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Bonded dimethylacrylamide as a permanent coating for capillary electrophoresis

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Abstract

A method for coating capillaries for capillary electrophoresis with chemically bonded polydimethylacrylamide has been developed, and the properties of the capillaries have been evaluated. The coated capillaries provided high separation efficiency, $12 \cdot 10^5$ theoretical plates/m was obtained for cytochrome *c*. The electroosmotic flow at pH 8.0 was $10 \cdot 10^{-10}$ to $6 \cdot 10^{-10}$ m^2 V^{-1} s^{-1}. The coated capillaries were quite stable at high pH. At least 150 runs could be done at pH 10 without appreciable performance deterioration. The excellent performance of the coated capillaries was illustrated by separation of basic proteins, acidic proteins, 9-fluorenylmethyl chloroformate-derivatized neurotransmitter amino acids, peptide reference mixtures and peptides digested from a bacteria protein.

Keywords: Coated capillaries; Polydimethylacrylamide; Proteins; Peptides; Amino acids

1. Introduction

It has for a long time been recognized that in order to approach ideal conditions for capillary electrophoresis (CE) electroosmotic flow (EOF) and analyte adsorption to the capillary wall should be eliminated [1–4]. The EOF depends on the ζ-potential of the surface double layer at the capillary wall. It can be shown that when the viscosity in a thin layer close to the capillary wall is increased, the ζ-potential will approach zero and thereby the EOF will be decreased [3]. Such an increased viscosity can readily be achieved by coating the capillary surface with a polymer. Coated capillaries have been widely used for the separation of biomolecules by techniques such as capillary zone electrophoresis, capillary gel electrophoresis and capillary isoelectric focusing [1–4]. The need for coated capillaries for CE is now increasing. Such columns would thus be beneficial in several new CE techniques. These include the partial filling technique, reversed migration micellar electrokinetic chromatography (RM-MEKC), sweeping injection for analyte enrichment and affinity CE. Further, coated capillaries open up new possibilities for cationic chiral selectors. Finally, the coated columns can be useful in non-aqueous systems and for on-column reactions. So far, a large number of different coatings has been described. The area has been extensively reviewed [5–12].

In fused-silica capillaries, the EOF driving force is the ionized silanol groups at the capillary surface. These groups are responsible also for the adsorption of analytes, in particular cationic analytes. Surface coating, in most cases, includes a partial saturation or blocking of these active sites. Thus the coating

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serves to diminish EOF as well as analyte adsorption at ionized silanol groups. However, other types of interactions between analyte and capillary surface should also be minimized. This concerns, for example, hydrophobic interaction between non-polar moieties of the analytes with the surface. For such analytes, typically proteins, the coating should have a hydrophilic surface.

Coating can be physically adsorbed or chemically bonded to the surface. A chemically bonded layer is, in general, more stable than a physically adsorbed layer. The adsorbed layer can, on the other hand, easily be rinsed out and then reformed. Chiari and co-workers [13–15] have presented a large number of different adsorbed surface coatings.

It is in many cases necessary to perform separations at a high pH. For chemically bonded coatings, the weakest point is often the linkage to the surface. Silicon–oxygen bonds are relatively easily hydrolyzed at high pH and therefore bonding by means of the hydrolytically more stable silicon–carbon bond has been attempted [7,16,17]. However, also the fused-silica bulk material is prone to hydrolysis at pH above 10.

Coating with polyacrylamide as proposed by Hjertén [2] is the most widely used method. A number of acrylamide derivatives has also been evaluated [18–23]. Chiari et al. presented a systematic investigation of the hydrolytic stability of some different derivatives of polyacrylamide [19]. Recently, a polyacrylamide derivative, polydimethylacrylamide (PDMA), has been successfully applied in CE as a dynamic coating for DNA sequencing [24–26]. This polymer added to the buffer at very low concentration can efficiently suppress EOF and make DNA separation possible using uncoated capillaries. Although dynamic coating is a simple method to eliminate the EOF, it may not be useful for applications such as CE–mass spectrometry (MS). In addition, such a dynamic coating, in general, becomes less effective at pH above 8.3 [19,25].

The aim of this work was to develop a new type of chemically bonded coating that has a good long-term stability, also at high pH, and good reproducibility for broad applications. Different applications with the new type of coated capillaries are demonstrated.

2. Experimental

2.1. Chemicals and reagents

Basic protein standards, α-chymotrypsinogen A (bovine pancreas), lysozyme (chicken egg white) and trypsinogen (bovine pancreas) were from Sigma (St. Louis, MO, USA), ribonuclease A (bovine pancreas) was from Fluka (Buchs, Switzerland), cytochrome *c* (horse heart muscle) was from Aldrich (Gillingham, UK). Acidic protein standards, trypsin inhibitor (soybean), α-lactalbumin (bovine milk), and β-lactalbumin (bovine milk) were from Sigma. HPLC peptide standard mixture H-2016: Gly–Tyr, Val–Tyr–Val, methionine enkephalin, leucine enkephalin, angiotensin II and peptide standard P-2693: bradykinin, bradykinin fragment 1-5, substance P, (Arg^8)-vasopressin, luteinizing hormone releasing hormone, bombesin, leucine enkephalin, methionine enkephalin, oxytocin were from Sigma. Ammonium persulfate (APS), γ-methacryloxypropyltrimethoxysilane (MPT) and *N*,*N*,*N′*,*N′*-trimethylethylenediamine (TEMED) were obtained from Sigma, 9-fluorenylmethyl chloroformate (FMOC) and sodium dodecyl sulfate (SDS) were from Fluka, *N*,*N*-dimethylacrylamide (DMA) (99%), boric acid and tris(hydroxymethyl)aminomethane (Tris) were from Aldrich. Polypropylene filters were from Arbor Technologies (Ann Arbor, MI, USA). Other chemicals used in this work were of analytical grade. Fused-silica capillaries, 50 μm I.D., were obtained from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Apparatus and separation conditions

