Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 800 (2004) 83–89
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Plastic substrates based separation channels in electromigration techniques

Jana Charvátová [a,*], Zdeněk Deyl [a], Miroslav Klevar [b], Ivan Mikšík [a], Adam Eckhardt [a]

[a] *Institute of Physiology, Academy of Science of the Czech Republic, Vídeňská 1083, 14220 Prague 4, Czech Republic*
[b] *Institute of Chemical Technology, Technická 5, 16628 Prague 6, Czech Republic*

Abstract

Three types of plastic materials (polyester, polyurethane and polymethylmethacrylate) were tested as materials for manufacturing separation columns (polyester and polyurethane capillaries were used) or separation channels (polymethylmethacrylate) in the chip format. A set of 11 fluorescein isothiocyanate amino acid derivatives was used as the test mixture. Using α-cyclodextrin additive to the background electrolyte in the case of the chip separation was also tested. The main problem with all plastic separation media was the selectivity of the separation. The best results, practically identical with bare fused silica capillary, were obtained with the polymethylmethacrylate chip, provided that α-cyclodextrin in a concentration 40 mmol/l was added to the background electrolyte. An important observation was that in SDS containing background electrolyte all the plastic materials used exhibited a distinct electroosmotic flow, which was ascribe to the sorption of the negatively charged constituents of the background electrolyte to the capillary wall. Regarding the order in which the individual components of the test mixture were brought to the detector only a single change was observed. Histidine migrated in the polystyrene and polymethylmethacrylate separation channels more slowly than in the bare silica or polyurethane based capillaries.

Keywords: Plastic separation channels; Fluorescein isothiocyanate amino acid derivatives

1. Introduction

Capillary channels used in standard electromigration techniques are routinely made of fused silica. The present trends in capillary electromigration techniques represented by chip technologies exploit to a considerable extent polymeric substrates for manufacturing the separation channels. Separation on silica or glass based chips requires a rather complicated technology ([1–9], for review see [10]); on the other hand, exploiting polymeric substrates for the preparation of microfluidic devices is much more simple, in particular if the moulding technique is used [11–13]. On the other hand, very little is known about the properties of plastic capillaries (channels) with respect to their behavior in electromigration separations. Typically, capillaries manufactured from uncharged polymeric substrates should be devoid of electroosmotic flow, which, however, is not the case [14]. This effect was assumed to be caused by the sorption of charged ions stemming from the background electrolyte to the plastic capillary wall. As stressed in recent review by Smith [15] amino acids continue to be used as model set of compounds for testing new approaches in separations. The reason for using this category of compounds is obviously the fact that the physico-chemical properties of these compounds are very closes indeed, which implies that if this set of compounds can be separated in a particular system it is rather likely that other categories of compounds could be separated in this system as well.

Capillary electrophoresis of amino acids can be effected both in the free and derivatized form; the separation of underivatized amino acids brings about two problems, namely detection and selectivity. Owing to the absence of a distinct chromophore in the molecule, detection has to be done at very short wavelengths, which is incompatible with most other materials used for the construction of the separation channel except fused silica. The selectivity problem arises from the fact that capillary zone electrophoresis (CZE) separations are based on the mass-to-charge ratio which does not differ too much with most members of the classical set of 20 amino acids. This was attempted to be overcome by using buffer additives like alkylsulfonic acids at acid pH [16,17].

* Corresponding author.

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.10.073

The detection problems are most easily overcome by derivatization. Of the numerous possibilities, separations of dinitrophenyl (DNP)-, dansyl-, and fluorescein isothiocyanate (FITC)-derivatives have been widely explored ([18–20], for review see [15]). However, detection by UV absorbance in channels manufactured from other materials than fused silica can be difficult even if the derivatized amino acids bear a distinct chromophore. The other problem is that most of the derivatization reagents yield side products upon the derivatization reaction the chemical nature of which is in most cases difficult to specify. Therefore, the reaction conditions for the derivatization have to be precisely specified [21]. The derivatized amino acids, owing to their limited solubility in water and involvement of the free amino groups in the dramatization reaction have to be separated in the micellar electrochromatography mode: typical examples for FITC and DNS derivatives separations have been described in [5,6]. Regarding FITC derivatives, microchip separation has been reported in [21]; the separation channel was represented by a simple cross manufactured in a glass plate; the channel was 7 cm long (4.8 cm separation length), rectangular profile 60 μm × 15 μm. The separations were done at 3000 V per the active length of the channel, detection by fluorescence at 470–490/520 nm (excitation/emission wavelength, respectively), using 100 mmol/l borate buffer containing 30 mmol/l SDS and 20 mmol/l γ-cyclodextrin (pH 9.4). The running times for different amino acids ranged from 60 to 110 s. Though nearly baseline separation of amino acid enantiomers was observed with all 21 amino acids tested, the separation of at least selected amino acids was not shown, obviously all the amino acids comigrated or showed only poor resolution. On the other hand, the authors declare that the enantioselectivity in all cases tested was better in the chip mode compared to standard capillary separation.

