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Journal of Chromatography A, 811 (1998) 225–232

JOURNAL OF CHROMATOGRAPHY A

Mixed-mode capillary electrokinetic separation of positional explosive isomers using sodium dodecyl sulfate and negative-β-cyclodextrin derivatives

John H.T. Luong*, Yong Guo

Biotechnology Research Institute, National Research Council Canada, 6100 Royalmount Avenue, Montreal, Quebec H4P 2R2, Canada

Received 26 January 1998; received in revised form 17 March 1998; accepted 6 April 1998

Abstract

A mixed-mode capillary electrophoretic technique has been developed for the separation of positional nitroaromatic explosive isomers. The procedure utilized two different buffer additives as pseudo-stationary phases with different selectivities towards the analytes. Sodium dodecyl sulfate (SDS) displayed selectivities for the explosives which were similar to C_{18} reversed-phase HPLC. The negatively charged sulfobutyl ether-β-cyclodextrin (SB-β-CD), sulfated-β-CD and succinylated-β-CD separated the explosives on the formation of inclusion complexes with the analytes, and exhibited different selectivities for the explosives compared to SDS. A mixed pseudo-stationary phase was then formulated by combining 10 m*M* SB-β-CD, 30 m*M* SDS and 10% acetonitrile in 20 m*M* borate at pH 9 to resolve the two most difficultly separated pairs: 1,3-dinitrobenzene/1,4-dinitrobenzene and 3-nitrobenzene/4-nitrobenzene.

Keywords: Buffer composition; Positional isomer separation; Explosives; Nitrobenzene; Nitrotoluenes

1. Introduction

Nitroaromatic compounds are an important class of environmental pollutants, particularly as explosive residuals in contaminated soil and water. Identification and determination of explosives are of importance in environmental analysis and remediation technology because there are growing concerns over the environmental fate and toxicity of such pollutants with the closures of several military bases throughout the world. There is also a high demand for an efficient and complimentary method to HPLC in forensic science for verification of explosives and other illicit materials.

Electrokinetic chromatography (EKC) is effective for the separation of both ionic and nonionic species. The separation of neutral compounds in EKC is dependent on the use of charged buffer additives such as micelles, bile salts, or charged cyclodextrin derivatives as a pseudo-stationary phase [1–4]. The EKC technique using micelles is more commonly called micellar electrokinetic chromatography (MEKC) in which micelles are formed by surfactants at concentrations exceeding a critical micelle concentration (CMC) in the running buffer [5]. Charged micelles migrate under the combined influence of electroosmotic and electrophoretic mobilities. Neu-

*Corresponding author.

0021-9673/98/$19.00
PII: S0021-9673(98)00284-2

tral compounds acquire effective mobilities through their association with the charged micelles, and the separation is based on differential partitioning of the analytes between the micellar and aqueous phases. Charged cyclodextrins have also been employed as the pseudo-stationary phase to separate polycyclic aromatic hydrocarbons (PAHs) compounds in EKC [6,7]. A neutral cyclodextrin, moving closely to the electroosmotic flow, is usually added to the running buffer to form a second cyclodextrin phase to enhance the separation resolution and efficiency. The separation of neutral compounds was attributed to differential partitioning of analytes between the neutral and charged cyclodextrin phases [6,7]. To date, several investigators have demonstrated the applicability of CE for the analysis of nitramine and nitroaromatic explosives [8–12].

In this study, the feasibility of using two pseudo-stationary phases with different selectivities for separating the positional explosive isomers with EKC has been demonstrated. Sodium dodecyl sulfate (SDS), a common negative surfactant, and three negative cyclodextrin derivatives were tested, respectively, as the potential pseudo-stationary phases in the EKC separation. The chromatographic performance of each phase in separating the ten selected positional explosive isomers was evaluated, and the selectivity was compared. Sulfobutyl ether-β-cyclodextrin (SB-β-CD) and SDS were then combined in the running buffer as a mixed pseudo-stationary phase for the separation of the explosives.

