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Stability of capillaries coated with highly charged polyelectrolyte monolayers and multilayers under various analytical conditions—Application to protein analysis

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ABSTRACT

The stability of capillaries coated with highly charged polyelectrolytes under various analytical conditions was studied, as well as their performance for the analysis of proteins by Capillary Electrophoreis (CE) over a wide range of pH (2.5–9.3). In this study, fused silica capillaries were modified either with a poly(diallyldimethylammonium) chloride (PDADMAC) monolayer or PDADMAC/poly(sodium 4-styrenesulfonate) (PSS) multilayer coatings, using optimal coating conditions previously determined [1–3]. Results show that the coated capillaries are remarkably stable and efficient to limit protein adsorption under a variety of extreme electrophoretic conditions even in the absence of the coating agent in the background electrolyte which is exceptional for non-covalent coatings. Monolayer coated capillaries were demonstrated for the first time to be stable to acidic rinses and to organic solvents which proves that the stability of the capillaries is highly dependent on the coating procedure used. In addition, PDADMAC/PSS multilayer coatings were found to be stable to alkaline treatments. PDADMAC/PSS coated capillaries gave excellent performances for the analysis of proteins covering a large range of p*I* (4–11) and of molecular weight (14–65 kDa) over a wide pH range (i.e. 2.5–9.3). Even at high pH 9.3, protein analysis was possible with very good repeatabilities ($RSD_{tm} < 1\%$ and $RSD_{CPA} < 2.6\%$ ($n \geq 8$)) and high peak efficiencies in the order of 700,000.

1. Introduction

Developments in the fields of proteomics and biotechnology have caused an increasing demand for sensitive and selective analytical tools for peptide and protein analysis, and for techniques that allow protein analysis under non-denaturizing conditions.

In the past decade, capillary electrophoresis (CE) has become one of the most powerful tools for qualitative and quantitative analysis of peptides and proteins [4–12]. CE provides some unique advantages such as very high resolution and efficiencies and, in particular, the possibility to study intact proteins without causing conformational changes, unlike in liquid chromatography (LC) where organic mobile phases and/or stationary phases may cause protein denaturation [5].

The separation of peptides and proteins by CE is strongly affected by adsorption onto bare fused-silica capillaries caused by electrostatic, hydrogen and hydrophobic interactions between the analytes and the silanol groups of the capillary surface [13,14]. Adsorption leads to poor repeatability and deterioration of separation performance [4,13,15,16]. Several approaches have been explored to overcome this problem [4,6,9,13,17–19]. Background electrolyte (BGE) solutions of extreme pH values [20,21] or high ionic strength [22,23] can be used. However, under these conditions most proteins are unstable and selectivity may be limited. Another approach consists in changing the properties of the inner capillary surface by various coating procedures. Capillary coating agents can be either covalently bound to the silanol groups or physically adsorbed to the capillary surface. For covalent coatings, complex and multiple time-consuming steps are required and coating reproducibility between capillaries is usually poor [13,17,24,25]. Physically adsorbed coatings are strongly attached to the capillary wall by electrostatic, hydrogen and hydrophobic interactions [13,18]. They present several advantages [17,26] like simplicity of the coating procedure, possibility of automation and regeneration of the coating as well as EOF modification [27,28]. Neutral polymers like poly(ethylene oxide) (PEO) [29,30] and poly(dimethyl acrylamide) (PDMA) [26] can be used for physical coatings. However, apart from PEO that was demonstrated to be stable till ~pH 7.7 [29,30], neutral coatings are usually not stable at pH values higher than 5 [31]. Cationic polymers are usually more stable than neutral polymers. However, capillaries coated with a monolayer of polycation are reported to have short endurance and to be unstable in the presence of extreme analytical conditions (highly acidic or basic solutions, presence of organic solvent, etc.). Regeneration of

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the coating between runs or addition of the coating agent in the BGE may therefore be necessary to obtain stable coatings [32–34]. The presence of the coating agent in the BGE may cause protein denaturation and may interfere with analyte detection, especially with MS detection. To further improve stability, reproducibility and lifetime of physical coatings, Katayama et al. [34,35] introduced a successive multiple ionic-polymer layer (SMIL) coating procedure, in which "a cationic polymer is sandwiched between an anionic polymer and the uncoated negative fused-silica capillary". Graul at al. [36] were the first to report the great ability of PDADMAC/PSS multilayer coatings to inhibit irreversible protein adsorption in CE as well as the influence of coating conditions on analytical performances. Multilayer coatings were found to be stable over a wide range of pH [34–36].

The higher efficiency of SMIL coatings compared to monolayers for protein analysis is mainly to be attributed to a better coverage of the silanol groups leading to a more hydrophilic surface and therefore reduced protein adsorption [34–36]. An important advantage of SMIL coatings is the possibility to use various coating agents (highly charged high molecular weight polyelectrolytes, small molecular mass peptides, proteins, DNA, etc.) which enables to introduce a characteristic selectivity based on interactions between the analytes and the coating. Kitagawa et al. [37] have successfully analyzed basic proteins in three-layer poly(ethyleneimine) (PEI)/dextran sulafate (DS)/Protamine coated capillaries. In the same study, the possibility to perform chiral OT-CEC in a three layer PEI/DNA/Poly(arginine) coated capillary is demonstrated, the DNA-Poly(arginine) complex playing a significant role in the enantiorecognition. Furthermore, the addition of various additives in the BGE, allows to improve selectivity in a very fine manner with excellent repeatability. Thus, Weinbauer et al. [38] realized the separation of closely related recombinant allergen variants in SMIL coated capillaries.

SMIL coatings were also recently successfully applied in PMMA or PDMS microchips for electrophoresis to improve the functionality of the polymeric material [39,40]. Improved performances in terms of EOF repeatability and peak efficiencies were observed in coated microchips and were comparable to those obtained in standard glass microchips [39].

The use of basic or organic rinses with monolayer or SMIL coatings with or without regeneration of the coatings between runs has been reported in several publications [34,41,42]. Lu et al. [42] have recently reported the possibility to perform non-aqueous capillary electrophoresis (NACE) in PDADMAC/PSS coated capillaries, and Steiner et al. [44] have reported the separation of inorganic and organic anions at highly basic pH (12). However, to the best of our knowledge, no systematic study has been yet conducted to investigate the endurance of SMIL coatings during protein analysis neither their ability to prevent adsorption under different analysis conditions.