In this investigation, we have tested three different polymeric substrates for the separation of an 11-membered test mixture of FITC derivatized amino acids. Polyurethane, polyester and polymethylmethacrylate materials were used for manufacturing the separation channels. The first two (polyurethane and polyester) were used as tubing mounted to a standard capillary electrophoresis apparatus; polymethylmethacrylate was used in a chip format.

2. Materials and methods

2.1. Chemicals

Boric acid, sodium hydroxide and model set of amino acids were obtained from Lachema (Brno, Czech Republic), sodium dodecylsulphate (SDS) and fluorescein isothiocyanate were purchased from Sigma–Aldrich (St. Louis, MO, USA). All solutions were prepared from purified water through the Millipore Q system (Bedford, MA, USA) and prior to analysis filtered again with the 45 μm filter (Bedford, MA, USA).

2.2. Derivatization procedure

The derivatization of amino acids with FITC was performed according to [21]. A 2.5×10^{-3} mol/l solution of FITC was prepared in acetone. Solutions containing 2×10^{-3} mol/l of each amino acid were prepared in a buffer composed of 20 mmol/l boric acid, pH 10, adjusted with 10 mol/l sodium hydroxide solution. A 100 μl of each of the amino acid solution was allowed to react with 50 μl of FITC solution overnight in the dark at room temperature. Derivatized amino acids were diluted in the run buffer 10 times prior to injection. The background electrolyte used in all separations was composed of 100 mmol/l boric acid, 30 mmol/l SDS, pH 9.4 adjusted with 10 mol sodium hydroxide solution.

2.3. Instrumentation

All experiments were performed using a Beckman P/ACE 5500 system (Fullerton, CA, USA) equipped with laser-induced fluorescence (LIF) detection. The excitation wavelength of the detector was 488 nm; the emission wavelength was 520 nm. The instrument was controlled, data collected and manipulated by Beckman P/ACE Station program Version 1.21. Specifications of separation systems

Table 1
Specification and arrangement of the separation systems used

Separation system	i.d./o.d. (μm)	Total length (cm)	Effective length (cm)	Arrangement	Note
Fused silica	50/375	47	40	Standard	–
Fused silica	75/375	47	40	Standard	–
Polyurethane (PU)/fused silica	65/420	43.1	36.3	6.5/6.8 cm silica extensions (inlet/outlet), 28 cm PU part	For tightening of silica extensions see Fig. 1
	75/375				
Polyester	80/400	26.0	19.2	Pure polyester	–
Polymethylmethacrylate (PMMA) chip		21.5	19		For arrangement see Fig. 2

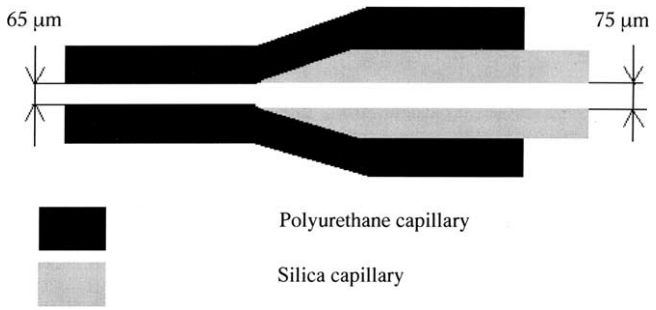

Fig. 1. Schematic representation of the joining point of the polyurethane capillary with the fused silica extensions.

used during experiments are shown in Table 1. Fused silica capillaries were obtained from Composite Metal Services (Ilkley, Yorkshire, UK). Plastic capillaries (polyurethane and polyester) were obtained from Polymicro Technologies Inc. (Phoenix, AZ, USA). The separations were performed at 20 kV or 10 kV, and 25 °C or 15 °C, respectively.

