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Received January 12, 2011
Revised March 7, 2011
Accepted March 11, 2011

Research Article

High-sensitive protein analysis by FESI-CE-MALDI-MS

Capillary zone electrophoresis (CZE) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) are two techniques highly suitable for the separation and detection of intact proteins. Herein, based on the use of a recently introduced iontophoretic fraction collection interface for the coupling of CE and MALDI-MS, the potential of the combination of both techniques for the analysis of intact proteins is assessed. To further provide a bioanalytical platform with high-sensitivity capabilities, field-enhanced sample injection is integrated as on online preconcentration strategy upstream from the electrokinetic separation. Under optimized conditions, more than 3200- and 4800-fold improvement, respectively in terms of peak height and peak area, as well as LODs ranging from 5 to 10 nM, has been achieved.

Keywords:
CE / Field-enhanced sample injection / Matrix-assisted laser desorption/ ionization mass spectrometry / On-line preconcentration
DOI 10.1002/elps.201100024

1 Introduction

Capillary zone electrophoresis (CZE) is a simple and fast separation technique combining very high separation efficiency with low sample volume requirement. In the last two decades, it has also been demonstrated in various studies that its combination with MS provides a very powerful analytical platform for the characterization of peptides and/or protein mixtures [1, 2].

As compared with chromatography-based techniques, CE, due to its miniaturized format, generally suffers from a lower loading capacity. To circumvent this drawback, sample preconcentration techniques can be integrated, which can be further classified into offline [3, 4] and online [5, 6] approaches.

The online sample preconcentration is considered as the most convenient approach for trace analysis of proteins. Indeed, it can be easily accomplished by simple manipulations of the experimental conditions such as composition of background electrolytes (BGEs) and/or sample matrices without the need for any modification of commercially available CE instruments. As a result, the development of these preconcentration approaches and the assessment of their performances and applicability generate a considerable interest.

A variety of online sample preconcentration approaches for proteins and peptides analysis by CE have been already reported and reviewed [7, 8]. The common approach is to modify the experimental parameters so as to enable the introduction of large amounts of materials in the separation capillary. As CE is a free solution separation technique, all of these approaches are based upon the variation, at a given point of the separation path, of the analyte velocities as a mean to induce the stacking phenomena.

Several electrophoretic-based preconcentration methods such as large-volume sample stacking (LVSS) [9], field-amplified sample stacking (FASS) [10] and field-enhanced sample injection (FESI) [11, 12] have been developed. These techniques are based on the reduction of the analyte migration velocities when they encounter a drop in electrical field strength at the boundary between the sample matrix and BGE zones. Therefore, FASS and FESI are generally applicable to sample matrices of low conductivity. By comparison, transient isotachophoresis (t-ITP) [13], where the sample is stacked between a leading and a terminating electrolyte, is more suitable to samples of high conductivity such as most of the biological samples.

Besides the existence of preconcentration techniques solely based on the electrokinetic process, other strategies more heavily rely on the physico-chemical properties of the considered analytes. For example, the acido-basicity or hydrophobicity characteristics of the considered analytes are used in the dynamic pH junction [14–17] and sweeping [18], respectively. To further extend the magnitude of the

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Abbreviations: FESI, field-enhanced sample injection; **SA,** sinapinic acid; **SEF,** signal enhancement factor

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preconcentration phenomena, hybrid techniques where at least two stacking strategies are combined have also been proposed and usually constitute the most powerful approaches. In this context, Quirino and Terabe proposed and further demonstrated, mainly for small molecules, the capabilities of the combination of sweeping with FESI, while Hirokawa et al. proposed electrokinetic supercharging (EKS) as a novel preconcentration technique [19, 20]. It combines the strengths of electrokinetic injection and those of t-ITP and has so far been applied to small ions [21], DNA strands [22], SDS denaturized proteins [23] as well as peptides [24].

