Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 800 (2004) 91–100
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Micellar electrokinetic chromatography with polyelectrolyte complexes as micellar pseudo-stationary phases

Alexey V. Shpak*, Andrey V. Pirogov, Oleg A. Shpigun

Chemistry Department, Moscow State University, GSP-2 Vorob'evy Hills, 119992 Moscow, Russia

Abstract

The separation of dansyl (DNS-AAs) and carbobenzoxy (CBZ-AAs) amino acids using micellar electrokinetic chromatography employing polyelectrolyte-surfactant complexes (PSC) formed in the reaction between polyacrylic acid (PAA) and dodecyltrimethylammonium bromide (DTAB) as pseudo-stationary phases was described. The PSCs were stabilized by hydrophobic interactions of alkyl chains of the surfactant ions and converted to an intramolecular micellar-like phase. The running buffer was a 50 mM solution of sodium phosphate (pH 6.0) containing 4.6–20.2 mM PSC, in which a part of carboxyl groups of PAA was blocked by aliphatic amines. For the systems with 7.9 mM of PAA/DTAB complex ($\varphi = 0.30$, φ-composition of water-soluble polyelectrolyte complex) as a pseudo-stationary phase, the peaks of six dansyl amino acids (DNS-AAs) were baseline resolved. The separation in this case is based on a complex distribution mechanism of the dansyl derivatives between the free buffer and the intramolecular micellar-like phase of the water-soluble PSC. On the other hand, the additives of PAA/DTAB complex ($\varphi = 0.30$) to the running buffer does not essentially affect on the electrophoretic behaviour of the CBZ-AAs, the variant MEKC is not realized. The influence of the concentration of the complex of PAA/DTAB on the electrophoretic behaviour of analytes was investigated. Relative retentions and relative selectivities were used for describing electrophoretic behaviour of the amino acid derivatives.

Keywords: Pseudo-stationary phases; Micellar electrokinetic chromatography; Polyelectrolyte complexes; Polyacrylic acid; Amino acids

1. Introduction

Micellar electrokinetic chromatography (MEKC) is one of the most widely used capillary electrophoresis modes. The separation by MEKC is based on the differential partitioning of analytes between the micelle and the surrounding aqueous phase. In the case of charged solutes, a combination of distribution between phases and electrophoretic mobility is the reason of their separation [1–3]. Conventional micelles are formed in the running buffer on adding the surfactants at concentrations higher than their critical micelle concentrations (CMC).

It is well known, that the most common surfactant employed is sodium dodecyl sulfate (SDS), which has a low CMC (8.1 mM in pure water at 25 °C) and provides good selectivity and efficiency. The CMC and aggregation number greatly depend on a number of factors, such as ionic strength, the presence of a co-solvent and temperature [4]. In general, the CMC decreases with the addition of electrolytes to the solution. The Krafft point, the temperature below which the solubility of the surfactant is lower than the CMC, is 16 °C for SDS in pure water. Hence, care must be taken not to let the temperature of the solution go below the Krafft point [5]. Migration behaviour and separation by MEKC can be easily modified by the addition of different salts, complexing agents and organic solvents (acetonitrile, methanol or isopropyl alcohol) to the separation buffer. These modifiers affect the interaction between the micelle and analytes. Hydrophobic analytes are difficult to separate because they tend to have migration times close to migration times of the micelle with very high retention factors, but adjustment of the retention factors by the addition of high amount of organic solvents disrupts micelle formation [6]. In addition, direct coupling of MEKC with conventional surfactants to mass spectrometry (MS) with the aim of selectivity enhancement in the separation step is hazardous because of the influence of the micelles in the running buffer on MS performance, resulting in a loss in sensitivity and ion source contamination [7,8].

* Corresponding author. Tel.: +7-916-638-43-88; fax: +7-095-939-25-79.
E-mail address: shpak@analyt.chem.msu.ru (A.V. Shpak).

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.10.072

The use of polymeric pseudo-stationary phases addresses many of the problems associated with conventional micelles: polymers provide very stable pseudo-stationary phases with zero CMC; the phases can be used at virtually any concentration; the primary covalent structure and concentration of the phase does not change with a change in the analytical conditions; the structures can be used in the presence of relatively high concentrations of organic modifiers; and they can be employed with mass spectrometric detection [6,9–12].

Palmer et al. [13] introduced the use of micelle polymers that are covalently bonded together and thus have a fixed primary structure. Ozaki et al. [14] reported on MEKC with two high molecular mass surfactants, butyl acrylate-butyl methacrylate-methacrylic acid copolymer sodium salt (BBMA) and butyl methacrylate-methacryloyloxyethyl-trimethylammonium chloride copolymer (BMAC). The CMC of BBMA and BMAC were found to be effectively zero. BBMA showed significantly different selectivity for naphthalene derivatives in comparison with SDS [15]. The introduction and characterization of polymeric pseudo-phases has been reviewed in detail [6,11,12,16,17].

The application of the mixture of a tri-block copolymer, poly(methyl methacrylate-ethyl acrylate-methacrylic acid), commercially known as Elvacite 2669, and SDS as a pseudo-stationary phase was reported by Leonard and Khaledi [18].

Zakaria et al. [19] reported on the use of a mixture of a soluble cationic polymer poly(diallydimethylammonium chloride) (PDADMA) and a amphiphilic anion (hexanesulfonate) as a pseudo-stationary phase. A migration model was derived that takes into account the ion-exchange interactions between the anions and PDADMA as well as ion-pair interactions between the samples (opiates) and the hexanesulfonate.

Recently, we have reported the use of polyelectrolyte-surfactant complexes (PSC) formed in the reaction between polyacrylic acid (PAA) and dodecyltrimethylammonium bromide (DTAB) as pseudo-stationary phases. PSCs are formed as a result of the ion-exchange reaction between surfactant ions and ionized units of a macromolecule. These compounds are stabilized by hydrophobic interactions of alkyl chains of surfactant ions and could be considered as a special class of surface-active polyelectrolytes [20]. The theoretical examination of polyelectrolyte-surfactant interactions shows that the necessary condition for the formation of PSC is a generation of an intramolecular micellar phase. It is known that surfactant ions in PSCs produce an intramolecular micellar phase and the morphology of such micelles is significantly different from that of corresponding “typical” surfactant micelles [21]. The free part of the polyion is responsible for the solubility of these compounds in water [22,23]. In a previous paper [24], we have demonstrated the separation of uncharged substituted phenols and dansyl aminoacids with polyelectrolyte-surfactant complexes PAA-DTAB as a buffer additive. PSC provided very different selectivity in comparison to SDS micelles for phenols.

This paper, the effect of the PSC of PAA/DTAB ($\varphi = 0.30$) on the separation of mixtures of CBZ and DNS-amino acids in these systems was investigated.