2.3.1. Adaptation of CE system for the use of the polyurethane capillary

As for the polyurethane capillary, it was not possible to record the fluorescence of the separated compounds directly on the column, the polyurethane capillary was provided by a fused silica extension of standard fused silica capillary on which the detection window was created by a standard procedure. Similar extension was used also on the inlet end of the plastic column. The joining point is schematically shown on Fig. 1. The separation length of the polyurethane separation column was 36.3 cm; the length of silica extensions was 6.5 cm on the inlet side, and 6.8 cm on the outlet side, respectively. The joining point was manufactured in such a way that disturbances in the background electrolyte flow were minimized.

2.3.2. Adaptation of the CE system for the use of polyester capillary

On the contrary to the polyurethane column, this capillary allowed direct fluorescence detection. However, the capillary was much too soft to allow penetration of the capillary through the septum of the sample and background electrolyte vials. Therefore, both the inlet and the outlet ends of the capillary were provided with rigid sleeves (457 μm i.d., Supelco, Bellefonte, PA).

2.3.3. Microchip system used

The channel configuration of the chip was a simple cross, which was operated under similar conditions as described by Chen and Chen [13]. Briefly, two identical home-made power sources were used to supply the loading and separation voltages. (Sample loading at 1.3 V; i.e. 60 V/cm, running conditions 7.5 kV, i.e. 350 V/cm.) The channel dimensions were roughly 250 μm × 75 μm, the depth of the cross was not assayed. Sample loading was effected by applying the sample loading voltage between positions 3 and 4 (Fig. 2) for 10 s, for separation the separation voltage was applied between positions 1 and 2 of the separation cross. Detection was done by an epifluorescence system using 50 W high-pressure mercury lamp (Hammamamtsu, Tokyo, Japan) as excitation and radiation source. A Leica episcopic fluorescence microscope (DAS Microscop DMLS, Leica, Heerbrugg, Switzerland) was used. The filter system used for excitation and emission measurements consisted of a 450–490 nm excitation filter, a dichroic mirror (510 nm) and a barrier filter (515 nm). Leica PL Fluotar long distance working objective (magnification: 63×, aperture: 0.70) was built in the detection system to collect the emitted fluorescence by a Hammamatsu silicon photodiode equipped with model HC 220-21 amplifier. Signal monitoring was recorded by a Shimadzu integrator C-R6A, Chromatopack, Shimadzu, Tokyo, Japan.

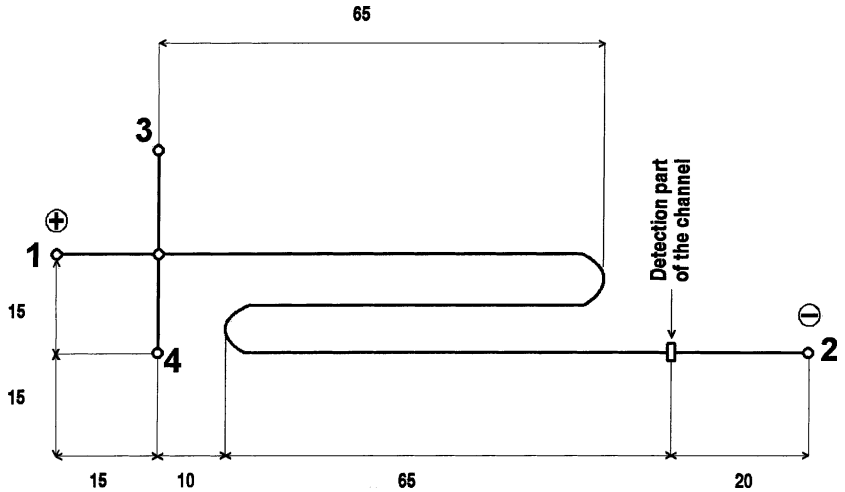

Fig. 2. Setup and dimensions (mm) of the polymethylmethacrylate separation assembly. (1, 2) Separation path and (3, 4) sample loading.