In addition, chromatography-based preconcentration methods such as solid-phase extraction (SPE) [25, 26] and membrane PreConcentration (mPC) [27] have also been employed to improve the sensitivity of the CE analysis. SPE-CE is efficient at concentrating hydrophobic compounds but it has some limitations such as loss of resolution, peak broadening, peak tailing and the increased backpressure that disturbs the electroosmotic flow (EOF) [5]. These limitations can be partially solved by mPC due to the limited volume of solvent required for elution that avoids the adverse effects of the large volumes of organic solvents on CE.

As compared with the aforementioned approaches, due to its simplicity and the other unique features, we have used FESI as the preconcentration approach to improve the sensitivity of the protein analysis by CE and CE-MALDI-MS.

Briefly, FESI is based on the fact that each given analyte presents a different velocity when in the high-conductivity BGE zone or in the low-conductivity sample zone. Indeed, considering that the current density has to remain constant throughout the separation capillary, the local electric field existing in the low conductivity sample zone is much higher than in the BGE zone. As a consequence, the analytes move rapidly from the sample vial into the capillary and once they reach the high conductivity BGE, their velocity drops significantly resulting in a stacking process at the sample matrix/BGE boundary. Such conditions thus allow the use of extensive electrokinetic injections, which strongly enhances the mass loading capabilities of the CE technique. Depending on the employed BGE and the analytes, FESI can yield signal enhancement factor (SEF) values between few hundreds and few thousands. However, some limiting factors such as volume and separation length of capillary should be considered. The volume of the sample injected into the capillary should be optimized up to the point where the highest sensitivity enrichment without any negative effect on resolution and efficiency is obtained. In this context, as compared with strong EOF systems, the use of neutrally coated capillaries is usually beneficial.

As a benchmark example, Law et al. employed FESI for online sample preconcentration as well as a bubble cell capillary to increase the optical path length for the analysis of four standard proteins. As a result, 5000- to 26 000-fold increase in peak area has been achieved from stacking process as compared with normal hydrodynamic injection, resulting in a 3–10 ng/mL detection limit of standard proteins [28].

Furthermore, due to the unique capabilities of MS as a detection tool, especially in proteomics studies, hyphenation of FESI-CE systems with MS could be considered as a very valuable alternative to UV detection systems. In this context, Monton and Terabe [29] as well as Yang et al. [30] reported high sensitivity analysis of peptides by FESI-CE followed by ESI-MS. In both cases, SEFs around 3000 leading to the achievement of LODs in the low nanomolar range was obtained for peptide analysis.

Because the relatively high-conductivity buffers typically used in FESI can affect the electrospray process, the hyphenation of FESI-CE with MALDI-MS may constitute a very valuable alternative for the sensitive analysis of protein mixtures by MS.

In the present study, after having optimized the experimental conditions required for the integration of an FESI preconcentration step for protein analysis in CZE with a conventional UV detector, its compatibility with MALDI-MS has been assessed. To this end, a recently introduced iontophoretic fraction collection device has been used [31] and the spotting process optimized. Very efficient sample stacking was obtained under the developed conditions as indicated by a significant sample depletion occurring during the injection. Depending on the considered proteins, SEFs between 3200 and 4800 were achieved, leading to the accomplishment of LODs in the very low nanomolar range. Moreover, it is shown that the coupling to a MALDI plate spotter does not alter significantly the preconcentration possibilities as well as the separation performances. When used with a conventional UV detector, the reported FESI preconcentration step showed an acceptable repeatability with relative standard deviations (RSD) below 0.5% for the migration times and below 18% for the peak area.

2 Materials and methods

2.1 Materials

All chemicals and proteins used were of analytical reagent grade and obtained from Sigma-Aldrich (Schnelldorf, Switzerland). All buffer and protein sample solutions were prepared with water produced by an alpha Q Millipore system (Zug, Switzerland). A stock solution of different proteins was made and different concentrations of the sample were prepared by a serial dilution of the stock solution.

