ELSEVIER

Journal of Chromatography A, 798 (1998) 269–273

JOURNAL OF CHROMATOGRAPHY A

Separation of neutral compounds by capillary electrokinetic chromatography with a replaceable charged linear polymer as pseudo-stationary phase

B. Potocek[a], B. Maichel[a], B. Gas[b], M. Chiari[c], E. Kenndler[a,*]

[a]*Institute for Analytical Chemistry, University of Vienna, Währingerstrasse 38, A 1090 Vienna, Austria*
[b]*Institute for Physical and Macromolecular Chemistry, Charles University, Prague, Czech Republic*
[c]*Istituto di Chimica degli Ormoni, Consiglio Nazionale delle Ricerche, Milan, Italy*

Abstract

Neutral substances are separated by capillary electrokinetic chromatography using a negatively charged linear polymer (partially hydrolyzed polyacrylamide), dissolved in the buffer (pH 7, at a concentration of 2%), as pseudo-stationary phase. The solutes are transported by the electroosmotic flow towards the cathode, whereas the charged polymer forms an electrically driven counterflow; the non-charged parts of the polymer interact with the solutes, thus leading to retardation and separation of the analytes (dimethyl sulfoxide, phenol, pyrogallol). Plate numbers are 20 000 to 24 000, and are not much lower than theoretically reachable, taking longitudinal diffusion as the only peak dispersion process into account. © 1998 Elsevier Science B.V.

Keywords: Pseudo-stationary phases; Electrokinetic chromatography

1. Introduction

Capillary electrokinetic chromatography (EKC) is a method which combines the selectivity obtained by chromatography (caused by the molecular interactions between the analytes with the mobile and the stationary phase, respectively) with the high separation efficiency of capillary zone electrophoresis (CZE), which is achieved in this case due to the plug-like radial velocity profile of the electroosmotic flow (EOF) [1,2]. It combines the same principles as capillary electrochromatography (CEC), whereas the latter usually employs a packed bed formed by particles normally used in reversed-phase (RP) HPLC. CEC has some disadvantages, that need to be overcome before this technique will be better accepted for routine application. Some practical problems of system reliability exist, e.g., caused by the frits positioned at the capillary ends to maintain the packed particles in the column.

There are, however, more fundamental problems using RP particles for the generation of the EOF on the one hand (non-derivatized silanol groups dissociate under the conditions of the experiment, forming an electric double layer), and implementing separation selectivity on the other hand. It is well known from CZE in fused-silica capillaries that the negatively charged surface is often undesirably modified due to the adsorption of sample or matrix components, resulting in low reproducible EOF. This effect is observed on the surface of the RP particles, too. Although this problem can be solved at least

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)00962-X

partially in the case of CZE with fused-silica capillaries, e.g., by rinsing with chemically aggressive washing solutions, this procedure cannot be applied to RP particles because of their very limited stability at extreme pH.

The number of chargeable free silanol groups is quite limited in the case of RP particles, because most of the groups are modified by chemically bonding the alkyl groups. The result of this limitation is a low charge density on the surface, and therefore a low velocity of the EOF is reachable. Together with the low chemical stability of the bonded phase (which prevents from the application of mobile phases with high pH, where the EOF velocity has its maximum) there is a considerable limited velocity of the mobile phase.

One solution of these problems may lie in the application of the stationary phase not bonded on silica particles, but in a continuous bed as demonstrated by Fujimoto and coworkers [3–5] and Liao et al. [6]. Here the stationary bed is formed by a rigid, three-dimensional porous gel, in that charged groups are located, generating an EOF within the pores. Selectivity is established by copolymerization of lipophilic groups. The bed is covalently fixed at the capillary wall, which makes the use of frits unnecessary and avoids the problems associated with them. Although showing very favorable aspects, these systems may lack, however, easy manipulation and simple replacement of the bed. They bear some of the disadvantages of the gel matrices, used as sieving media in capillaries for the size-specific separation of DNA fragments and proteins by capillary electrophoresis (CE).