In previous studies [1,2], we demonstrated that the coating conditions of the capillaries have a dramatic influence on the stability of poly(diallyldimethylammonium) chloride (PDADMAC) monolayer and PDADMAC/poly(sodium 4-styrenesulfonate) (PSS) SMIL coatings and on their ability to prevent peptide and protein adsorption. The experimental parameters that were systematically investigated were the type and concentration of polyelectrolytes, the ionic strength of coating and dipping/stabilizing solutions, and the procedures used for coating and capillary storage. Optimal coating conditions were determined, and the PDADMAC monolayer and PDADMAC/PSS SMIL coated capillaries performed very well for the analysis of peptides and proteins at acidic pH (i.e. 2.5) [1–3].

In this study, the ability of PDADMAC monolayer and PDADMAC/PSS multilayer coated capillaries (under optimal coating conditions) to be used under various analysis conditions was studied. The performance of these coatings for the analysis of proteins over a wide pH range, i.e. 2.5–9.3, as well as their stability to intensive rinses (acidic, alkaline, organic solvent) between analyses was examined. Coating ability to prevent protein adsorption was investigated under the different analysis conditions by monitoring peak efficiencies, and repeatability of migration times and time-corrected peak areas (CPAs) of various test proteins.

2. Materials and methods

2.1. Chemicals and materials

Doubly distilled water, produced in house from a glass apparatus, was used throughout. PDADMAC (High Molecular Weight: $M_r \sim 4.10^5$–5.10^5) 20% (w/w) in water, PSS (average $M_r \sim 10^6$) 10% (w/w) in water, carbonic anhydrase (CA) from bovin erythrocytes, cytochrome c (Cyt c) from bovine heart, α-lactalbumin (α-Lac) from bovine milk, lysozyme (Lys) from chicken egg white, myoglobin (Myo) from horse heart, ribonuclease A (Rib A) from bovine pancreas and phosphate buffered saline solution (PBS; pH 7.2) were purchased from Sigma–Aldrich (Lyon, France). Sodium tetra-borate $10H_2O$ ($Na_2B_4O_7{\cdot}10H_2O$, purity ≥99.5%) and Tri(hydroxymethyl)aminomethane (TRIS, purity ≥99.7%) were from Fluka (Buchs, Switzerland). Acetonitrile (CH_3CN), N,N-dimethylformamide (DMF), hydrochloric acid (HCl, 37% (w/w) in water), methanol (CH_3OH) and sodium hydroxide (NaOH, purity ≥97%) were from Carlo Erba (Val de Reuil, France). Di-sodium hydrogen phosphate dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$, purity ≥98%), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$, purity ≥98%) and orthophosphoric acid (H_3PO_4, 85% (w/w) in water) were from Prolabo (Paris, France). Sodium chloride (NaCl, purity ≥99.5%) was purchased from Prolabo (Paris, France), Carlo-Erba (Val de Reuil, France) or Fluka (Buchs, Switzerland). All chemicals were used as received.

Nylon Puradisc™ Syringe Filters, pore size 0.45 μm were purchased from Whatman (Versailles, France).

2.2. Solutions

Coating solutions were prepared by dissolving the cationic (PDADMAC) or the anionic (PSS) polyelectrolyte at 0.2% (w/v) in a 20 mM TRIS aqueous solution adjusted to pH 8.3 with 0.01 M HCl. The ionic strength (I) of this solution was 0.01 M, neglecting the polyelectrolyte concentration. It was set to 1.5 M by NaCl addition. Coating solutions were used within one week and stored at 4 °C when not in use.

Different aqueous background electrolyte solutions (BGE) were used to cover a wide pH range [2.6–9].

- For analysis at pH 2.5, a 100 mM TRIS-phosphate buffer was prepared by mixing 100 mM H_3PO_4 with 73.64 mM TRIS, without pH adjustment. The ionic strength of this solution is 0.07 M.
- For analysis at pH 7.0, a 100 mM phosphate buffer was prepared by mixing 61.35 mM NaH_2PO_4, $2H_2O$ with 38.65 mM Na_2HPO_4, $12H_2O$. The pH of this solution was adjusted to 7.0 with NaOH 1 M. The ionic strength of this solution is approximately 0.18 M.
- For analysis at pH 9.3, a 50 mM $Na_2B_4O_7$, $10H_2O$ buffer was prepared by dissolving the appropriate quantity in distilled water, without pH adjustment. This corresponds to 100 mM borate buffer. The ionic strength of this solution is 0.1 M.

The EOF marker solution was a 0.05% (v/v) DMF solution in water.

Test protein solutions used to evaluate the coating performance in terms of peak efficiency and adsorption prevention consisted of different acidic and basic proteins. Stock individual solutions 0.5 g/L CA (pI 6.2), 0.5 g/L Rib A (pI 8.7) and 0.1 g/L Myo (pI 7.3) were

Table 1
Capillary conditioning and protein analytical procedures used with mono- and multi-layer coated capillaries.

		L = 31.2 cm; i.d. 50 μm	L = 81.2 cm; i.d. 25 μm
Pressure for rinse cycles		20 psi	60 psi
Capillary conditioning procedure	1. Rinse NaOH 1 M	15 min	30 min
	2. Rinse NaOH 0.1 M	15 min	30 min
	3. Rinse distilled water	2 min	4 min
Number of coated layers		1 and 5 (cationic coating)	4 (anionic coating)
Analytical procedure	1. Injection side	Cathode	Anode
	2. Injection volume	DMF: 2.37 nL Protein: 9.88 nL	DMF: 2.37 nL CA, Myo, RibA: 10.19 nL Hb: 1.19 nL
	3. Separation voltage	−10 kV (reverse polarity)	+30 kV (normal polarity)
	4. Separation temperature	25 °C	25 °C
	5. Detection wavelength	214 nm	CA, Myo, RibA: 214 nm Hb: 415 nm

prepared in 0.07 M PBS. A stock mixed solution containing 0.2 g/L α-Lac (p*I* 4.3), 0.1 g/L Myo (p*I* 7.3), 0.5 g/L Rib A (p*I* 8.7), 0.1 g/L Cyt c (p*I* 10.5) and 0.1 g/L Lys (p*I* 11) was also prepared in 0.07 M PBS solution. Gentle agitation was used for protein dissolution to avoid any possible denaturation [45]. Protein solutions were filtrated through 0.45 μm pore size filters, divided into aliquots and then stored at −20 °C. Each aliquot was defrosted at a constant temperature of 37 °C (bain-marie) for 25 min, heated at 50 °C (bain-marie) for 25 min and then allowed to stand at 25 °C for 30 min before analysis [2]. Protein solutions were used within three days and stored at 4 °C when not in use. Injection of individual proteins enabled peak identification through mobility matching.