2.2 Standard protein mixture

A standard protein solution of cytochrome *c* (Cyt. *c*), lysozyme (Lys), ribonuclease A (RNase A), S-ribonuclease (S-RNase), α-lactalbumin (α-lac), β-lactoglobulin A and B (β-lac A and B), lactoferrin, bovine serum albumin (BSA), aldolase and myoglobin (Myo) have been prepared in water

and depending on the case, different dilutions of all or some of these proteins have been considered as sample and used for FESI-CE experiments.

2.3 CE-UV

CE experiments were performed with a Hewlett-Packard^{3D} CE System (Waldbronn, Germany). Fused-silica capillaries (50 μm id, 41.5 cm effective length, 50 cm total length) were obtained from BGB analytik AG (Boeckten, Switzerland) and coated with 5% hydroxypropyl cellulose (HPC) in the laboratory with a procedure described earlier by Shen and Smith [32]. After optimization, 83.3 mM ionic strength ammonium acetate (pH 4.0) has been used as BGE. Between different separations in the same BGE, water and buffer rinses were successively performed. Samples were introduced into the capillary by electrokinetic injections (3 kV, 8 min), and the separation of proteins was performed by applying 20 kV across the capillary (0.4 kV/cm) while monitoring the UV absorbance at 200 nm (Table 1).

2.4 Iontophoretic spotting

At one end of the neutrally coated capillaries, about 10 cm of the 41.5 cm-long capillary was first painted with a silver ink from Ercon (Wareham, MA, USA) after what the ink was cured at 80°C for at least 60 min. The capillary was then placed in a CE-MS cassette and the painted extremity placed in a ceramic holder, being an integrated part of a lab-made robotic system able to move in all three axes and computer controlled via a Labview program (National Instruments, Austin, TX, USA). The details of the custom-built robotic CE-MALDI interface have been already described in a previous paper [31]. The protein sample was injected into the capillary by applying 3 kV for 8 min, and the separation was performed by applying 16.6 kV across the capillary (0.4 kV/cm). To allow the collection of the CE-separated proteins, the silver-painted extremity of the capillary was sequentially moved during the separation through various positions of an AnchorChip MALDI target (Bruker, Bremen, Germany), a given volume of BGE being present on each position for a constant delivery of the separation current.

Table 1. SEF values for six different proteins obtained from UV absorbance of proteins at 200 nm

Protein	SEF by peak height	SEF by peak area
Cyt. *c*	4547	7432
Lys	4869	8189
Myo	2606	3346
RNase A	3680	5159
S-RNase	2385	3931
α-Lac	1344	1056
Average values	3239	4852

2.5 MALDI-TOF MS analysis

Prior to start the separation, 7–8 μL of ammonium acetate solution (pH 4.0, ionic strength = 83 mM) was placed on each position of the AnchorChip MALDI target. After sample collection, the droplets were first dried in vacuum. Then, 1 μL of a 2 mg/mL sinapinic acid (SA) in 0.1% trifluoroacetic acid/acetonitrile (0.1% TFA/ACN, 30:70) was deposited on each position and subsequently allowed to dry. MALDI-TOF analysis has been performed using a Bruker Microflex equipped with a nitrogen laser operating at 337 nm.

3 Results and discussion

3.1 Effect of pH and ionic strength of BGE

The properties of a BGE used for a CE separation, including its pH and ionic strength, have significant impact on efficiency, resolution and sensitivity of FESI-CE separation. Since the electrophoretic mobility of a protein changes with the pH of the BGE, pH is certainly the most important separation parameter as it defines the selectivity of the system [33]. As a first step, various ammonium acetate BGEs with pHs 3.5, 3.75, 4.0, 4.25, 4.5, 4.75 and 5.0 were preliminary used to determine the optimum separation pH. From the electropherograms (data not shown), the best resolution was obtained when a pH of 4.0 was used.