During the development of analogue systems we decided to investigate the possibilities of separation systems based on replaceable polymers. These polymers can interact with the analytes due to molecular interactions and may thus act as a kind of stationary phase. If they were electrically neutral like the separands, they would move through the capillary with the same velocity of the EOF as they were not fixed in the system, and no contribution to separation selectivity will be induced. One possibility to generate a finite velocity difference in view of the separands, a prerequisite for separation, is to charge the polymer chains, establishing in this way a flow of the polymer strands against the sample components when voltage is applied. So, e.g., negatively charged polymer chains (which must contain uncharged groups for molecular interactions with the neutral sample components) will migrate towards the anode whilst the EOF raised by the charged capillary wall flows into the opposite direction to the cathode together with the sample.

This principle is similar to micellar electrokinetic chromatography (MEKC), and like this technique there will exist a retention window: no neutral analyte can migrate faster than the EOF, and slower than with the net velocity of the polymer chains. In contrast to MEKC, however, there is no need to select the concentration of the additive higher than above a certain critical value. In addition, it is not restricted to micelle forming additives.

Charged polymers have been already used as pseudo-stationary phase for the separation of charged analytes due to an ion-exchange or ion-pair mechanism by Terabe and coworkers [7–10] named ion-exchange EKC. Stathakis and Cassidy [11,12] and Erim [13] used charged polymers to enhance the selectivity for the separation of fully and partially charged solutes. In a strict sense these papers apply two techniques simultaneously: CZE of the ions is superimposed by a kind of chromatography due to the ion–polymer interaction.

In the present study, in contrast, no electrophoresis occurs, and the non-charged solutes are transported by the EOF and retarded by the pseudo-stationary phase by a chromatographic mechanism. Thus it is in fact EKC as termed by Terabe and Isemura [7]. It should be pointed out that in some of the papers cited above such a mechanism, which was named aromatic adsorption in the context of CZE by Hjerten et al. [14], is anticipated to describe the selectivity for the separation of the ions obtained.

During the investigation of the applicability of easily available polymers as pseudophases (e.g., agarose with negatively charged groups, so-called high EOF agarose, or polyethyleneimine [13]) linear polyacrylamide (PAA) was also applied as possible candidate material, used in previous work for size-specific separation of protein–SDS complexes [15]. The present paper describes the preliminary results of successful separation of neutral compounds with partially charged PAA used as pseudo-stationary phase.

2. Experimental

2.1. Chemicals

Acrylamide and ammonium persulfate were purchased from Serva (Heidelberg, Germany). N,N,N′,N′-Tetramethylethylenediamine (TEMED) was obtained from Sigma (St. Louis, MO, USA). Phenol, pyrogallol, sodium dihydrogenphosphate monohydrate and disodium hydrogenphosphate-12-hydrate were obtained from E. Merck (Darmstadt, Germany). Dimethyl sulfoxide was purchased from Loba (Fischamend, Austria).

2.2. Apparatus

All analysis were performed on a CE system (P/ACE 2100 Beckman, Fullerton, CA, USA) equipped with an on-column UV absorbance detector (214 nm). Uncoated fused-silica capillaries of 27.0 cm (effective length 20.0 cm)×100 μm I.D. were used. Samples were introduced into the capillary by electrokinetic injection (5 s; 1 kV). The capillary cartridge was thermostatted at 20.0°C. A constant voltage of 2 kV was applied. The resulting current was 100 μA.

2.3. Procedures

The separation medium was made as follows. A linear polyacrylamide formed by polymerization of 100 mg of acrylamide, 10 μl of ammonium persulfate solution (10%) and 1 μl of TEMED in 1 ml deionized water was partly hydrolysed by 200 μl of sodium hydroxide (7.5 mol/l). After heating on the water bath for 20 min the polymer solution was allowed to cool to room temperature. The solution was neutralized by adding 300 μl of sulfuric acid (10%). The polymer solution was diluted with 4 ml of a phosphate buffer (5 mmol/l, pH 7.0), degassed and filled into the capillary using the high pressure mode of the instrument. As the anodic electrolyte vessel was also filled with this solution, it was constantly delivered into the capillary during the separation.