2.3. Instrumentation and operating conditions

All experiments were performed on a P/ACE MDQ instrument (Beckman, Fullerton, CA, USA) equipped with a photodiode array detection system.

Capillaries used for all experiments were prepared from bare fused-silica tubing (TSP, Composite Metal Services, Hallow, UK) and housed in a cartridge with a (100 × 800 μm) detection window. Capillaries of 31.2 cm total length (21 cm to the detector window) and 50 μm internal diameter (i.d.) were used all through this study except for the analyses of proteins at pH 9.3 where capillaries of 81.2 cm total length (71 cm to the detector window) and 25 μm i.d. were employed.

A new capillary was prepared for each set of experiments. New capillaries were initially conditioned with alkaline solutions to produce a surface capable to bind homogeneously polyelectrolytes. Conditioning procedure for the different capillaries is summarized in Table 1. PDADMAC and PSS were respectively chosen as the polycation and the polyanion coating agents.

For analysis at pH 2.5 and 7.0, cationic PDADMAC monolayer and 5-layer PDADMAC/PSS coated capillaries were used, i.e. the last deposited layer was a PDADMAC layer. The cationic monolayer was deposited by rinsing the capillary for 10 min with the polycation solution, preceded by a 2-min water rinse. Then, a 2-min rinse was carried out with the BGE before applying a +10 kV potential for 10 min in the BGE to stabilize the coating. For multilayer (5-layer) coatings, cationic and anionic polyelectrolyte layers were alternatively deposited following, for each layer deposition, the procedure described above for the PDADMAC monolayer, and introducing a 2-min rinse step with the BGE between layers. Coating stabilization – similar to that of monolayer stabilization – followed by a 10 min wait time in BGE was carried out after film deposition (for details see references [1,2]). In the case of these cationic coatings, cathodic injections were done since the EOF was reversed.

For analysis at pH 9.3, anionic 4-layer PDADMAC/PSS coated capillaries were used, i.e. the last deposited layer was a PSS layer. In this case, the capillaries used are longer than the ones employed for analysis at pH 2.5 and at pH 7.0 (81.2 cm *vs.* 31.2 cm total length) and had a smaller internal diameter (25 μm *vs.* 50 μm). Thus, cationic and anionic polyelectrolyte layers were alternatively deposited by rinsing the capillary for 25 min with the appropriate coating solution, preceded by a 4-min water rinse. Then, a 5 min wait time in the coating solution followed by a 4-min rinse with the BGE was carried out. To stabilize the coating after film deposition, a +30 kV potential for 15 min was applied in the BGE followed by a 10 min wait time in the BGE. In this case, the EOF was normal and anodic injections were done.

Coated capillaries were stored in water. Stored coated capillaries of 31.2 cm total length were regenerated by a 5-min rinse with water followed by a 5-min rinse with BGE and 10-min equilibration at +10 kV in the BGE. For 81.2 cm total length capillaries, the regeneration procedure consisted of a 10-min rinse with water followed by a 10-min rinse with BGE and 15-min equilibration at +30 kV in the BGE.

Analysis conditions are listed in Table 1. For protein analysis, the BGE solution in the separation vials were changed every five runs. Between runs, capillaries were flushed with the BGE.

2.4. Measurements and calculations

2.4.1. Electroosmotic mobility determination

Electroosmotic mobility (μ_{eo}) for coated capillaries was calculated from the migration time of DMF used as neutral marker. For slow EOF (<10^{-5} cm^2 V^{-1} s^{-1}), the method described by Williams and Vigh [46] was used (for details see [1]).

2.4.2. Repeatability studies

Coating stability was controlled directly after coating deposition by repeated injections of EOF marker solution, and in the course of protein analysis by EOF marker co-injection. Possible analyte adsorption was monitored by repeated measurements of protein t_m and CPAs, and EOF mobility. Repeatability was expressed as RSDs, where "*n*" is the number of repeated injections.

2.4.3. Coating chemical stability

PDADMAC/PSS coating stability towards acidic, alkaline and organic rinses was studied by measuring the EOF mobility immediately after the capillary coating (μ_{eo1}), and after each destabilizing treatment (μ_{eo2}) using a 100 mM TRIS-phosphate buffer pH 2.5 as running electrolyte. The stability of the coating was evaluated by EOF mobility RSD and by measuring the % of variation of the EOF mobility before and after the destabilizing treatment, $|(\mu_{eo1} - \mu_{eo2})/\mu_{eo1}| \times 100$ (%).

3. Results and discussion

In the first part of this work, the stability of PDADMAC monolayer and PDADMAC/PSS multilayer coated capillaries towards different destabilizing treatments, i.e. acidic, alkaline and organic

Table 2
Chemical stability of PDADMAC monolayer coatings.

Destabilizing treatment	Monolayer coatings				
	EOF_1 before treatment		EOF_2 after treatment		Variation of EOF mobility
	μ_{eo1} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)	μ_{eo2} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)	(%)
(a) Rinse 1 M HCl	−2.86	0.15	−2.92	0.20	2.10
(b) Rinse 1 M NaOH	−2.94	0.06	−0.16	20.7	94.56
(c) Rinse CH_3CN	−3.16	0.28	−3.07	1.35	2.85
(d) Rinse CH_3OH	−2.94	0.15	−2.86	0.62	2.72
(f): Successive rinses: 1 M HCl/1 M NaOH	−2.86	0.15	+0.03	32.00	101.05
(g): Successive rinses: 1 M HCl/1 M NaOH/CH_3CN/CH_3OH	−2.90	0.11	−0.40	13.77	86.21
(h): (g) repeated 3 times	−2.90	0.11	+0.05	23.50	101.72

Table 3
Chemical stability of 5-layer PDADMAC/PSS coatings (last deposited layer is cationic).

