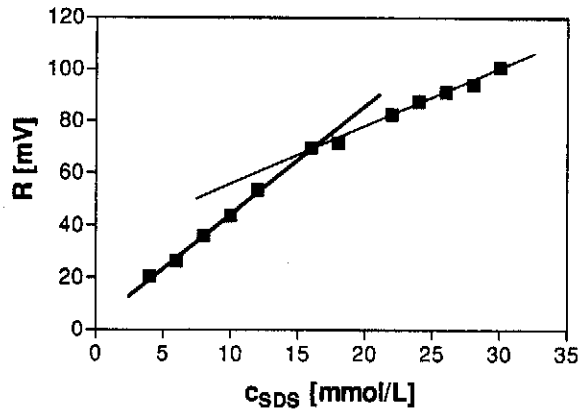


Fig. 4 Evaluation of critical micelle concentration of sodium dodecyl sulphate in 25 mmol l^{-1} borate background electrolyte pH 8.5 with addition of 5 % acetonitrile at 35 °C. R – response of refractive index detector

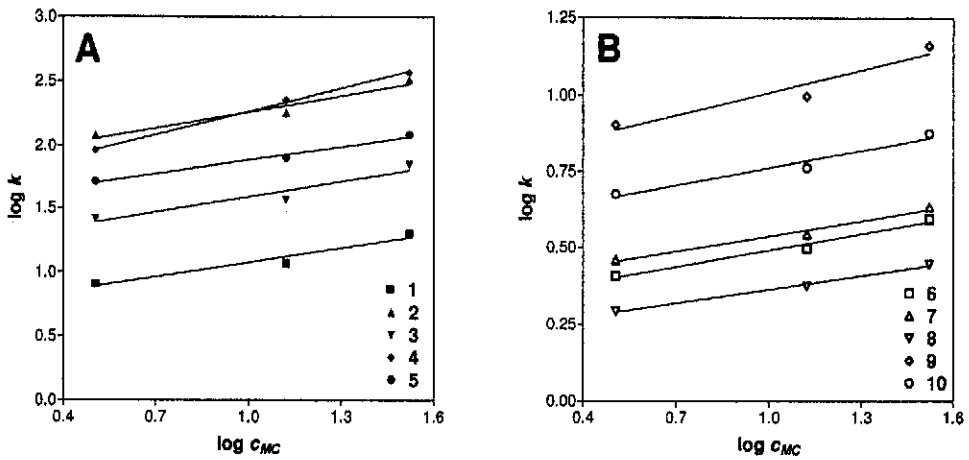


Fig. 5 Retention factors, k, of anthraquinone dye intermediates (A) and anthraquinone dyes (B) in 25 mmol l^{-1} borate background electrolyte pH 8.5 with 20 % acetonitrile and various concentrations of micelles, c_{MC}

Table I Critical micelle concentration, CMC, of sodium dodecyl sulphate in 25 mmol l^{-1} borate background electrolyte pH 8.5 with addition of acetonitrile, determined using refractive index measurement at 35 °C

Concentration of acetonitrile, % (v/v)	CMC, mmol l^{-1}
0	8.8
5	15.7
10	22.3
20	36.8

The calculated retention indices of anthraquinone dyes and intermediates are shown in Table II. With acetonitrile added into the background electrolyte, the solvophobic interactions are becoming weaker than in pure water or background electrolyte. In background electrolytes without organic modifier, the polar indices q_i are close to unity as theoretically expected and the main mechanism of separation of tested anthraquinone intermediates is based on the hydrophobic interactions with SDS micelles. Addition of the organic modifier (AcN) affects less the retention of *n*-alkylbenzene calibration standards than the tested antraquinones, so it leads to decreasing of n_{ce} values of anthraqiunones in electrolytes with AcN in comparison to those without AcN. In background micellar electrolytes with AcN, the values of polar indices q_i are significantly lower than unity which indicates possible interactions of anthraquinones with AcN in aqueous phase. The results presented in Table II suggest that the retention of tested solutes in SDS micelles can be described by Eq. (1). Further research should be focused on the selection of calibration standard series covering the whole retention range.

Table II Lipophilicity, n_{CE}, and polar indices, q_i, of antraquinone dyes and antraquinone dye intermediates in MEKC. Background electrolyte 25 mmol l^{-1} borate pH 8.5 with various concentrations of SDS (30, 40, 50 and 70 mmol l^{-1}) and acetonitrile. Linear *n*-alkylbenzenes used as standards for calibration of retention

Compound	SDS, 0 % ACN		SDS, 20 % ACN	
	n_{ce}	q_i	n_{ce}	q_i
C.I. Solvent Blue 36	-	-	4.71	0.52
C.I. Solvent Blue 104	-	-	8.05	0.64
C.I. Solvent Violet 13	-	-	5.98	0.61
C.I. Solvent Violet 14	-	-	7.75	0.75
C.I. Solvent Yellow 163	-	-	7.03	0.56

Table II – Continued

Compound	SDS, 0 % ACN		SDS, 20 % ACN	
	n_{ce}	q_i	n_{ce}	q_i
Antraquinone	5.10	0.92	3.57	0.27
1-Aminoantraquinone	5.39	1.01	3.75	0.26
2-Aminoantraquinone	4.74	0.91	3.27	0.24
2,6-Diaminoantraquinone	5.10	1.31	4.97	0.35
1,2-Dichloroantraquinone	5.10	1.31	4.36	0.29

Conclusion

Five non-polar anthraquinone dyes and five anthraquinone dye intermediates were separated by micellar electrokinetic chromatography. The best separation of anthraquinone dye intermediates was achieved using 25 mmol l^{-1} borate background electrolyte pH 8.5 with addition of 50 mmol l^{-1} SDS and 20 % acetonitrile. Optimal separation of both groups of analytes was achieved using addition of 50 mmol l^{-1} SDS and 20 % acetonitrile to the background electrolyte, where all the anthraquinone intermediates and four dyes were baseline separated. The retention of tested compounds in MEKC was characterized using the lipophilic and polar indices concept earlier used in RP-HPLC and micellar LC. The calculated indices in micellar background electrolytes without acetonitrile indicate the main role of hydrophobic interactions in separation of anthraquinone dye intermediates. The retention mechanism of very hydrophobic anthraquinone samples in micellar mobile phases is significantly affected by addition of polar organic solvent to the background electrolyte and competitive equilibria of solutes between the micelles and organic solvent should be taken in the account.

Acknowledgements

This work was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic, project No. MSM0021627502.

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