3. Results and discussion

Two phenols and dimethyl sulfoxide (DMSO) were used as sample components. Like DMSO, the phenols are uncharged under the conditions of capillary EKC (pH 7.0). This can be assumed considering the pK_a values of the phenols, which are between 9 and 10. A further confirmation that a separation is not obtained without the interaction with a (pseudo)stationary phase is given by the capillary zone electropherogram obtained with the phosphate buffer (pH 7.0, 5 mmol/l) without additive (data not shown), where all three sample components comigrate with the same velocity (that of the EOF generated by the electrical double layer at the capillary wall) and are forming a single peak. Obviously the application of a solution of an electrically neutral polymer, e.g., about 2% linear polyacrylamide (the matrix modified as described in Section 2.3) does not lead to separation, because the polymeric network migrates with the same velocity as the separands under these conditions.

When amide groups of the linear polyacrylamide are partially substituted by negatively charged carboxylic groups the situation is more favorable for a potential separation, as the polymer chains are now migrating under the influence of the electrical field against the EOF and against the sample zone towards the anode (they are moving with a mobility lower than that of the EOF), forming a kind of a counterflow of the pseudo-stationary phase. It can be seen from Fig. 1 that the interaction between the analytes and the phase are sufficiently strong and selective to separate the analytes: all three uncharged separands are retarded and migrate slower than the EOF. As pointed out, the charged parts of the polymer chains are used to form an electrically driven counterflow of the network, and other parts of the chains offer molecular interaction to generate sufficient selectivity for separation.

With the restrictions concerning the migration of concentration boundaries [16], an estimate of the capacity factors (taking the negative jump of the signal at 13 min as EOF marker, which may be of limited adequacy) lead to the following values: DMSO 0.11, phenol 0.16, pyrogallol 0.26.

The plate numbers, N, observed are in the range of 20 000 and higher (N for pyrogallol is 24 000).

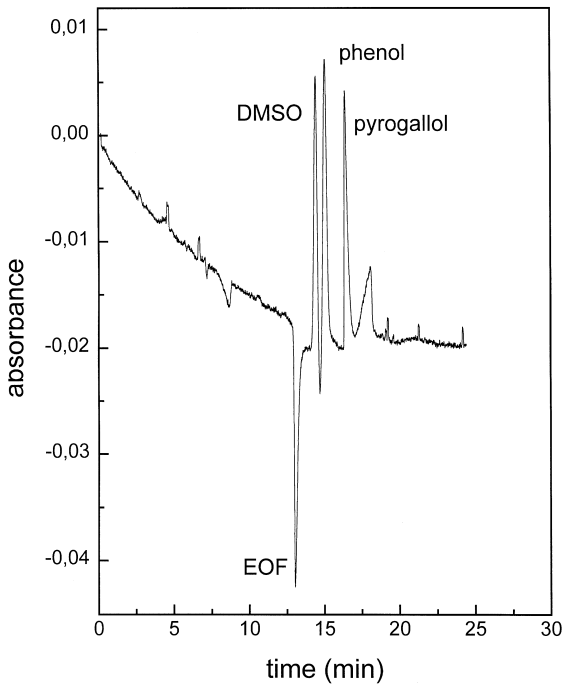

Fig. 1. Capillary electrokinetic chromatogram with partly negatively charged PAA as pseudo-stationary phase for separation of the uncharged analytes at pH 7.0. Conditions: uncoated fused-silica capillary (27.0 cm (20.0 cm effective length)×100 μm I.D.). Electrokinetic injection (5 s; 1 kV). Temperature 20.0°C. Voltage 2 kV. UV absorbance detector (214 nm) placed at the cathodic end of the capillary.

These values are not far from those theoretically predicted, taken that only longitudinal diffusion is considered as process causing peak broadening. For this theoretical case plate numbers in the range of 28 000 are expected according to the Einstein equation of the variance as function of time available for diffusion, when a value of about $8 \cdot 10^{-6}$ cm^2/s is taken for the diffusion coefficient and 900 s for the time available for diffusion. We take here the diffusion coefficient typical for aqueous solutions, because the mobility (and therefore the diffusion coefficient) in polymer solutions like that under consideration is changed to a much smaller extent compared to pure aqueous solutions, than the bulk phase viscosity. Whereas a 3% linear PAA solution has a fourteen-fold viscosity compared to water, the mobility of certain ions is reduced only by about 30%, and this reduction is related rather to molecular interactions between the polymer and the solute (as we relate it in the present paper) than to viscosity changes of the solution [17].

