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Novel applications for organic polymer capillaries

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Abstract

Applications of CE separations with organic polymer capillaries are shown. Capillaries made from poly(methyl methacrylate) (PMMA) and polyether ether ketone (PEEK) show extremely good hydrolytic stabilities. Separations of sugars, amino acids and peptides could be achieved at high pH values. With a fluorescence detector it was possible to detect the analytes on-column, whereas for UV detection and with PEEK capillaries a detection window of fused silica had to be installed. With those capillaries it was possible to apply very high field strengths in the separation of inorganic cations via indirect UV detection. © 1998 Elsevier Science B.V.

Keywords: Capillary columns; Poly(methyl methacrylate) capillaries; Polyether ether ketone capillaries; Derivatization, electrophoresis; Detection, electrophoresis; Polymer capillaries; Sugars; Amino acids; Peptides

1. Introduction

Fused-silica capillaries have become the standard capillaries used for capillary electrophoresis, although some inconveniences have to be coped with. Besides their fragility, the affinity for protein adsorption can cause some problems in the analysis of biopolymers. Permanent wall coatings have been developed to improve these separations [1,2]. Therefore organic polymer capillaries have been proposed for use in CE [3,4]. It has been shown that hydrophilic organic polymer capillaries gave higher efficiencies in protein separations than untreated fused silica. After surface modification with poly(vinyl alcohol), hydroxyethylcellulose or poly(ethylene glycol), even higher efficiencies were achieved [5]. Contrary to materials like nylon or polyesters, that allow facile coating procedures, polyether ether ketone (PEEK) and poly(methyl methacrylate) (PMMA) are extremely difficult to modify chemically. Therefore in this work, a different approach was chosen. The drawback of small reactivity to derivatization reactions was used for separations under extreme conditions. As especially PMMA has shown to be extremely stable in aqueous systems (hydrolysis only occurs at very high pressures and temperatures [6,7]), separations in highly alkaline media were investigated. The second topic of interest in this work was the application of other detection methods to organic polymer capillaries. With the exception of PMMA and PTFE, which are not completely opaque in the low UV region, no organic polymer capillaries can be used for on-column UV detection. To use these capillaries with the standard CE equipment, a fused-silica detection window has to be coupled with the polymer capillary [8].

In addition to this well-established detection method, sensitive on-column detection was of great interest. We examined the application of on-column fluorescence detection.

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2. Conditions

2.1. Instrumentation

Separations using UV detection were performed in a Beckman P/ACE 2050 capillary electrophoresis system (Beckman Instruments, Munich, Germany) with Beckman Gold V 7.12 instrument control software. For the fluorescence experiments a modular system was used consisting of a Lauerlabs Prince CE system (Lauerlabs, Emmen, The Netherlands) and an HP 1046A fluorescence detector (Hewlett-Packard, Waldbronn, Germany), whose detection cell was adapted to capillary electrophoresis. Data were collected and evaluated using HP Chem Station software.

2.2. Materials

All buffer substances as well as the cation samples, amino acids, peptides, 2-aminoanthracene, glucose and maltose were purchased from Fluka (Buchs, Switzerland). The oligomaltosides were purchased from Sigma (St. Louis, MO, USA). The dansylated amino acids were purchased from Serva (Heidelberg, Germany).

2.3. Capillaries

PMMA capillaries (365 μm O.D.×50 μm I.D.) were obtained from Drummond Scientific (Broomall, USA). They could be used without further modification for fluorescence detection.

PEEK capillaries (500 μm O.D.×65 μm I.D.) were purchased from Upchurch Scientific (Oak Harbor, USA). A fused-silica detection window had to be introduced to allow UV detection [5].

New capillaries were treated with 1 *M* NaOH and water for 15 min prior to use.

2.4. Pre-column derivatization

Amino acids and peptides were derivatized with *o*-phthaldialdehyde (OPA) and mercaptoethanol to form fluorescent isoindole derivatives [9]. Typically 300 ppm solutions of the amino acids and peptides were derivatized with 2 mmol OPA dissolved in 5 ml of isopropanol and 50 ml of 10 m*M* sodium tetraborate in water (pH 10.5 adjusted with sodium hydroxide), to which 200 μl of mercaptoethanol was added 10 min before derivatization. Samples were injected after 2 min reaction time at ambient temperature.