Later, if the Joule heating is kept at a moderate level, increasing the ionic strength of a BGE usually yields higher resolutions in a CE separation because the loading capacity of the BGE increases together with this parameter. Furthermore, based on the FESI principles, the sample preconcentration ability will also increase with the BGE ionic strength as long as the Joule heating remains not significant [34]. In our study, to optimize the ionic strength of the BGE (ammonium acetate, pH 4.0), various separations were performed while varying the ionic strength of the buffer from 12.5 to 125 mM. By increasing the ionic strength from 12.5 to 83.3 mM, an improvement of the separation resolution was first observed. At ionic strengths higher than 100 mM, a significant decrease in the resolution occurred supposedly because of an excessive Joule heating. For ionic strength values of 83.3 and 100 mM, the achieved resolutions were comparable; however, a higher reproducibility was obtained at the lower value, especially when long injection times were used. As a result, 83.3 mM ionic strength ammonium acetate buffer at pH 4.0 was thus used for subsequent analyses.

3.2 Injection parameters

The amount of protein injected into the capillary is related to the applied injection voltage and time. To investigate the effect of the sample injection time on the sensitivity and

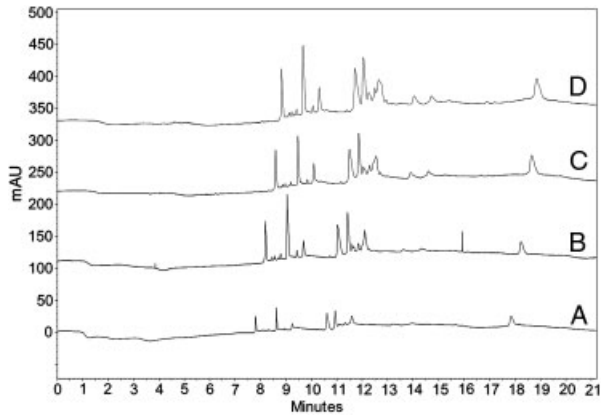

Figure 1. Effects of increasing injection voltage on protein separation by FESI-CE-UV. (A) 1 kV, (B) 2 kV, (C) 3 kV and (D) 4 kV. Injection time: 480 s, BGE: 83.3 mM ammonium acetate (pH 4.0). Sample: standard proteins containing Cyt. *c* (35 nM), Lys (28 nM), RNase A (35 nM), S-RNase (29 nM), α-lac (113 nM), β-lac (88 nM), lactoferrin (106 nM), BSA (24 nM), Myo (24 nM) and aldolase (50 nM).

resolution of the protein separations, a 83-mM ionic strength ammonium acetate buffer was used and the proteins were injected into the capillary using injection times ranging from 30 and 540 s while the injection voltage was kept constant. The best electropherogram concerning both sensitivity and resolution was obtained when proteins were injected for 480 s. No significant sensitivity improvement was observed when the injection time was longer than 480 s.

Subsequently, to further optimize the injection voltage, a protein test mixture with concentration ranging from 6 to 25 nM was considered. The injection time was fixed at 480 s while the injection voltage was increased from 1 to 4 kV. As shown in Fig. 1, by increasing the injection voltage up to 3 kV, the sensitivity of the analysis was increased due to the larger amount of proteins introduced into the capillary. At higher voltages however, a reduced separation resolution was observed, presumably due to an increased joule heating and/or a BGE overloading effect. Therefore, an electrokinetic injection performed at 3 kV for 480 s was considered as optimal injection conditions and used for further experiments.

3.3 Sensitivity and detection limit

Since the detection sensitivity of CE with an absorbance detector is not very high (μM for most UV absorbing analytes), it is of utmost importance to demonstrate that the modified CE technique developed here is compatible with the analysis of low concentration samples.

To evaluate the detection limit of proteins by optimized FESI-CE-UV, the optimized method was employed to analyze low concentrated standard protein samples. Figure 2 shows the electropherograms obtained from a 600-μL large test protein mixture sample. Based on the presented electropherograms, different proteins are separated and detectable at about 1 nM concentration level.

