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Noncovalently bilayer-coated capillaries for efficient and reproducible analysis of proteins by capillary electrophoresis

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Abstract

The suitability of noncovalently bilayer-coated capillaries for the analysis of proteins by capillary electrophoresis (CE) at medium pH was investigated. Fused-silica capillaries were coated simply by successively flushing with a polybrene (PB) and a poly(vinyl sulfonate) (PVS) solution. A protein test mixture was used to evaluate the performance of the coated capillaries. Comparisons with bare fused-silica capillaries were made. Several background electrolytes (BGEs) were tested in combination with the PB-PVS coating, showing that optimum performance was obtained for the proteins using high BGE concentrations. With a 300 mM Tris phosphate buffer (pH 7.0), good plate numbers (150,000–300,000), symmetrical peaks, and favorable migration-time repeatabilities (RSDs below 0.8%) were obtained for the proteins. Using bare fused-silica capillaries, the protein peaks were significantly broadened and the migration-time RSDs often exceeded 5%. It is concluded that the PB-PVS coating effectively minimizes adverse protein adsorption and provides a very stable electroosmotic flow (EOF). We also investigated the potential of a commercially available bilayer coating (CEofix™) for protein analysis. It is demonstrated that with this coating, good plate numbers and peak symmetries for proteins can be achieved when the CEofix BGE ("accelerator") is replaced by a common BGE such as sodium or Tris phosphate. Apparently, the negatively charged polymer present in the "accelerator" interacts with the proteins causing band broadening. The utility of the bilayer coatings is further illustrated by the separation of proteins such as interferon-α 2b, myoglobin and carbonic anhydrase, by the analysis of a degraded insulin sample in time, and by the profiling of the glycoprotein ovalbumin. In addition, it is demonstrated that even in the presence of concentrations of human serum albumin in the sample of up to 60 mg/mL, the PB-PVS coating still provides reproducible protein separations of good performance.

Keywords: Capillary electrophoresis; Proteins; Capillary coating; Reproducibility; Efficiency

1. Introduction

In the on-going research on understanding and unraveling biological processes, proteins have shifted more and more to the center of attention. Furthermore, in modern drug development there is a clear trend towards biopharmaceuticals, i.e. the use of (recombinantly produced) proteins for medical purposes [1,2]. As a result, there is a growing demand for efficient analytical techniques capable of separating intact proteins [3,4], preferably under nondenaturing conditions. Capillary electrophoresis (CE) with its charge-to-size ratio based separation mechanism can be a powerful tool for the analysis of proteins [5–7]. CE can perform fast separations and needs only minute amounts of sample, which is an important characteristic when sample availability is an issue. Moreover, electrically driven separation methods provide high peak efficiencies, potentially achieving plate numbers of several hundreds of thousands or more. However, the achievement of high efficiencies may not be as straightforward for proteins as for small molecules and peptides. Proteins are infinitely more complex, with positively and negatively charged groups, hydrophobic sites, and distinct three-dimensional conformations, which may change with pH and/or ionic strength. With such a level of heterogeneity, adverse interactions of protein molecules with silanol groups on the inner wall of the fused-silica capillary can easily occur. These may cause (irreversible) adsorption, band broadening

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and serious peak tailing when proteins are analyzed by CE. Moreover, adsorption of proteins may lead to changes in the electroosmotic flow (EOF) and thus to a poor migration-time reproducibility.

Adsorption of proteins to the capillary wall might be prevented by selecting background electrolytes (BGEs) with very high or low pH values [8,9]. However, at such extreme pHs, proteins can undergo conformational changes or even degradation. There are situations in which separations are preferably carried out at medium pH, so that proteins are analyzed in their natural state. Another approach to minimize protein-wall interactions is to mask the silanol groups by coating the inner capillary surface [10,11]. This can be accomplished by covalently binding a polymeric material such as poly(vinyl alcohol) or poly(acrylamide) to the capillary [12–14]. The preparation of a permanent capillary coating often is labor intensive, comprising of several chemical steps; therefore, reproducibility (capillary to capillary) might be an issue. Another way to coat capillaries is by adding coating agents to the BGE that physically adsorb to the capillary surface (i.e. dynamic coating) [15,16]. These agents, usually low-molecular weight compounds such as amines and surfactants, can be quite effective, but they can easily change the CE separation. A simple and elegant concept of capillary coating is by treating the capillary with one or more charged polymers that are physically adsorbed to the internal wall [17–25]. The preparation of these coatings is straightforward and fast, consisting of rinsing the capillary with one or more polymer solutions. Examples of positively-charged monolayer coatings used for analysis of proteins are polybrene [19], polyethyleneimine [20], and poly(diallyldimethylammonium chloride) [17]. Using this noncovalent-coating concept, bilayers [21], triple layers [22], or even multiple layers [23] can be formed by polymers of opposite charge being alternately adsorbed on each other. Noncovalently bilayer-coated capillaries have shown to yield a strong and stable EOF over a large pH range (2–11) and until now have mainly been used for the CE analysis of low molecular-weight compounds such as basic drugs [24,25]. The use of bilayer coatings for protein analysis has been quite limited so far. Katayama et al. [21] reported on the application of a polymeric bilayer of polybrene and dextran sulfate for the separation of some model proteins. Several other papers describe the use of a dedicated, commercially available kit for the analysis of the protein carbohydrate-deficient transferring (CDT) [26–30]. This kit employs a polymeric bilayer coating allowing the separation of CDT isoforms. Unfortunately, the exact composition of the coating polymers used in the kit is undisclosed.