2. Experimental

2.1. Instrumentation

All the experiments were carried out on a HP^{3D} CE capillary electrophoresis system (Agilent, Waldbronn, Germany) equipped with a diode-array detector (DAD). Agilent Technologies (Waldbronn, Germany) standard fused silica capillary, 80.5 cm × 50 μm i.d., with a detection window at 72 cm and Polymicro Technologies (Phoenix, AZ, USA) fused silica capillary with an effective length of 60 cm, an inner diameter of 50 μm and a detection window at a position of 8.5 cm from the end were used throughout. Injection was performed by applying a 50 mbar pressure for 3 s to the anodic side of the capillary. Separations were performed in a positive polarity mode. All the separations were performed at +25 kV, except where otherwise indicated. The capillaries were thermostated at 25 ± 0.1 °C. Peaks were registered at 210, 230, 254 and 270 nm simultaneously and the electropherograms shown in this paper were recorded at 210 and 254 nm. An Agilent CE ChemStation was used for the system control, data acquisition and postrun processing.

2.2. Reagents and materials

A fraction of polyacrylic acid (PAA) with average mass degree of polymerisation of $P_W = 1800$ was taken from the Division of High Molecular Compounds of Moscow State University as a 30% (w/v) aqueous solution and used without further purification. Dodecyltrimethylammonium bromide (DTAB), tetrabutylammonium bromide (TBAB) and Sudan III were obtained from Merck (Darmstadt, Germany). Standard dansyl (DNS-AAs) and carbobenzoxy (CBZ-AAs) amino acids, sodium dihydrogenphosphate, sodium hydroxide, hydrochloric acid, 2-naphthol and phenanthrene of high purity were obtained from Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), and Aldrich (Milwaukee, WI, USA). Acetonitrile, acetone, methanol were spectral and HPLC grade and used without further purification. All the solutes were prepared using a water for HPCE (Agilent Technologies, Waldbronn, Germany) and the water was purified using a Milli-Q Elix Millipore System (Milford, MA, USA). A sodium phosphate buffer 50 mM pH 7.0 was obtained from Agilent (Agilent Technologies, Waldbronn, German). All the used solutions were filtered through a 0.45 μm membrane filter prior to use. Acetone and methanol were served as markers of the electroosmotic flow. Sudan III, 2-naphthol, 2-naphthalenemethanol and phenanthrene were used as tracers of the micelles.

2.3. Preparation of water-soluble polyelectrolyte-surfactant complexes

Polyelectrolyte complexes formed in the reaction between salts of polycarboxylic acids and alkyltrimethylammonium bromides represent compounds stabilised by a co-operative system of salt bond and hydrophobic interactions. The reaction mixture composition Z is determined as a ratio of the surfactant molar concentration to the molar concentration of polyanion units in the solution:

$$Z = \frac{[\text{surf}]}{[\text{polymer}]}$$

A composition of a water-soluble polyelectrolyte complex (φ) is determined as a ratio of the number of surfactant ions to the number of polyacrylic acid monomer units in the complex particle:

$$\varphi = \frac{n(\text{surf})}{n(\text{polymer})}$$

Depending on the conditions (pH, ionic strength, chemical nature of the components), the yielding polyelectrolyte-surfactant complexes are either soluble or insoluble in water. The possibility of the formation of water-soluble nonstoichiometric complexes is defined by the degree of the polyelectrolyte polymerization and the length of the surfactant ion alkyl chain, with other parameters being equal [25].

A 300 mM stock solutions of polyacrylic acids were prepared by dissolving their appropriate amounts in water followed with adjustion of pH to 6.0 by a 3 M NaOH solution. Polyelectrolyte-surfactant complexes were obtained by the addition of the corresponding amount of DTAB to the stock solution of PAA under stirring. The reaction mixture becomes opaque after the addition of the DTAB solution but in 1–2 min becomes transparent again. Apparently, the interaction of a polyacrylic acid with a DTAB solution leads to the formation of local zones of an insoluble complex. If Z is in the range from 0 to Z_{lim} (for our conditions Z_{lim} was about 0.35) than the precipitation