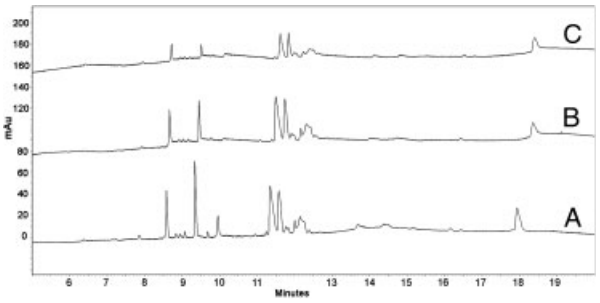

Figure 2. Electropherograms obtained from the analysis of very low concentrated standard protein sample by FESI-CE-UV. (A) 3.2–12.8 nM; (B) 1.6–6.4 nM; (C) 0.8–3.2 nM. BGE: 83.3 mM ammonium acetate (pH 4.0). Injection parameters: 3 kV, 480 s. Other experimental conditions are given in the legend of Fig. 1.

Table 2. Repeatability (RSD%) of migration time and peak area in FESI-CE obtained from UV absorbance at 200 nm

RSD% ($n = 5$)	Cyt. *c*	Lysozyme	Myoglobin	RNA	RNase
Migration time	0.48	0.49	0.29	0.40	0.27
Peak area	16.77	5.57	18.27	14.14	11.53

To estimate the SEF allowed by the described strategy, a protein sample of 1920-fold higher concentration was analyzed under conventional CZE conditions where 0.47% of the total capillary volume was hydrodynamically filled (30 mbar for 10 s) by the sample at the beginning of the separation. In these conditions, approximately 4.6 nL of sample solution (assumed viscosity of 1 cP) is introduced into the capillary during the injection. SEF values achieved by the integration of an FESI preconcentration step were then calculated as follows for six different proteins [35]:

$$\mathrm{SEF} = \frac{\text{peak parameters obtained withpreconcentrain}}{\text{peak parameters obtained without preconcentration}} \times \text{dilution factor} \quad (1)$$

The calculated average SEF values for the six considered proteins are 3200 and 4800 for peak height and area, respectively.

3.3.1 Repeatability and linearity

In order to study the repeatability allowed by the developed FESI-CE method, five replicate experiments were achieved under a given set of conditions and the RSD for migration times and peak area further calculated for the five different proteins. The results are summarized in Table 2. The run-to-run repeatability of protein analysis under optimized FESI-CE conditions was excellent with average RSDs of 0.38% for migration time and 13% for peak area.

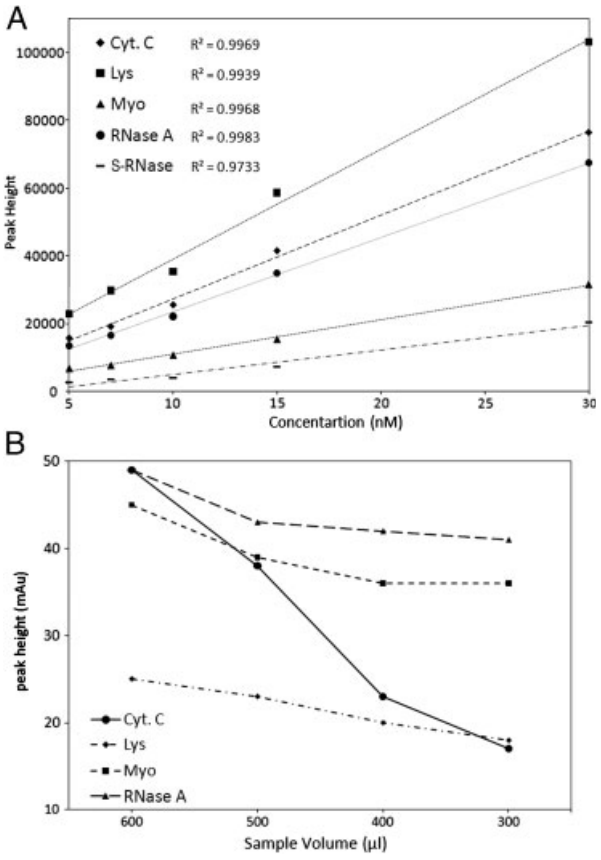

Figure 3. (A) Linearity: Peak heights increase linearly with increasing the concentration. (B) Sample depletion: Peak heights decrease by decreasing the sample vial volume. (The peaks corresponding to each number is indicated in Fig. 2. Protein sample concentration: 0.8–3.2 nM; BGE: 83.3 mM ammonium acetate (pH 4.0); injection parameters: 3 kV, 480 s. Other experimental conditions as in Fig. 1.