Carbohydrates were tagged using the classical reductive amination procedure [10–12] for the derivatization with 2-aminoanthracene. Two hundred and fifty μl of 500-ppm solutions of the sugars were treated with 125 μl of 1000 ppm of 2-aminoanthracene in MeOH–HOAc (92:8, v/v) and 250 μl of 10 000 ppm $NaBH_3CN$. The reaction mixture was treated in a microwave oven for 5 min at 130 W and was injected after dilution (1:10) with 4 *M* urea.

3. Results and discussion

3.1. UV detection using a fused-silica detection window

3.1.1. Indirect UV detection of cations with PEEK capillaries

For the separation of alkaline and alkaline-earth metal cations including ammonium, an indirect detection scheme based on pyridine as background electrolyte was chosen [13]. To improve the selectivity for the alkaline-earth elements, glycolic acid was added as complexing agent. To improve the ammonium potassium separation 18-crown-6 ether was added. Different separation voltages were applied to demonstrate whether Joule heating influences the separation efficiency. PEEK has a significantly lower thermal conductivity than fused silica (2500 vs. 6000 W/mK), thus problems with diffusion due to poor heat dissipation might be expected at relatively high voltages. As can be seen from Fig. 1, increasing separation voltage led neither to lower efficiencies nor to deterioration of the capillary material. Therefore the analysis time could be reduced by a factor of 2 in doubling the field strength. As can also be seen, no additional phantom peaks appeared indicating that no influence of polymer additives from the preparation and extrusion process could be observed.

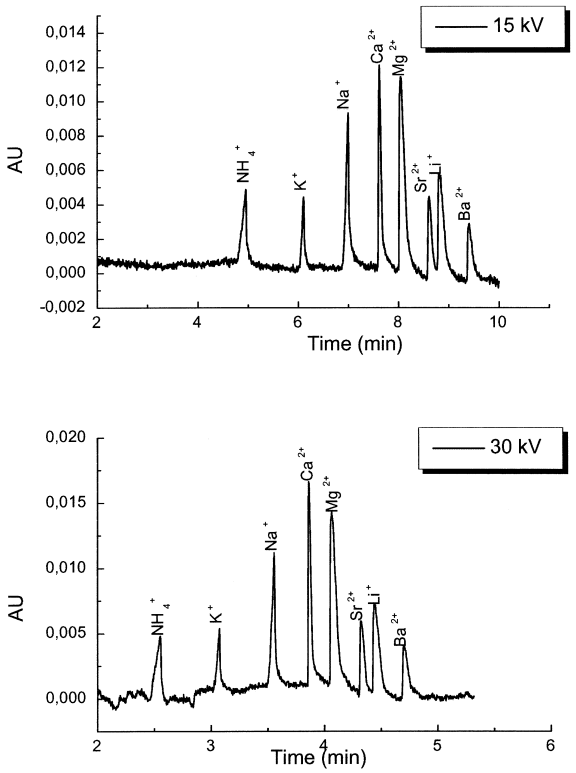

Fig. 1. Indirect detection of cations: capillary, PEEK 57/50 cm×75 μm; detection, 254 nm; cations 20 ppm each; buffer, 9 m*M* pyridine, 12 m*M* glycolic acid, 5 m*M* 18-crown-6, pH 3.6.

3.1.2. Separation of peptides under highly alkaline conditions

Theoretically two separation modes can be used for the analysis of amino acids and peptides by capillary zone electrophoresis: cationic mode at low pH and anionic mode in alkaline buffers. As has been demonstrated for the separation of amino acids, anionic mode leads to higher selectivities [14]. This effect could also be observed for di- and tripeptides. In acidic buffers, three peaks of the seven peptides in the sample could be observed, indicating acidic neutral and basic compounds. As can be seen in Fig. 2, with an ammonia-based buffer with a pH of 11.7, a good separation of six of the seven peptides was achieved, although Phe–Gly could not be separated entirely from Phe–Ala. It was observed that no deterioration of the capillary material occurred at this high pH, making this electrolyte system ideally suited for peptide separation using CE–electrospray