Recently, we have demonstrated the use of capillaries coated with a bilayer of polybrene (PB) and poly(vinyl sulfonate) (PVS) for the fast, highly reproducible and efficient analysis of peptides at low pH [31]. The migration-time repeatability for the peptides was less than 0.5% (relative standard deviation, RSD) and plate numbers exceeded 500,000. In the present work, we examined the suitability of our PB-PVS coating for the analysis of acidic proteins at medium pH (7.0–8.5), i.e. under non-denaturing conditions. As the nature and charge of the proteins is quite different from the peptides studied previously [31], a fully new optimization of the system was required. Therefore, parameters such as type of BGE, ionic strength and presence of PVS in the run buffer were studied. Comparisons with uncoated capillaries were made based on peak efficiencies, peak asymmetries and migration-time repeatabilities. The feasibility of using a commercial bilayer capillary coating (CEofix) for protein analysis was also tested, and the results were compared with the PB-PVS system. The applicability of the coated capillaries is shown by a few relevant examples.

2. Material and methods

2.1. Chemicals

Polybrene (hexadimethrine bromide) and PVS sodium salt were purchased form Sigma–Aldrich (Steinheim, Germany). The BGE constituents Tris, phosphoric acid, and disodium hydrogen phosphate were from Merck (Darmstadt, Germany). BGEs were prepared using deionized water and filtered using a 0.22 μm hydrophilic filter from Sartorius (Göttingen, Germany). The proteins α-lactalbumin (bovine milk), β-lactoglobulin A and B (bovine milk), carbonic anhydrase, human serum albumin, myoglobin and ovalbumin (chicken egg) were from Sigma–Aldrich (Steinheim, Germany). Humanized porcine insulin (donation from Prince Technologies, Emmen, The Netherlands) and recombinant human interferon-α 2b (PhEur, CRS) were used as test samples. Fresh protein solutions (1 mg/mL) in water were weekly made. The insulin stock solution also contained 0.2% (v/v) acetic acid. Test mixtures were prepared by diluting each protein stock solution to 250 μg/mL with deionized water, unless otherwise stated. A terbutaline (Holland Pharmaceutical Supply, Alphen aan de Rijn, The Netherlands) solution was prepared (1 mg/mL) and diluted to 50 μg/mL with deionized water prior to analysis. For EOF determinations, formamide was added to the test samples to final concentrations of 0.03–0.3% (v/v). BGEs were prepared by weighing disodium hydrogen phosphate or Tris to the desired concentration and titrated to pH 7.0–8.5 with phosphoric acid (5 M). These BGEs will be referred to as sodium phosphate or Tris phosphate with the respective concentration and pH given.

2.2. CE system

The experiments were carried out on a P/ACE MDQ capillary electrophoresis instrument equipped with a DAD detector (Beckman Coulter, Fullerton, CA, USA). Fused-silica capillaries were from Composite Metal Services (The Chase, Hallow, UK), having a total length of 60 cm, an effective length of 50 cm and an internal diameter of

50 μm. Injections were performed hydrodynamically for 5 s at 34.5 mbar (0.5 psi). The separation voltage was 30 kV, the capillary temperature was 25 °C and detection was performed at 205 nm. Electropherograms were analyzed using 32 Karat Software, version 4.01 (Beckman Coulter). Plate numbers were based on the peak widths at half maximum and peak asymmetry factors, on the peak widths at 10% height.

