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Capillary electrophoresis-mass spectrometry of basic proteins using a new physically adsorbed polymer coating. Some applications in food analysis

A new physically adsorbed capillary coating for capillary electrophoresis-mass spectrometry (CE-MS) of basic proteins is presented, which is easily obtained by flushing the capillary with a polymer aqueous solution for two min. This coating significantly reduces the electrostatic adsorption of a group of basic proteins (*i.e.*, cytochrome *c*, lysozyme, and ribonuclease A) onto the capillary wall allowing their analysis by CE-MS. The coating protocol is compatible with electrospray inonization (ESI)-MS *via* the reproducible separation of the standard basic proteins (%RSD values ($n = 5$) < 1% for analysis time reproducibility and < 5% for peak heights, measured from the total ion electropherograms (TIEs) within the same day). The LODs determined using cytochrome *c* with total ion current and extracted ion current defection were 24.5 and 2.9 fmol, respectively. Using this new coating lysozymes from chicken and turkey egg white could be easily distinguished by CE-MS, demonstrating the usefulness of this method to differentiate animal species. Even after sterilization at 120°C for 30 min, lysozyme could be detected, as well as in wines at concentrations much lower than the limit marked by the EC Commission Regulation. Adulteration of minced meat with 5% of egg-white could also be analysed by our CE-MS protocol.

Keywords: Capillary electrophoresis-mass spectrometry / Food analysis / Ion trap / Polymer coating
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1 Introduction

It is already well known that separation of proteins by capillary electrophoresis (CE) using fused-silica capillaries has strongly been hampered by solute adsorption onto the capillary wall [1–3]. Thus, adsorption of proteins onto capillary wall seems to be the main reason for observed efficiency loss [4–6], poor reproducibility in migration times and low protein recovery rates [7]. Electrostatic interactions between positively charged residues of the proteins and negatively charged silanol groups are responsible for such adsorption effects [8]. So far, different approaches have been proposed to reduce the noxious interaction between proteins and silica surface [2, 3, 6–18].

In the last years, the use of CE-MS has demonstrated remarkable possibilities for the analysis of whole proteins, due to on-line coupling of CE to electrospray ionization-mass spectrometry (ESI-MS) [19, 20], in which CE offers high resolution separations, while ESI-MS allows for the characterization of a wide molecular mass range of proteins, providing important information about their mass, structure, and conformational changes. The use of CE-MS to analyze whole proteins has been thoroughly described in different reviews and book chapters (see, *e.g.*, [19–21]) demonstrating the great possibilities of CE-MS to analyze this type of biomolecules. For instance, different standard proteins [22–27], glycosylated proteins [28–30], bovine and equine proteins [31, 32], metalloproteins [33–35], histones [36], recombinant human proteins [37, 38], proteins from aqueous humor [39], from whole blood [40], from bacteria [41, 42] or from cerebrospinal fluid [43] have been analyzed by CE-MS, showing the wide applicability of this technique.

However, analysis of proteins by CE-MS encounters the same problem of adsorption. Consequently, different strategies have been applied in CE-MS to overcome this limitation [19, 44]. In general, these approaches are using coatings covalently bonded to the capillary wall that can bear positive [45–47] neutral [36, 48, 49] or negative charge [50]. However, their major drawback is the time required to prepare the coating and its low stability at extreme pH. Also, the high price of some coated capil-

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Abbreviations: AIBN, 2,2'-azobisisobutyronitrile; **EIE**, extracted ion electropherogram; **EPyM-DMA**, *N,N*-dimethylacrylamide-ethylpyrrolidine methacrylate copolymer; **TIE**, total ion electropherogram

laries commercially available is an important drawback that should be considered. Other procedures used in CE-MS to reduce protein adsorption using noncovalent coatings on the capillary wall as a single [51] or multiple layer [52]. However, in this case mostly commercial kits have been used for CE-MS of proteins [51, 53].

The goal of this work was the development of a new, simple, fast, and reproducible physically adsorbed coating applicable to CE-MS of basic proteins. In order to make it compatible with CE-MS, the coating does not uses polymer added into the running buffer, regenerating it just by flushing the capillary between injections with a solution containing the macromolecule. Moreover, since the new procedure does not need the use of organic solvents, high-viscous solutions or elevated temperatures, its future application to CE separations of proteins in microchips can be expected. Finally, the usefulness of this new CE-MS protocol for analyzing proteins in real samples is demonstrated by addressing different problems of interest in food analysis.