2. Experimental

2.1. Materials

SDS, hydroxypropyl-β-cyclodextrin (HP-β-CD) with an average degree of substitution (DS) of 0.8 hydroxypropyl groups per cyclodextrin ring, sulfated-β-cyclodextrin (SULF-β-CD, DS 7-11) and nitroaromatic compounds were purchased from Aldrich (Milwaukee, WI, USA). Trinitrotoluene (TNT) was purchased from ChemService (West Chester, PA, USA). SB-β-CD (DS 4) was kindly given by Dr. John Staubaugh of Higuchi Bioscience Center for Drug Delivery Research (University of Kansas, Lawrence, KS, USA). Succinyl-β-cyclodextrin (SUC-β-CD, DS 0.4) was a gift from Wacker–Chemie (Burghausen, Germany). Acetonitrile (ACN) was HPLC grade (Fisher, Nepean, Canada) and water was purified using a Zenopure Quadra 90 filtration system (Zenon Environmental, Burlington, Canada) with a specific resistivity over 15 MΩ cm. Table 1 provides the names of common abbreviations for the ten nitroaromatic explosives used in this paper.

2.2. Equipment

All the CE experiments were performed on a P/ACE 5500 capillary electrophoresis system (Beckman, Fullerton, CA, USA). The separation capillary (50 μm I.D. and 360 μm O.D., Chromatographic Specialties, Brockville, Canada) was installed in a capillary cartridge. The separation capillary had a total length of 47 cm with an effective length of 40 cm. On-column UV detection was performed with a Beckman modular UV detector operated at 214 nm. Data acquisition and analysis were facilitated by using P/ACE Station software (Version 1.0, Beckman).

The HPLC system consisted of two Waters Model 590 pumps, a Waters WISP 710B auto-injector, and a Waters LC spectrophotometer (Model 481, Waters, Milford, MA, USA) operated at 229 nm. The system was operated isocratically at a flow-rate of 1.0 ml min^{-1} using (1:2) ACN–water mixture as the mobile phase. The stock mobile phase solution was degassed by helium prior to use. An HPLC column (Adsorbosphere XL C_{18}U, 250×4.6 mm) containing C_{18} phase (5 μm) was purchased from Alltech

Table 1
Names and abbreviations of the explosives analyzed by EKC

Name	Abbreviations	Peak No. (in all electropherograms)
2-Nitrotoluene	2-NT	1
3-Nitrotoluene	3-NT	2
4-Nitrotoluene	4-NT	3
1,2-Dinitrobenzene	1,2-DNB	4
1,3-Dinitrobenzene	1,3-DNB	5
1,4-Dinitrobenzene	1,4-DNB	6
2,3-Dinitrotoluene	2,3-DNT	7
2,4-Dinitrotoluene	2,4-DNT	8
2,6-Dinitrotoluene	2,6-DNT	9
3,4-Dinitrotoluene	3,4-DNT	10

(Deerfield, IL, USA). The HPLC column was not thermostated during operation and all injections were made in duplicate with a injection volume of 15 μl.

2.3. Procedures

The stock sodium borate solution (20 m*M*) was buffered to pH 9. SDS solutions were prepared by dissolving appropriate amount of SDS in a 20 m*M* borate buffer, pH 9. The cyclodextrin solutions were prepared by dissolving cyclodextrin powders in stock borate buffer, and adjusting to pH 9 with NaOH where necessary. All the solutions were sonicated and filtered through 0.22 μm filters before use. New capillaries were conditioned with 1 *M* NaOH for about 30 min and then washed with water and the running buffer, respectively for another 30 min each. Before daily operation, the capillary was cleaned with 0.1 *M* NaOH for about 10 min, and then with water and the running buffer for 10 min each. The capillary was further conditioned by applying 20 kV voltage for about 10 min before the first injection. The sample stock solution was prepared by dissolving the explosives in methanol (100 mg l^{-1} or 100 ppm each). The stock sample solution was then diluted into the running buffer (dilution factor 1:10) and analyzed by CE. Sample injection was performed by applying a small pressure (3.44 kPa or 0.5 p.s.i.) at the inlet of the capillary for 3 s. When using the SDS solution, the capillary was rinsed with ACN for 1 min and then with the running buffer for 2 min between injections. The separation voltage was applied over a 3-min ramp to prevent any possible current break-down.