Specification of the individual separation systems is summarized in Table 1.

3. Results and discussion

Using bare fused silica capillary (as the basis for evaluation of the separation in a plastic capillary) the results shown in Fig. 3 were consistently obtained. Eleven standard amino acids were baseline separated with bare fused silica capillary (50 μm i.d.); the separation window spread between 5.5 and 14 min running time, which is practically the same as that reported in [2]. Similar, but slightly worse results were obtained using silica capillary with the 75 μm inner diameter (Fig. 4). As can bee seen, the separation window slightly narrowed (5–12 min) and also the resolution became lower as compared to the 50 μm silica column. FITC amino acid derivatives Trp and His comigrated and were only partly resolved from the preceding peak of Ile. Resolution of Ala form Ser was worse in 75 μm capillary as well.

With polyester capillary the separation obtained (Fig. 5) was considerably faster, however, the separation window

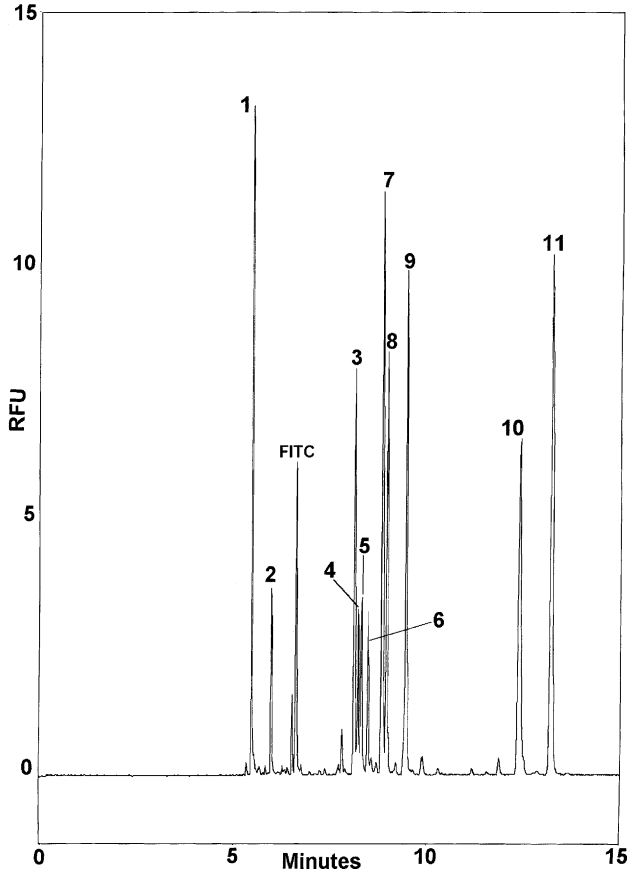

Fig. 3. Typical separation of the standard mixture of 11 FITC amino acid derivatives. Separation conditions: fused silica capillary, 47 cm (40 cm to the detector), 50 μm i.d., 20 kV, 100 mmol/l borate buffer, pH 9.4, 30 mmol/l SDS. Peak identification: 1-L-Arg, 2-L-Lys, 3-L-Ile, 4-Trp, 5-His, 6-Thr, 7-Ser, 8-Ala, 9-Gly, 10-Glu, 11-Asp.