2 Materials and methods

2.1 Chemicals

2-Ethyl-(2-pyrrolidine) methacrylate (EPyM) was synthesized by reaction of *N*-(2-hydroxyethyl)-2-pyrrolidine with methacryloyl chloride, both from Fluka (Buchs, Switzerland) and purified by column chromatography as previously described [54]. *N*,*N*-Dimethylacrylamide (DMA), purchased from Aldrich (Milwaukee, WI, USA), was vacuum-distilled. 2,2'-Azobisisobutyronitrile (AIBN) from Fluka was purified by fractional crystallization from ethanol. Tetrahydrofuran (THF) from Fluka was distilled and dried over molecular sieves. Other reagents were used as received. The proteins (lysozyme from turkey egg white and from chicken egg white, cytochrome C from bovine heart and ribonuclease A from bovine pancreas) were all obtained from Sigma (St. Louis, MO, USA). Acetic acid, formic acid, ammonium hydroxide, ammonium acetate, and sodium hydroxide were from Merck (Darmstadt, Germany) and used without further purification in different running buffers. Distilled water was deionized with a Milli-Q system (Millipore, Bedford, MA, USA). Six different buffer solutions have been used in this work: 0.9 M sodium formate/formic acid, pH 2.2; 100 mM sodium acetate/acetic acid, pH 5.0; 75 mM ammonium acetate/acetic acid, pH 5.5; 89 mM Tris, 20 mM ortophosphoric acid, and 2 mM EDTA, pH 7.3; 150 mM sodium tetraborate/boric acid, pH 8.5; and 100 mM sodium tetraborate/boric acid, pH 10.0.

2.2 Polymer synthesis and characterization: capillary coating

The monomers EPyM and DMA were copolymerized by free radical polymerization at 50°C, using AIBN ([I] = $1.5 \times 10\text{–}2$ mol·L^{-1}) as a radical initiator, THF ([M] = 1.0 mol·L^{-1}) as solvent, and a feed molar fraction of 19% EPyM. All conditions for EPyM-DMA copolymer synthesis and characterization are described elsewhere [55]. The chemical structure of the copolymer EPyM-DMA (ethylpyrrolidine methacrylate-*N*,*N*-dimethylacrylamide) is given in Fig. 1. A simple coating strategy was used consisting of flushing the capillary, prior to each run, with a diluted polymer solution (0.1 mg/mL in water) for 2 min and next replacing this solution by flushing the capillary with the separation buffer for 2 min.

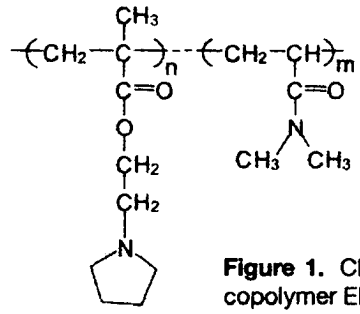

Figure 1. Chemical structure of the copolymer EPyM-DMA.

2.3 EOF measurements

EOF measurements were carried out on a Beckman P/ACE 2100 System (Fullerton, CA, USA) controlled by GOLD Software, with a UV/Vis detector working at 254 nm. Bare fused-silica capillaries were purchased from Composite Metal Services (Worcester, UK). Injections were made at the cathodic end using a N_2 pressure of 0.5 psi for 3 s. Acetone was used as a noncharged marker to determine the EOF of bare silica and polymer-coated capillaries. Running buffers were used at different pH for the EOF measurements. Before first use, uncoated capillaries were conditioned by a 20 min rinse with 0.1 M NaOH, followed by water rinse for 20 min. Between injections, uncoated capillaries were rinsed with water for 2 min, and buffer solutions for 2 min. In the case of polymer-coated capillaries, this routine was changed to 2 min of water, 2 min of diluted polymer solution (0.1 mg/mL) and 2 min of buffer solution.

2.4 Samples

Basic proteins (lysozymes, cytochrome *c*, ribonuclease A) were dissolved in Milli-Q water (0.5 and 0.05 mg/mL of each protein) and separated in two different capillaries

(bare silica and EPyM-DMA-coated capillary) both with 47 cm of total length (40 cm of effective length) and 50 μm ID. Chicken eggs, white wine, and minced meat from beef were bought in a local market. White egg was separated from yolk egg, lyophilized and stored at −4°C. Lyophilized white egg was reconstituted with distilled water at a concentration of 13 mg/mL or directly added to minced meat (*vide infra*). The sample solution was filtered on a 0.45 μm PVDF membrane (from Millex-HV; Millipore, Beford, MA, USA) before injection in CE-MS. Aqueous aliquots of chicken egg-white lysozyme (500 μg/mL) were treated for 30 min at different temperatures, (25, 60, 80, 100, and 120°C) in order to simulate the different thermal treatments used for food (*e.g.*, pasteurization, sterilization). Samples of white wine containing lysozyme were prepared by dissolving 50 μg/mL of egg-white lysozyme in wine. Samples of white wine and white wine containing lysozyme were directly injected in CE-MS without further treatment. Minced meat was adulterated by adding 5% of lyophilized egg-white followed by mixture homogenization. Extraction of proteins from minced meat and adulterated minced meat was carried out as follows: nine grams of minced meat were homogenized and mixed with 30 mL of a buffer composed of 90 mM EDTA, 3 mM Tris adjusted with boric acid to pH 8.5, for 90 min. The mixture was centrifigued at 12 000 × *g* for 20 min. The supernatant was filtered on a 0.45 μm PVDF membrane and injected in the CE-MS instrument.