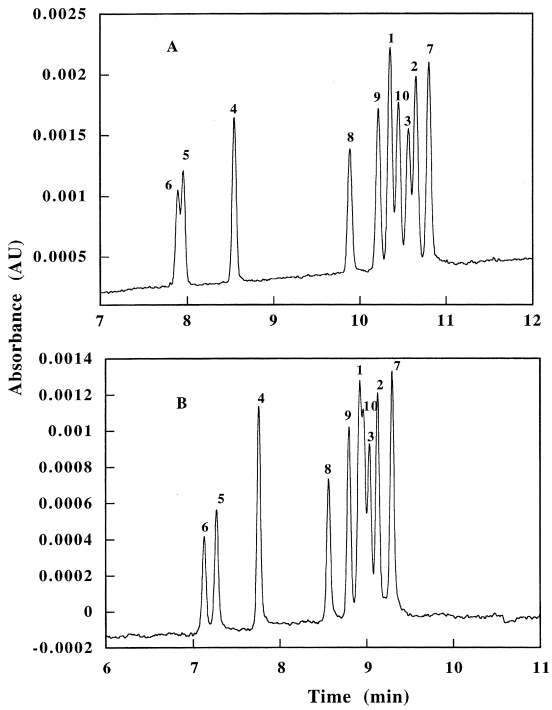

Fig. 1. (A) Separation of a mixture of ten explosives using 50 m*M* SDS in 20 m*M* borate buffer pH 9, the potential applied: 10 kV, resultant current: 8 μA and the electroosmotic migration time (t_{eof})=5.13 min. (B) Separation of a mixture of 10 explosives using 50 m*M* SDS/10 m*M* HP-β-CD in 20 m*M* borate buffer pH 9, potential applied: 10 kV, resultant current: 9.1 μA and t_{eof}= 5.37 min. Pressure injection for 3 s at 3.44 kPa or 0.5 p.s.i. and UV detection at 214 nm. Peak identification: (1) 2-nitrotoluene, (2) 3-nitrotoluene, (3) 4-nitrotoluene, (4) 1,2-dinitrobenzene, (5) 1,3-dinitrobenzene, (6) 1,4-dinitrobenzene, (7) 2,3-dinitrotoluene,(8) 2,4-dinitrotoluene, (9) 2,6-dinitrotoluene and (10) 3,4-dinitrotoluene.

3. Results and discussion

3.1. Separation of the explosives using SDS as the pseudo-stationary phase

Fig. 1A shows the electropherogram of the ten explosives using 50 m*M* SDS in 20 m*M* borate, pH 9. 1,2-DNB (peak 4) could be easily separated from 1,3-DNB (peak 5) and 1,4-DNB (peak 6), respectively and the other three dinitrotoluene isomers (peaks 7, 8, and 9) were completely resolved. However, 1,3-DNB was only partially resolved from 1,4-DNB (peaks 5 and 6) whereas 2-NT, 3-NT, 4-NT and 3,4-DNT (peaks 1,2,3 and 10) practically eluted as a cluster. Further optimization of the separation conditions with respect to pH and separation potential failed to improve the resolution of these two clusters. The 50 m*M* SDS, 25 m*M* phosphate, pH 7 electrolyte was first attempted by Oehrle [8,9] to separate 14 common nitramine and nitroaromatic explosives in less than 14 min. However, when a 10 m*M* or 25 m*M* phosphate buffer, pH 7.1 containing 50 m*M* SDS was initially attempted to separate the ten explosive model, only eight peaks resulted in the electropherogram and the last peak emerging only after 21.45 min even though the

separation potential was applied at 30 kV (figure not shown).