All CE separations were performed on a HP^{3D} CE capillary electrophoresis instrument (Hewlett-Packard). In uncoated capillaries, a normal polarity, e.g., positive high voltage was applied. In coated capillaries, a reversed polarity (negative high voltage) was applied for separation of negatively charged compounds and a positive high voltage was applied for positively charged analytes. Detection was by direct UV at 204 nm. The diode array detector was scanned from 190 to 400 nm to confirm peak identification.

2.3. Capillary coating procedures

2.3.1. Pretreatment of capillary inner surface

New bare fused-silica capillaries were first cleaned with acetone for 10 min, then flushed with 1 *M* NaOH for 30 min, followed by 0.1 *M* HCl for 10 min, finally washed with water for 10 min. After these washing steps, the capillaries were filled with a solution of 10% (v/v) MPT in acetic acid–acetone (100 μl MPT+450 μl acetic acid+450 μl acetone) and the ends were closed with rubber septum and then kept overnight in room temperature. The capillary was washed with acetone.

2.3.2. Coating procedures

A starting DMA solution was made by mixing DMA with Milli-Q water, then purified by degassing under reduced pressure for 20 min at 45°C in a water bath. This solution could, without deterioration, be kept in freezer for use for at least a week. To a diluted DMA solution, a small volume of 2-propanol (IPA) was added, 50 μl of 10% (v/v) aqueous TEMED and 50 μl of 10% (w/v) aqueous APS, the capillary was then filled with this solution. After closing the ends, the capillary was placed in an oven and mildly heated, 50°C for 2 h for polymerization. After cooling, unreacted monomer was removed by washing with water for 10 min. The capillary was then ready for a test. Newly coated capillaries were flushed with Milli-Q water for 10 min prior to separation and then flushed only with water and buffer between runs. Samples were normally introduced by pressure (50 mbar, 10 s). Other separation conditions are given in the corresponding figure legends and tables.

2.4. Measurement of electroosmotic flow

The EOF was measured from pH 2 to 10 using a 20 m*M* phosphate–NaOH buffer. 0.1% (v/v) aqueous DMF was used as a neutral maker and detected at 200 nm. A new fused-silica capillary was first flushed with 0.5 *M* NaOH for 60 min, then with water for 20 min. Before measuring EOF at different pH values, the capillary was flushed with 0.5 *M* NaOH for 2 min and 1 min with water and 10 min with buffer. In the case of a coated capillary, it was only flushed with buffer for 10 min. The direct measurement of EOF on the coated capillary was first attempted as for the uncoated capillary. However, no EOF was observed within 4 h, even injecting DMF from the detector side using reversed polarity mode did not give any peak, thus indicating a very small EOF in the coated capillaries. In order to measure such small EOF, a rapid indirect method, described by Williams and Vigh [27], was used. This method can be used for the rapid measurement of EOF values as low as $5\cdot10^{-10}$ m^2 V^{-1} s^{-1}. The EOF was calculated from Eq. (1) using data obtained by injecting the neutral marker three times:

$$\mu_{eo} = \frac{L_t L_d (t_{N_3} + t_{N_1} - 2t_{N_2})}{V(t_{mig} - t_{ramp\text{-}up}/2 - t_{ramp\text{-}down}/2)(t_{N_3} + t_{inj}/2 - t_d)} \quad (1)$$

Here t_{N_1}, t_{N_2}, t_{N_3}, are three neutral marker bands passing detector window by pressure mobilization, L_t and L_d are total capillary length and the length from injection point to detector, respectively, V is the high voltage applied for electrophoretic separation, t_{mig} is the time during electrophoretic separation, $t_{ramp\text{-}up}$ and $t_{ramp\text{-}down}$ are the times it takes to linearly change the applied potential between 0 and V, t_d is the time delay from the pressure mobilization step to the start of the data acquisition step.

3. Results and discussion

3.1. Synthesis

Polyacrylamide has for a long time been used as a medium in electrophoresis. In this context the length of the polymer chains influence the performance and synthesis variables affecting chain length have been studied. Thus in order to prepare low-viscosity entangled polymer networks Grossman used IPA as a chain transfer agent to reduce polyacrylamide chain length [28]. Gelfi et al. used 2-propanol and elevated reaction temperatures to prepare relatively short length polyacrylamide chains for use as molecular sieves in capillary zone electrophoresis [29]. Also

the monomer concentration in the reaction mixture has a great influence on the polymer chain length. However, the viscosity of the reaction solution limits the useful concentration. A too viscous solution cannot be injected or extruded from the capillary.

For the coating of capillary walls with polyacrylamide, Barberi et al. conclude that longer chains would be preferable [30], these would cover the surface more completely and the viscosity at the wall would be higher. Cifuentes et al. made an investigation of how the properties of polyacrylamide depended on the synthesis of the polymer. It was experimentally found that for a given ratio of TEMED and APS, there was a minimum effective concentration of monomer above, which all the coatings obtained behaved similarly. Below this concentration polyacrylamide coatings of very poor CE performance were obtained [31]. It seems that the poor coatings suffered from non-uniformity and incomplete coverage of the silica surface [30,32].

