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Short communication

New selectivity in electrokinetic chromatography using a polymeric dye as novel separation carrier

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Abstract

A water-soluble polymeric dye was used as separation carrier in electrokinetic chromatography. For separations of aromatic analytes, alternative selectivities as compared to a sodium dodecyl sulfate system were obtained. Separation performance in such a system was further influenced and controlled by the application of a counter pressure from the cathodic side and by varying the modifier content over a wide range. © 1997 Elsevier Science B.V.

Keywords: Selectivity; Buffer composition; Aromatic compounds

1. Introduction

In micellar electrokinetic chromatography (MEKC) using sodium dodecyl sulfate (SDS), as it was originally introduced by Terabe et al. in 1984 [1], separation of neutral analytes is mainly based on hydrophobic interactions/partitioning between the SDS micelle's interior and the buffer environment. The SDS buffer system offers relatively little possibilities to change selectivity because the required stability of the micelles precludes drastic changes in the organic modifier content. Several investigations have been reported dealing with the use of alternative separation buffer carriers in EKC [2–10]. Unfortunately, most of these additives hardly offer completely new selectivities compared to SDS. Nevertheless, they have other advantages, such as better stability or reduced microheterogeneity of the micelles [2–4] or enlarged migration window [4–10].

We recently reported on the approach of using π-electron rich anions — e.g. tetraphenyl porphyrin tetrasulfonate (TPPS) and Cardiogreen (CG) — as separation carriers in EKC [11]. For the separation of aromatic compounds, these dyes showed alternative selectivities compared to SDS. This may attributed to weak charge transfer (CT) interactions between the analytes and the carrier. However, since these carriers are UV active, a different working principle has to be applied to allow detection [11,12]. Briefly, this principle is based on partial filling of the capillary with the separation containing buffer, combined with the application of pressure from the cathodic side to further delay this carrier from entering the detection window. Such a working principle influences separation performance as well: as a consequence of the flow profile distortion efficiency is reduced [12]. But at the same time the migration time ratio (determined by the ratio of the migration time of the electroosmotic flow marker and the migration time of the

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separation carrier t_0/t_{MC}) is significantly decreased resulting in an improved separation performance at moderate pressures [14].

In this paper we report on the use of a polymeric dye as a new charge transfer interacting separation carrier which offers alternative selectivities to SDS and tolerates higher organic modifier contents.

2. Experimental

2.1. Instrumentation

All experiments were performed with a Prince instrument (LauerLabs, Emmen, Netherlands), equipped with a Jasco 875-CE UV detector (Jasco, Tokyo, Japan) and the personal computer-based integration software GYNKOSOFT (Gynkotek, Munich, Germany).

For these studies we used fused-silica capillaries with 75 cm×50 μm I.D. (MicroQuartz, Munich, Germany). The effective capillary length from inlet to detector was 47 cm. UV detection was performed on-capillary at 254 nm.

2.2. Chemicals

Buffer solutions were prepared from analytical-grade chemicals (Merck, Darmstadt, Germany). A stock solution of borate buffer was prepared at a concentration of 0.05 *M* with sodium tetraborate and adjusted to a pH of 9.4 with hydrochloric acid. For the different experiments it was diluted with water and methanol as organic modifier.

Acetone (Fluka, Buchs, Switzerland) was used as electroosmotic flow marker. The nitroaromatic compounds for the EKC separations were obtained from Promochem (Wesel, Germany) and were used at concentrations of 5–80 μg/ml in buffer with modifier.

The separation carrier poly(vinylamine) sulfonate anthrapyridone (Poly R-478) was obtained from Aldrich (Steinheim, Germany). SDS was purchased from Roth (Karlsruhe, Germany). Solutions were prepared by dissolving them in the respective buffer–modifier mixture. For all experiments in this work concentrations were 10 mg/ml Poly R-478 or 0.025 *M* SDS.

2.3. Procedures

Before each run, the capillaries were flushed with 0.1 *M* NaOH for 2.5 min at 1000 mbar. Subsequently, the capillaries were rinsed with buffer (without the separation carrier) for 2.5 min at the same pressure. For the run the outlet-vial of the buffer was changed to use only fresh buffer. The rinsing procedures and injections were carried out hydrodynamically [11,12]. The capillary was only partially filled with the additive by hydrodynamically injecting the carrier containing buffer as it is described elsewhere [11,12]. In this work, the separation zone was 30% of the effective capillary length.