When 10 m*M* HP-β-CD was added to 50 m*M* SDS, the elution order was the same as in the separation using only SDS (Fig. 1B) although the run was shorter. It was noted, however, that the electroosmotic migration time was slightly longer (5.37 min vs. 5.13 min). In addition to the possibility of being adsorbed to the capillary wall via hydrophobic interactions to shield surface charge, the addition of neutral HP-β-CD caused a noticeable increase in viscosity (not measured) and thus decreased electroosmotic flow. The rationale for introducing this neutral cyclodextrin to the running buffer could be explained by the fact that the explosives were anticipated to form a complex with this neutral cyclodextrin to affect change in their apparent molecular weight. HP-β-CD is electrically neutral and its outside surface is hydrophilic, therefore, it was not anticipated to interact with the micelle and moves along with the buffer phase. The addition of this neutral cyclodextrin effected the baseline resolution between 1,3-DNB and 1,4-DNB (peaks 5 and 6), but the resolution of the NT cluster remained practically unchanged. In fact, the resolution between 2-NT and 3,4-DNT was slightly decreased (peaks 1 and 10). It should be noted that neutral cyclodextrin derivatives have been used to improve the separation of PAHs [13,14]. The nonionic compounds are expected to partition between the micellar phase and neutral cyclodextrin phase.

The selectivity of SDS for the explosives was first compared to that of a C_{18} phase in reversed-phase HPLC. To compare the selectivity of SDS for the explosives, the migration times of the explosives were transformed into range-rescaling factors (X_a), defined as $(t_i-t_o)/(t_f-t_o)$ where t_i is a solute's migration time, t_f the migration time of the last-eluting compound, and t_o the electroosmotic migration time. This transformation allows a direct comparison of the migration time of the explosives in SDS and the C_{18} HPLC phase on the same scale [15]. If the selectivities are identical, all the data points must fall in the diagonal region whereas a resulting scattered plot implies a difference in the selectivities. Linear regression of the data points in Fig. 2 resulted in a straight line (slope of 1.064, RDS of 0.023, $R^2=0.92$) which indicated that the selectivity of SDS for the explosives was very similar to that of the C_{18} phase. Such a result was not totally unexpected because the separation of the explosives in SDS was based on the hydrophobic interaction between the explosives and hydrophobic micelles of SDS. This interaction was analogous to that between the solutes and the C_{18} phase in reversed-phase HPLC. The more hydrophobic solute associated more strongly with the hydrophobic micelles of SDS, and migrated more slowly. Similarly, the more hydrophobic solute would spend more time than the smaller one in the C_{18} phase. As expected from the elution patterns obtained in Fig. 1A and Fig. 1B, the addition of 10 m*M* HP-β-CD in SDS did not affect the selectivities of SDS for the ten explosives. The range rescaling plot between SDS versus SDS/HP-β-CD resulted in a straight line with a slope of unity and a correlation coefficient of 0.992 (figure not shown).

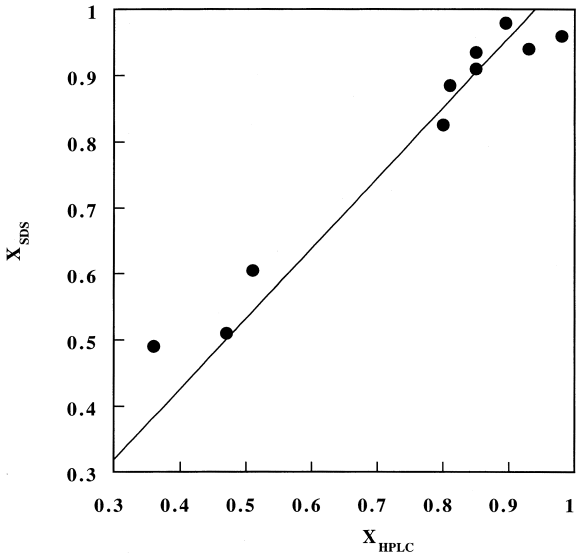

Fig. 2. Plot of rescaling factors of the ten explosives in EKC using SDS (50 m*M*) vs. rescaling factors of the explosives in reversed-phase HPLC using a C_{18} phase.