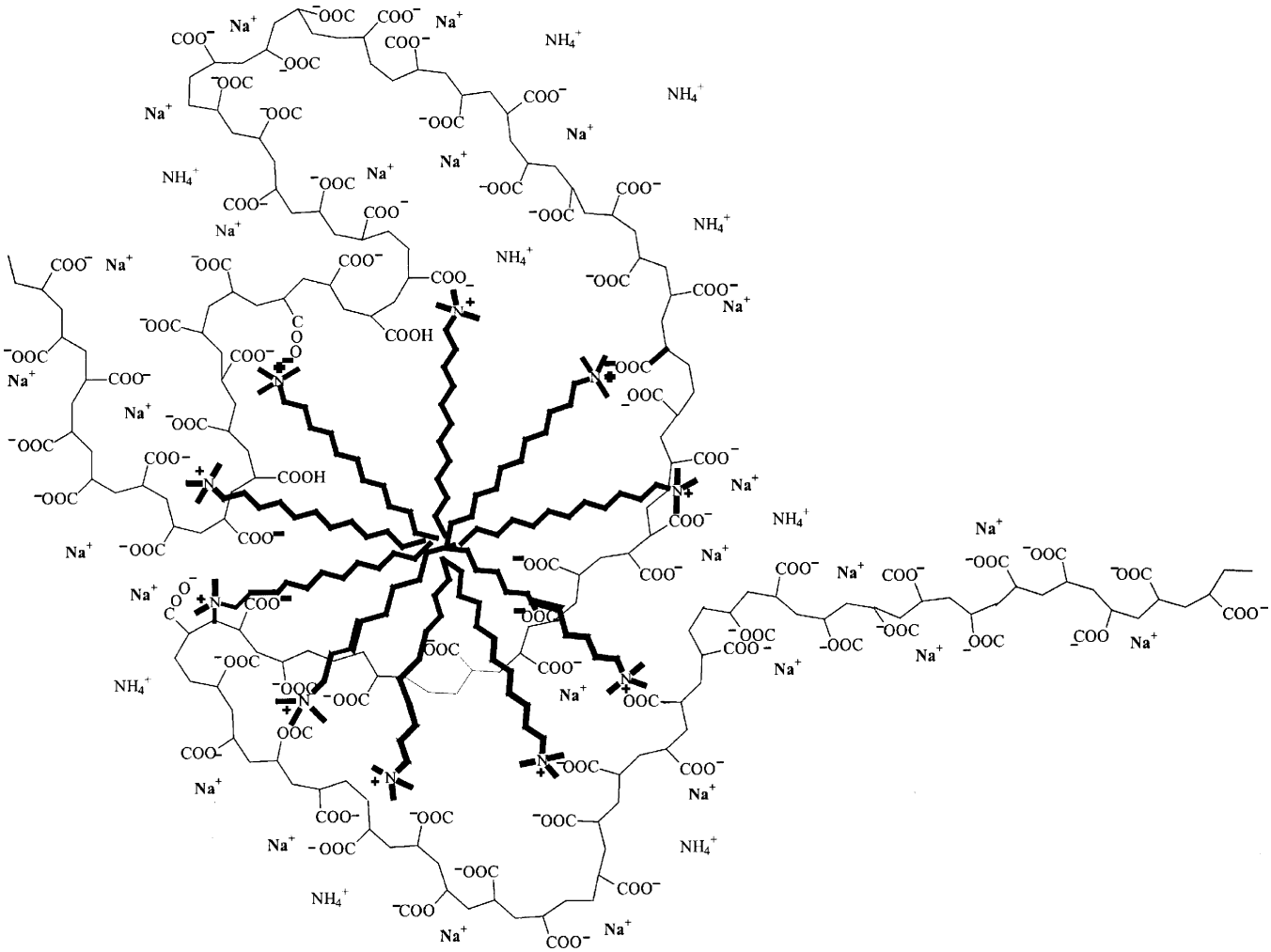

Fig. 1. Scheme of water-soluble polyelectrolyte complex between polyacrylic acid and alkyltrimethylammonium salts. The aggregation number does not represent reality.

does not take place and the system remains externally homogeneous. A schematic structrure of a PAA-DTAB complex is presented in Fig. 1. The produced complexes were kept in darkness at room temperature for a day before use.

A precipitation of the water-insoluble polyelectrolyte complex takes place on adding a solution of DTAB to a solution of PAA when Z exceeds Z_{lim}. For this reason, the complexes with Z above 0.35 were not used further in the work as a pseudo-stationary phase in MEKC.

2.4. Sample and buffer preparation

Initially, the capillary was flushed with a 1 M NaOH for 5 min and, next with deionized water for 5 min. When changing buffers, the capillary was firstly rinsed with a 50 mM phosphate buffer (pH 6.0) without any PSC for 7 min, next with a new buffer with PSC for 5 min. Between sample injections, the capillary was rinsed with a running buffer for 4 min.

The pH of the buffers was adjusted by a titration of a commercial available 50 mM sodium phosphate buffer pH 7.0 (Agilent Technologies, Waldbronn, Germany) with a 3 M solution of hydrochloric acid.

Structures of seven dansyl and nine carbobenzoxy amino acids used in this study are shown in Fig. 2. Standard stock solutions of the analytes were prepared in water–methanol media (40% (v/v) of methanol) at a concentration range from 0.4 to 2.0 mg/ml. A sample stock solution containing all the dansyl or all the carbobenzoxy amino acids (30 μg/ml of each compound) was prepared from individual standards by diluting with water.

3. Results and discussion

When cationic surfactants such as DTAB are added to a background electrolyte, the capillary surface becomes coated with some amount of the surfactant. This phenomenon results in a recharge of the capillary surface from negative to positive and in an opposite direction of the EOF. Thus, cationic surfactants included in a PSC could be adsorbed by capillary walls. But the data obtained show that this does not occur or occurs insignificantly. The EOF decreases by 5% (from 7.00 ± 0.06 to 7.37 ± 0.09 min of the migration times, $n = 8$), in our opinion, which could be caused by an increase in the viscosity of the buffer solution. If PAA alone, without cationic surfactants is added to the background electrolyte, the EOF decreases by about 5% also. Any evidences that the complex or its components are adsorbing by capillary walls were not found.

It should be noted that the interaction of linear polyelectrolytes with oppositely charged surfactant ions proceeds as a cooperative binding at the surfactant concentrations that are two to three orders lower than the CMC of the surfactant. Nevertheless, the cationic surfactant (DTAB) included in the polyelectrolyte-surfactant complex does not adsorb by capillary walls.

3.1. Separation of dansyl amino acids

Seven dansyl amino acids (Fig. 2) were used as test components: dansyl threonine (DNS-Thr), dansyl serine (DNS-Ser), dansyl valine (DNS-Val), dansyl methionine (DNS-Met), dansyl leucine (DNS-Leu), dansyl norleucine (DNS-NorLeu), and dansyl phenylalanine (DNS-Phe). Considering pK_a values of the carboxyl group of the amino acids (which are between 2.2 and 2.6), amino acid derivatives are negatively charged under the applied conditions (pH 6.0). This also becomes apparent from the electropherogram shown in Fig. 3A obtained with a 50 mM phosphate buffer (pH 6.0) without a PSC additive. All the seven DNS-AAs comigrate practically together against the electroosmotic flow (EOF) and give a group of poorly resolved peaks. A similar picture was observed when we used a mixture of a 50 mM solution of sodium phosphate and a 8 mM polyacrylic acid as a running buffer (pH 6.0). Based on this phenomenon, it is possible to assume that at the selected experimental conditions PAA does not form micelle-like aggregates shared in the buffer solution. An insignificant difference in the migration times is caused by own mobilities of the derivatized amino acids. When a polyelectrolyte-surfactant complexe of PAA-DTAB is added to the running buffer, negatively charged PSCs migrate under the effect of the electrical field against the EOF towards the anode (the inject end). As seen in Fig. 3B, this polyelectrolyte-surfactant complex, contains an intramolecular micellar phase and leads to a retardation of the DNS-AAs, which then migrate at a lower velocity. In this case the separation results from various distribution of dansyl derivatives between the free buffer and an intramolecular micellar-like phase of water-soluble PSCs. The migration times of the DNS-AAs is incerased at the increase of a concentration of the PSC in a running buffer (Fig. 3C). In this case the MEKC mode is realized.

The order of migration of the DNS-AAs was following: DNS-Thr < DNS-Ser < DNS-Val < DNS-Met < DNS-Leu < DNS-NorLeu. In comparison with a SDS micelles the used PSC had the same selectivity for the dansyl amino acids. Even for the systems with a 5.0 mM of PAA/DTAB complex ($\varphi = 0.30$) as a pseudo-stationary phase, the peaks of the six DNS-AAs are well resolved. But it should be noted that the peak of DNS-Phe was not observed when PSC was used as a pseudo-stationary phase. Apparently, very hydrophobic dansyl phenylalanine could be incorporated into PSC micelles. In this case, dansyl phenylalanine could act as a tracer of the micelles. But we did not obtain a peak that corresponds to the migration time of the pseudo-stationary phase. On the other hand, the dansyl phenylalanine is not probable incorporated into the PSC micelles due to its size and hydrophobity.