4. Conclusions

The application of a charged polymer in fused-silica capillaries allows the separation of non-charged analytes, which migrate by the EOF against the electrophoretic flow of the pseudo-stationary phase. The advantage of this EKC system is clear:

(i) The chemical nature of the uncharged part of the polymer chain allows to adjust a wide range of selectivity, including e.g., chiral recognition; the charged part may be used even for ion-exchange with charged analytes.

(ii) It enables to establish higher speed of analysis compared to CEC with RP particles, where limiting values of about 2.5 mm/s are typical for a field strength as high as 60 000 V/m [18]. Under those electrical conditions fused-silica capillaries produce a three times faster EOF [19]. Besides short analysis time, a broad retention window may be reached. If the mobility of the EOF caused by the capillary wall has the same value (but opposite sign) than the polymer, the latter will in fact be stationary with zero net velocity.

(iii) As the system consists of a solution (with more or less favorable viscosity), it is easily replaceable and simple in manipulation; this means that for each run a novel column can be established.

(iv) As there are no Si–O–C bonds involved, buffers at high pH with fast EOF can be applied.

(v) As the separation is carried out in open capillaries, the tubes can be rinsed in between the analytical runs even with solutions with extreme pH values without destruction.

It must be pointed out, however, that at this preliminary level of development the medium range stability of the polymer as made by the procedure described above is not as high as desirable. Therefore the polymer solution must be replaced after several runs by a freshly prepared one. Another limitation caused by the procedure is the relatively high final salt concentration of the background electrolyte, limiting the applied voltage due to thermal heating. Although this limitation can be easily

overcome, a contribution of the charged polymers to the total current will still remain. The better characterization of the polymer and an improved method for its formation is the topic of current work.

References

[1] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135–143.
[2] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317–328.
[3] C. Fujimoto, Anal. Chem. 67 (1995) 2050–2053.
[4] C. Fujimoto, J. Kino, H. Sawada, J. Chromatogr. A 716 (1995) 107–113.
[5] C. Fujimoto, Y. Fujise, Anal. Chem. 68 (1996) 2753–2757.
[6] J.-L. Liao, N. Chen, C. Ericson, S. Hjerten, Anal. Chem. 68 (1996) 3468–3472.
[7] S. Terabe, T. Isemura, Anal. Chem. 62 (1990) 650–653.
[8] S. Terabe, T. Isemura, J. Chromatogr. 515 (1990) 667–676.
[9] M. Tanaka, T. Ishida, T. Araki, A. Masuyama, Y. Nakatsuji, M. Okahara, S. Terabe, J. Chromatogr. 648 (1993) 469.
[10] M. Castagnola, L. Cassiano, A. Lupi, I. Messana, M. Patamia, R. Rabino, D.V. Rossetti, B. Giardina, J. Chromatogr. A 694 (1995) 463–469.
[11] C. Stathakis, R.M. Cassidy, Anal. Chem. 66 (1994) 2110–2115.
[12] C. Stathakis, R.M. Cassidy, J. Chromatogr. A 699 (1995) 353–361.
[13] B.F. Erim, J. Chromatogr. A 768 (1997) 161–167.
[14] S. Hjerten, L. Valtcheva, K. Elenbring, D. Eaker, J. Liq. Chromatogr. 12 (1989) 2471–2499.
[15] A. Widhalm, C. Schwer, D. Blaas, E. Kenndler, J. Chromatogr. 549 (1991) 446–451.
[16] K. Kenndler-Blachkolm, S. Popelka, B. Gas, E. Kenndler, J. Chromatogr. A 734 (1996) 351–356.
[17] W. Schützner, S. Fanali, A. Rizzi, E. Kenndler, Anal. Chem. 67 (1995) 3866–3870.
[18] S.E. Van den Bosch, S. Heemstra, J.C. Kraak, H. Poppe, J. Chromatogr. A 755 (1996) 165–177.
[19] C. Schwer, E. Kenndler, Anal. Chem. 63 (1991) 1801–1807.