2.5 CE-MS conditions

The analyses were carried out on a P/ACE 5500 CE apparatus (Beckman Instruments, Fullerton, CA, USA), equipped with a UV-Vis detector working at 200 nm and coupled to the mass spectrometer (Esquire 2000TM ion-trap mass spectrometer from Bruker Daltonik, Bremen, Germany). The bare fused-silica capillary with 50 μm ID was purchased from Composite Metal Services (Worcester, England). The detection length to the UV detector was 20 cm, the total length 87 cm (corresponding to the MS detection length). Injections were made at the anodic end using N_2 pressure of 0.5 psi for a given time (1 psi = 6894.76 Pa). The CE instrument was controlled by a PC running the System Gold software from Beckman. Electrical contact at the electrospray needle tip was established *via* sheath liquid consisting of methanol–water (50:50 v/v) containing 0.05% v/v acetic acid delivered at a flow rate of 4 μL/min by a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL, USA). The mass spectrometer operated in the positive ion mode. The spectrometer was set for scanning at 800–2200 *m/z* range at 13 000 u/s during separation and detection. For the connection between the CE system and the electrospray ion source of the mass spectrometer, the outlet of the separation capillary was fitted into the electrospray needle of the ion source and a flow of conductive sheath liquid established electrical contact between capillary effluent and water for the electrospray needle. MS operating conditions were optimized by adjusting the needle-counter electrode distance, liquid sheath flow rate, and applied electrospray potentials while the standard basic proteins (lysozyme, ribonuclease A, and cytochrome *c*), were injected by direct infusion in the CE–ESI-MS system. The nebulizer/drying gas conditions were 4 psi nitrogen, 3 L/min nitrogen at 200°C. The instrument was controlled by a PC running the Esquire NT software from Bruker Daltonik. Charge assignment in the MS spectra was done by using the "Charge deconvolution" utility available in the DataAnalysis V. 3 program of the Bruker Daltonik Esquire software. The coating strategy between injections was slightly modified in order to take into consideration the longer capillary used in CE-MS. In this case, the strategy consisted of flushing the capillary, prior to each run, with a diluted polymer solution (0.1 mg/mL in water) for 2 min (with the nebulizing gas stopped) and replacing this solution by flushing the capillary with the separation buffer for 3.7 min. Before first use, uncoated capillaries were conditioned as above (*i.e.*, a 20 min rinse with 0.1 M NaOH followed by a water rinse for other 20 min).

3 Results and discussion

3.1 Capillary coating: characterization by CE-UV and CE-MS

EPyM-DMA copolymer bears amine groups belonging to the ethylpyrrolidine methacrylate monomer (Fig. 1). Based on its similarity with other copolymers already described in [56] (*e.g.*, diethylaminoethylmethacrylate-acrylamide), the p*K* of this amine group is expected to be ~9, providing a positive electrical charge to this macromolecule at pH values < 9. However, the EPyM content in the copolymer is not too high (only 19% of EPyM was initially used as feed molar fraction) and, therefore, a variable behavior of the coated capillary depending on the pH can be expected in CE. In order to study this effect, the EOF of a EPyM-DMA-coated capillary was measured employing running buffers at different pH values, and compared to a bare fused-silica capillary (Table 1). The EPyM-DMA-coated capillary shows an anodic EOF (*i.e.*, negative values) at low pH, a nearly zero EOF at pH ~ 6 and a low cathodic EOF at pH > 8. This behavior can be explained [14] considering that the global electrical charge onto the capillary wall is due to both the amine groups of the polymer (bearing a positive electrical charge) and the remaining silanol groups onto the silica wall (bearing a negative

Table 1. EOF as a function of pH

pH	EOF (10^{-8} $m^2s^{-1}V^{-1}$)	
	EPyM-DMA-coated capillary	Bare fused-silica capillary
2.2	−1.84[a)]	0.19
5.0	−0.41	2.13
7.3	0.25	3.90
8.5	0.51	5.63
10.0	1.30	4.56

a) The negative value means anodic EOF.