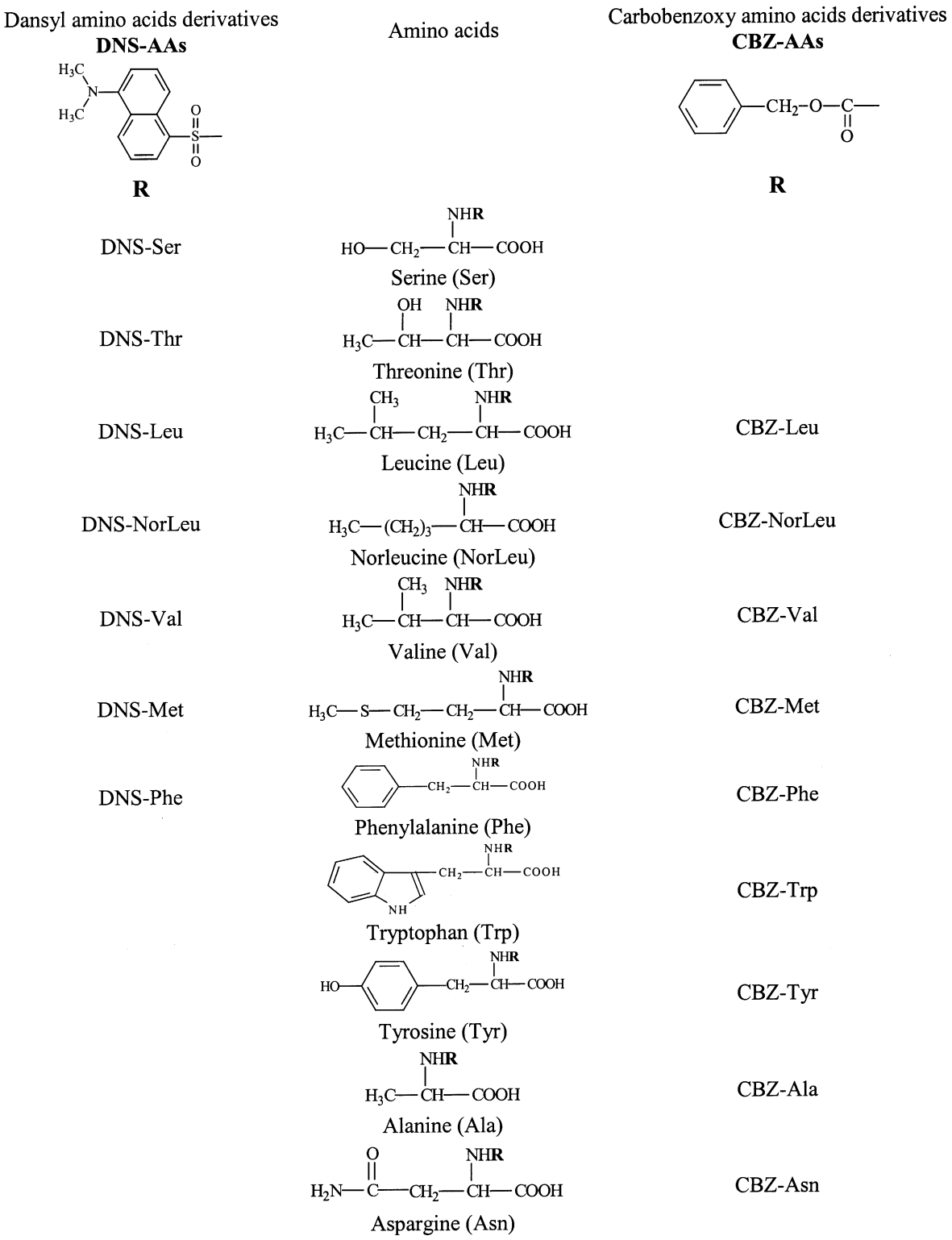

Fig. 2. Structures of the dansyl and carbobenzoxy amino acids analytes.

3.2. Separation of carbobenzoxy amino acids

The separation of nine CBZ amino acids (Fig. 2) was performed in the same separation buffers as for the dansyl amino acids, but the complexes pseudo-stationary phase concentration range was 0–15.8 mM. CBZ amino acid derivatives are negatively charged under the applied conditions. A separation of the amino acids with two different 50 mM phosphate buffers (pH 6.0), with and without additives a 7.9 of mM PAA/DTAB ($\varphi = 0.3$) is shown in Fig. 4. When a 50 mM