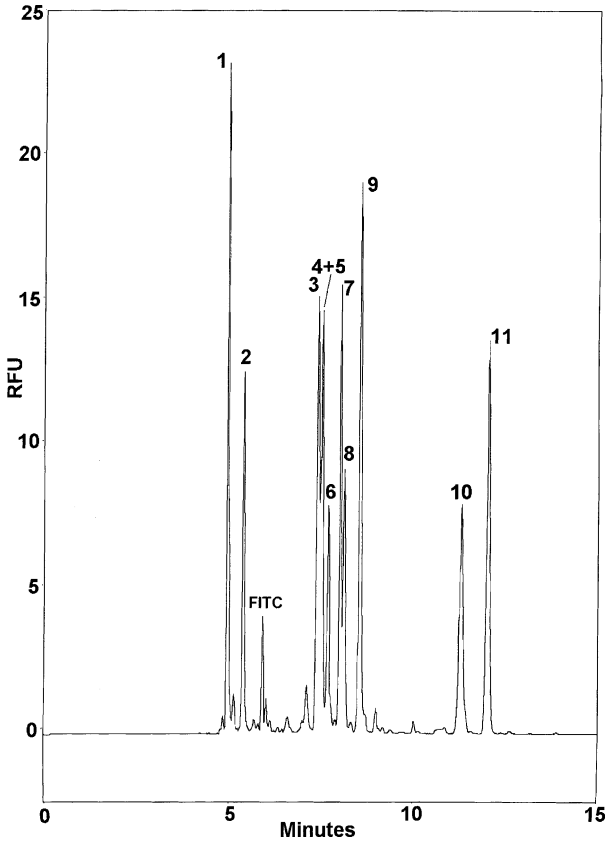

Fig. 4. Typical separation of the standard mixture of 11 FITC amino acid derivatives. Separation conditions: fused silica capillary, 47 cm (40 cm to the detector), 75 μm i.d. Other conditions as in Fig. 3.

represented only 85% of that seen with bare fused silica. This apparently reflects the fact that with the polyurethane capillary we had to use only half of the running voltage (10 kV per capillary as compared to 20 kV in bare silica) in order to avoid distortion of the capillary channel owing to excessive Joule heat production. Another factor involved is that the capillary used was for technical reasons only 19.2 cm long (effective length). The selectivity achieved was considerably lower as Ile + Trp, Ser + Ala and Gly + His were fused in single peaks. The faster appearance of the first peak in the profile (Arg) reflects the fact that a shorter capillary was used. On the other hand, it has to be emphasized that the polyester capillary exhibited distinct electroosmotic flow though it did not bear any chargeable residues on its surface. The only explanation for this effect (as already mentioned in Introduction) is that the surface charge of the capillary resulted from the sorption of charged anions present in the background electrolyte (e.g. SDS). If there was no electroosmotic flow in the polyester capillary system, there should be no peaks visible if the normal polarity mode (i.e. plus pole attached to the inlet side of the column) was used, as the SDS micelles possess abundant negative charge and migrate with a high speed towards the anode. If we compare the sequence, in which the individual peaks came in front of the detector window in the bare silica and

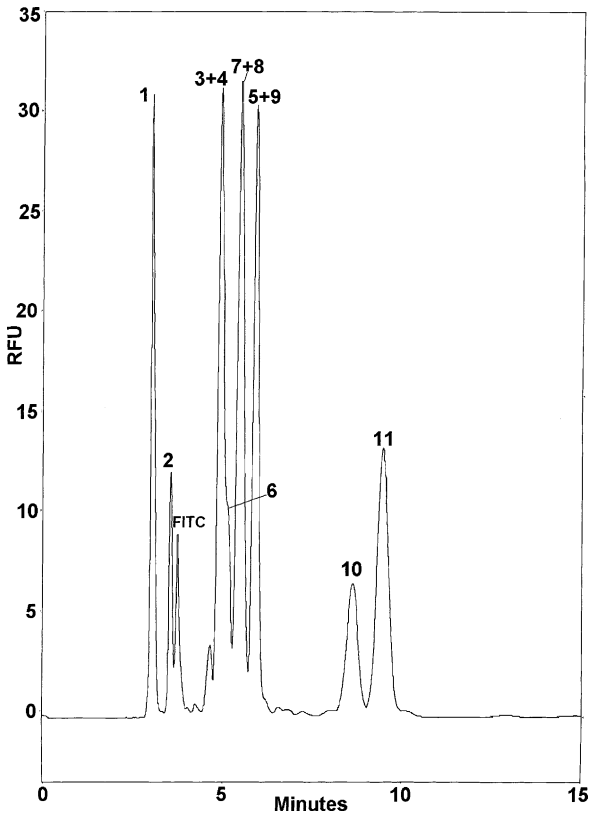

Fig. 5. Separation of the standard mixture of 11 FITC amino acid derivatives. Separation conditions: polyester capillary (26 cm, 19.2 cm to the detector, 80 μm i.d.). Other conditions and peak identification as in Fig. 3 except that 10 kV was applied during separation.