New bare fused-silica capillaries were rinsed with water for 10 min at 1380 mbar followed by 1 M NaOH for 15 min at 1380 mbar, and water for 5 min at 1380 mbar. After this treatment, capillaries were used as such, or coated with PB-PVS or CEofix using the procedures described below. Before each analysis, capillaries were flushed with BGE for 2 min at 1380 mbar. Between runs, the coated capillaries were flushed with a solution containing the appropriate negatively-charged polymer for 2 min at 1380 mbar. When samples contained human serum albumin an additional rinse with 100 mM sodium hydroxide (5 min at 1380 mbar) was incorporated between runs.

2.3. PB-PVS coating procedure

PB was dissolved in deionized water to a final concentration of 1% (m/v), and a solution 25% (v/v) of PVS was diluted to 1% (v/v) with deionized water. Coating was performed by rinsing first for 30 min at 34.5 mbar with 1% (m/v) PB and then with water for 5 min at 1380 mbar. Subsequently, the capillary was flushed with 1% (v/v) PVS for 30 min at 34.5 mbar, and again water for 5 min at 1380 mbar. The capillary was then ready for CE analysis with the BGE of choice.

The integrity of the coating was examined by analyzing an aqueous solution of terbutaline (50 μg/mL) in triplicate as described previously [31]. The coating was considered well when the relative standard deviation (RSD) of the migration time of terbutaline was lower than 0.5% and the plate number of terbutaline exceeded 500,000. This integrity procedure was also used to test the stability of the coating during protein analysis. Unwanted adsorption or damage to the coating caused by proteins will be revealed by changes in the migration time and/or plate number of terbutaline.

2.4. CEofix coating procedure

Capillaries were also coated with a commercial coating kit (CEofix™ Method Development Kit) from Analis S.A. (Namur, Belgium), which contains the coating solutions and BGEs. Uncoated capillaries were first rinsed with water for 2 min at 1380 mbar, followed by 1 M NaOH for 1 min at 1380 mbar. Subsequently, the capillary was rinsed with so-called "initiator" solution (which contains a positively charged polymer) for 1 min at 1380 mbar, followed by a rinse with so-called "accelerator" (pH 8.2) (which contains a negatively charged polymer) for 1 min at 1380 mbar. After this, the capillary was filled with BGE of choice for CE analysis. The integrity of the CEofix coating was checked as described above using terbutaline.

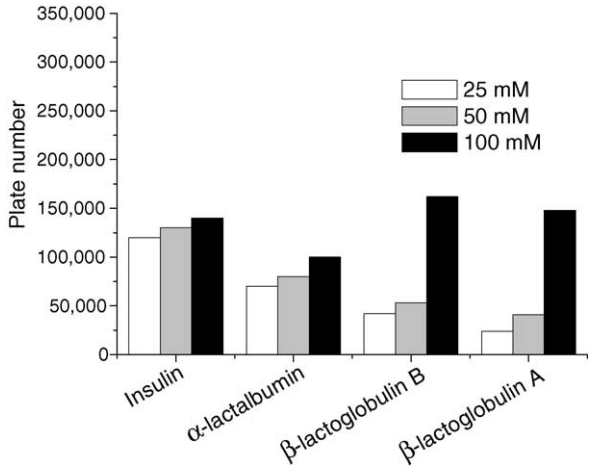

Fig. 1. Plate numbers obtained for test proteins analyzed by CE using a PB-PVS coated capillary with various concentrations of sodium phosphate (pH 7.0).

3. Results and discussion

3.1. PB-PVS coating

The potential of PB-PVS coated capillaries for the analysis of proteins at medium pH was studied using a test mixture of insulin, α-lactalbumin, and β-lactoglobulin A and B. Under various conditions, efficiency (plate numbers), peak asymmetry, and migration-time repeatability of the proteins were evaluated. In the first experiments, the protein test mixture was analyzed using a PB-PVS coated capillary and 25 mM sodium phosphate (pH 7.0) as BGE. Plate numbers for insulin were satisfactory (ca. 130,000 plates), whereas α-lactabumin and the β-lactoglobulins presented lower plate numbers (<70,000). The performance could be improved by increasing the ionic strength of the BGE, i.e. by raising the sodium phosphate concentration to 50 and 100 mM (Fig. 1). The largest gain in plate number was obtained for the β-lactoglobulins. As can be expected, the EOF was reduced at higher BGE concentrations and the overall protein migration times increased. With a BGE of 25 mM phosphate, the total analysis time was less than 5 min and with 100 mM phosphate, it was ca. 10 min. Nevertheless, at each sodium phosphate concentration the EOF was quite stable with RSDs of migration times being always less than 0.8% ($n = 5$).