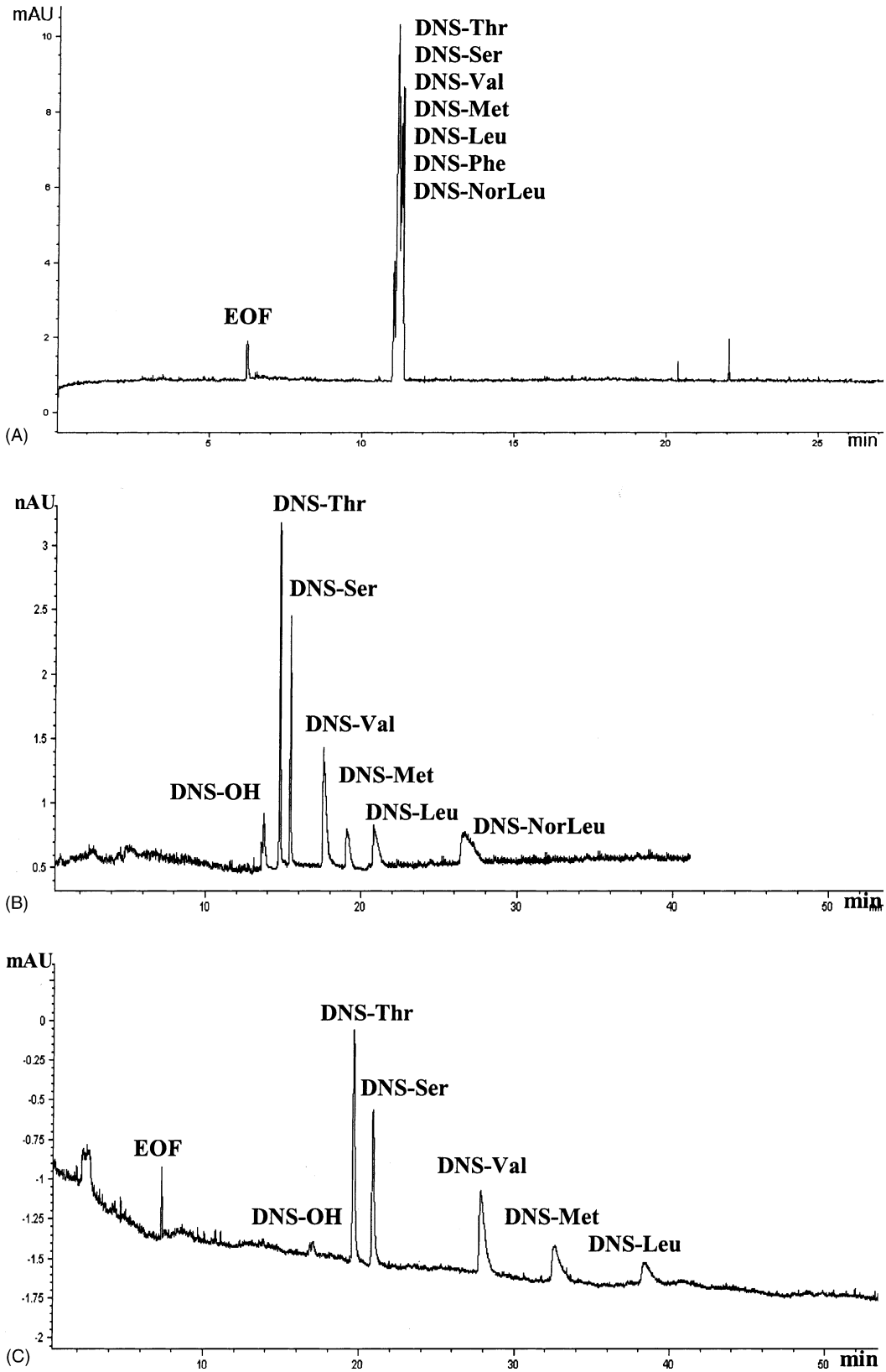

Fig. 3. Separations of DNS amino acids. Buffer: (A) a 50 mM phosphate buffer pH 6.0; (B) a 50 mM phosphate buffer, 7.9 mM of PAA/DTAB ($\varphi = 0.3$) pH 6.0; (C) a 50 mM phosphate buffer, 15.8 mM of PAA/DTAB ($\varphi = 0.3$) pH 6.0. Capillary: 80.5 cm (72 cm to the detector) × 50 μm i.d.; voltage: 30 kV; injection: 150 mbar s; detection 254 nm; current: 64 μA.

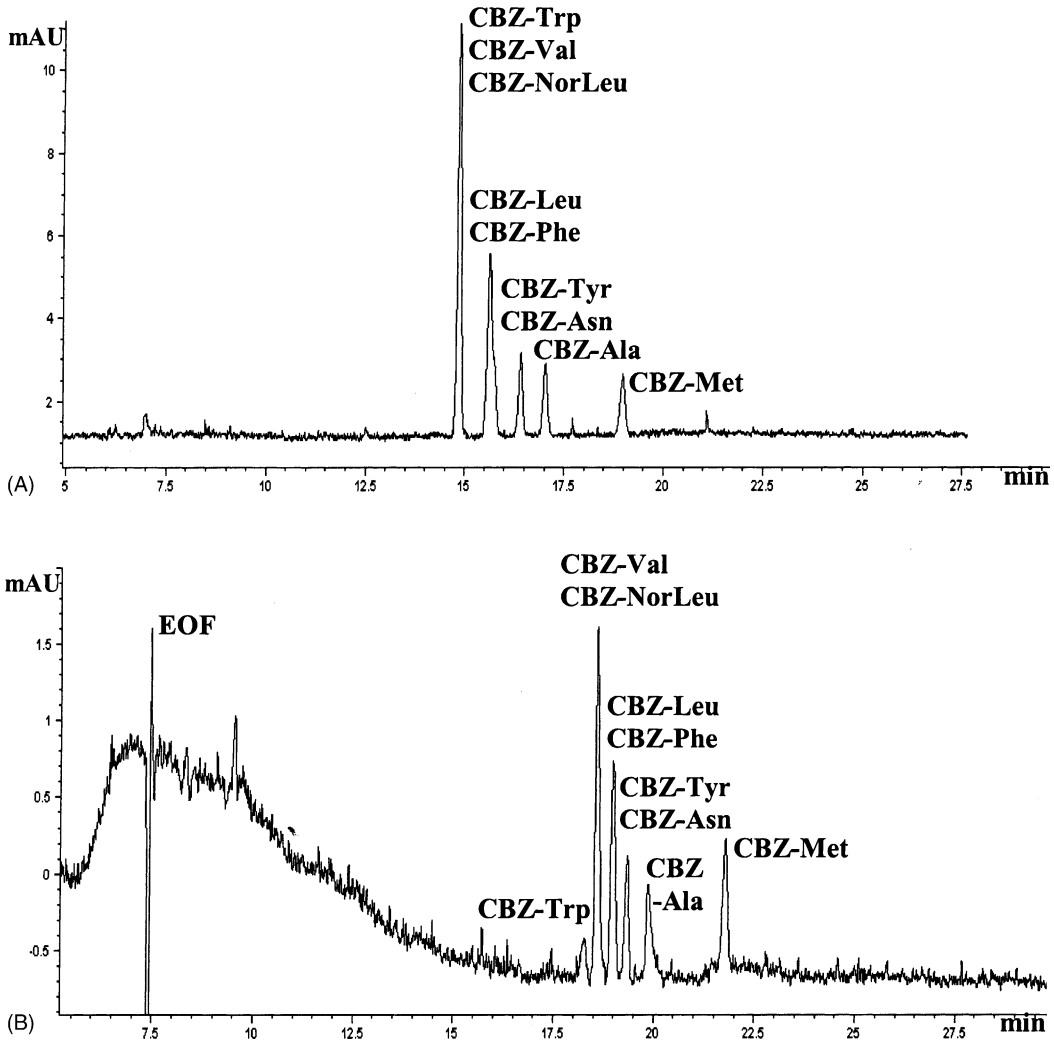

Fig. 4. Separations of CBZ amino acids. Buffer: (A) a 50 mM phosphate buffer pH 6.0; (B) a 50 mM phosphate buffer, 7.9 mM PAA/DTAB ($\varphi = 0.3$) pH 6.0. Capillary: 80.5 cm (72 cm to the detector) × 50 μm i.d.; voltage: 30 kV; injection: 150 mbar s; detection 210 nm; current: 62 μA.

sodium phosphate solution without the additives of PSC, was used as a buffer, nine derivatives of amino acids are detected as five peaks, several derivatives migrate as single zone, and times of migration are close to each other, from 14.9 to 18.9 min (Fig. 4A). Separation is based on differences in the migration velocities of CBZ ions, which is a combination of electrophoretic and electroosmotic migrations. The capillary zone electrophoresis (CZE) mode is realized. Due to the contribution of a neutral fragment, practically identical to all the analytes, the electrophoretic mobility of derivatives is very similar and, thus, their good separation is impossible.