By applying counter pressures up to 40 mbar, reproducibility of migration times (standard deviation 1–2%) and plate numbers (2–3%) are hardly affected, as was established by earlier experiments [11].

3. Results and discussion

As described in [11], π-electron rich anions capable to charge transfer interactions with aromatic analytes offer alternative selectivities to the SDS system. Unpublished experiments performed with several sulfonated dyes without (e.g. hydroxynaphthol blue or eriochrome black T) and with (e.g. Cardiogreen) a hydrocarbon chain spacer between the π-electron systems and the anionic moiety of a separation carrier, showed that separation performance is significantly enhanced for the latter.

One of the possible candidates which meets this structural requirements is Poly R-478, shown in Fig. 1. Selectivity differences between the Poly R-478 system were initially investigated in comparable buffer systems (0.02 *M* borate, pH 9.4, 10% methanol). Due to reasons stated above a counterpressure above 20 mbar had to be applied in the case of the UV-active Poly R-478. The different migration order of a mixture of amino–nitroaomatic compounds in both systems is depicted in Table 1. The plate numbers in the Poly R-478 system are lower compared with the corresponding values in the SDS system. First, flow profile distortion by the counterpressure contributes to reduced efficiency in the Poly

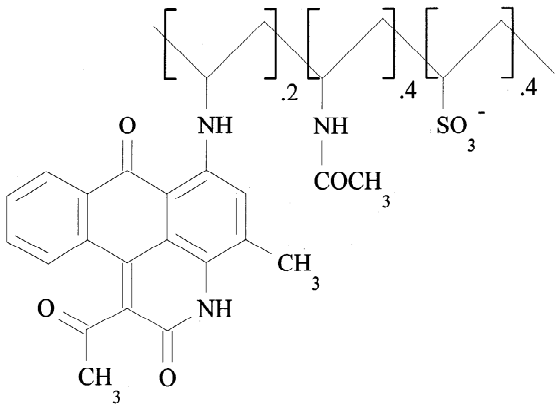

Fig. 1. Structure of Poly R-478.

R-478 system. Second, additional band broadening occurs at the interface between the carrier containing buffer zone and the pure buffer zone in partial filling EKC [13]. However, the comigrating compounds 3-nitroaniline/4-nitroaniline and 4-nitrotoluene/diphenylamine in the SDS system could be separated in the Poly R-478 system as a consequence of a different selectivity. The fact that only aromatic compounds show significant retention supports the assumption that the selectivity behaviour of Poly R-478 is governed by charge transfer interactions.

In contrast to the micellar SDS system, the interactions between the analytes and the 'charge transfer' separation carriers are one-on-one type interactions. Therefore, selectivity and resolution in such a system may not only be influenced and controlled by a counterpressure (as it is the case in the SDS system [14]), but also by variation in the content of the organic modifier over a wide range. An additional influence of both means on the efficiency via the flow profile distortion, the migration time window, the viscosity and the diffusion coefficients makes it nearly impossible to predict changes in resolution when varying any of these parameters. The multitude of experimental results obtained in the Poly R-478 system (some of which are given in Fig. 2 and Table 2) eludes straightforward interpretation at this point. Nevertheless, some statements can be made:

Already counterpressures above 5 mbar successfully keep the separation carrier out of the detection window. But increasing pressure effectively improves resolution for analytes eluting with longer migration times, due to a decreased migration time ratio t_0/t_{MC}. This is demonstrated in Fig. 2 where the resolution of the peak pair 2-aminonaphthalene/2-amino-4,6-dinitrotoluene is improved as the counterpressure is increased. Unfortunately, the reduced efficiency causes a loss of resolution for faster migrating compounds. A way out of this dilemma could be the application of a counterpressure gradient during the electrophoretic run. Investigations about the feasibility of such an approach are currently underway.