The high conductivity of the 100 mM sodium phosphate buffer caused relatively high currents (ca. 180 μA), which might be problematic causing excessive Joule heating. In order to allow higher ionic strengths at reduced currents, we tested Tris phosphate as BGE in combination with the PB-PVS coated capillary. Fig. 2 shows the effect of the Tris phosphate concentration on the plate numbers of the tested proteins. At higher ionic strengths, increased peak efficiencies were obtained. At 400 mM Tris phosphate (pH 7.0) the maximum plate numbers ranged from 125,000 (α-lactalbumin) to 340,000 (insulin), whereas the observed current was 160 μA. Nevertheless, some adverse effects were observed for the

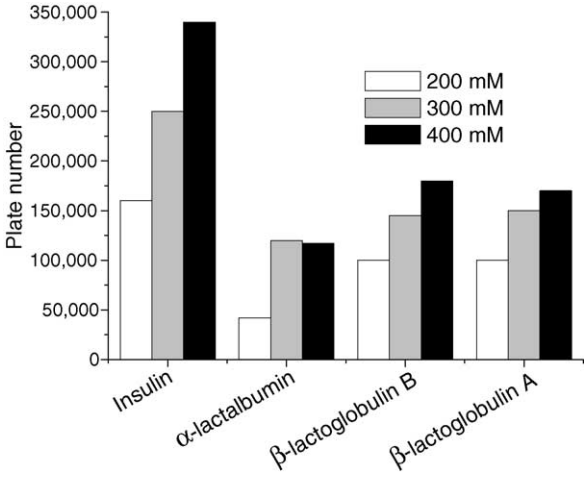

Fig. 2. Plate numbers obtained for test proteins analyzed by CE using a PB-PVS coated capillary and BGEs with various concentrations of Tris phosphate (pH 7.0).

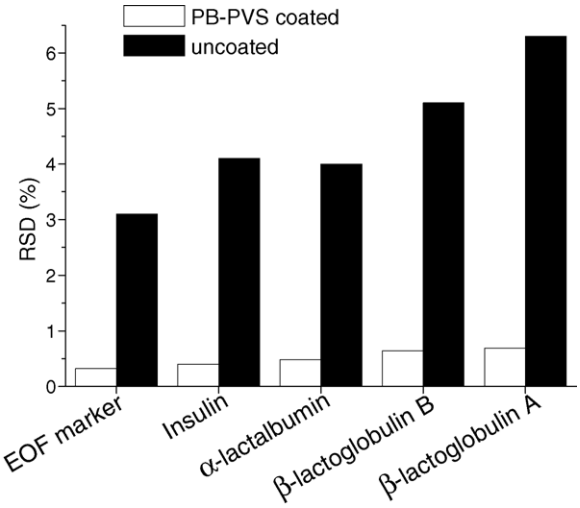

Fig. 4. Migration-time repeatability (% RSD) obtained during the analysis of protein test mixture using a PB-PVS coated and a bare fused-silica capillary.

400 mM Tris phosphate buffer: the migration-time RSDs increased to ca. 1.5%, the peaks were less symmetrical than with 300 mM (asymmetry factors of 1.6 and 1.0, respectively), and the baseline showed an increase of noise. On further experiments, we used 300 mM Tris phosphate (pH 7.0) as BGE because it provided a more stable system (migration-time RSDs < 0.8%) and good efficiencies (plate numbers of 150,000–250,000), at currents below 120 μA (Fig. 3). An Ohm's plot (current versus applied voltage) recorded for this BGE showed good linearity up to 30 kV, indicating that Joule heating is not critical and still within acceptable levels.

In order to verify the utility of the bilayer coatings, the protein test mixture was also analyzed in bare fused-silica capillaries and compared to results obtained with PB-PVS coated capillaries using 300 mM Tris phosphate (pH 7.0) as BGE. Overall, the CE performance obtained with the uncoated capillary was less favorable, even when using the high ionic strength BGE. For α-lactalbumin and the β-lactoglobulins plate numbers were 70,000–100,000 with the bare capillary,

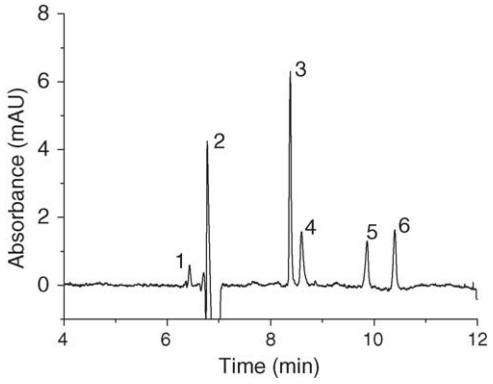

Fig. 3. CE of a protein test mixture using a PB-PVS coated capillary and a BGE of 300 mM Tris phosphate (pH 7.0). Peaks: 1, EOF marker; 2, system peak; 3, insulin; 4, α-lactalbumin; 5, β-lactoglobulin B; 6, β-lactoglobulin A.