In 1998 Madabhushi presented quite successful results regarding DNA separations on capillaries dynamically coated with polydimethylacrylamide [24]. This was later shown also by Ren et al. [25]. For the synthesis, Madabushi and Ren et al. used methods that possibly would lead to relatively short chain lengths. Reactions were thus at 50°C in the presence of 2-propanol. This approach was followed in this work the difference was that we attempted to achieve chemical bonding to the surface.

The influence of polymer chain length on capillary performance was studied. For that purpose we prepared a set of capillaries with different concentrations of monomer and 2-propanol. The number of theoretical plates/m was used as a response; the plate number is indicative of possible band broadening. We found that the highest plate numbers were achieved when a low monomer concentration in the presence of IPA had been used in the polymer synthesis, Table 1. However, the lowest EOF was achieved when the polymer consisted of longer chains, see Section 3.3.

3.2. Separation efficiency

3.2.1. Comparison with literature data

A large number of coating methods for capillaries intended for protein separation has been presented. Most applications are for basic proteins where plate numbers/m typically are in the range 400 000 to 1 200 000 [18,21,31,33,34]. Our columns show efficiencies in the upper part of this range, Table 1. In general, acidic proteins give somewhat lower plate numbers. Here, trypsin inhibitor can give high plate numbers but lactoglobulins and lactalbumin give broader peaks. Typically, Chiari et al. achieved 732 000 plates/m for soybean trypsin inhibitor at pH 8.8, while β-lactoglobulin B gave 88 000 plates/m [17]. In this work N/m for trypsin inhibitor was 44 000.

3.2.2. Efficiency as a function of voltage

According to Hjertén [3] the plate height (H) can be written as:

$$H = \frac{\Delta x_0^2}{12 L_D} + \frac{2D}{\mu_i E} \quad (2)$$

where Δx_0 is width of the starting zone (cm); L_D is migration distance (cm); D is diffusion constant (cm^2

Table 1
Effects of DMA concentration and IPA on migration times and separation efficiency

DMA concentration (%)	IPA (%, v/v)	Migration time (min)					Δt (min)	Separation efficiency (plates/m·10^5)				
		Ly	Cy	Ri	α-Ch	Tr		Ly	Cy	Ri	α-Ch	Tr
1	3.5	6.100	6.819	8.572	10.321	10.666	4.566	9.58	12.0	7.67	7.42	7.18
2	3.5	5.498	5.673	7.732	9.429	9.695	4.197	7.60	8.82	5.06	6.85	7.58
5	3.5	4.751	4.902	6.57	7.924	8.138	3.387	6.14	7.09	5.70	4.91	5.23
1	0	5.505	5.683	7.707	9.415	9.703	4.189	7.32	8.33	7.11	7.25	7.46
2	0	5.568	5.748	7.848	9.559	9.822	4.254	5.15	7.67	6.73	6.77	6.82

Δt = migration window. Capillary: 40.5 cm (32.5 cm to detector) × 50 μm I.D. Sample concentration: 0.1 mg/ml. Injection: 50 mbar, 10 s. Buffer: 50 m*M* acetic acid–Tris, pH 4.80, 20 kV, 21 μA.

s^{-1}); μ_i is electrophoretic mobility ($cm^2\ s^{-1}\ V^{-1}$); E is field strength ($V\ cm^{-1}$). Here the first term is for width of injection plug, and the second is for diffusion. Eq. (2) is valid provided there is no band broadening from Joule heating and adsorption. A plot of $H=f(1/E)$ will give a straight line when there is no band broadening from Joule heating or adsorption [3]. Such plots for three proteins are shown in Fig. 1. The plots are fairly linear up to about 20 kV, at higher voltage band broadening from Joule heating becomes noticeable. The regression R^2 for ribonuclease A was 0.9720, for α-chymotrypsinogen it was 0.9868, and for trypsinogen 0.9998. This may indicate that trypsinogen has the lowest adsorption.

3.2.3. Surface adsorption

A simple expression for protein interaction with the capillary wall was derived by Cifuentes et al. [35]:

$$\frac{1}{t}=\frac{\mu_p}{l}\cdot E-\frac{\mu_p Q_c}{l\varepsilon x^2} \tag{3}$$

Here t is the time needed for uniform movement of a particle a distance l from the injection to the detection points, μ_p is the electrophoretic mobility of the particle; E is the field strength; Q_c is the electric charge of the particle; ε is the dielectric constant of the buffer; x is the average distance between surface and particle. A plot of $1/t=f(E)$ will give a straight line where the intercept is a measure of the degree of electrostatic interaction between capillary surface and analyte. The higher the absolute value of the intercept, the stronger is the electrostatic interaction with the wall. Fig. 2 shows plots for four proteins. Intercepts and slopes are given in Table 2. As noted before [36], there is a strong correlation between the isoelectric point (pI) value and the intercept value, Table 2. Trypsinogen gives the lowest absolute intercept value; thus this protein would have the lowest degree of surface adsorption. This is in agreement with the regression data obtained from the plots in Fig. 1.