3.2. Separation of the explosives using a negative cyclodextrin and a neutral cyclodextrin

Like micelles, the negatively charged CD migrates at a noticeably slower rate than the buffer phase, and

functions as a pseudo-stationary phase in analogy with MEKC. Formation of the inclusion complex is facile on the electrophoretic time scale and the apparent mean mobility of a solute can be related to the intrinsic mobility of the solute (μ_A) and the solute-cyclodextrin inclusion complex (μ_{ACD}) as $(\mu_A+\mu_{ACD}K[CD])/(1+K[CD])$. The formation constant of the inclusion complex, K, is defined as [ACD]/([A]+[CD]) where [CD], [A], and [ACD] are the concentration of cyclodextrin, solute, and inclusion complex, respectively. This model was originally developed for optimization of cyclodextrin modified capillary electrophoresis for separation of enantiomers [16]. For the separation of neutral compounds, a negatively charged cyclodextrin was mandatory. In addition, a neutral cyclodextrin was also required for the explosives to establish equilibrium between the two cyclodextrin phases [6,7]. The separation mechanism was attributed to the formation of inclusion complexes between the two cyclodextrins and the explosives. For neutral hydrophobic solutes [14,17], if the aqueous concentration of the solute is considered negligible, and the mobility of the neutral CD is considered zero, the mobility of the neutral hydrophobic (μ_{nh}) can be written as $\mu_2 K_i CD_2/[CD_1+K_i CD_2]$ where $K_i=K_2/K_1$. In this equation, μ_2 is the mobility of the charged complex, CD_1 and CD_2 are the concentrations of the neutral and the charged CDs, respectively. Therefore, the quality of a resolution and sensitivity are governed by the two cyclodextrin concentrations in the electrolyte solution and the selectivity is dependent upon whether the solute will form a strong or weak complex with the neutral or negatively charged cyclodextrin [6,14,17].

A series of experiments was therefore conducted to separate the above ten explosives using a 20 m*M* borate buffer pH 9 containing 30 m*M* SULF-β-CD and 10 m*M* HP-β-CD. However, all of the ten explosives eluted very closely as a big cluster, indicating low selectivities of SULF-β-CD for the explosives (Fig. 3A). 2,3-DNT was co-eluted with 2,6-DNT (peak 7 and 9) whereas 4-NT migrated together with 2,4-DNT (peaks 3 and 8). In comparison to the separation using SDS (Fig. 1A,B), there was also a reversal of the elution order for the two pairs: 1,3-DNB/1,4-DNB (peaks 5 and 6) and 1,3-NT/1,4-NT (peaks 2 and 3). Surprisingly, 2-NT