When a polyelectrolyte complex of PAA/DTAB was used as an additive to the buffer in the concentration range from 4.6 to 15.8 mM, a similar picture was observed. The elution order of the analytes with PSC pseudo-stationary phases is CBZ-Trp < CBZ-Val = CBZ-NorLeu < CBZ-Leu = CBZ-Phe < CBZ-Tyr = CBZ-Asn < CBZ-Ala < CBZ-Met. The same elution order was also observed in the case of 50 mM of phosphate (pH 6.0) as a running buffer: CBZ-Trp = CBZ-Val = CBZ-NorLeu < CBZ-Leu = CBZ-Phe < CBZ-Tyr = CBZ-Asn < CBZ-Ala < CBZ-Met, except that CBZ-Trp eluted with the CBZ-Val and CBZ-NorLeu.

The migration time of CBZ-AAs increases insignificantly with an increase in PAA/DTAB concentration. In our opinion this is caused by an increase in the viscosity of the buffer solution, the EOF decreases cent from 7.00 ± 0.06 to 7.37 ± 0.09 min, $n = 8$ (Fig. 4). If polyacrylic acid alone, without cationic surfactants, is added to the background electrolyte, then migration times of CBZ derivatives increase also. The results indicate that the additives of pseudo-stationary of PAA/DTAB to the running buffer does not affect the electrophoretic behaviour of CBZ amino acids.

3.3. Effect of the concentration of polyelectrolyte-surfactant complex

The separation mechanism of neutral solutes in MEKC is essentially chromatographic and usually described using modified chromatographic relationships. The ratio of

the total moles of solute in the micelle (i.e., the pseudo-stationary phase) to those in the mobile phase, the capacity factor, k', is given by

$$k' = \frac{t_r - t_o}{t_o(1 - t_r)/t_m}$$

where t_r is the retention time of the solute, t_o and t_m are the migration times of a compound which does not interact with the pseudo-stationary phase, and the migration time of the micelle, respectively. The selectivity coefficient, α, defined as a ratio of capacity factors, k', would serve as the most appropriate parameter to express the separation selectivity. The calculation of the capacity factor requires the measurement of the migration velocity of the solutes, of the bulk solution (EOF), and of the pseudo-stationary phase. Thus, the micelle time is necessary for the calculation of α. However, the determination of t_m is uncertain in some cases; for example, if the buffer contains cyclodextrins [26].

The measurement of the migration time of the polyelectrolyte-surfactant pseudo-phase is a very intricate procedure. Sudan III, 2-naphthol, 2-naphthalenemethanol and phenanthrene were tested as micellar markers of different concentration of PAA/DTAB. In all the cases, we did not obtain a peak that corresponds to the migration time of the micelle for PSC. It was found that the additives in the running buffer of 10% (v/v) of acetonitrile provided the determination migration time of the pseudo-stationary phase t_m without any micellar marker (Fig. 5). We assumed that this peak can be attributed to migration time of the pseudo-phase. The relative standard deviation (R.S.D. = 0.93%, $n = 5$) values of the migration time of PSCs were obtained from five replicate analyses of the DNS-AAs analytes. We did not use the data of t_m for systems that did not contain an organic modifier. A pseudo-stationary phase and, hence, the time of its migration in such a case can be essentially different.

Except for the case mentioned above, dansyl amino acids are negatively charged under the selected conditions (pH 6.0) and a combination of the distribution between phases and electrophoretic mobility is the reason of their separation.

In order to solve these problems, we used other parameters to describe the selectivity of the separation at least qualitatively, namely retention factors or relative retentions p'; and relative selectivities α'. For the calculation of p' and α', migration velocities of solutes and DNS-Thr (CBZ-Val for the CBZ derivatives) were taken as references:

$$p' = \frac{t_r - t_o}{t_o}, \qquad \alpha' = \frac{t_r - t_o}{t_{\text{DNS-Thr}} - t_o}$$

The effect of the concentration of the complex of PAA/DTAB in the range from 4.6 to 20.2 mM on the electrophoretic behaviour of analytes was investigated. The use of pseudo-stationary phase concentrations higher than 20 mM, results in an increase in the generated current and the time of the analysis. Except for that the high concentration of PSC in the running buffer leads to a local change φ and, as consequence a formation of a zone of a water-insoluble complex. On the other hand, the addition of the complex with a concentration less than 2 mM almost does not affect the separation.

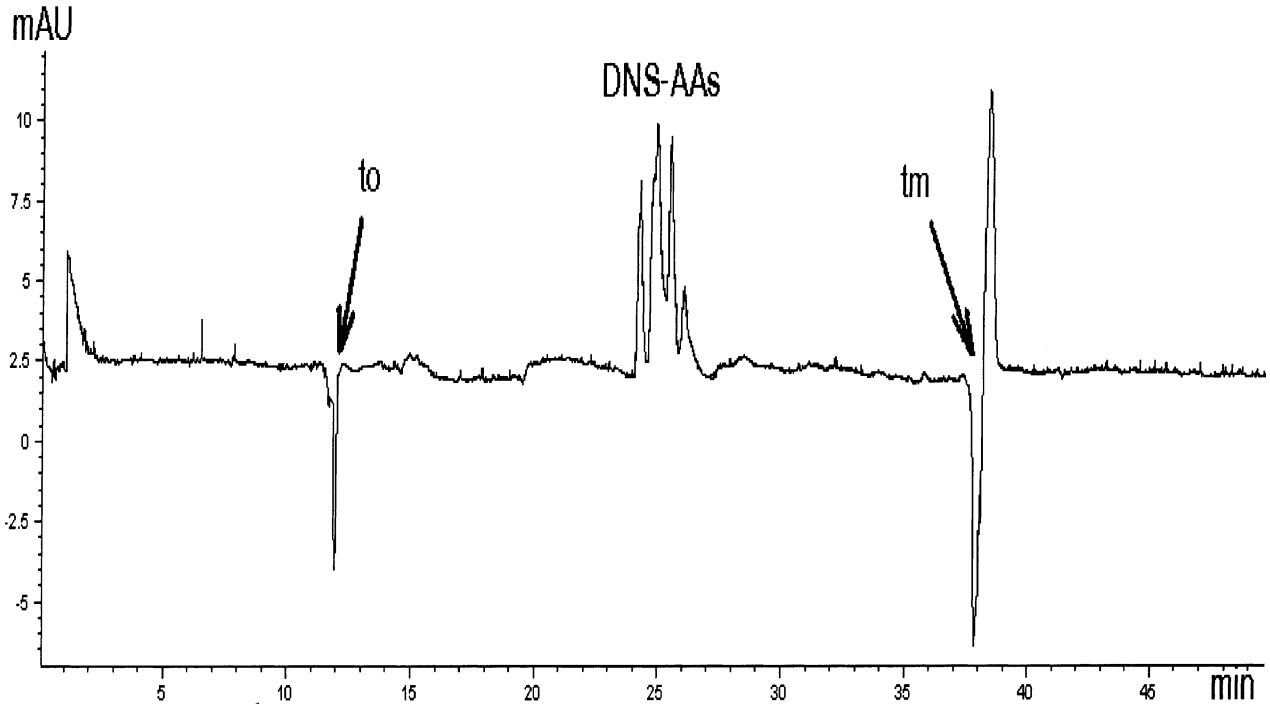

Fig. 5. Effect of the additing of 10% (v/v) of acetonitrile to the running buffer. Buffer: a 50 mM phosphate buffer, 8.7 mM PAA/DTAB ($\varphi = 0.3$), 10% (v/v) of acetonitrile pH 6.0. Capillary: 80.5 cm (72 cm to the detector) × 50 μm i.d.; voltage: 20 kV; injection: 100 mbar s; detection 210 nm; current: 25 μA.