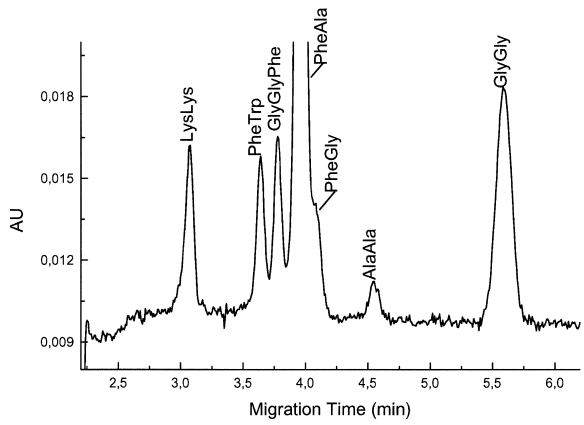

Fig. 2. Separation of seven peptides: capillary, PMMA 40/47 cm×95 μm; buffer, 100 m*M* NH_3, pH 11.7; detection, 214 nm; *U*=25 kV.

ionization (ESI)-MS, since the volatile systems presented so far do not give the necessary selectivities.

3.2. Detection without detection window

It has been shown that a detection window does not noticeably influence either separation or overall electroosmotic flow [15]. However, the installation of a detection window may be inconvenient. Therefore, other detection methods which do not imply modifications of the capillary were examined. The most promising approaches were on-column fluorescence [16], laser-induced fluorescence and end-column CE–ESI-MS. PMMA capillaries are not transparent in the UV region, however at wavelengths above 250 nm excitation for fluorescence detection is possible. In the following, three applications for direct on-column fluorescence detection with PMMA capillaries are shown.

3.2.1. Fluorescence detection of amino acids and peptides

Derivatization with orthophthaldialdehyde has the advantage that the fluorophore is only formed in the derivatization reaction [17]. Thereby the derivatization reagent does not need to be removed from the products. The isoindole derivative formed could be detected through PMMA, as demonstrated in Fig. 3 by the corresponding excitation and emission spec-

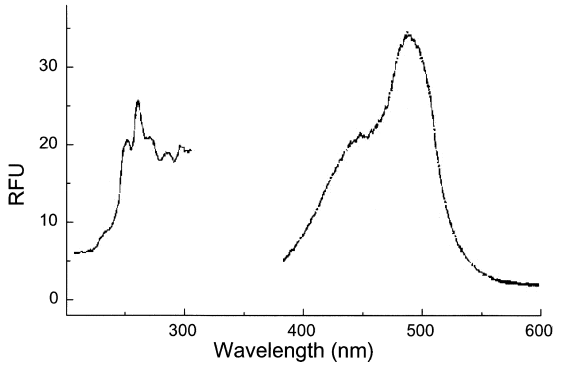

Fig. 3. Fluorescence spectra for OPA amino acids in PMMA capillaries.

tra. Using micellar electrokinetic chromatography, the derivatized amino acids could be separated and detected on-column at λ_{ex}=255 nm and λ_{em}=486 nm, as shown in Fig. 4. A sample of four dipeptides was derivatized and analyzed in the same way as shown in Fig. 5. Due to lower reactivities, peptides gave lower signals than amino acids. Of course, dansylated amino acids can also be detected on-column at λ_{ex}=257 nm and λ_{em}=494 nm. Fifteen dansylated amino acids were separated. The corresponding electropherogram is shown in Fig. 6. Even the separation of leucine and isoleucine, which is often difficult to achieve, was possible using this method. Isoleucine gave a second peak due to impurities in the reagent.

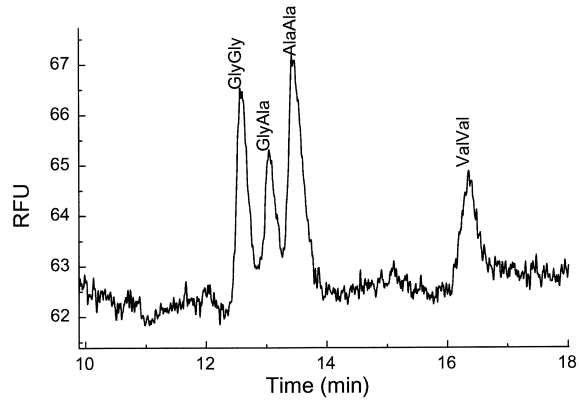

Fig. 5. Separation of four OPA dipeptides in PMMA. Buffer, 50 m*M* borate, 200 m*M* SDS, pH 8.5; *U*=15 kV; *L*=94/80 cm. Detection: fluorescence at λ_{ex}=255 nm; λ_{em}=486 nm.