whereas for insulin the efficiency was comparable to the one obtained with the PB-PVS coating. However, with bare capillaries the performance was much less stable. For instance, the peak width of insulin showed great variability on bare capillary, whereas constant peak widths were obtained on the coated capillary. The peak areas (corrected for migration time) of the proteins analyzed with the uncoated capillary also greatly varied (RSDs > 30%), indicating irreversible adsorption of proteins. On the PB-PVS coated capillary, corrected peak areas for the proteins were quite constant (RSDs < 5%) and showed no gradual change in time. The migration-time repeatability was also much better on the bilayer-coated capillaries than on bare fused-silica capillaries (Fig. 4). For the test proteins, migration-time RSDs were always lower than 0.8% using the coated capillary, whereas the corresponding RSDs were often above 5% for the bare capillary. These observations suggest that the PB-PVS coating effectively prevents adverse protein-wall interactions. It provides a stable and constant EOF which, in turn leads to satisfactory migration-time and peak area repeatabilities. Also in the longer term the PB-PVS coating showed good stability. Using the same capillary for days, RSDs for the migration times of the test proteins were always within 1%. When a new piece of capillary was installed, a small shift in absolute migration time could be observed for the proteins (cf. Figs. 3 and 6 for insulin), but for each individual capillary, migration times were very stable (Fig. 5).

Using a PB-dextran sulfate bilayer coating, Katayama et al. [21] also found good efficiencies and repeatabilities for insulin, α-lactalbumin and β-lactoglobulins A and B. It should be noted that with our PB-PVS system using 300 mM Tris phosphate the peak resolution for the same proteins is significantly better. Besides different characteristics of our coating, this also could be due to the higher ionic strength of our BGE. A high ionic strength provides improved plate numbers and a somewhat slower EOF, which overall enhances

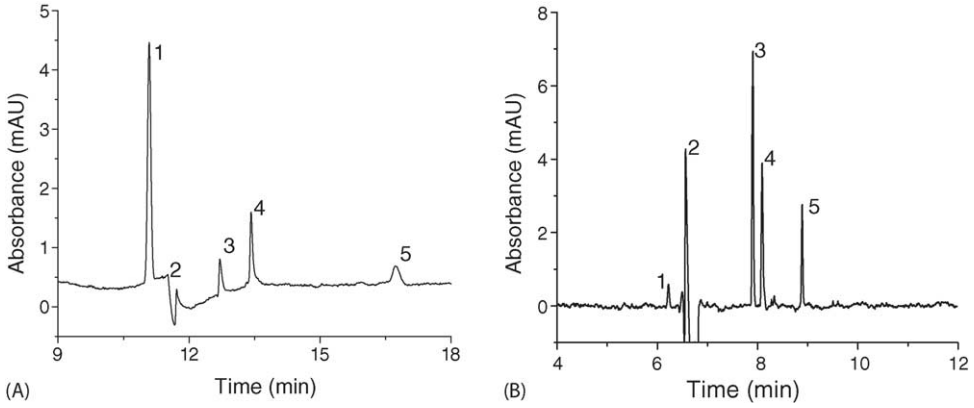

Fig. 5. CE of a protein test mixture using a capillary coated according to the CEofix procedure and a BGE comprising (A) accelerator (pH 8.2) or (B) 300 mM Tris phosphate (pH 7.0). Peaks: 1, EOF marker; 2, system peak; 3, insulin; 4, α-lactalbumin; 5, β-lactoglobulin B.

the resolution. As we used a low-conductivity buffer and a smaller internal capillary diameter, we also could apply a higher field strength than Katayama et al., which also adds to a better resolution.

As shown above, the use of high ionic strength BGEs causes an overall increase in the peak efficiency of proteins. As the proteins were dissolved in water, a difference in conductivity exists between the injection zone and the BGE, which might induce sample stacking and thus improved peak efficiency. To check the effect of stacking, the protein mixture was also dissolved 300 mM Tris phosphate (pH 7.0) and analyzed. Overall, using deionized water as sample solvent had a positive effect on the plate numbers. For insulin dissolved in buffer and water, plate numbers were 180,000 and 250,000, respectively. For α-lactalbumin, the gain caused by stacking was lower (ca. 30,000) and for the lactoglobulins no significant difference in plate numbers was observed. These results show that although stacking contributes to the enhancement of the separation efficiency of the studied proteins, the favorable plate numbers can be largely ascribed to the bilayer coating in combination with a BGE of high ionic strength.