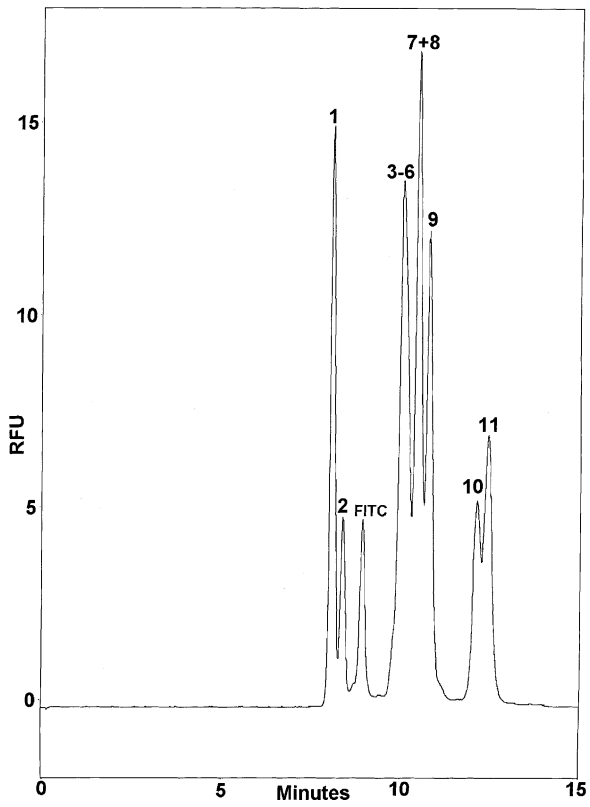

Fig. 6. Separation of the standard mixture of 11 FITC amino acid derivatives. Separation conditions: polyurethane capillary 28 cm, two fused silica extensions (6.5/6.8 cm, inlet/outlet, 75 μm i.d.), effective length of the system 36.3 cm. Other conditions and peak identification as in Fig. 3 except that 10 kV was applied during separation.

polyester capillary, a distinct retardation of histidine peak can be seen in the polyester column. All the other peaks (though some of them were fused) were brought in front of the detector in the same order in both capillary types.

In the polyurethane capillary provided with the silica capillary extensions, the speed with which the individual analytes came to the detector is the slowest of all the three systems tested (Fig. 6). To what extent this is due to a similar ion-sorption as found with the polyester system and to what extent this is due to the fused silica extensions and longer total length of the capillary is difficult to evaluate. The selectivity of this arrangement is rather poor: Lys is only partly resolved from Arg, Thr, Ile, His and Trp form a fused peak, the same situation was found with Ala + Ser, glycine is only partly resolved from the preceding fused peaks of Ala + Ser and also glutamate and aspartate are only partly separated. The separation window (comparing the differences in migration of the fastest and slowest peaks) represents only 62% of that seen with bare silica capillary and 85% of that observed with the polyester column.

Obviously, the main problem with our set of amino acids when using polymeric capillaries is selectivity. It seams feasible to propose that the poor selectivity in plastic based capillaries stems from the fact that a portion of the SDS micelles was adsorbed to the capillary wall and distorted the separation equilibrium between the solute–free micelles–adsorbed micelles resulting in a decreased selectivity. Admittedly, separations run in fused silica and plastic capillaries were run at different voltages per cm of the capillary, however, increasing the run voltage in the plastic capillaries was limited by adverse heating effects (Table 2).

Polymethylmethacrylate was tested in a chip format. It is worthwhile to emphasize that the length of the channel was very close to that of the polyester capillary. The results are seen if Fig. 7. Arg and Lys were clearly separated from each other as well as from the FITC peak, it should be

Table 2
Separation voltages per centimeter of individual separation systems

Separation system	Total length (cm)	Applied voltage (kV)	Voltage/length (V/cm)
Fused silica (50 μm i.d.)	47	20	425
Fused silica (75 μm i.d.)	47	20	425
Combined PU/fused silica	43.1	10	232
Polyester	26	10	385
PMMA chip	21.5	7.5	350

PU: polyurethane; and PMMA: polymethylmethacrylate.