electrical charge). Thus, under acidic pH the amine groups are the main charged groups on the capillary wall resulting in a global positive charge and, as a consequence, an anodic EOF. Under very basic pH, the number of positive charges on the polymer decreases and the negative silanol groups become predominant on the capillary wall, resulting in a cathodic EOF. It is noteworthy that this EOF is clearly reduced using the EPyM-DMA capillary (*e.g.*, as shown in Table 1 at pH 8.5, the EOF in the coated capillary is 10 times lower than in the bare silica capillary) being a good indication of the shielding effect of the EPyM-DMA coating even at these basic pH. From these EOF values, it can be deduced that the capillary is indeed coated by this copolymer. It is interesting to mention here that the coating is easily obtained just by flushing the capillary with a dilute solution containing the polymer. The applicability and reproducibility of this coating in CE-MS was next studied using a group of basic proteins.

Likewise CE, the development of CE-MS for protein analysis has been hampered by the tendency of highly charged proteins to adsorb onto the wall of the fused-silica capillary. In CE-MS, this effect is even more critical because the buffer cannot be modified as freely as in CE (*e.g.*, using additives or extreme pH values) due to the harmful effects that such modifications could have on the MS signal. An example of this noxious effect is shown in Fig. 2A, where the CE-MS electropherogram obtained