3.2. CEofix coating

We also checked the suitability of a commercially available capillary coating (CEofix) for the analysis of proteins. We tested the CEofix Development Kit, which is predominantly intended for the analysis of low-molecular weight compounds such as basic drugs [25,32–34]. Similar to the PB-PVS coating described above, with CEofix a bilayer coating is obtained. The coating procedure comprises a rinse of the capillary with a solution containing a positively-charged polymer ("initiator") followed by a rinse with a solution containing a negatively-charged polymer ("accelerator"). A protein test mixture of insulin, α-lactalbumin, and β-lactoglobulin B (250 μg/mL each) was initially analyzed in CEofix-coated capillaries using the accelerator (pH 8.2) as BGE (according to the provider's procedure). This CE system presented good migration-time RSDs for the EOF marker and the proteins; however, peak efficiencies were rather low and peaks quite asymmetric (Table 1). Furthermore, the total analysis time was relatively long (ca. 18 min).

In order to improve the performance of the proteins on the CEofix coating, we studied the possibility of using these capillaries in combination with Tris phosphate BGEs without added polymer. So, after coating the capillary using CEofix initiator and accelerator, analyses were carried out with 300 mM Tris phosphate (pH 8.2 or 7.0) as BGE. Between runs, capillaries were conditioned by rinsing (1380 mbar) with the accelerator for 2 min, followed by 2 min with Tris phosphate. With both Tris phosphate buffers, much better efficiencies and peak shapes were obtained for the proteins (Table 1). In addition, the EOF was faster, leading to shorter analysis times. The migration-time repeatabilities remained very good (<1% RSD) when the accelerator was replaced with Tris phosphate BGEs. Somewhat higher plate numbers were observed for the proteins analyzed using the pH 7.0 buffer, which might be caused by the higher ionic strength of

Table 1
Results obtained with various BGEs for the CE analysis of a protein test mixture[a] using capillaries coated using the CEofix procedure

Parameter	Accelerator (pH 8.2)	300 mM Tris phosphate	
		pH 8.2	pH 7.0
$RSD_{migration\ time}$ (%)	0.3–0.6	0.4–0.8	0.4–0.6
Plate number	50000–80000	200000–270000	250000–310000
Asymmetry factor	1.4–2.0	1.1	1.0
Analysis time (min)	18	11	10

[a] Insulin, α-lactalbumin and β-lactoglobulin B.

this buffer with respect to the buffer with pH 8.2. Overall, the results obtained with the CEofix-coated capillaries in combination with Tris phosphate BGEs, were comparable in terms of efficiency and repeatability to the results obtained for the PB-PVS coated capillaries.

The low plate numbers observed for the proteins when analyzed using the CEofix coating with the accelerator BGE, suggests that the negatively-charged polymer present in the accelerator interacts with the proteins causing band broadening. We observed similar effects for proteins analyzed on PB-PVS coated capillaries when various concentrations of PVS (0.001–0.01%, v/v) were added to the 300 mM Tris phosphate (pH 7.0) BGE. In the presence of only 0.005% PVS in the BGE, plate numbers of the proteins deteriorated dramatically. This is in line with our previous work [31], where we observed a decrease of plate numbers for peptides analyzed with PB-PVS coated capillaries using PVS-containing BGEs.

3.3. Applicability

As shown above and by others, one common problem in analyzing proteins using bare fused-silica capillaries is the irreproducibility of migration times. This is especially problematic when sample and/or stability profiles have to be analyzed in time. For the reliable comparison of profiles and to be able to observe small changes in sample composition, migration-time stability is of crucial importance. One example of such a situation is during the stability monitoring of therapeutic proteins. Fig. 6 shows the repeated analysis of a sample of insulin stored at 80 °C. Each 15 min, aliquots were taken and analyzed by CE using both bare and PB-PVS coated capillaries with 300 mM Tris phosphate (pH 7.0) as BGE. Both systems reveal the gradual deamidation of insulin (at Arg21), leading to an increase in the net negative-charge of the protein. Clearly, with the PB-PVS coated capillary the position of the peaks are much more stable. In fact, the migration-time RSDs for insulin and desamido-insulin with this system were lower than 0.5%, whereas with the bare fused-silica the RSDs exceeded 3.5%. The differences in performance are also evident: for the coated capillary, plate numbers were up to 300,000 and the peak asymmetry factors close to 1.0, whereas with the bare-fused silica much lower plate numbers and asymmetric peaks were obtained. Clearly, the PB-PVS coated capillary shows good potential for the reliable monitoring of degradation products of biopharmaceuticals.