The intercept is a function of analyte mobility and analyte surface adsorption. Thus, the intercept depends on the pH, and therefore it may be difficult to compare data taken with different buffers. Our data for lysozyme at pH 4.80 do not agree with data from Cifuentes et al. [35] for lysozyme on a polyacrylamide-coated capillary at pH 5.5. Ren et al. [36]

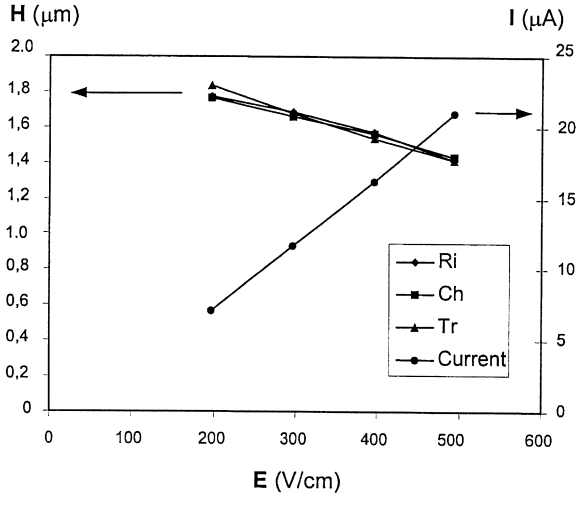

Fig. 1. Characterization of surface adsorption by efficiency as a function of voltage over an MPT–PDMA coated capillary. Conditions: capillary 40.5 cm (32.5 cm to detection window)×50 μm I.D.; buffer, 50 m*M* acetic acid–Tris (pH 4.8); UV detection at 204 nm. Sample, a mixture of three basic proteins.

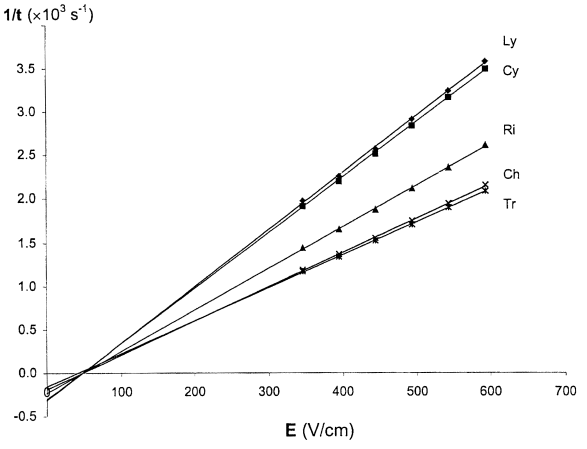

Fig. 2. Relationship between $1/t$ (migration time) and field strength on an MPT–PDMA coated capillary. Conditions: capillary 40.5 cm (32.5 cm to detection window)×50 μm I.D.; buffer, 50 m*M* acetic acid–Tris (pH 4.8); UV detection at 204 nm. Sample, a mixture of five basic proteins, lysozyme (Ly), cytochrome *c* (Cy), ribonuclease A (Ri), α-chymotrypsinogen (Ch), trypsinogen (Tr); concentration, 0.1 mg/ml; injection: 50 mbar, 5 s.

Table 2
Interaction strength of basic proteins with MPT–PDMA coated capillary surface

Protein	p*I*	M_r	Intercept (*A*)	Slope (*B*)	Linear regression coefficients (r^2)
Lysozyme	11.0	14 000	−0.31	0.0066	0.9995
Cytochrome *c*	10.2	12 500	−0.31	0.0064	0.9994
Ribonuclease A	9.3	13 700	−0.23	0.0048	0.9996
α-Chymotrypsinogen A	9.2	25 000	−0.19	0.0039	0.9996
Trypsinogen	8.7	23 700	−0.16	0.0038	0.9997

p*I* and M_r from Refs. [16,36,33,37].

used the same pH as we did but the coating was totally different, poly(ethylene–propylene glycol), they obtained an intercept of lysozyme at −0.35, which may indicate a similar electrostatic interaction as, compared to our capillaries.

The plate numbers correlate with p*I* and intercept except for cytochrome *c* which has the highest plate count, thus other factors than electrostatic interaction with the surface also play a role for the band width.

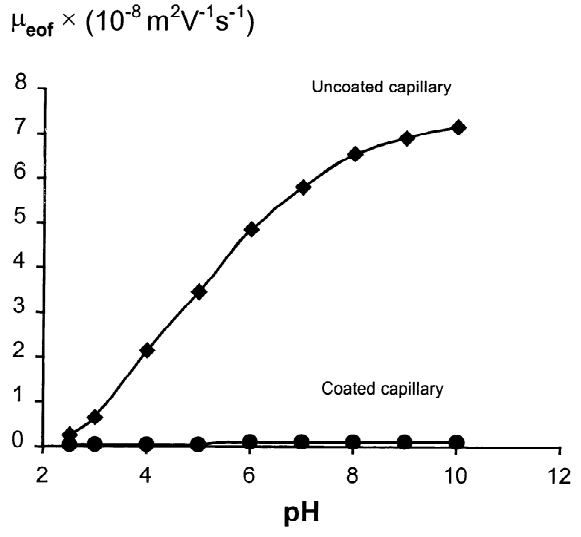

Fig. 3. EOF as a function of pH on an uncoated and an MPT–PDMA coated capillary. Conditions: capillary 40 cm (32 cm to detection window)×50 μm I.D.; buffer, 20 m*M* phosphate–Tris (pH 2.5–10); 20 kV; UV detection at 204 nm. EOF for uncoated capillary was directly measured by injecting DMF as a neutral marker; EOF from coated capillary was indirectly measured according to Williams and Vigh [26].