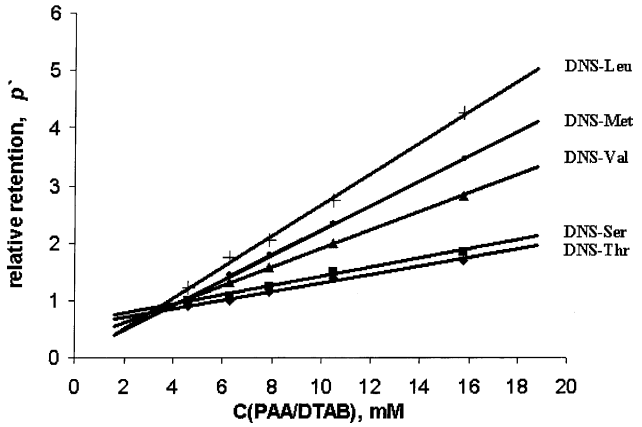

Fig. 6. Influence of the polyelectrolyte complex PAA/DTAB ($\varphi = 0.3$) concentration on the relative retention of dansyl amino acids. Buffer: a 50 mM phosphate buffer and PAA/DTAB. Other conditions as in Fig. 3.

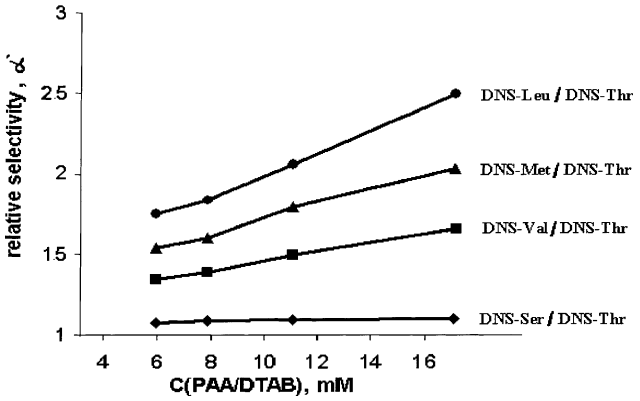

Fig. 7. Selectivity of the separation (α') of DNS-Thr and others dansyl derivatives as a function of the concentration of polyelectrolyte complex of PAA-DTAB ($\varphi = 0.3$). Buffer: a 50 mM phosphate buffer and PAA/DTAB. Other conditions as in Fig. 3.

3.4. Dansyl amino acids

Fig. 3B and C shows the influence of the concentration of PSC on the migration time and the separation of dansyl amino acids. As seen from Fig. 3B and C all the sample components are baseline separated. Fig. 6 shows the dependence of the relative retention of the dansyl derivatives on the concentration of PAA/DTAB. A good linear relationship was obtained in the range of 4.6–20.2 mM for each amino acids (correlation coefficient range: 0.995–0.999).

The relative retention increases significantly for DNS-AAs while the pseudo-stationary phase concentration is increased. As expected, the retardation becomes stronger with an increase in the concentration of PAA/DTAB. All the curves for dansyl amino acids are crossed in a point with a concentration of a PSC about 3.2 mM. Hence, adding a complex pseudo-stationary phase to the buffer at a level of 3 mM or less do not affect the separation of the derivatives. This assumption was confirmed experimentally. A mixture of derivatized amino acids was tested with a 1 mM of a polyelectrolyte complex of PAA/DTAB ($\varphi = 0.3$) in a 50 mM phosphate running buffer, pH 6.0. The same picture was observed in the case of a solution of 50 mM of phosphate (pH 6.0) as a buffer.

The relative retention of the all dansyl derivatives for a buffer without the adding a PSC pseudo-stationary phase is equal and is about 0.9. From Fig. 6 one can see that relative retention for DNS-AAs in the point of crossing of all the curves at 3.2 mM is 0.85. Hence, the contribution of own mobilities of analytes to relative retentions makes about 0.85. It is not clear why curves are crossed in the point with the concentration of the complex of 3.2 mM, and not in the origin of coordinates. The interaction of linear polyelectrolytes with oppositely charged surfactant ions proceeds as a cooperative binding at surfactant concentrations one to three orders lower than CMC. In theory, the "critical micelle concentration" of the intramolecular micellar-like phase is nearly zero.

The relative retention (used instead of the capacity factor) increases linearly with the concentration. Hence, the separation mechanism of dansyl amino acids is essentially chromatographic. Fig. 7 shows the selectivity of the separation of DNS-Thr and others dansyl derivatives. The selectivity increases with an increase in concentration of the complex pseudo-stationary phase. But with an increase in the concentration of the complex, the efficiency of the separation decreased, and the most important, the analysis time essentially grows. It has been established, that the optimum conditions of the separation are achieved for buffer solutions containing 6 mM of the complex PAA/DTAB ($\varphi = 0.3$) in a 50 mM sodium phosphate (pH 6.0) as a running buffer. Six dansyl amino acids were separated in less than 24 min.

In was observed that the selectivity separation of DNS-Thr and DNS-Ser did not change with a change in the concentration of the PAA/DTAB. Treonine (Thr) and serine (Ser) amino acids contain hydroxyl groups in their molecules and, therefore, are less hydrophobic then other tested amino acids. Less hydrophobic compounds interact less strongly with a intramolecular micellar phase and migrate faster. The efficiency and selectivity of the separation was good, although some peak tailing was observed. The observed plate numbers, N, are about 40,000 m^{-1} for DNS-Val, DNS-Met, DNS-Leu and DNS-NorLeu. For DNS-Thr and DNS-Ser, even higher theoretical plate numbers (300,000 m^{-1}) we achieved. But the actual reason for a significant difference in the efficiency and electrophoretic behavior for such big molecules in the presence of hydroxyl groups still is not clear.