Destabilizing treatment	5-layer coatings				
	EOF_1 before treatment		EOF_2 after treatment		Variation of EOF mobility
	μ_{eo1} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)	μ_{eo2} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)	(%)
(a) Rinse 1 M HCl	−2.98	0.15	−2.92	0.08	2.01
(b) Rinse 1 M NaOH	−2.99	0.14	−2.98	0.08	0.33
(c) Rinse CH_3CN	−2.92	0.10	−2.84	0.98	2.74
(d) Rinse CH_3OH	−2.90	0.18	−2.83	0.76	2.41
(f): Successive rinses: 1 M HCl/1 M NaOH	−2.98	0.08	−2.99	0.12	0.34
(g): Successive rinses: 1 M HCl/1 M NaOH/CH_3CN/CH_3OH	−2.92	0.08	−2.73	0.10	6.51
(h): (g) repeated 3 times	−2.92	0.08	−1.90	0.39	34.93

rinses was studied. In the second part, the possibility to use these coatings for protein analysis over a wide range of pH, i.e. 2.5–9.3, was investigated. Several proteins covering a wide range of p*I* (4–11) and of molecular weight (14–65 kDa) were used to evaluate the capillary coating performance.

3.1. PDADMAC/PSS coating stability towards acidic, alkaline or organic media

Different destabilizing treatments were performed on PDADMAC monolayer and PDADMAC/PSS multilayer coated capillaries to investigate their stability towards acidic, alkaline and organic media. The different treatments operated and their effects on coating stability are summarized in Tables 2–4. Coated capillaries were rinsed for 30 min (i.e. 240 capillary volumes) with either 1 M HCl, 1 M NaOH, CH_3OH or CH_3CN (see Tables 2–4, treatments a–d). The stability of monolayer and multilayer coated capillaries to the application of successive destabilizing treatments such as acidic rinses followed by alkaline ones (Table 2a–c, treatments f–h) was also evaluated. The BGE used was a TRIS-phosphate buffer (100 mM) at pH 2.5.

3.1.1. PDADMAC monolayer coatings

PDADMAC monolayer coatings were found to be highly stable towards 1 M HCl, CH_3OH or CH_3CN rinses (variation of EOF mobility is less than 3%). The PDADMAC monolayer coated capillaries also exhibited very long life time since more than 100 analyses were performed on the same capillary during 2 months and after several storage periods (every week) without any noticeable deterioration of the analytical performances.

These results are very important since monolayer coated capillaries were found so far to be unstable to acidic and organic media and to have short life time as reported by Katayama et al. [34,35] for polybrene monolayer coatings. The strong stability of the PDADMAC monolayer coatings can be attributed to an extremely good adherence of PDADMAC to the silica surface due to the strong electrostatic interactions between the ammonium groups of the polymer and the silica groups [44], and also to the optimized coating conditions used in this study (for details see references [1,2]). More specifically, the presence of a high content of salts (1.5 M) in the coating solution results in thicker and more stable capillary coatings [1,2].

Table 4
Chemical stability of 4-layer PDADMAC/PSS coatings (last deposited layer is anionic).

Destabilizing treatment	4-layer coatings				
	EOF_1 before treatment		EOF_2 after treatment		Variation of EOF mobility
	μ_{eo1} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)	μ_{eo2} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)	(%)
(a) Rinse 1 M HCl	3.02	0.24	2.95	0.51	2.32
(b) Rinse 1 M NaOH	2.93	1.15	1.62	4.50	53.58
(c) Rinse CH_3CN	3.05	0.38	2.98	0.59	2.30
(d) Rinse CH_3OH	3.06	0.59	2.95	0.87	3.59
(f): Successive rinses: 1 M HCl/1 M NaOH	3.03	0.61	2.97	0.30	1.98
(g): Successive rinses: 1 M HCl/1 M NaOH/CH_3CN/CH_3OH	3.05	0.50	2.79	2.18	8.52
(h): (g) repeated 3 times	3.04	0.42	1.62	4.56	46.71

Table 5
EOF of monolayer and multilayer coatings at different pH values of BGE.

pH	Number of layers	Immediately after capillary coating		During or after protein analysis	
		μ_{eo} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)	μ_{eo} (10^{-4} cm^2 V^{-1} s^{-1})	RSD (%)
2.5	1	−2.88	0.15 (n = 9)	−2.83	0.99 (n = 13)
	5	−2.96	0.10 (n = 9)	−3.03	0.59 (n = 13)
7.0	1	−2.18	0.64 (n = 15)	−1.59	4.47 (n = 20)
	1[a]	−2.29	0.19 (n = 20)	−2.25	0.22 (n = 9)
	5[a]	−2.25	0.16 (n = 11)	−2.25	0.09 (n = 9)
9.3	4	+3.99	0.12 (n = 8)	+3.93	0.13 (n = 6)

[a] Coating regeneration (0.5 min PDADMAC rinse step between runs).

However, PDADMAC monolayers were found to be not stable when submitted to alkaline treatments, since about 95% of the monolayer was removed with 1 M NaOH. These results are in accordance with previous studies from Katayama et al. [34,35] on polybrene monolayer coatings. After rinsing the capillary first with 1 M HCl, followed by 1 M NaOH (Table 2a, treatment f), the coating is even fully removed since the EOF measured after this latter treatment is cathodic, and its magnitude is similar to that obtained in uncoated fused-silica capillaries (+0.03 × 10^{-4} cm^2 V^{-1} s^{-1}).

3.1.2. PDADMAC/PSS multilayer coatings

Similar results were obtained for the stability of both 5-layer (last deposited layer is cationic) and 4-layer (last deposited layer is anionic) coated capillaries. As for monolayers, multilayer coated capillaries were found to be very stable towards acidic and organic rinses. Furthermore, while alkaline rinses dramatically destabilized monolayer coatings, multilayer coatings proved to be highly stable as the variation of the EOF mobility observed after applying the treatment is less than 0.4% for cationic 5-layer coatings and 2% for anionic 4-layer coatings.

Application of successive sequences of alkaline, acidic and organic rinses (Table 2b and c, treatments g and h) lead to an alteration of the coatings. However, the % of variation observed for the EOF mobility is much smaller than in the case of monolayer coatings which confirms the better stability of multilayer coatings. This can be explain by an extensive interpenetrations of the different polyelectrolyte layers [34–36], an irreversible complexation between PDADMAC and PSS [47–51] and an increase of the thickness of the coating which inhibit protein interactions with the capillary. Although an alteration of the coatings was observed, this deterioration only concerned the last deposited layer. Indeed, when a simple rinse with the polyelectrolyte corresponding to the last deposited layer (i.e. PDADMAC for the 5-layer and PSS for the 4-layer coated capillaries respectively) was applied after successive sequences of alkaline, acidic and organic rinses, a coating of the capillary with an EOF that had statistically the same characteristics as the EOF obtained before the destabilizing treatment was obtained. These results confirm the very good stability of the PDADMAC/PSS multilayer coatings compared for instance to Polybrene/DS coatings that were found to be not stable to acidic rinses [34,35].