CE can be very useful for the analysis of glycoproteins [4,35,36], providing characteristic profiles showing the difference in glycosilation of the various isoforms. In order to study changes in the heterogeneity of glycoforms, reproducible performance, obviously, is essential. Fig. 7 shows the CE analysis of a sample of the glycoprotein ovalbumin using a PB-PVS coated capillary and 300 mM Tris phosphate (pH 7.0) as BGE. Ovalbumin is known to be heterogeneous in nature with at least nine different carbohydrate structures giving rise to a specific glycoform pattern when analyzed by CE [37]. The analysis was repeated five times with 1 h intervals between the runs. The first and fifth analysis is shown in Fig. 7, demonstrating an excellent stability of the CE system. Carrying out the same repeated analysis on a bare fused-silica capillary, the time per analysis increased and, more importantly, the glycopattern showed a considerable variation, both in peak migration times and in peak widths. These results indicate that adverse interactions of the glycoprotein isoforms with the internal capillary wall are effectively minimized when using the bilayer coating.

The ability to perform protein analysis with the PB-PVS coating was further evaluated by the CE separation of a mixture containing recombinant interferon-α 2b (a

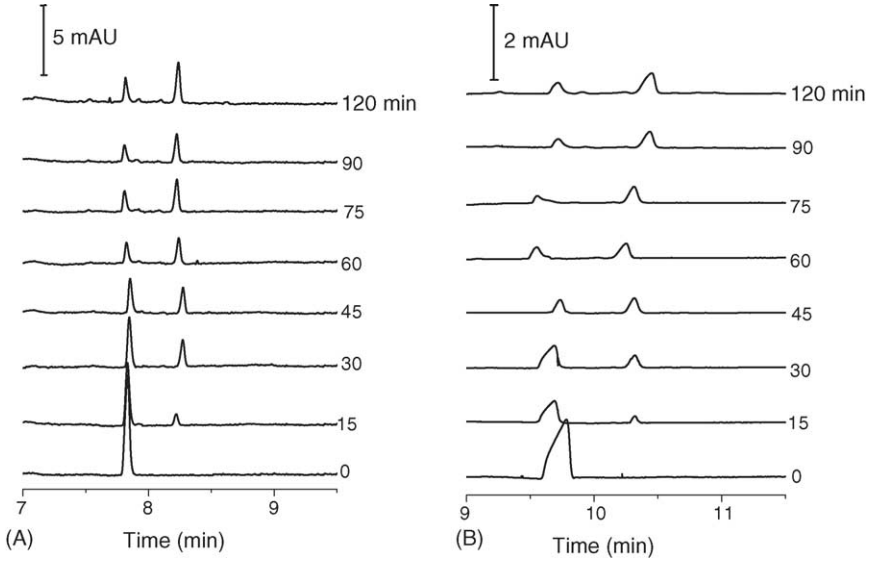

Fig. 6. CE of aliquots of an insulin sample (100 μg/mL in water) using 300 mM Tris phosphate (pH 7.0) as BGE with (A) a PB-PVS coated capillary, or (B) a bare fused-silica capillary. Aliquots of the sample were taken and analyzed at the indicated times after storing the sample at 80 °C.

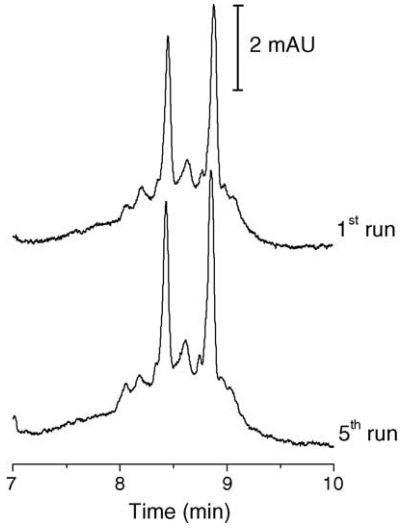

Fig. 7. Repeated CE analysis of ovalbumin from chicken egg (1.0 mg/mL in water) using a PB-PVS coated capillary and 300 mM Tris phosphate (pH 7.0) as BGE.