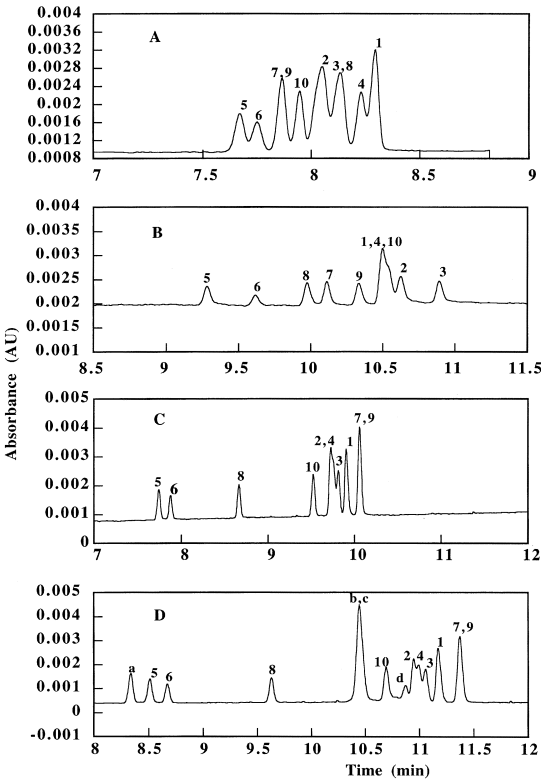

Fig. 3. (A) Separation using 20 m*M* borate buffer pH 9 containing 30 m*M* sulfated-β-cyclodextrin and 10 m*M* hydroxypropyl-β-CD (HP-β-CD), resultant current: 89.5 μA and $t_{eof}=6.84$ min. (B) Separation using 20 m*M* borate buffer pH 9 containing 30 m*M* succinylated-β-cyclodextrin and 10 m*M* HP-β-CD, resultant current: 46.3 μA and $t_{eof}=6.95$ min. (C) Separation using 20 m*M* borate buffer pH 9 containing 30 m*M* sulfobutylether-β-cyclodextrin and 10 m*M* HP-β-CD, resultant current: 39.8 μA and $t_{eof}=5.75$ min. (D) Separation of trinitrotoluene (peak a), 1,3-dinitronaphthalene (DNN, peak b), nitrobenzene (peak c) and 1,5-DNN (peak d) from the above ten explosive model using 20 m*M* borate buffer pH 9 containing 30 m*M* sulfobutylether-β-cyclodextrin and 10 m*M* HP-β-CD, resultant current: 39.8 μA and $t_{eof}=5.81$ min. Other conditions and peak identification are the same as in Fig. 1 except that the potential applied was 15 kV.

formed a very strong complex with SULF-β-CD instead of 2,3-DNT and emerged last. Separation was noticeably improved using a buffer containing 30 m*M* SUC-β-CD and 10 m*M* HP-β-CD in 20 m*M* borate, pH 9 (Fig. 3B). The separation window was widened to enable the complete separation of seven explosive compounds. However, 2-NT, 1,2-DNB and 3,4-DNT (peaks 1,4, and 10) co-migrated and moved

as a cluster. It should also be noted that 3-NT (peak 2) was only partially separated from this cluster. In contrast to SULF-β-CD, the degree of substitution of this negative cyclodextrin is very low (DS=0.4) and was not effective. Perhaps, SUC-β-CD with a higher degree of substitution which has not been developed may be more effective in separating these explosive isomers.

The separation of the ten explosive model using 20 m*M* SB-β-CD and 10 m*M* HP-β-CD in EKC was attempted. It should be noted that this separation buffer was reported to resolve the 16 priority US Environmental Protection Agency (EPA) PAHs [6,7]. The electropherogram obtained (Fig. 3C) exhibited two co-eluted pairs: 2,3-DNT/2,6-DNT (peaks 7 and 9) and 3-NT/1,2-DNB (peaks 2 and 4). Further optimization of the separation conditions with respect to the dual cyclodextrin concentration ratio failed to improve the resolution of these two clusters. It is of importance to note that this type of buffer system could easily separate trinitrotoluene (TNT, peak a), 1,3-dinitronaphthalene (DNN, peak b) and 1,5-DNN (peak d) from the above ten explosive model (Fig. 2D). However, nitrobenzene was observed co-eluted with 1,3-DNN (peak b/c). In this series of experiments the potential applied was increased to 15 kV so that the separation time was somewhat comparable to that of SDS (Fig. 1A,B).

A comparison of the separation of the explosives using SDS and SB-β-CD/HP-β-CD in EKC revealed quite a different elution pattern as evidenced by the resulting scattered data (Fig. 4), indicating different selectivities of SDS and SB-β-CD/HP-β-CD for the explosives in EKC. The selectivities of SULF-β-CD were somewhat similar to those of SUC-β-CD, however, they were completely different from those of SB-β-CD. The range rescaling plot also revealed that SDS exhibited different selectivities toward the explosives in comparison to SULF-β-CD/HP-β-CD and SUC-β-CD/HP-β-CD (figure not shown). The migration time of the negatively charged cyclodextrins could not be determined since these cyclodextrins contain a mixture of differently substituted cyclodextrins. Consequently, the capacity factors for the explosives in the negatively charged cyclodextrins could not be obtained directly from the electropherogram.

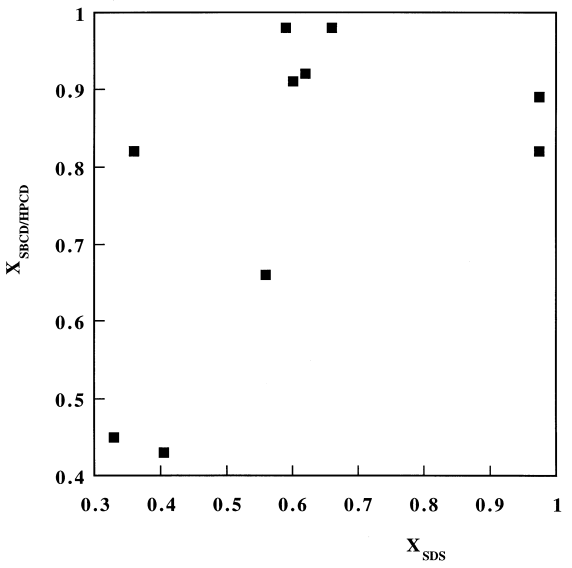

Fig. 4. Plot of range-scaling factors of the ten explosives in EKC, SB-β-CD/HP-β-CD vs. SDS.