The stability of PDADMAC monolayer and PDADMAC/PSS multilayer coatings towards organic solvents (CH_3OH and CH_3CN) offers the possibility to analyze samples containing organic solvent and predicts their ability to be used with electrolytes containing high percentage of organic modifiers. Recently, Lu et al. [43] have recently successfully analyzed phenolic acids in non-aqueous capillary electrophoresis (NACE) using a three layers PDADMAC/PSS coated capillary. However, it should be noted that EOF may need some time to stabilize when using a non-aqueous BGE or after organic rinses (RSD obtained for EOF mobility after organic rinses are superior to that obtained before the treatment, see Table 2). Indeed, organic solvent may induce an alteration of the polyelectrolyte chain configuration and some time is therefore needed for reequilibration in the presence of BGE.

The stability of PDADMAC/PSS multilayer coatings to acidic and alkaline rinses, is also a very important issue since this type of rinse cycles is very efficient to improve protein analysis repeatability [18,35] when analyte adsorption is encountered. Another advantage of applying acidic and alkaline rinses with these highly charged polyelectrolyte coatings is that strong pH-hysteresis effects are suppressed, unlike for uncoated fused silica capillaries [52]. Stability of SMIL coatings towards extreme pH suggests possibility to use them for analysis over a wide pH range.

3.2. Stability of PDADMAC monolayer and PDADMAC/PSS multilayer coatings as a function of the pH of the BGE solution

The possibility to perform electrophoretic analysis of proteins with PDADMAC monolayer, and 4- or 5-layer PDADMAC/PSS coated capillaries over a wide pH range (2.5–9.3) was examined. Since ionization and conformation of proteins can dramatically change depending on their environment, i.e. composition and pH of the BGE [45,53], no comparison can be made between the results obtained under the different analysis conditions. At low and neutral pH (i.e. 2.5 and 7.0), the test proteins (Lys, Cyt c, α-Lac, Myo and Rib A) were analyzed with cationic coatings (mono and 5-layer coatings) since they are all positively charged (CA that is also positively charged was not analyzed in these conditions since it co-migrates with other proteins). On the opposite, at alkaline pH, only negatively charged proteins (Myo, RibA, CA) were analyzed with anionic 4-layer coating.

Table 6
Protein analysis repeatability obtained at different pH values of BGE solution with PDADMAC monolayer and 5-layer PDADMAC/PSS coatings (n = 13).

pH	Number of layers			
	1		5	
	RSD % range for t_m	RSD % range for CPAs	RSD % range for t_m	RSD % range for CPAs
2.5	0.66–1.12	3.58–10.61	0.37–0.48	4.55–8.02
7.0[a]	0.18–0.49	6.12–7.72	0.53–0.64	5.22–7.79

[a] Results given for α-Lac, Cytc and Lys. For protein analysis conditions see Table 1.

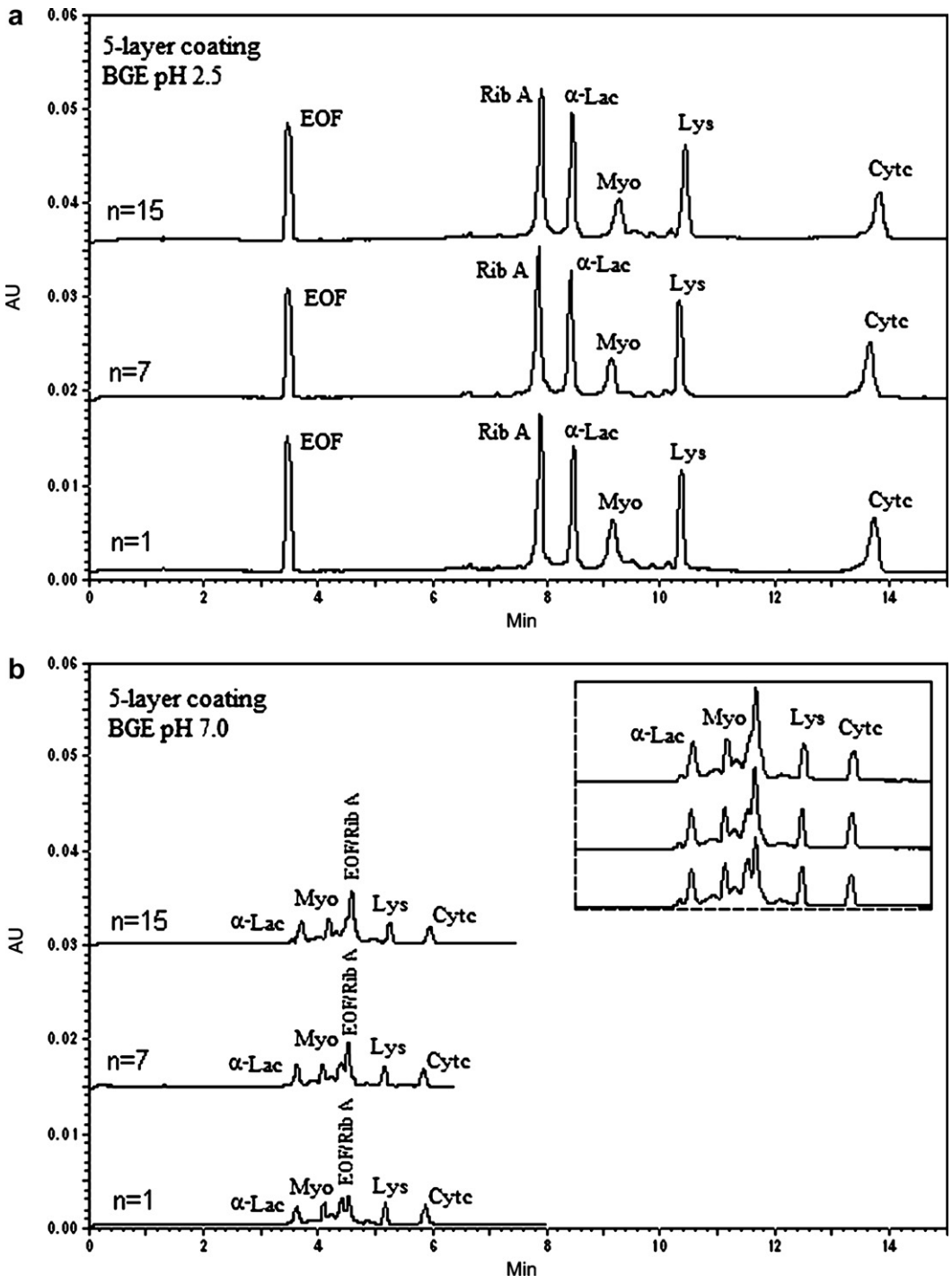

Fig. 1. Repeatability of protein analysis using 5-layer PDADMAC/PSS coated capillaries (31.2 cm total length, 50 μm i.d.). Electropherograms obtained for the 1st, 7th and 15th injection (a) at pH 2.5 (100 mM TRIS-phosphate buffer) and (b) at pH 7.0 (100 mM phosphate buffer). Peak identification: Rib A (ribonuclease A); α-Lac (α-lactalbumine); Myo (myoglobin); Lys (lysine) and Cytc (cytochrome C). For capillary coating and electrophoretic conditions see Sections 2.2, 2.3 and Table 1, respectively.