3.3. Electroosmotic flow

Fig. 3 shows EOF as a function of pH for a coated and a non-coated capillary. EOF data for non-coated and two types of coated capillaries are given in Table 3. The lowest EOFs were obtained with capillaries in which the dimethylacryl amide polymerization had been performed with 1% DMA and no IPA. Thus, it seems that this polymerization condition gives the most complete surface coverage.

The EOF values of our coated capillaries are similar to what has been reported earlier. For a commercially available capillary, eCAP (Beckman Instruments, Fullerton, CA, USA) an EOF of $4.7 \cdot 10^{-10}$ m^2 V^{-1} s^{-1} at pH 6.5 was reported [27]. For capillaries coated with adsorbed epoxy poly(DMA) the EOF at pH 8.5 was $8 \cdot 10^{-10}$ m^2 V^{-1} s^{-1} [13].

Under the applied conditions, there is a slight EOF in uncoated fused-silica capillaries also at pH 2.5, Table 3. This means that there is a risk for surface adsorption of cationic analytes at pH 2.5. In view of

Table 3
Electroosmotic flows measured on uncoated and coated capillaries

pH	Uncoated	Coated (with 3.5% IPA)	Coated (without IPA)
2.5	0.268	0.0524	0.0263
3.0	0.648	0.0478	0.0265
4.0	2.14	0.0521	0.0318
5.0	3.43	0.0575	0.0409
6.0	4.84	0.0833	0.0428
7.0	5.82	0.0938	0.0722
8.0	6.55	0.0983	0.0638
9.0	6.88	0.0896	0.0615
10.0	7.15	0.105	0.0886

Unit: $\cdot 10^{-8}$ m^2 V^{-1} s^{-1}.

this, we conclude that the use of coated capillaries can be beneficial also at low pH.

3.4. Stability of the coating

3.4.1. Stability on solvent rinsing

The effects of rinsing the coated capillary with solvent were evaluated. For evaluation, a test mixture of five basic proteins was separated before and after extensive solvent rinsing, Fig. 4. Rinsing led to somewhat decreasing migration times; efficiencies were, slightly decreased. It seems that the coated capillaries would be suitable also for non-aqueous CE.

3.4.2. Stability at high pH

The coated capillaries are quite stable at high pH. This is illustrated by the separation of five FMOC-derivatized amino acids at pH 10 before and after 50 runs at this pH, Fig. 5. Migration times become slightly decreased after running the capillary for some time at pH 10, but the plate numbers are not changed. It should be noted that it is possible to successfully run many more separations than 50 at

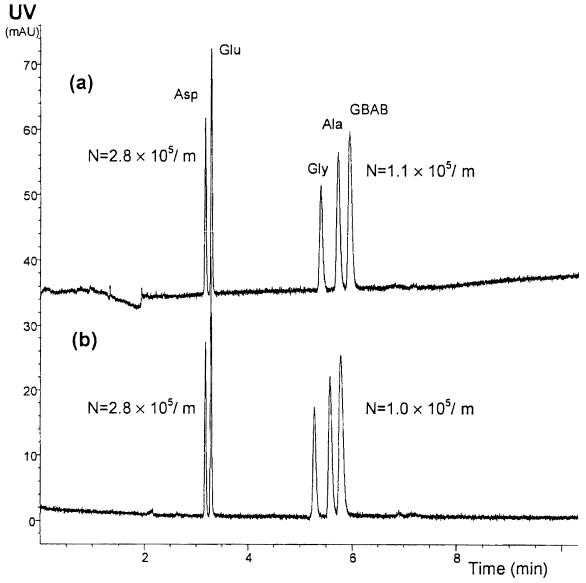

Fig. 5. Separation of mixed neurotransmitter amino acids at high pH on a coated capillary. Conditions: capillary 35 cm (27 cm to detection window)×50 μm I.D.; buffer, 50 m*M* boric acid–NaOH (pH 10.0); −15 kV; current, 34 μA; UV detection at 204 nm. Sample, five mixed amino acid standards; concentration, 100 μ*M*. (a) First run, (b) 50th run.