3.3. Separation of the explosives using two pseudo-stationary phases in mixed-mode EKC

Mixed-mode separation involving two phases with different selectivities has been used in liquid chromatography [18–21]. A column can contain two different phases which separate the analytes based on different mechanisms. Peak capacity and resolution can be improved using this approach. For example, a C_{18} phase and a β-CD phase have been combined in a mixed-bed column to separate some simple PAHs [22]. The selectivity in the mixed-mode was different from that using each individual phase, and changed with the ratio of the two phases. The different selectivities of SDS and SB-β-CD/HP-β-CD for the ten explosives provide a basis for combining the two systems in the mixed-mode EKC separation, i.e. both SDS and SB-β-CD were used in the running buffer as the pseudo-stationary phases. As in the mixed-bed column in HPLC, the ratio of the two phases (SDS/SB-β-CD) is important to achieve the optimal resolution and neutral cyclodextrins or organic solvents such as acetonitrile and methanol are normally needed to improve the separation.

A series of experiments was conducted to optimize

Table 2
Concentrations of five additives used in the mixed-mode capillary electrophoretic technique (the potential applied was 10 kV)

Run	SB-β-CD (m*M*)	SDS (m*M*)	HP-β-CD (m*M*)	Borate (m*M*)	Acetonitrile (m*M*)
1	10	30	10	10	10
2	20	20	10	10	10
3	20	30	10	20	0
4	20	30	0	10	0
5	10	30	0	20	10
6	10	20	10	20	0
7	20	20	0	20	10
8	10	20	0	10	0

the concentrations of borate, SB-β-CD, SDS, HP-β-CD and acetonitrile in the separation buffer (Table 2). Only five explosives were selected for the test, 1,3-DNB, 1,4-DNB, 3-NT, 4-NT and 2,3-DNT. The resolution between 1,3-DNB and 1,4-DNB was referred to as R_{s_1} and this pair was selected since these two compounds tended to co-elute as shown earlier. Similarly, 3-NT was shown to co-elute with 4-NT and the resolution of this pair was designated as R_{s_2}. The last explosive, 2,3-DNT was chosen because it eluted after the above four explosives and its migration time served as t_f which enabled the estimation of the resolution and the separation efficiency.

Fig. 5A presents the separation of the five explosive model using 20 m*M* SDS and 10 m*M* SB-β-CD in the mixed-mode EKC (run 8). The electropherogram exhibited only three peaks and the least peak was identified as 2,3-DNT as expected. The first and second peak, respectively were then identified as 1,3-DNB/1,4-DNB and 3-NT/4-NT. Increasing both SDS and SB-β-CD to 30 m*M* and 20 m*M*, respectively did not resolve these two isomer pairs (run 4, Table 3). Similarly, increasing the borate concentration up to 20 m*M* in the running buffer containing both SDS and SB-β-CD in any combination did not have any effect on the resolution of these two pairs.

The addition of HP-β-CD up to 10 m*M* only exhibited a noticeable effect on the resolution of 3-NT and 4-NT whereas the pair 1,3-DNB and 1,4-DNB was practically unresolved (runs 3 and 6, Table 3). Similarly, the resolution of 1,3-DNB and 1,4-