To study the linearity, six individual protein solutions at different concentrations (from 5 to 30 nM) were prepared, and the peak heights corresponding to each protein at different concentrations were compared. In Fig. 3A, the peak heights for five proteins present in the sample are plotted versus their concentration. This figure shows that the peak height increases linearly within the concentration range tested and that as it is expected, the slope is higher for the proteins with a higher mobility.

3.4 Sample depletion

During the electrokinetic injection, positively charged analytes and buffer components enter the capillary. With prolonged injection times, theoretically it is possible to significantly deplete the sample from analytes [36]. To study the ability of the developed FESI-CE methodology to deplete the proteins from the sample solution, different sample volumes of 3–12 nM standard protein in water were analyzed under similar conditions by using optimized

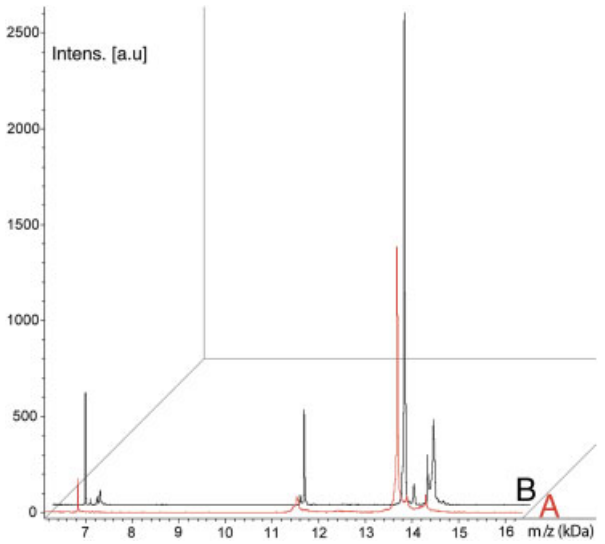

Figure 4. Effect of ammonium acetate on MALDI-MS spectra. (A) Protein sample dissolved in 83 mM ammonium acetate (pH 4.0). (B) Protein sample dissolved in deionized water. Sample: 0.3–1 pmol of RNase A (11.5 kDa), S-RNase (13.6 kDa), α-lac (14.2 kDa) and Lys (14.3 kDa). MALDI matrix: 2.5 mg/mL sinapinic acid in 0.1% TFA/50% ACN.

FESI-CE system. Figure 3B shows the peak height variations when injecting from samples presenting identical composition and protein concentrations but different total volumes. The decrease in the peak heights by reducing the sample volume illustrates that the developed FESI-CE system is an efficient approach for injecting and concentrating dilute levels of proteins from relatively large volume of sample as it shows that analytes can be exhaustively injected from volumes much larger than the total volume of the used capillaries. Furthermore, it shows as expected that FESI preconcentration is less effective for proteins with relatively low electrophoretic mobilities.

3.5 Hyphenation of FESI-CE to MALDI-MS

Based on the FESI preconcentration principle, high ionic strength BGEs are usually used to achieve efficient preconcentration prior to CE separation. Since high ionic strength buffers are not tolerable in MS, the hyphenation of FESI-CE with ESI-MS is difficult. On the other hand, the hyphenation of FESI-CE with MALDI-MS could be realized if care is taken regarding the compatibility of the BGE with MS.