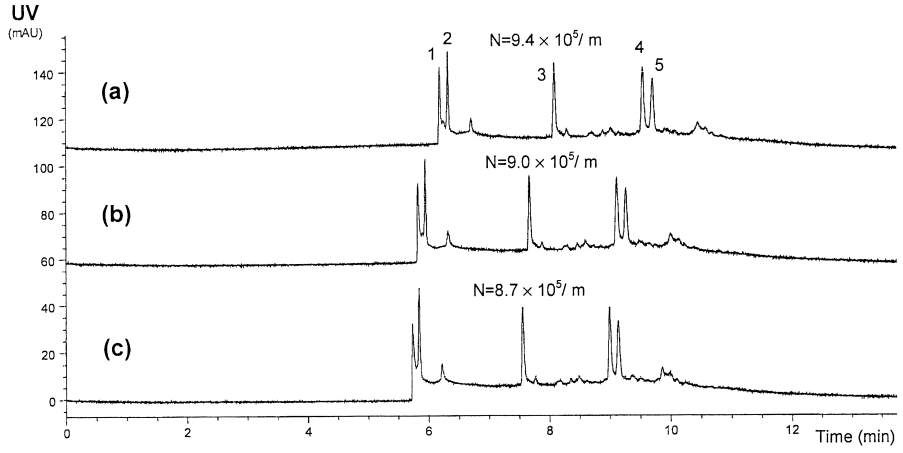

Fig. 4. Examination of organic solvent resistance of MPT–PDMA permanent coating. (a) Newly coated capillary 35 cm (27 cm to detection window)×50 μm I.D.; buffer, 50 m*M* acetic acid–Tris (pH 4.8); 17 kV; current, 19 μA; UV detection at 204 nm. Sample, five basic protein standards: 1=lysozyme, 2=cytochrome *c*, 3=ribonuclease A, 4=α-chymotrypsinogen, 5=trypsinogen; concentration, 0.1 mg/ml; injection: 50 mbar, 5 s. (b) After washing with acetonitrile for 30 min with an applied pressure 940 mbar. (c) After washing with IPA for 30 min with an applied pressure 940 mbar.

pH 10. Also after 150 runs at pH 10 the system worked in a satisfactory way.

The weak points of the coating are the –Si–O–Si– linkages; these are susceptible to hydrolysis at high pH. It seems, however, that the observed stability depends on the hindered accessibility of the Si–O–Si bonds by the polymer layer.

3.4.3. Reproducibility of migration time

For 10 consecutive runs on a given capillary the RSDs of migration times were: α-chymotrypsinogen A, 0.68%; lysozyme, 0.75%; trypsinogen, 0.66%; ribonuclease A, 0.61%; cytochrome *c*, 0.74%. The migration times were decreasing slightly. Thus in a series of runs of the five-component protein mixture

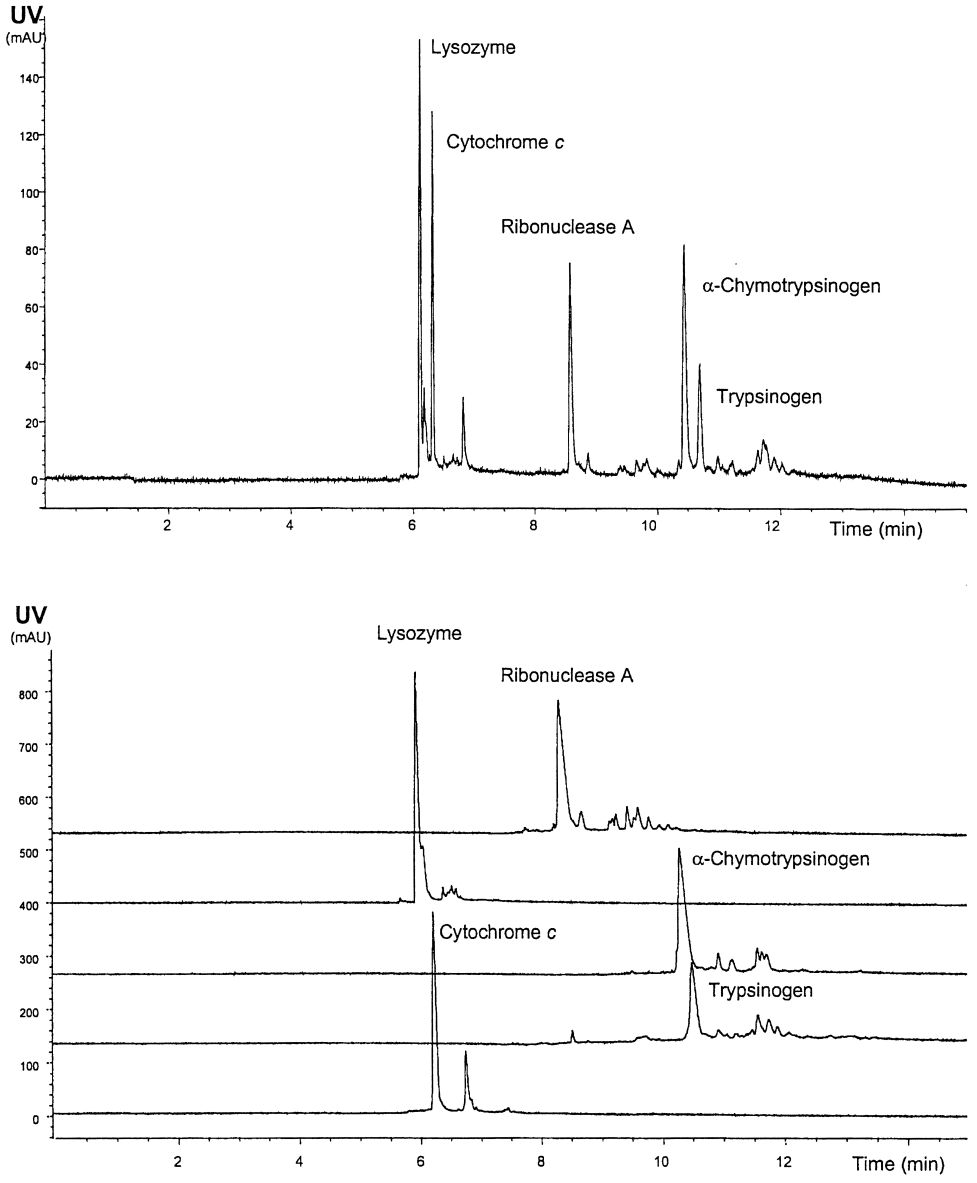

Fig. 6. Separation of five mixed basic proteins and impurities on MPT–PDMA coated capillary. Conditions: capillary 43 cm (35 cm to detection window)×50 μm I.D.; buffer, 50 m*M* acetic acid–Tris (pH 4.8); 22 kV; current, 21 μA; UV detection at 204 nm. Sample, five basic protein standards; concentration, 0.1 mg/ml; injection: 50 mbar, 5 s.

the migration time of α-chymotrypsinogen A was 6.10 min in the 14th run and 5.82 min in the 34th run. For three different capillaries, the RSDs of migration times were: α-chymotrypsinogen A, 0.51% and for cytochrome *c*, 4.10%.