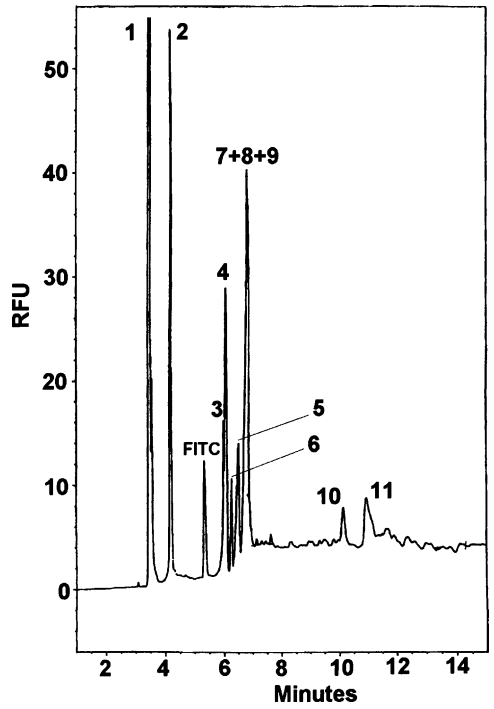

Fig. 7. Separation of the standard mixture of 11 FITC amino acid derivatives on a polymethylmethacrylate chip (for the setup of the chip see Section 2). Conditions: 100 mmol/l borate, pH 9.4 containing 30 mmol/l SDS, 10 kV per chip, active length of the channel 19 cm, total length 21.5 cm. Peak identification as in Fig. 3.

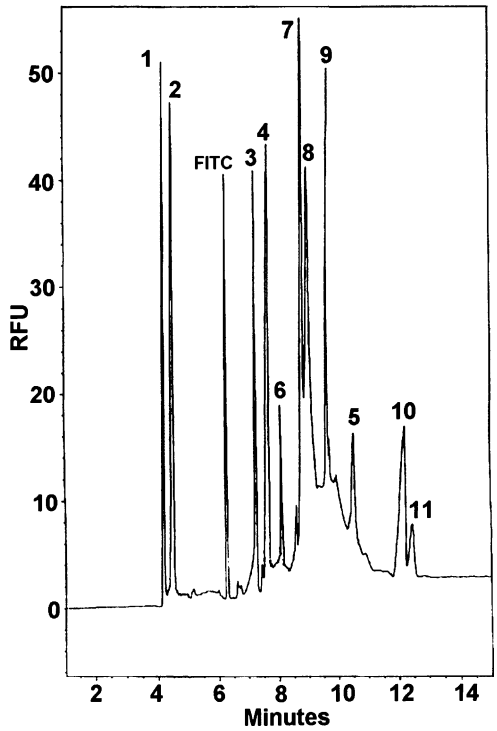

Fig. 8. The separation obtained with background electrolyte containing α-CD. 100 mmol/l borate, pH 9.3, 20 mmol/l SDS and 40 mmol/l α-CD. Peak identification and other conditions as in Fig. 7.

noted that the separation of FITC from Lys was much better than that obtained with polyester capillary. In the section of the electropherogram densely populated with peaks, Ile and Trp were partly separated (comigrated in the polyester capillary), Thr was separated clearly from the neighbouring peaks (in the polyester capillary it was only partly separated from the fused peak of Ile and Trp). The peak of His was clearly observed ahead of the fused peaks of Ala, Gly and Ser. Glu an Asp acid peaks were the last coming in front of the detector window. This separation selectivity was considerably better than that seen if the polyester tubing was used for manufacturing of the separation channel, however, it was still worse than the separation obtained in bare silica capillary. With the polymethylmethacrylate chip a further improvement was achieved by adding α-cyclodextrin (Fig. 8) as reported in [21]. The only difference to the published separation conditions was that the background electrolyte used for the α-cyclodextrin runs was 100 mmol/l pH 9.3 borate buffer to which 20 mmol/l SDS and 40 mmol/l cyclodextrin was added. In the case of the front of the electropherogram, the first three peaks offered practically identical pattern no matter whether α-cyclodextrin was present in the background electrolyte or not. The main difference regarding the central heavily populated part of the electropherogram, namely Ile, Gly and Thr were baseline separated, Ser and Ala were only partly separated and the rest of the electropherogram was separated quite clearly. The result was comparable to that obtained with bare silica except the position of the His peak which was displaced to longer migration time as seen with polystyrene (but no polyurethane) separation column. This location of the His peak persisted no matter whether α-cyclodextrin was present in the background electrolyte or not. The separation window was in the system exploiting polymethylmethacrylate slightly shorter (than that seen in the bare silica column). As expected, after the experience with other plastic-made separation columns also the polymethylmethacrylate channel displayed a distinct electroosmotic flow (actually the separation was even faster than in the bare silica or in the polyurethane made capillaries and was comparable with the speed seen in the polyester column).