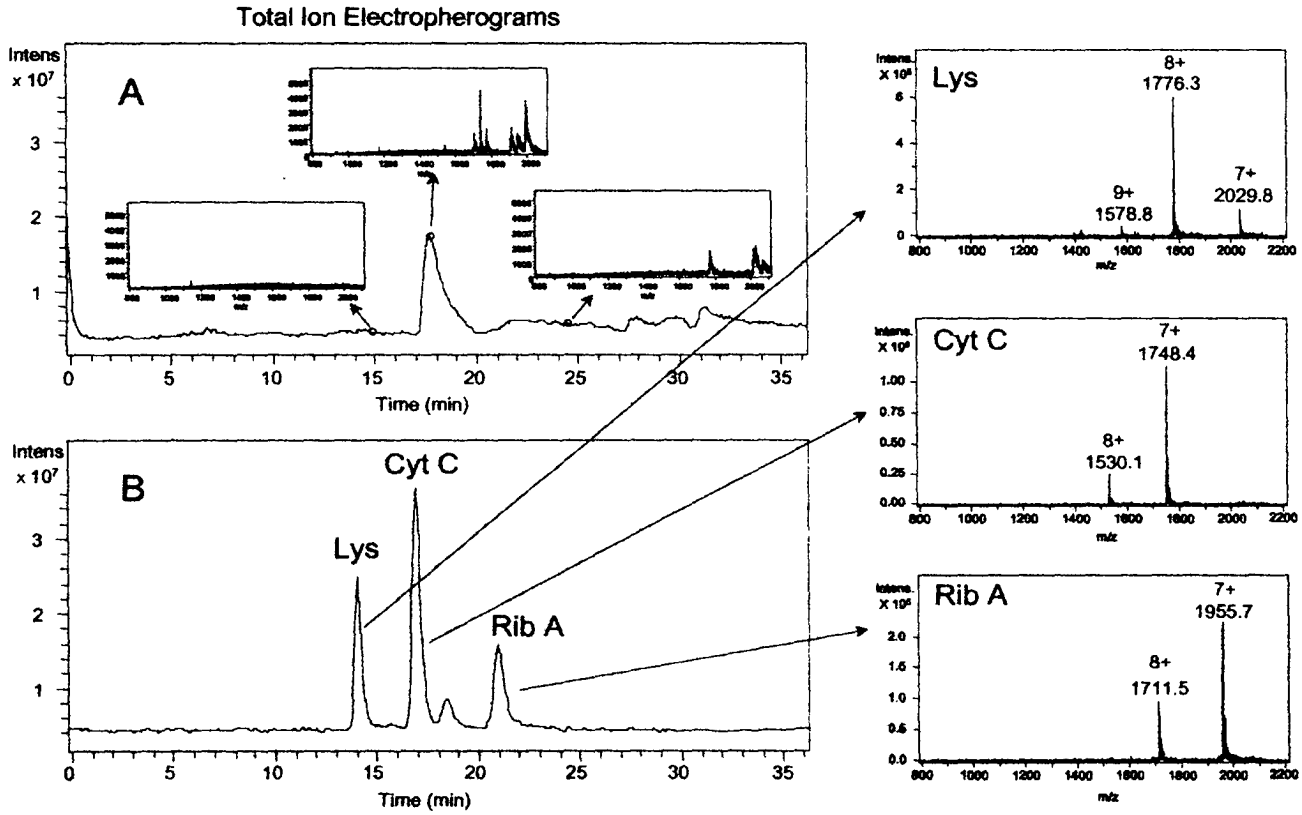

Figure 2. CE-MS TIEs of three basic proteins using (A) a bare fused-silica capillary and (B) an EPyM-DMA-coated capillary. Running buffer, 75 mM ammonium acetate/acetic acid, pH 5.5. Injection at 0.5 psi for 30 s of lysozyme from turkey egg-white (Lys); cytochrome *c* (Cyt C), and ribonuclease A (Rib A) (0.5 mg/mL of each protein). Fused-silica capillary, 50 μm ID, 87 cm total length; running voltage, 25 kV; temperature, 25°C. MS conditions: sheath liquid, methanol–water (50:50 v/v) containing 0.05% v/v acetic acid; sheath flow, 4 μL/min; nebulizer gas, 3 psi, 4 L/min at 200°C; scan range, *m/z* 800–2200 (target mass: 1500 *m/z*).

of three standard basic proteins (*i.e.*, lysozyme, cytochrome *c*, and ribonuclease A is shown. A separation buffer at pH 5.5 was used because at this pH a high electrostatic interaction between the basic proteins and the bare silica capillary wall could be expected [57]; under these conditions, a good separation of basic proteins would be an indication upon the usefulness of our approach. The MS spectra obtained from the electropherogram depicted in Fig. 2A from 20 min to 35 min showed in all cases a typical proteinaceous profile, giving an idea of the important adsorption effect. As can be deduced from Fig. 2B, the use of a EPyM-DMA-coated capillary under identical separation conditions enables an adequate CE-MS separation of the three basic proteins.

In order to demonstrate that the polymer used during the coating protocol has no effect on the MS signal, five analyses were consecutively carried out applying the coating protocol described in Section 2.2. Under these conditions, no significant variation of the MS sensitivity and noise was observed, obtaining%RSD values ($n = 5$) < 1% for the analysis time of the three proteins and < 5% for the peak heights measured from the total ion electropherograms (TIEs) within the same day. Interestingly, the same experiment carried out without any coating regeneration provided $\%RSD_{n=5}$ up to 7.8% was obtained for analysis time reproducibility, showing the importance of the polymer regeneration between injections. These results corroborate the good reproducibility and compatibility of this coating procedure with ESI-MS analysis.

Moreover, this coating protocol is clearly compatible with CE-MS since it allows to obtain adequate MS spectra of these proteins (Fig. 2). Thus, from the MS spectra in Fig. 2, the relative molecular masses (M_r) of the proteins were calculated as of 14203, 12233, and 13682 Da for lysozyme, cytochrome *c*, and ribonuclease A, respectively. These values are in a good agreement with the theoretical ones (*i.e.*, 14192, 12232, and 13682 Da, respectively, demonstrating the usefulness of this approach. Besides, an additional small peak migrating between cytochrome *c*, and ribonuclease A (Fig. 2B) was assigned to an additional form of cytochrome *c*, as could be deduced from the MS spectra obtained for this compound which was the same than that from the cytochrome *c* peak. Although a further study about the nature of this compound is out of the scope of this work, it is interesting to mention that this additional compound has been previously observed by CE in aqueous solutions of cytochrome *c* [12].

The sensitivity of the CE-MS procedure using an EPyM-DMA-coated capillary was determined by injecting a 0.05 mg/mL solution of cytochrome *c* and calculating the limit of detection (LOD; using a signal/noise ratio = 3) directly from the TIE and extracted ion electropherogram (EIE, using a value of 1748.7 ± 0.5 Da as target ion). The LOD obtained in terms of concentration of protein injected was 1.2 μM using the TIE mode and 0.14 μM using the EIE mode, corresponding to 24.5 fmol and 2.9 fmol of protein detected, respectively.

3.2 CE-MS of proteins in food analysis

The potential of this procedure was further demonstrated by carrying out the separation of lysozymes (*i.e.*, strongly basic proteins with p*I* values > 9.5) from two different species, namely from chicken and turkey egg-white. Proteins from egg white can be used, among other applications [58, 59], for detecting different adulterations in foods [60] (*e.g.*, adulteration of foie gras adding turkey or chicken egg-white [61]). Figure 3A shows the CE-MS TIE of the two standard proteins, demonstrating that the use of an EPyM-DMA-coated capillary brings about a correct separation of these two strongly basic proteins. Figure 3B shows the CE-MS TIE of lysozyme detected in a real sample from chicken egg white, together with its corresponding MS spectrum. The procedure allows the easy analysis of this protein with a simple sample treatment and free of interference with other egg-white proteins (*e.g.*, ovoalbumin, ovomucoid, ovoglobulins, and ovotransferrin [62]). This can be explained by (i) the separation pH chosen for the CE-MS analysis (5.5); (ii) the use of the coated capillary (resulting in a very low EOF at that pH), and (iii) the isoelectric points of these proteins (ranging from 3.5 to 6.6 for ovoalbumin, ovomucoid, ovoglobulins, and ovotransferrin, and > 9.5 for lysozyme [63–65]). In comparison with other egg-white proteins, at the separation pH lysozyme bears a much higher positive charge that carries it to the detection point within reasonable times since the EOF of the coated capillary at that pH is practically zero (see Table 1). It is interesting to be mentioned that, according to the MS spectra (Figs. 2 and 3B) obtained from lysozyme of chicken and turkey egg-white, the M_r of lysozyme from chicken is 14 307 Da (theoretically 14 304 Da), which is significantly different from that of turkey lysozyme (*i.e.*, M_r = 14 203 Da). Therefore, their migration times in CE together with their specific MS spectra provide two complementary dimensions for the adequate discrimination and characterization of these proteins.