3.5. CBZ amino acids

The dependence of the relative retention, p', of CBZ amino acids VS the concentration of the polyelectrolyte complex of PAA/DTAB ($\varphi = 0.3$) was also to be investigated with an aim to study the interaction of a pseudo-stationary phase and analytes (Fig. 8.). Five CBZ amino acids completely

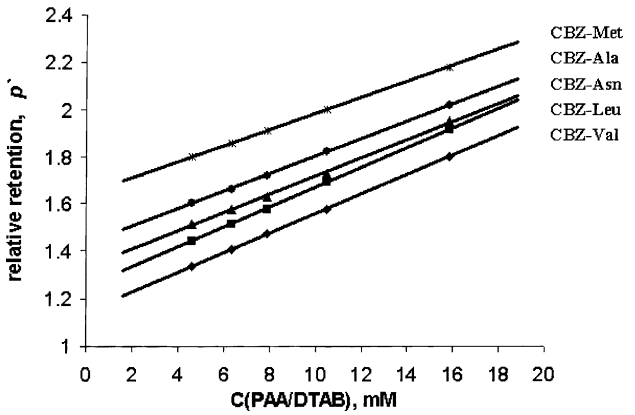

Fig. 8. Influence of the polyelectrolyte complex PAA/DTAB ($\varphi = 0.3$) concentration on the relative retention of CBZ amino acids. Buffer: a 50 mM phosphate buffer and PAA/DTAB. Other conditions as in Fig. 4.

separated in a variant of capillary zone electrophoresis (separation is caused by different own mobilities of derivatized amino acids) were used as test components: CBZ-Val, CBZ-Leu, CBZ-Asn, CBZ-Ala and CBZ-Met.

Fig. 4B shows that the addition of PAA/DTAB to the background electrolyte seems to influence poorly the selectivity at all concentrations of PAA/DTAB.

The relative retention increase insignificantly for CBZ-AAs with an increase in the pseudo-stationary phase concentration. In was found that the relative selectivity separation of CBZ amino remains constant with a change in the concentration of the PAA/DTAB. The results indicate that adding pseudo-stationary PAA/DTAB to the running buffer does not affect the electrophoretic behaviour of CBZ amino acids.

4. Conclusions

Polyelectrolyte-surfactant complexes of polycarboxylic acids and alkyltrimethylammonium salts look very promising as a new type of pseudo-stationary phases in MEKC. PSC produce an intramolecular micellar phase and the morphology of such micelles is significantly different from that of the corresponding surfactant "typical" micelles. Pseudo-stationary phases on the basis of polyelectrolyte-surfactant complexes show unique selectivity to various derivatives of amino acids. The peaks of DNS-AAs are baseline resolved, hence, differential partitioning of dansyl amino acids derivatives between micellar-like aggregates of PSC and the surrounding aqueous phase. To the contrary, adding pseudo-stationary PAA/DTAB to the running buffer does not affect the electrophoretic behaviour of CBZ amino acids.

A very promising property of the PSC pseudo-phases is that the nature and the structure of micellar-like units could be easy modified by changing the degree of polyelectrolyte polymerization, molecular weight distribution of the used polyacid, and the length of the surfactant ion alkyl chains. Also, pseudo-stationary phases based on PSCs could be used in the presence of relatively high amounts of organic modifies for the separation of very hydrophobic analytes.

The zero CMC, high electrophoretic mobility, low surface activity, suitable performance at low concentrations, and the absence of signal in the mass region (for direct coupling with a mass spectrometric detector) make these phases very promising.

Acknowledgements

This work was made under the financial support of the Russian Foundation of Basic Research, grant No. 01-03-32202. We are grateful to Division of High Molecular Compounds of Moscow State University and personally to Dr. Yu.A. Zakharova for providing samples of the polyacrylic acids.

References

[1] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111.
[2] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[3] J.P. Qurino, S. Terabe, J. Chromatogr. A 856 (1999) 465.
[4] M.G. Khaledi, J. Chromatogr. A 780 (1997) 3.
[5] P.G. Muijselaar, K. Otsuka, S. Terabe, J. Chromatogr. A 780 (1997) 41.
[6] C.P. Palmer, N. Tanaka, J. Chromatogr. A 792 (1997) 105.
[7] J. Varghese, R.B. Cole, J. Chromatogr. A 652 (1993) 369.
[8] M.N. Lamoree, U.R. Tjaden, J. van der Greef, J. Chromatogr. A 712 (1995) 219.
[9] C.P. Palmer, J. Chromatogr. A 780 (1997) 75.
[10] H. Ozaki, N. Itou, S. Terabe, Y. Takada, M. Sakairi, H. Koizumi, J. Chromatogr. A 716 (1995) 69.
[11] B. Maichel, E. Kenndler, Electrophoresis 21 (2000) 3160.
[12] S.A. Shamsi, C.P. Palmer, I.M. Warner, Anal. Chem. 73 (2001) 140a.
[13] C.P. Palmer, M.Y. Khaled, H.M. McNair, J. High Resolut. Chromatogr. 15 (1992) 756.
[14] H. Ozaki, A. Ichihara, S. Terabe, J. Chromatogr. A 709 (1995) 3.
[15] H. Ozaki, S. Terabe, A. Ichihara, J. Chromatogr. A 680 (1994) 117.
[16] C.P. Palmer, Electrophoresis 23 (2002) 3993.
[17] H.H. Yarabe, E. Billiot, I.M. Warner, J. Chromatogr. A 875 (2000) 179.
[18] M.S. Leonard, M.G. Khaledi, Electrophoresis 25 (2002) 1019.
[19] P. Zakaria, M. Macka, J.S. Fritz, P.R. Haddad, Electrophoresis 23 (2002) 2821.
[20] E.D. Goddard, Colloid Surf. 19 (1986) 301.
[21] Yu.A. Zakharova, A.D. Kolbanovskiy, L.A. Krinitskaya, V.A. Kasaikin, A.M. Wasserman, Polym. Sci. Ser. B 37 (1995) 439.
[22] V.A. Kasaikin, V.A. Efremov, Y.A. Zakharova, A.V. Zezin, V.A. Kabanov, Doklady Akademii Nauk (Russ.) 354 (1997) 498.
[23] V.A. Kasaikin, E.A. Litmanovich, A.B. Zezin, V.A. Kabanov, Doklady Akademii Nauk (Russ.) 367 (1999) 359.
[24] A.V. Pirogov, A.V. Shpak, O.A. Shpigun, Anal. Bioanal. Chem. 375 (2003) 1199.
[25] V.A. Kasaikin, J.A. Zakharova, Colloids Surf. A: Physicochem. Eng. Aspects 147 (1999) 107.
[26] H. Wan, P.E. Anderson, A. Engstrom, L.G. Blomberg, J. Chromatogr. A 704 (1995) 179.