As mentioned above, the separation carrier net movement towards the cathode cannot only be slowed down by a counterpressure but also by an increased methanol content. For 50% methanol in the buffer the separation carrier has become quasi-stationary. For that case the filling degree could even be increased to 90% of the effective capillary length. Similar as with an increased counterpressure later eluting analyte pairs display better resolution. On the other hand, resolution of early eluting pairs is diminished by both reduced efficiency and a complexation equilibrium which is shifted to the free analytes due to the higher methanol content.

4. Conclusions

By the application of a polymeric dye, Poly R-478, as a new separation carrier in MEKC different selectivities for aromatic compounds as compared to SDS-MEKC could be observed. The use of a counterpressure from the cathodic side, originally intended to prevent the UV-active carrier from entering the detection window, proved to be a valuable tool to influence and control resolution. Additionally, the polymeric dye allows a large range of organic modifier content in the buffer. This shows to be a further means to influence and manipulate separation performance. Since it is very difficult to establish a model for the observed dependencies, optimization of separation has to be done empirically. Both counterpressure gradients and modifier gradients have to be taken into consideration to achieve this goal.

Table 1
Comparison of migration order and plate numbers in a Poly R-478 system (left columns) and a SDS system (right columns) for a mixture of amino/nitro aromatics

Poly R-478			SDS		
Plate number	**Compound**	**Migration order**	**Migration order**	**Compound**	**Plate number**
66 000	2,6-Diaminotoluene	1	1	2,6-Diaminotoluene	63 000
88 500	2,3-Dinitrotoluene	2	2	3-Nitroaniline	145 000
			2	4-Nitroaniline	131 500
102 000	2,6-Dinitrotoluene	3	3	2-Nitroaniline	153 000
90 200	3-Nitroaniline	3			
63 000	Diphenylamine	4	4	2,4-Dinitrotoluene	194 400
38 000	4-Nitrotoluene	5	5	2,6-Dinitrotoluene	161 300
39 000	2,4-Dinitrotoluene	6	6	2,3-Dinitrotoluene	180 500
38 800	4-Amino-2,6-dinitrotoluene	6			
50 000	4-Nitroaniline	7	7	2-Amino-4,6-dinitrotoluene	172 000
40 100	2-Nitroaniline	7			
15 400	2-Amino-4,6-dinitrotoluene	8	8	4-Amino-2,6-dinitrotoluene	162 000
12 500	2-Aminonaphthalene	9	9	2-Aminonaphthalene	140 000
			10	4-Nitrotoluene	33 000
			10	Diphenylamine	162 000