CE-MS of basic proteins can be used to study different problems in food analysis. For example, lysozyme is added during wine making to control growth and activity of the bacteria responsible for malolactic fermentation, allowing this way the production of quality wines containing lower levels of sulfur dioxide. However, since this protein can produce allergic reactions [66], the European Union has established in the final product a maxi-

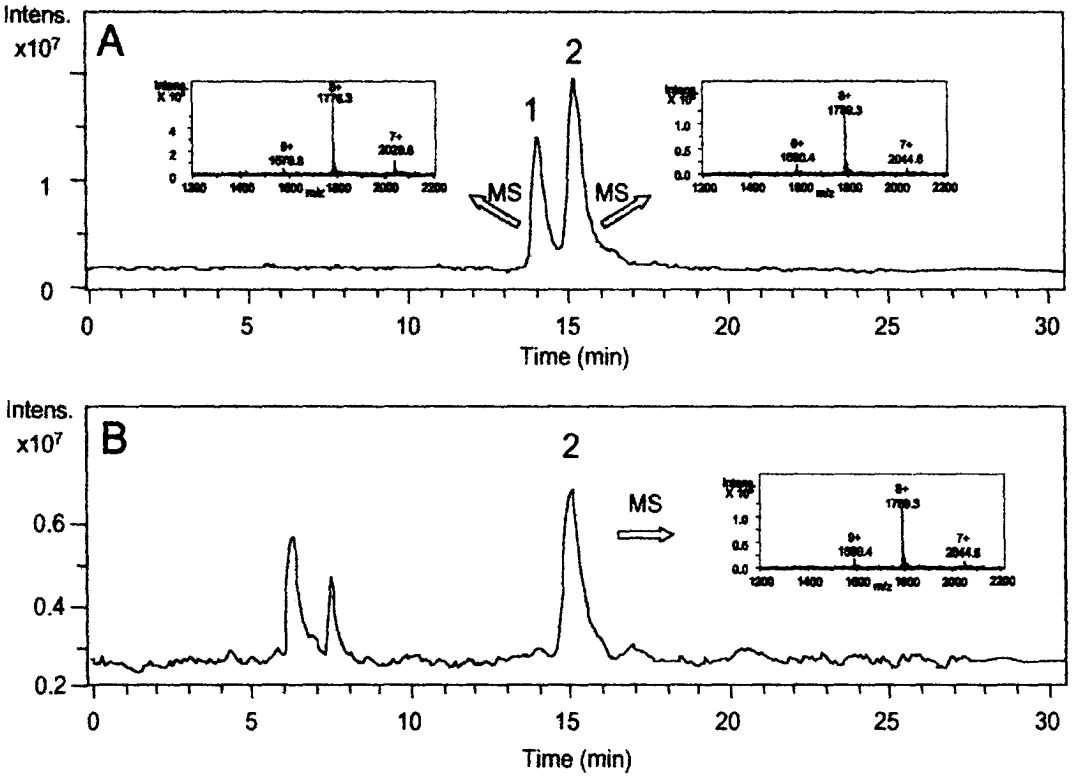

Figure 3. CE-MS TIE of (A) standard lysozyme from turkey (1) and chicken (2) egg white, and (B) lysozyme detected in a chicken egg-white sample. Conditions as in Fig. 2.