3.5. Applications

The separation of five basic proteins is shown in Fig. 6. Standards were not particularly pure as shown in Fig. 6B. As mentioned above, acidic proteins may, in many cases, give broader peaks than basic proteins. Separation of a test mixture containing two acidic proteins at pH 8.8 is shown in Fig. 7. Plate numbers were lower than with basic proteins. A further example on applications in high pH buffers is the separation of FMOC-derivatized neurotransmitter amino acids at pH 10.0, Fig. 5.

Fig. 8 shows the separation of nine peptides in a test mixture at pH 2.5. Separation of peptides, digested from a bacteria protein, is shown in Fig. 9.

4. Conclusions

Capillaries coated with a chemically bonded film of polydimethylacrylamide show excellent performance in CE. Very high separation efficiencies can easily be obtained when analyzing basic proteins. This indicates that adsorption of these analytes at the capillary wall is negligible. Further, the residual electroosmotic flow is extremely low, and that indicates a good surface coverage. Stability of the coated layer at a high pH is good. We attribute this to the hindered accessibility of the Si–O–Si bonds by the polymer layer. Finally, the coated capillaries can withstand rinsing with organic solvents such as acetonitrile and 2-propanol, this indicates that the columns may be useful also in non-aqueous CE.

Acknowledgements

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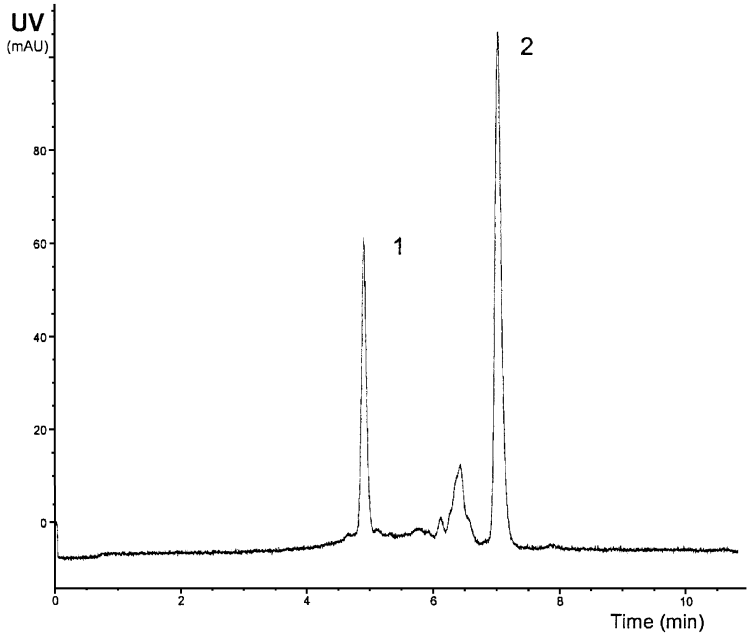

Fig. 7. Separation of acidic proteins on a coated capillary. Conditions: capillary 40 cm (32 cm to detection window)×50 μm I.D.; buffer, 50 m*M* glycine–Tris (pH 8.80); −20 kV; current, 2.8 μA; UV detection at 204 nm. Peaks: 1, trypsin inhibitor; 2, α-lactalbumin; concentration, 100 μ*M*. Number of theoretical plates/m: 1, $0.44 \cdot 10^5$; 2, $0.58 \cdot 10^5$.