3.2.1. EOF repeatability for mono- and multi-layer coatings

For cationic PDADMAC monolayer and 5-layer PDADMAC/PSS coated capillaries, an anodic EOF was obtained at pH 2.5 and 7.0. Its magnitude was slightly higher at pH 2.5 (Table 5) which is mainly to be attributed to the difference in buffer composition (100 mM TRIS-phosphate at pH 2.5 *vs.* 100 mM phosphate buffer at pH 7.0) and to the higher ionic strength of the electrolyte at pH 7.0 (0.18 M *vs.* 0.07 M at pH 2.5) [54]. Small changes in the chain configuration of PDADMAC and PSS between pH 2.5 and pH 7.0 are also possible [55]. However, as already reported in the literature [55], the EOF obtained in cationic coated capillaries can be considered as almost independent of the BGE pH in the range 2.5–7, in contrast to the EOF obtained in uncoated fused-silica capillaries [54]. At pH 2.5, very stable EOF was obtained after coating as well as during protein analysis for both monolayer and multilayer coatings (RSD $\mu_{eo} < 1\%$). At pH 7.0, regeneration of the last deposited layer, i.e. PDADMAC, between runs is necessary to obtain a stable EOF. Without any coating regeneration between runs, the EOF drifted progressively with time and protein analysis was not possible (Table 5). This result shows the importance of monitoring the

coating stability not only after its deposition but also in the course of the protein analysis. Since the variations of EOF mobility were only observed during protein analysis, this phenomenon is to be attributed to the gradual adsorption of some of the test proteins that are negatively charged at neutral pH and can therefore interact via electrostatic interactions with the cationic PDADMAC coating, and not to the alteration of the coating. This assumption was confirmed by the fact that in the absence of coating regeneration, CPAs of the proteins decreased between runs which is characteristic of adsorption phenomena. When the capillary was rinsed between analyses for 0.5 min with the PDADMAC coating solution, extremely stable EOF was achieved (RSD μ_{eo} < 0.3%). Indeed, since PDADMAC has a very high molecular weight, it can remove lower molecular weight proteins from the coating surface by a so called "stripping effect" as it is reported in the literature [56].

At pH 9.3, a 100 mM borate BGE was used. The EOF obtained for the 4-layer coated capillaries was cathodic with a high mobility of $+3.99 \times 10^{-4}$ cm^2 V^{-1} s^{-1} (Table 5). It was highly stable immediately after coating and even after several protein analyses (approximately 100 runs) with a RSD μ_{eo} < 0.20%. Unlike for cationic coatings at pH 7.0, no regeneration between runs was needed to maintain a stable EOF. Indeed, the possible adsorption of negatively charged protein previously observed at pH 7.0 in the cationic coated capillary is probably limited in this case by repulsive interactions with the anionic coated capillary.

This study shows that PDADMAC monolayer and PDADMAC/PSS multilayer coatings are stable over a large pH range from 2.5 to 9.3 with a long lifetime (>100 protein analyses). Furthermore, possible adsorption of the proteins during analysis can be limited by: (i) using a coated capillary with a last deposited polyelectrolyte layer carrying a charge similar to that of the proteins or (ii) simply rinsing the coated capillary between runs with a solution of polyelectrolyte.

3.2.2. *Protein analysis with mono- and multi-layer coatings*

Results are given in Tables 4 and 5, and representative electropherograms obtained with 5-layer and 4-layer coated capillaries are shown in Figs. 1 and 2, respectively.

3.2.2.1. Cationic PDADMAC monolayer and 5-layer PDADMAC/PSS coatings. A test mixture of Lys, Cyt c, α-Lac, Myo and Rib A, was analyzed with cationic coatings at acidic and neutral pHs. As expected, the migration order of the proteins as well as the resolution changed with pH. At acid pH (2.5), the five proteins have a global positive charge (pH < p*I*) and were detected after the EOF marker (Fig. 1a). Repeatable analyses were obtained for both monolayer and multilayer coatings with RSD_{tm} < 1.2% (n = 13). At pH 7.0, only the Lys and the Cyt c are positively charged whereas the α-Lac and the Myo have a negative net charge and migrated faster than the EOF marker. The RibA is almost neutral and co-migrated with the EOF (Fig. 1b). To obtain repeatable analyses of the proteins, regeneration of the last deposited layer of PDADMAC was necessary. A step rinse with PDADMAC coating solution for 0.5 min appeared to be very efficient to eliminate the adsorbed proteins. Repeatability results at pH 7.0 (Table 6) refers only to α-Lac, Lys and Cyt c since the low resolution between Myo, Rib A and the EOF marker made inaccurate the protein peak integration. In these conditions, RSD_{tm} were less than 1% (n = 13).

For α-Lac and Lys, better peak efficiencies were obtained at pH 2.5 while similar results were obtained when analyzing Cyt c under acidic and neutral conditions.

3.2.2.2. Anionic 4-layer PDADMAC/PSS coatings. Myo, Rib A and CA were individually analyzed using 100 mM borate BGE at pH 9.3.