mum level of lysozyme of 500 μg/mL (Commission Regulation No. 2066/2001). Standard procedures for detection of lysozyme in wine involve the use of antigen-antibody reactions, SDS-PAGE or HPLC [67]. The general limitations of these procedures are long analysis times and intensive labor. Besides, other more specific drawbacks as, *e.g.*, low sensitivity for SDS-PAGE and risk of false positives/negatives for immunochemical reactions, make necessary the development of new analytical procedures. The utility of our CE-MS protocol to address this problem was tested. Samples of white wine without and with 50 μg/mL of lysozyme (*i.e.*, a concentration ten fold lower that the detection level marked by the regulation) were directly injected without further purification (Fig. 4). Comparing the electropherograms from pure white wine (Fig. 4A) and white wine containing 50 μg/mL of lysozyme (Fig. 4B), it can be noticed that the CE-MS protocol allows for the easy detection of lysozyme without interference from any sample compounds. Logically, in the case that any wine compound could interfere with lysozyme (*i.e.*, migrating at the same time), the use of the extracted ion current as MS detection would solve this limitation (see Fig. 4C). Moreover, as can be seen in Fig. 4C, using this MS mode (*i.e.*, EIE with *m/z* of 1789.3 and 2044.6) a clear improvement of the signal/noise ratio is obtained, with a LOD of 1.3 mg/L for lysozyme calculated for a signal/noise ratio = 3. This value is 5–30 times better than the LOD provided by the different methods employing HPLC with UV detection [67, 68].

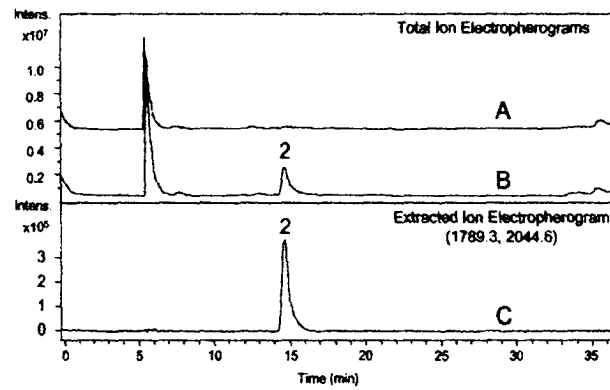

Figure 4. (A) CE-MS TIE of white wine; (B) CE-MS TIE of white wine with 50 μg/mL of lysozyme; (C) CE-MS EIE (using ions of *m/z* 1789.3 and 2044.6) of white wine with 50 μg/mL of lysozyme. MS scan range, *m/z* 1400–2200 (target mass: 1800 *m/z*). Conditions as in Fig. 2.

A general concern during the development of any food analytical method based on protein determination is the possible effect of different thermal treatments (*e.g.*, pasteurization, sterilization, *etc.*) on stability of proteins. Since the publications about thermal stability of lysozyme do not agree [69, 70], the behavior in CE-MS of this protein after different thermal treatments was analyzed. Aliquots of a lysozyme solution in water were heated for 30 min at 25, 60, 80, 90, 100, and 120°C (Fig. 5). After the strongest treatment (*i.e.*, 120°C for 30 min, corresponding to sterilization) this protein can still be detected by CE-MS as a small peak at the concentration used (*i.e.*, 500 μg/mL). At this temperature, however, a clear decrease of the peak intensity was observed as a result of the thermal denaturation of this protein. On the other hand, it was also observed, that neither the electrophoretic behavior nor peak intensity of this protein changed using middle to low temperatures from 25 to 80°C, the latter corresponding to the maximum temperature typically used for pasteurization. Although it has been shown that the dissolution pH has some effect on the thermal denaturation of egg-white proteins [71], our results indicate that the procedure based on CE-MS can be less influenced by thermal degradation than those procedures based on antigen-antibody reactions [69, 70, 72]. Moreover, although the sensitivity provided by immunodiagnostic techniques is in general better than those one obtained by CE-MS, some other aspects, like the longer analysis times usually required for these immunotests (typically hours for ELISA *vs.* 30 min for CE-MS) and the risk of obtaining false positives due to cross reactions, have also to be kept in mind. Besides, the results provided by CE-MS can be considered unequivocal since it provides a double confirmation about the identity of any compound (*i.e.*, migration time and mass spectrum).

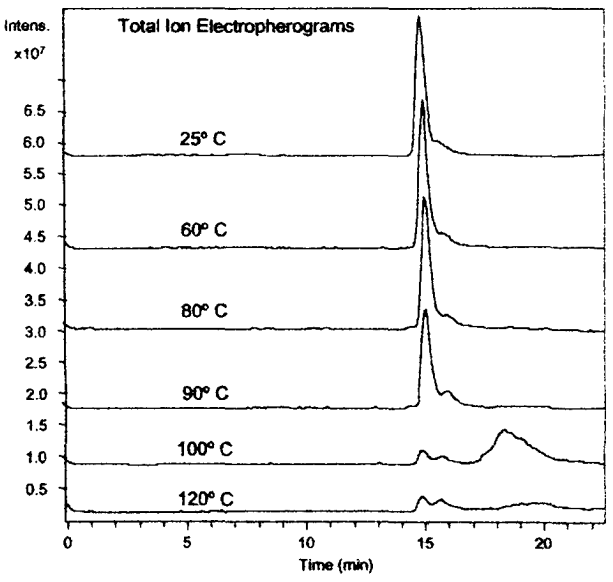

Figure 5. CE-MS TIEs of chicken egg-white lysozyme treated for 30 min at the temperatures indicated. Conditions as in Fig. 4.