biopharmaceutical), myoglobin, carbonic anhydrase and human serum albumin (HSA). Using a BGE of 300 mM Tris phosphate (pH 8.5) the proteins were well separated showing narrow peaks except for HSA (Fig. 8A). HSA samples often are heterogeneous (comprising various isoforms), and yield a profile rather than a discrete band when analysed by high-performance CE [38]. Plate numbers for the other proteins (which are not heterogeneous) ranged from 125,000 (interferon-α 2b) to over 300,000 (myoglobin and carbonic anhydrase). When the protein mixture contained HSA (1 mg/mL) and was analysed repeatedly, increasing migration times were observed for the proteins. These shifts most likely were caused by adsorption of HSA – a notorious protein in this respect – to the capillary wall. The problem could be solved by introducing an extra rinse of the capillary between runs with 100 mM sodium hydroxide in order to flush the adsorbed protein out of the capillary. The integrity and performance of the PB-PVS coating was not affected by the alkaline rinses, and the good migration-time stability was restored achieving RSDs of less than 0.5% ($n = 5$) for each protein. We further tested the capability of the PB-PVS system to deal with high concentrations of HSA. Fig. 8B and C show the separation of the protein mixture with HSA present at a level of 10 and 60 mg/mL, respectively. Despite the huge overloading with HSA, the system showed a remarkable stability with very constant migration times (RSDs < 0.5%) for the proteins. When the high HSA concentrations were co-injected the plate numbers of myoglobin and carbonic anhydrase somewhat declined and, as a consequence, their peak heights decreased. The lower efficiency is most probably caused by the relatively high ionic strength and conductivity of the HSA-rich samples leading to reduced stacking, and not by deterioration of the capillary coating. In fact, after these experiments the protein mixture without HSA was analysed using the same PB-PVS coating yielding again plate numbers for myoglobin and carbonic anhydrase in the 300,000–400,000 range.

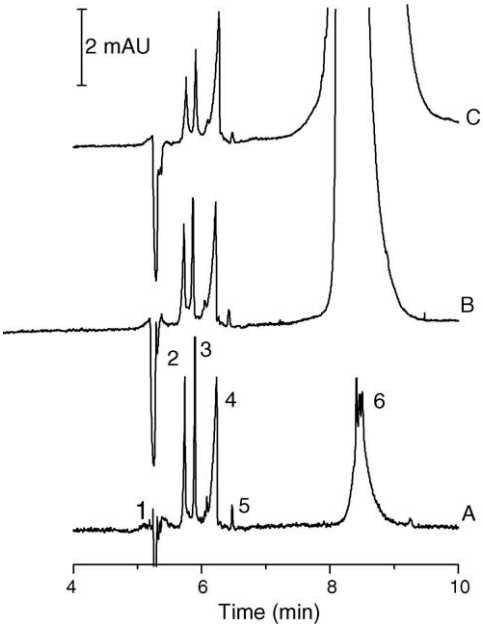

Fig. 8. CE of a protein test mixture containing HSA at a concentration of (A) 1 mg/mL, (B) 10 mg/mL, and (C) 60 mg/mL using a PB-PVS coated capillary and a BGE of 300 mM Tris phosphate (pH 8.5). Peaks: 1, system peak; 2, myoglobin; 3, carbonic anhydrase; 4, interferon-α 2b; 5, impurity from carbonic anhydrase; 6, HSA.

4. Conclusions

This work demonstrates the utility of noncovalent bilayer coatings for the CE analysis of proteins. The preparation of the coating is simple and straightforward, comprising only two short rinsing steps with a positively-charged polymer and a negatively-charged polymer, respectively. CE with PB-PVS coatings provides highly efficient and repeatable results for proteins. This excellent performance, which cannot be obtained with bare-fused silica capillaries, derives from the stable (and fast) EOF provided by the coatings, and the minimization of protein-capillary wall interactions. The potential of the PB-PVS system is demonstrated by the analysis of a degraded insulin sample and of the glycoprotein ovalbumin comprising various isoforms. Furthermore, it is shown that the PB-PVS coating can handle protein samples with high concentrations of HSA and still provides stable migration times. This can be advantageous for the analysis of biopharmaceutical formulations in which HSA is often used as stabilising agent. The possibility to use a commercial kit (CEofix) for preparing bilayer coatings suitable for proteins is also shown. Prerequisite for a good performance is that the BGE provided by the kit is replaced by a common buffer as sodium or Tris phosphate.

As demonstrated in this work, no polymeric coating agents have to be added to the BGE to achieve good performances

with bilayer CE coatings. This is a clear advantage when coupling such CE systems with mass spectrometry (MS) in order to gain mass and/or structural information on peptides and proteins. Currently, we are working on the development of CE–MS systems applying bilayer-coated capillaries. In that respect, we are also studying the possibility of using volatile BGEs in combination with PB-PVS coatings. The stable and relatively high EOF mobility provided by the coating is a definite advantage when performing CE–MS, especially with sheathless interfacing.

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