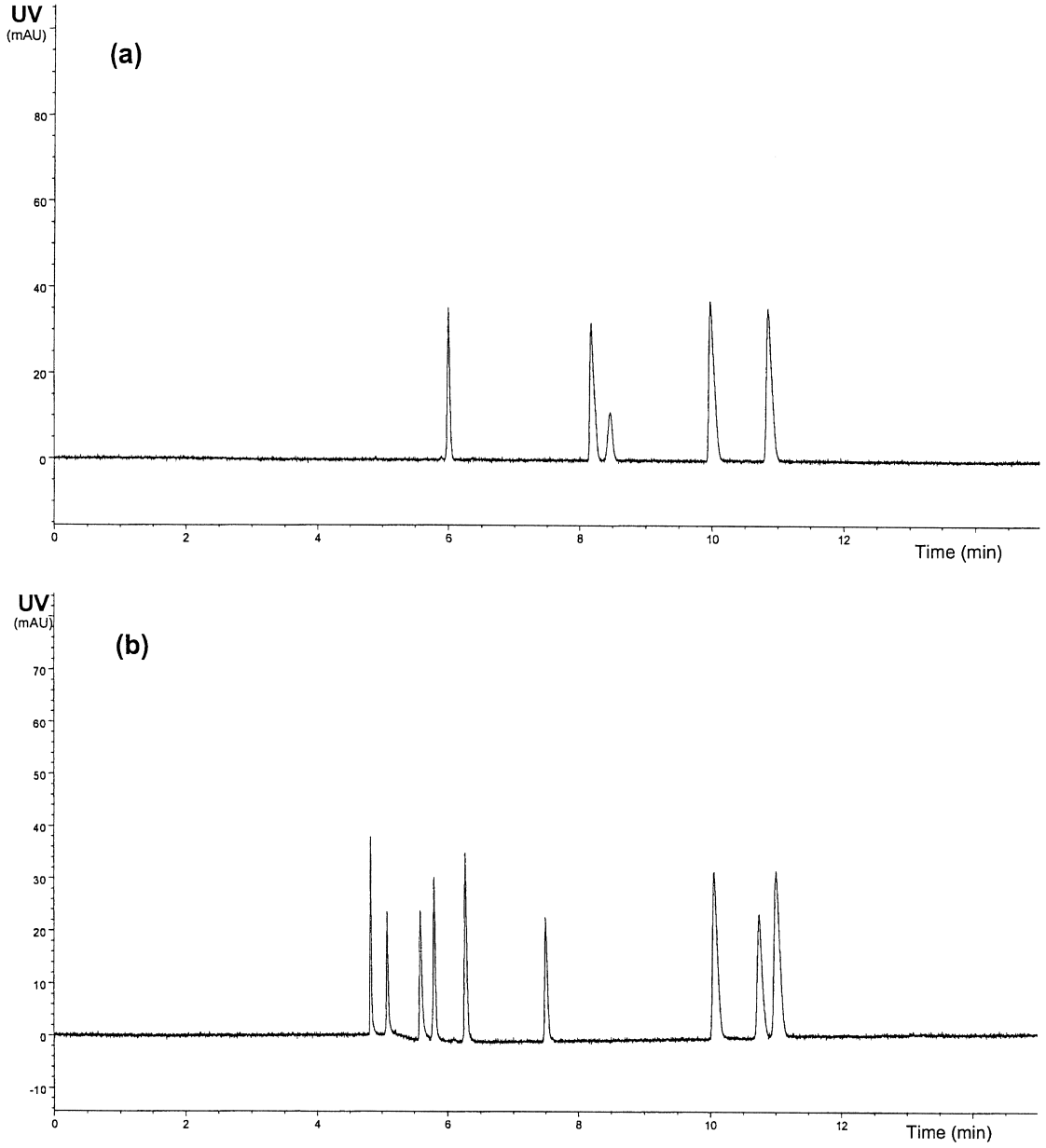

Fig. 8. Separation of peptides on MPT–PDMA coated capillary. Conditions: capillary 40.5 cm (32.5 cm to detection window)×50 μm I.D.; buffer, 25 m*M* phosphorus acid–Tris (pH 2.5); 20 kV; current, 17.6 μA; UV detection at 204 nm. Sample, (a) HPLC peptide standard mixture H-2016; (b) peptide standard mixture P-2693; concentration, ca. 0.06 mg/ml; injection: 50 mbar, 5 s.

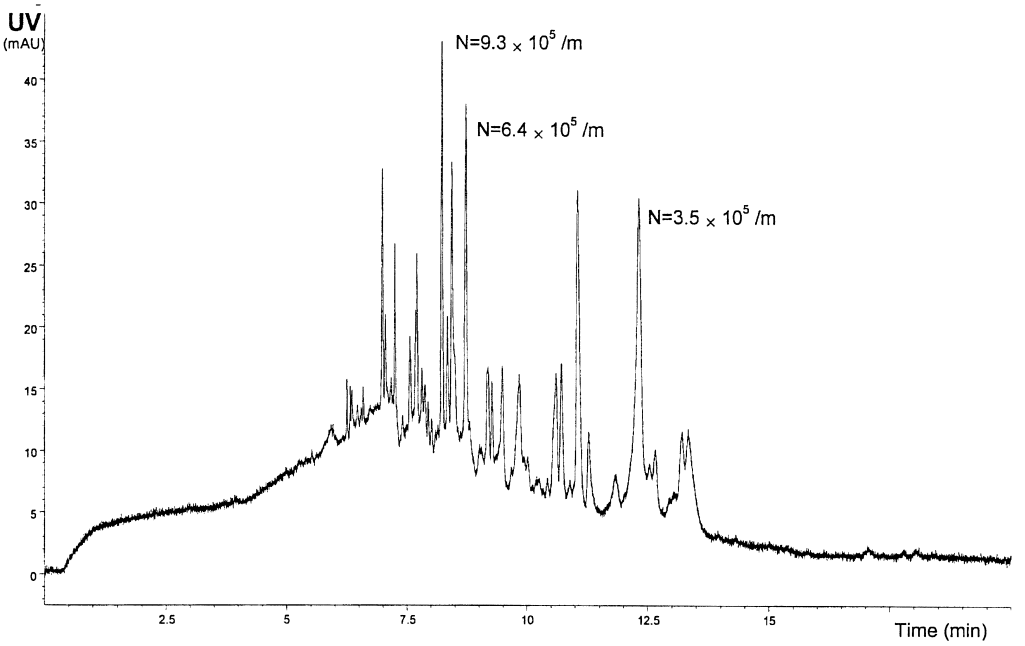

Fig. 9. Separation of the peptide digest of a bacteria protein extracted from wastewater, CE injection with sample stacking. Conditions: buffer, 40 m*M* phosphorus acid–Tris (pH 2.5); 20 kV; current, 16 μA; other conditions as in Fig. 8. Sample concentration, very diluted, injection, 50 mbar, 240 s.

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