Chicken egg-white is practically ubiquitous in food industry (*e.g.*, in dairy products, confectionery, salad dressings, noodles *etc.*) [73]. Although in general the addition of egg-white is indicated in the food label, in some cases egg contamination or adulteration is not declared with the subsequent risk of allergic reactions and/or mislabeling. Thus, chicken egg-white can appear as a contaminant [72], undeclared in the label [74], or it can be misused for improving texture and/or for economical reasons in, *e.g.*, seafood, minced meat, "foie gras", *etc.* [60, 75–77]. Immunochemical methods [60, 72] or classical gel electrophoresis [78] have been mainly developed to detect these contaminations/adulterations. However, the mentioned drawbacks of immunochemical tests (long analysis times, risk of false positives, strong effect of the thermal treatment) and those of classical gel electrophoresis (labor-intensive, low sensitivity) make necessary the development of new analytical protocols. In this work, CE-MS has been applied for detection of adulteration of minced meat from beef with chicken egg-white, employing as target the detection of lysozyme. Protein samples from minced meat and minced meat containing 5% of chicken egg-white were obtained as indicated in Section 2.4. The detection of lysozyme under these conditions is highly demanding since only a 3.5% of the total proteins in dry egg-white consists of lysozyme and, therefore, the final percentage of lysozyme in the adulterated mince meat is nearly 0.18%. For this reason, the most sensitive MS detection mode based on extracted ion current was used. Figure 6 shows the CE-MS EIEs of the two

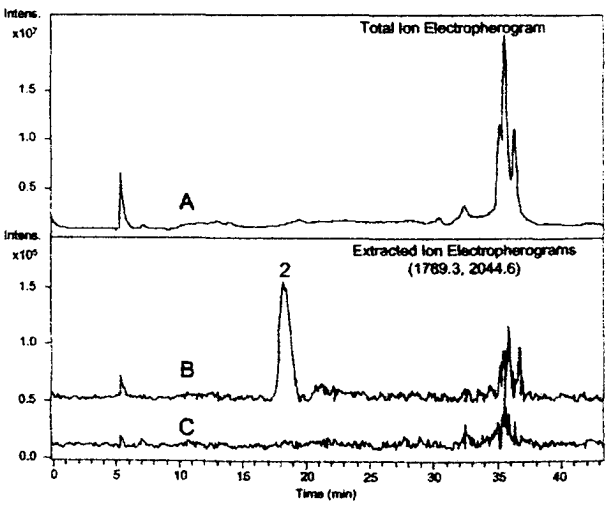

Figure 6. (A) CE-MS TIE of minced meat from beef. CE-MS TIEs (using *m/z* ions of 1789.3 and 2044.6) of (B) minced meat adulterated with 5% of chicken egg-white and (C) minced meat from beef. Conditions as in Fig. 4.

samples, minced meat in Fig. 6C and minced meat containing 5% of chicken egg-white in Fig. 6B. For comparison, Fig. 6A shows the CE-MS TIE obtained by injecting the same sample as in Fig. 6C (*i.e.*, clean minced meat). This method can detect in a simple and easy way this adulteration and without any interference from the rest of proteins belonging to the meat that migrate between 35 and 40 min (Fig. 6A). However, although theoretically the expected concentration of lysozyme in the adulterated minced meat ($\sim 10^{-5}$ M) should provide an adequate MS peak, the signal obtained for lysozyme is close to the detection limit (see Fig. 6B). Therefore, the fast and simple extraction procedure of proteins used in our work should be modified in order to improve the lysozyme yield. The migration time for lysozyme in this sample increased significantly, which is probably due to both the higher ionic strength and pH of the buffer used to extract the protein from meat.

The applications demonstrated in this section based on CE-MS of proteins are, to our knowledge, among the first ones in food analysis and are one of the few examples that can be found in literature demonstrating the possibilities of CE-MS to analyze whole proteins in "real samples", since, in general, most of the works published are related to mixtures of standard proteins [21, 44].

4 Concluding remarks

A new, simple, fast, and reproducible polymer coating for CE-MS of basic proteins has been developed. The coating is physically adsorbed onto the capillary wall and it is regenerated only by flushing the capillary between injections with a dilute solution containing the polymer. In this CE procedure, separation of proteins is achieved with no polymer added to the running buffer, making it compatible with CE-MS. Moreover, the good possibilities of CE-MS of proteins to solve real problems in food analysis have been demonstrated.

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