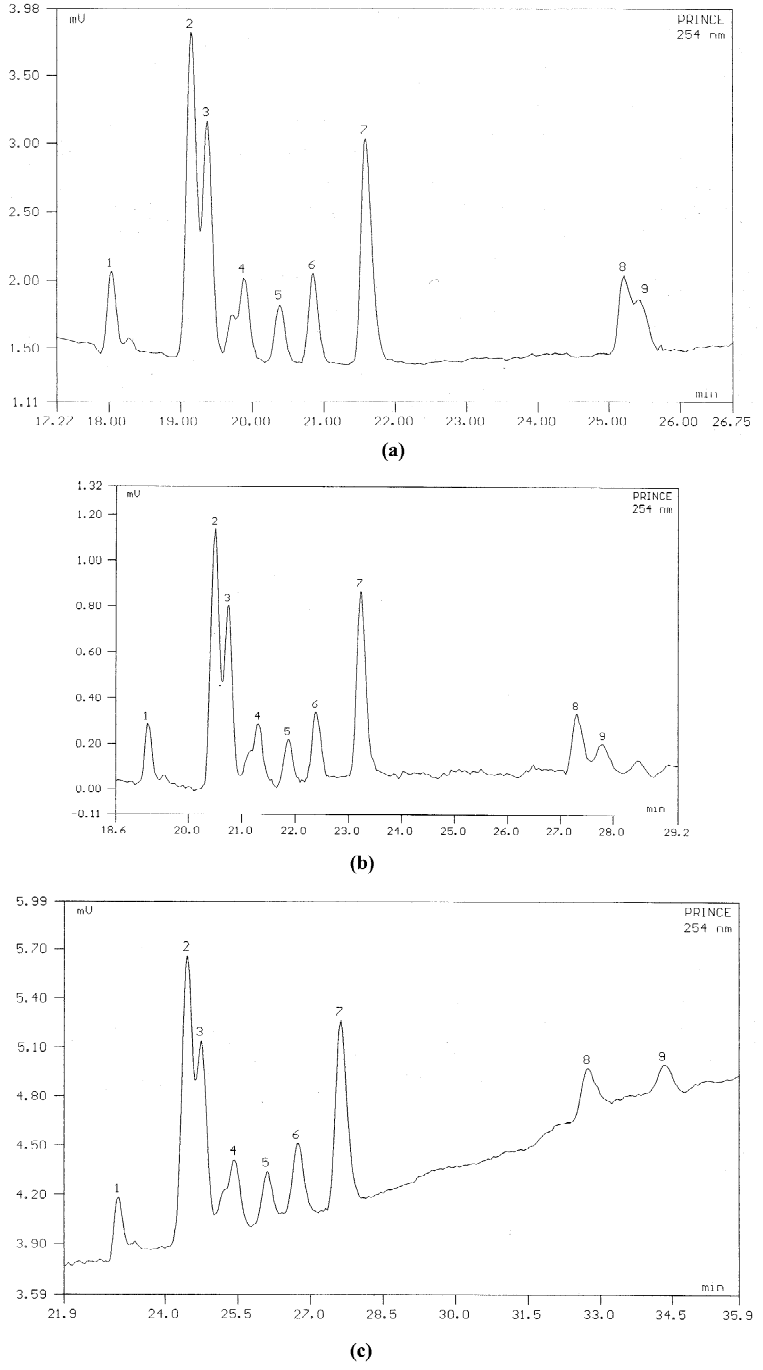

Fig. 2. Effect of increased counterpressure on separation performance in a Poly R-478 system for a mixture of amino/nitro aromatics (a) 10 mbar; (b) 15 mbar; (c) 25 mbar. Conditions: 0.02 *M* borate buffer (pH 9.4), 40% methanol; 10 mg/ml Poly R-478, 30 kV; Injection procedure: hydrodynamic injection (10 mbar, 6 s, injection volume 1.2 nl), from the anodic side. Peak assignment: 1=2,6-diaminotoluene (50.0 μg/ml); 2=2,3-dinitrotoluene (42.0 μg/ml)/3-nitroaniline (20.0 μg/ml); 3=2,6-dinitrotoluene (10.8 μg/ml); 4=2-nitroaniline (4.8 μg/ml)/4-nitroaniline (5.0 μg/ml); 5=4-nitrotoluene (30.0 μg/ml); 6=diphenylamine (50.0 μg/ml); 7=2,4-dinitrotoluene (76.8 μg/ml); 8=2-amino-4,6-dinitrotoluene (18.2 μg/ml); 9=2-aminonaphthalene (50.0 μg/ml).

Table 2
Influence of methanol content and counterpressure on plate numbers and resolution in a Poly R-478 system

Methanol content (%)	Counter pressure (mbar)	N (2,6-DAT) $t_M=t_0$	N (4-NT)	t_M (2,3-DNT) (min)	t_M (2,6-DNT) (min)	R_S (2,6-DNT/2,3-DNT)	t_M (2-AN) (min)	t_M (2-A-4,6-DNT) (min)	R_S (2-AN/2-A-4,6-DNT)
10	40	59 000	23 100	0.71	0.98	1.3	5.38	6.43	>5.5
20	10	85 000	76 500	0.52	0.68	1.1	3.99	5.25	5.5
30	10	105 000	67 200	0.96	1.22	1.0	6.74	7.05	0.86
40	10	71 800	65 700	1.11	1.34	Unresolved	7.18	7.18	Unresolved
40	15	65 000	64 600	1.28	1.52	Unresolved	8.60	8.08	0.96
40	25	54 000	52 800	1.41	1.69	Unresolved	11.26	9.69	2.43
50	0	74 000	32 000	0.57	0.57	Unresolved	4.66	3.17	5.08
60	0	65 000	Unresolved	0.69	0.69	Unresolved	4.94	3.60	3.31
70	0	30 000	Unresolved	0.54	0.54	Unresolved	6.64	4.22	3.24

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