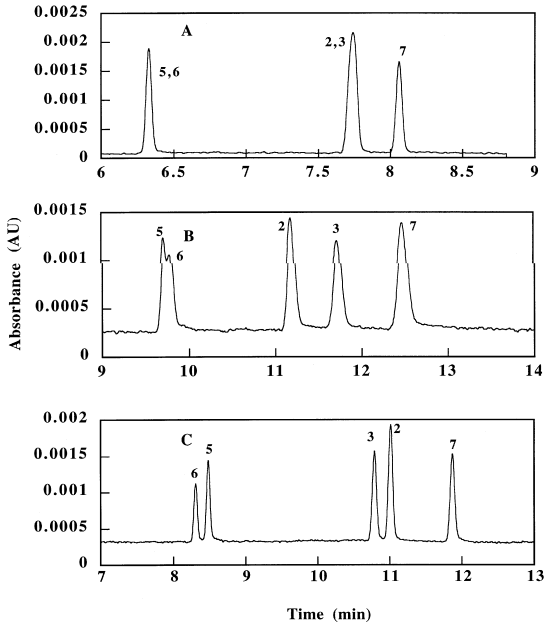

Fig. 5. (A) Separation of five selected explosives using two pseudo-stationary phases, 20 m*M* SDS and 10 m*M* SB-β-CD in 10 m*M* borate pH 9. (B) The running buffer contains 20 m*M* SDS, 20 m*M* SB-β-CD, 10% acetonitrile in 20 m*M* borate, pH 9. (C) The running buffer contains 30 m*M* SDS, 10 m*M* SB-β-CD, 10% acetonitrile in 20 m*M* borate, pH 9. Other conditions and peak identification are the same as in Fig. 1.

DNB ($R_{s_2}=2.95$) was greatly improved by adding 10% acetonitrile in a 20 m*M* borate buffer, pH 9 containing SB-β-CD and SDS, 20 m*M* each (Fig. 5B, run 7). Unfortunately, this mixed-mode buffer system could not resolve 1,3-DNB from 1.4-DNB

Table 3
Migration time and peak resolution in the mixed-mode capillary electrophoretic technique

Run	t_o (min)	t_f (min)	R_{s_1}	R_{s_2}	Resultant current (μA)
1	6.52	9.26	0.56	0.34	16.2
2	7.42	10.49	−0.58[a]	0	21.7
3	6.63	9.33	−0.65[a]	−1.62[a]	33.1
4	6.27	11.23	0	0	26.4
5	6.31	12.01	1.89	1.96	17.3
6	6.64	6.93	0	−1.15[a]	16.5
7	7.63	11.02	−0.27[a]	−2.95[a]	22.3
8	5.29	8.13	0	0	15.8

[a]Negative sign indicates the reversal of elution order obtained in Fig. 1A,B (6, 5, 3, 2 and 7). Each run was performed in triplicate.

($R_{s_1} = 0.27$). The five explosive compounds were finally resolved using a 20 m*M* borate buffer, pH 9 containing 30 m*M* SDS, 10 m*M* SB-β-CD and 10% acetonitrile (run 5, Fig. 5C). As shown in Table 3, the explosives were baseline separated with the resolution of 1.89 for 1,3-NT and 4-NT and 1.96 for 1,3-DNB and 1,4-DNB, respectively. In comparison to the separation running buffer used in Fig. 5B, there was a reversal of the elution order since 1,4-DNB eluted before 1,3-D NB and the 4-NT peak emerged earlier than the 3-NT peak. The efficiencies for the five explosives range from 116 000 to 162 000 theoretical plates m^{-1}. Reproducibility for the migration time and the peak area was about 1.5–3% and 3.2% R.S.D., respectively for all the five explosives over ten electropherograms. In this series of experiments, after each run, the capillary was cleaned with 0.1 *M* NaOH for about 10 min, and then with acetonitrile and the running buffer for 10 min each. The capillary was further conditioned by applying 20 kV voltage for about 10 min before the next injection.

4. Conclusions

SDS and SB-β-CD, respectively, were selected as the pseudo-stationary phases to separate the nitro-aromatic positional isomers and the selectivity of SDS for the ten explosive model was similar to that of the C_{18} phase in HPLC. Neither SDS nor SB-β-CD could provide complete separation of all the ten explosives even under optimized conditions. The different selectivities of SDS and SB-β-CD for the explosives provided the basis for combining the two systems. In the presence of acetonitrile, both SDS and SB-β-CD were effectively used as the pseudo-stationary phases to attain baseline separation for the two most difficultly separated pairs in the mixed-mode EKC.

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