3.2.2. Fluorescence detection of carbohydrates

Carbohydrates cannot be detected at wavelengths above 190 nm. They are, however, ionized at high pH values and can be separated with highly alkaline buffers. By either deprotonation of the hydroxyl functions or borate complexation, selectivities can be improved considerably using pH values of 11 and higher [18]. In our case the sugars were derivatized with 2-aminoanthracene prior to separation in a borate buffer. A pH of 11.1 was applied to the separation of glucose, maltose and several maltooligoside derivatives, as shown in Fig. 7. The long analysis time stems in part from the required capil-

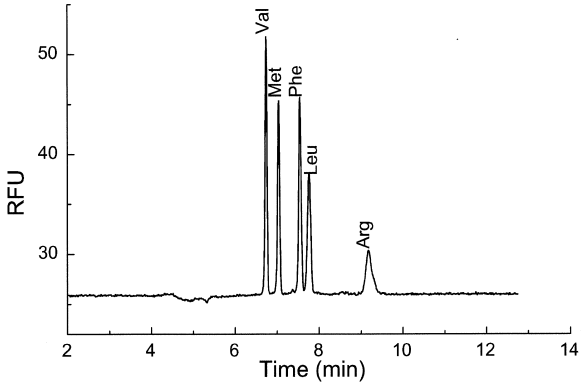

Fig. 4. Separation of five OPA amino acids in PMMA. Buffer, 50 m*M* borate, 100 m*M* sodium dodecyl sulfate (SDS), pH 8.5; *U*=20 kV; *L*=94/80 cm. Detection: fluorescence at λ_{ex}=255 nm; λ_{em}=486 nm.

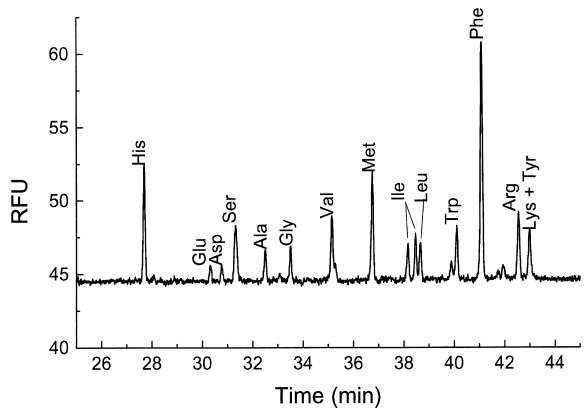

Fig. 6. Separation of 15 dansylated amino acids in PMMA. Buffer, 50 m*M* borate, 200 m*M* SDS, pH 8.5; *U*=18 kV; *L*=104/90 cm. Detection: fluorescence at λ_{ex}=257 nm; λ_{em}=494 nm.

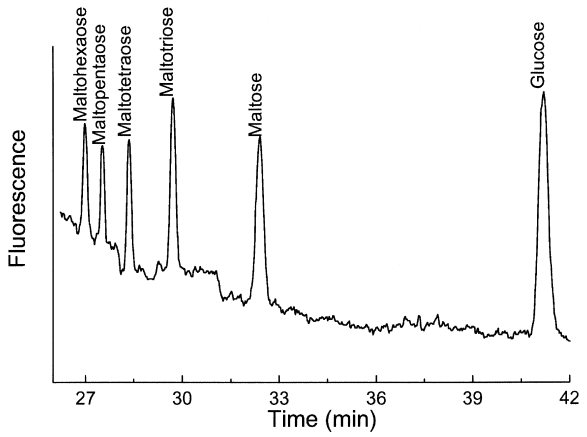

Fig. 7. Separation of oligomaltosides in PMMA. Buffer, 200 m*M* borate, pH 11.1; U=15 kV; capillary, PMMA 60/46 cm×50 μm. Detection: fluorescence at λ_{ex}=263 nm; λ_{em}=495 nm; derivatization with 2-aminoanthracene.

lary length (60 cm) for the connection to the fluorescence detector. In the same way different beer samples were analyzed. As can be seen in Fig. 8, typical patterns of oligomaltoside distribution were found for each sample. Non-alcoholic malt beverages do not show the typical fingerprint of the other beers with maltose as main source of carbohydrates. In this case glucose represents the major sugar compound.