4. Conclusions

1. At least polyester and polyurethane capillaries can be used in capillary electrophoretic systems.
2. Generally, the selectivity with plastic based capillaries is worse in comparison to bare silica.
3. At least with polyester based capillary system a distinct electroosmotic flow was observed which was ascribed to the sorption of negatively charged constituents (SDS) of the background electrolyte to the capillary wall.
4. In specified separations (in our case one amino acid, i.e. histidine) out of 11 members of the test mixture

was displaced in its elution in the polyester capillary exploiting system.
5. It is proposed that the decreased selectivity results from the sorption of the micellar pseudophase to the capillary wall (see also item 3).
6. Separation channel created in the polymethylmethacrylate plate displayed also a distinct electroosmotic flow comparable to that seen in the polyester tubing.
7. Addition of α-cyclodextrin to the background electrolyte in the polymethylmethacrylate exploiting system resulted in a considerable improvement of selectivity.

Acknowledgements

This work was supported by the Grant Agency of the Czech Republic (Grant No. 203/01/0288) and by the Academy of Sciences of the Czech Republic (AVOZ 5011 922).

References

[1] A.T. Woolley, R.A. Mathies, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 11348.
[2] K. Arlt, S. Brandt, J. Kehr, J. Chromatogr. A 926 (2001) 319.
[3] Z.H. Fan, D.J. Harrison, Anal. Chem. 66 (1994) 177.
[4] S.C. Jacobson, R. Hergenroder, A.W. Moore, J.M. Ramsey, Anal. Chem. 66 (1994) 4127.
[5] L.B. Koutny, D. Schmalzing, T.A. Taylor, M. Fuchs, Anal. Chem. 68 (1996) 18.
[6] S.C. Jacobson, R. Hergenroder, L.B. Koutny, J.M. Ramsey, Anal. Chem. 66 (1994) 1107.
[7] K. Fluri, G. Fitzpatrick, N. Chiem, D.J. Harrison, Anal. Chem. 68 (1996) 4285.
[8] P.C. Simpson, D. Roach, A.T. Woolley, T. Thorsen, R. Johnston, G.F. Sensabaugh, R.H. Mathies, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 2256.
[9] K.L.K. Liu, K.L. Davis, M.D. Morris, Anal. Chem. 66 (1994) 3744.
[10] V. Dolnik, S. Lin, S. Jovanovich, Electrophoresis 21 (2000) 41.
[11] C.S. Effenhauser, G.J.M. Bruin, A. Paulus, M. Ehrat, in: Proceedings of the 2nd International Symposium on Miniaturized Total Analysis System, μTAS'96, Basel, 19–22 November 1996.
[12] R.M. McCormick, R.J. Nelson, M.G. Alonso-Amigo, D.J. Benvegnu, H.H. Hooper, Anal. Chem. 69 (1997) 2626.
[13] Y.H. Chen, S.H. Chen, Electrophoresis 21 (2000) 165.
[14] Z. Deyl, unpublished results.
[15] J.T. Smith, Electrophoresis 20 (1999) 3078.
[16] M.J. Thornton, J.S. Fritz, C.W. Klampf, J. High Resolut. Chromatogr. 20 (1997) 647.
[17] H. Wang, J.-L. Gu, H.-F. Hu, T.H. Ding, R.N. Fu, Fresenius J. Anal. Chem. 361 (1998) 419.
[18] K. Takizawa, H. Nakamura, Anal. Sci. 14 (1998) 925.
[19] B. Soontornniomleij, S. Chen, E. Minnoor, D. Pietrzyk, J. Liq. Chromatogr. Relat. Technol. 21 (1998) 2957.
[20] E. Skocir, M. Prosek, M. Oskomic, Chromatographia 44 (1997) 267.
[21] I. Rodríguez, L.J. Lin, S.F.Y. Li, Electrophoresis 21 (2000) 211.