Results are presented in Table 7 and Fig. 2a–c. As shown in Table 5, very good repeatabilities were obtained for the different

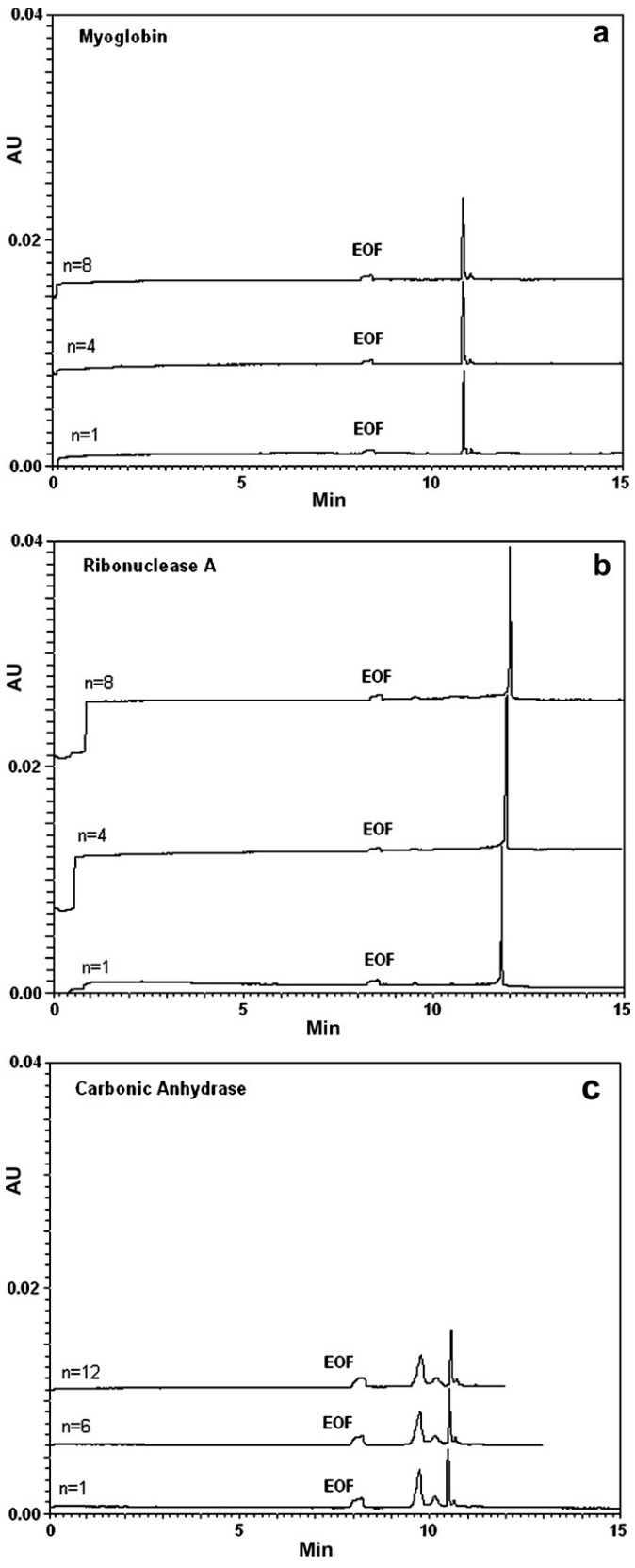

Fig. 2. Repeatability of protein analysis using 4-layer PDADMAC/PSS coated capillaries (81.2 cm total length, 25 μm i.d.) and 100 mM borate BGE pH 9.3. Electropherograms obtained for (a) Myo (myoglobin); (b) Rib A (ribonulease a) and (c) CA (carbonic anhydrase). For capillary coating and electrophoretic conditions see Sections 2.2, 2.3 and Table 1, respectively.

Table 7
Protein analysis with anionic 4-layer PDADMAC/PSS coated capillaries, pH 9.3.

	RSD_{tm} (%)	RSD_{CPA} (%)	N
Myo ($n=8$)	0.12	1.09	~715,000
Rib A ($n=8$)	0.90	2.53	~700,000
CA ($n=12$) (4 peaks detected)			
1st peak	0.05	1.44	~20,000
3rd peak	0.06	2.06	~400,000

For protein analysis conditions see Table 1.

proteins with $RSD_{tm} < 1\%$ and $RSD_{CPA} < 2.6\%$ ($n \geq 8$). No regeneration of the capillaries between analyses was necessary, and high peak efficiencies in the order of 700,000 were obtained for Myo and CA.

4. Conclusion

The chemical stability of PDADMAC monolayer and PDADMAC/PSS multilayer coated capillaries towards acidic, alkaline and organic destabilizing treatments was investigated.

This is a very important concern since the performances of physically adsorbed coatings have often been reported to degrade when working at basic pH or in the presence of organic solvent for instance [31,34]. A non-covalent monolayer coating is for the first time demonstrated to be stable and to have a long lifetime when analyses are performed at high pH values and in the presence of organic solvents. Furthermore, the multilayer coatings we used also proved to be stable to successive sequences of acidic, alkaline and organic rinses. The stability of these coatings is demonstrated not only after film deposition but also during protein analysis. This study demonstrates that the stability of the coating depends on the coating conditions used and that the optimal coating procedure proposed in previous studies [1,2] allows obtaining highly stable coatings.

The possibility to use PDADMAC/PSS coated capillaries under broad analytical conditions has very important issues in CE especially for the analysis of proteins:

- The strong chemical stability of these coatings under various analytical conditions in the absence of the coating agent in the BGE, especially with SMIL coatings, predicts their possible use with MS detection. In fact, for CE-MS, the coating agent must not migrate into the mass spectrometer because the presence of non-volatile buffer constituents may deteriorate the ionization of the analytes thus the detection sensibility.
- The stability of the PDADMAC/PSS coatings towards alkaline/acidic and organic rinses allows the use of corrosive rinse cycles between analyses which can fully remove analytes that may be adsorbed on the coatings.
- The possibility to analyze proteins in PDADMAC/PSS coated capillaries over a wide range of pH can be very useful to alter separation selectivity in CE.