4. Conclusions

Organic polymer capillaries were used for applications under extreme pH conditions, without deterioration of the capillary material or loss of separation efficiency. In addition to UV detection, fluorescence detection could be applied without modification of the capillaries. An important aspect in working with

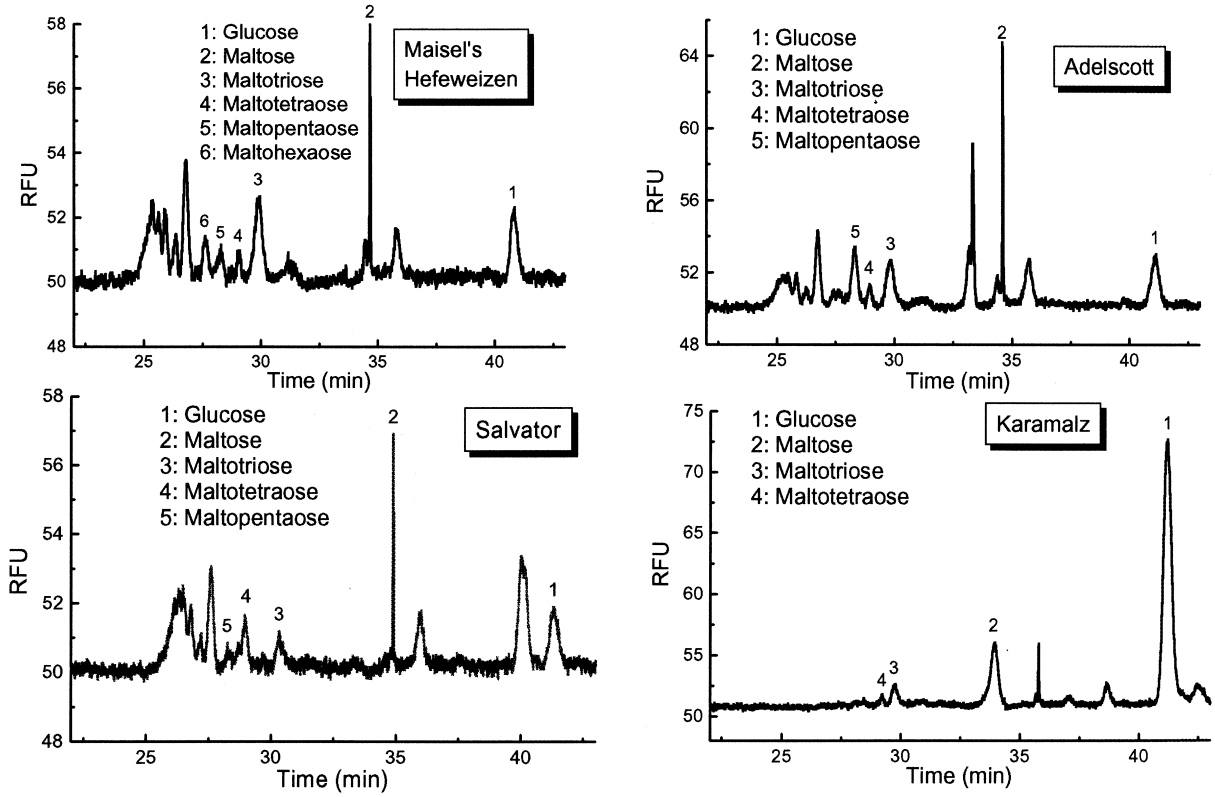

Fig. 8. Determination of oligomaltosides in beers. Buffer, 200 m*M* borate, pH 11.1; U=15 kV; capillary, PMMA 60/46 cm×50 μm. Detection: fluorescence at λ_{ex}=263 nm; λ_{em}=495 nm; derivatization with 2-aminoanthracene.

these capillaries is their use in combination with high pH values and MS coupling.

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