References

[1] R. Nehmé, C. Perrin, H. Cottet, M.D. Blanchin, H. Fabre, Electrophoresis 29 (2008) 3013.
[2] R. Nehmé, C. Perrin, H. Cottet, M.D. Blanchin, H. Fabre, Electrophoresis 30 (2009) 1888.
[3] R. Nehmé, C. Perrin, V. Guerlavais, J.-A. Fehrentz, H. Cottet, J. Martinez, H. Fabre, Electrophoresis 30 (2009) 3772.
[4] J.M. Hempe, in: J.P. Landers (Ed.), Capillary and Microchip Electrophoresis and Associated Microtechniques, CRC Press, New York, 2008, p. p75.
[5] N.J. Munro, A. Huhmer, J.P. Landers, in: R.E. Reid (Ed.), Peptide and Protein Drug Analysis, CRC Press, New York, 2000, p. p345.
[6] V. Dolník, Electrophoresis 29 (2008) 143.
[7] V. Dolník, Electrophoresis 27 (2006) 126.
[8] K. Hutterer, V. Dolník, Electrophoresis 24 (2003) 3998.
[9] V. Kašička, Electrophoresis 20 (1999) 3084.
[10] J.P. Landers, in: J.P. Landers (Ed.), Capillary and Microchip Electrophoresis and Associated Microtechniques, CRC Press, New York, 2008, p. p4.
[11] L. Cruz, S.A. Shippy, J.V. Sweedler, in: M.G. Khaledi (Ed.), High Performance Capillary Electrophoresis: Theory, Techniques, and Applications, Wiley, New York, 1998, p. 303.
[12] A. Elhamili, M. Wetterhall, B. Arvidsson, R. Sebastiano, P.G. Righetti, J. Bergquist, Electrophoresis 29 (2008) 1619.
[13] Z. El Rassi, W. Nashabeh, in: N.A. Guzman (Ed.), Capillary Electrophoresis Technology, CRC Press, New York, 1993, p. 383.
[14] D.S. Salloum, J.B. Schlenoff, Biomacromolecules 5 (2004) 1089.
[15] T. Wehr, R. Rodriguez-Diaz, M. Zhu, in: T. Wehr (Ed.), Capillary Electrophoresis of Proteins, CRC Press, New York, 1999, p. 37.
[16] E.A.S. Doherty, R.J. Meagher, M.N. Albarghouthi, A.E. Barron, Electrophoresis 24 (2003) 34.
[17] J. Horvath, V. Dolník, Electrophoresis 22 (2001) 644.
[18] P.G. Righetti, C. Gelfi, B. Verzola, L. Castelletti, Electrophoresis 22 (2001) 603.
[19] V. Dolník, Electrophoresis 18 (1997) 2353.
[20] H.H. Lauer, D. McManigill, Anal. Chem. 58 (1986) 166.
[21] R.M. McCormick, Anal. Chem. 60 (1988) 2322.
[22] H.H. Lauer, D. McManigill, TrAC 5 (1986) 11.
[23] J.S. Green, J.W. Jorgenson, J. Chromatogr. 478 (1989) 63.
[24] X. Huang, L. Doneski, M.J. Wirth, Anal. Chem. 70 (1998) 4023.
[25] R. Barberi, J.J. Bonvent, R. Bartolino, J. Roeraade, L. Capelli, P.G. Righetti, J. Chromatogr. B 683 (1996) 3.
[26] E.A.S. Doherty, K.D. Berglund, B.A. Buchholz, I.V. Kourkine, T.M. Przybycien, R.D. Tilton, A.E. Barron, Electrophoresis 23 (2002) 2766.
[27] G. Danger, M. Ramonda, H. Cottet, Electrophoresis 28 (2007) 925.
[28] G. Danger, R. Pascal, H. Cottet, Electrophoresis 29 (2008) 4226.
[29] C. Preisler, E.S. Yeung, Anal. Chem. 68 (1996) 2885.
[30] N. Iki, E.S. Yeung, J. Chromatogr. A 731 (1996) 273.
[31] G.R. Joppien, J. Phys. Chem. 82 (1978) 2210.
[32] M.E. Roche, M.A. Anderson, R.P. Oda, L.B. Riggs, M.A. Strausbauch, R. Okazaki, P.J. Wettstein, J.P. Landers, Anal. Biochem. 258 (1998) 87.
[33] J.J. Pesek, M.T. Matyska, S. Swedberg, S. Udivar, Electrophoresis 20 (1999) 2343.
[34] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 2254.
[35] H. Katayama, Y. Ishihama, N. Asakawa, Anal. Chem. 70 (1998) 5272.
[36] T. Graul, J. Schlenoff, Anal. Chem. 71 (1999) 4007.
[37] F. Kitagawa, M. Kamiya, Y. Okamoto, H. Taji, S. Onoue, y. Tsuda, K. Otsuka, Anal. Bioanal. Chem. 386 (2006) 594–601.
[38] M. Weinbauer, H. Stutz, Electrophoresis 31 (2010) 1805–1812.
[39] C.A. Currie, J.S. Shim, S.H. Lee, C. Ahn, P.A. Limbach, H.B. Halsall, W.R. Heineman, Electrophoresis 30 (2009) 4245–4250.
[40] K. Boonsong, M.M. Caulum, B.M. Dressen, O. Chailapakul, D.M. Cropek, C.S. Henry, Electrophoresis 29 (2008) 3128–3134.
[41] J.R. Catai, Electrophoresis 25 (2004) 817–824.
[42] J.R. Catai, H. Tervahauta, G.D. Jong, G. Somsen, J. Chromatogr. A 1073 (2005) 185–192.
[43] Y. Lu, M.C. Breadmore, J. Sep. Sci. 33 (2010) 2140–2144.
[44] J.S. Fritz, S.A. Steiner, J. Chromatogr. A 934 (2001) 87–93.
[45] L. Hovgaard, L. Skiver, S. Frokjaer, in: S. Frokjaer, L. Hovgaard (Eds.), Pharmaceutical Formulation Development of Peptides and Proteins, CRC Press, Philadelphia, 2000, p. 33.
[46] B. Williams, G. Vigh, Anal. Chem. 68 (1996) 1174.
[47] G. Decher, Science 277 (1997) 1232.
[48] J.B. Schlenoff, H. Ly, M. Li, J. Am. Chem. Soc. 120 (1998) 7626.
[49] M. Castelnovo, J.-F. Joanny, Langmuir 16 (2000) 7524.
[50] M. Lösche, J. Schmitt, G. Decher, W.G. Bouwman, K. Kjaer, Macromolecules 31 (1998) 8893.
[51] G. Decher, J.B. Schlenoff, in: G. Decher, J.B. Schlenoff (Eds.), Multilayer Thin Films, Wiley-VCH, Weinheim, 2003, p. 99.
[52] W.J. Lambert, D.L. Middleton, Anal. Chem. 62 (1990) 1585.
[53] T. Pritchett, F.A. Robey, in: J.P. Landers (Ed.), Capillary Electrophoresis of Proteins in Handbook of Capillary Electrophoresis, CRC Press, Florida, 1997, p. p259.
[54] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice, Springer-Verlag, Berlin, 1993, p. 22.
[55] Y. Wang, P.L. Dubin, Anal. Chem. 71 (1999) 3463.
[56] Z. Sui, D. Salloum, J.B. Schlenoff, Langmuir 19 (2003) 2491.