Although the ammonium acetate is recognized as a MALDI compatible buffer, at relatively high concentrations, it has a deleterious effect on both the sensitivity and resolution of the protein analysis. Figure 4 demonstrates the ion suppression phenomena induced by the presence of a high concentration of ammonium acetate on the sensitivity of protein analysis by MALDI-MS. As it is shown the sensitivity of MALDI-MS analysis of standard proteins, when dissolved in 83 mM ionic strength ammonium acetate (mass

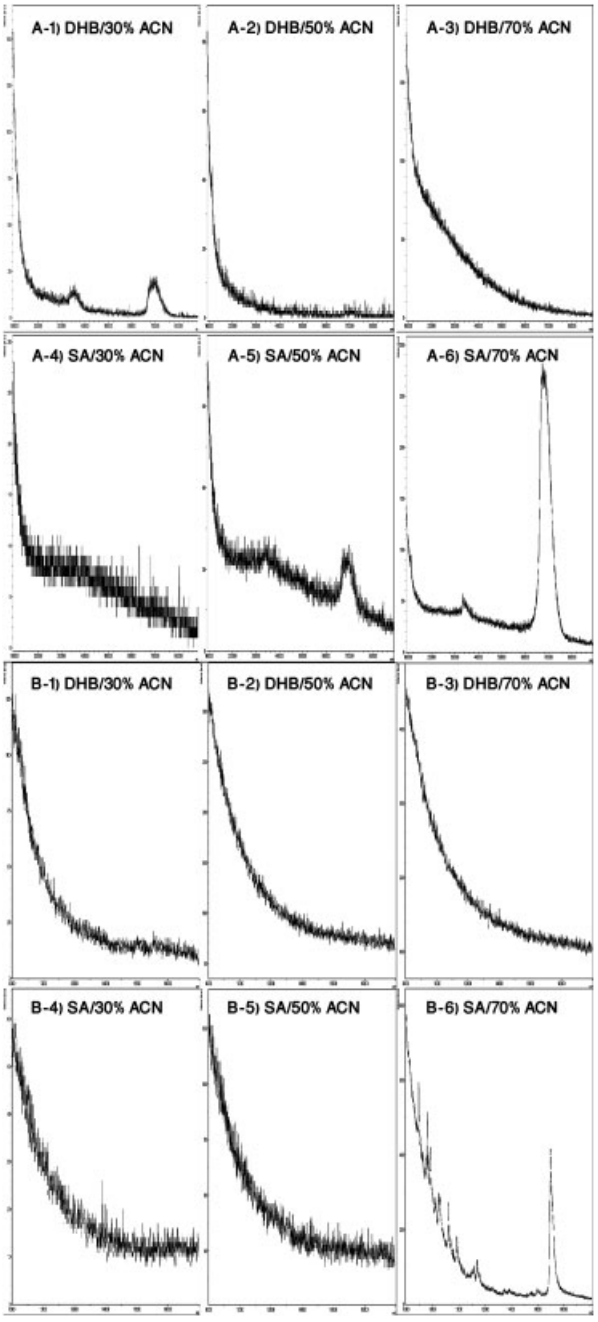

Figure 5. Comparison of different matrixes/solvents for MALDI-MS protein analysis in the presence of ammonium acetate. (A) 540 fmol BSA and (B) 580 fmol myoglobin. The matrix/solvent used in each case is written on the figure. The concentration of DHB and SA is 10 and 2 mg/mL, respectively.

spectrum A) is low as compared with the typical conditions where they are dissolved in water (mass spectrum B). Nevertheless, optimizing parameters like sample spotting method, MALDI matrix and matrix solvents may increase the sensitivity of the protein analysis in the presence of ammonium acetate. For these reasons, to reach the highest possible sensitivity in protein analysis in the presence of ammonium acetate, different parameters such as sample spotting method, matrix nature and matrix solvent have been optimized in the present study.

Different sample spotting approaches on the MALDI plate including 'dried droplet', 'sandwich' and 'thin layer' have been tried. Highest sensitivities were obtained while the MALDI matrix was simply added to the plate after the protein solution had been fully dried under gentle vacuum.

To find the MALDI matrix/solvent more compatible with the presence of ammonium acetate, a comprehensive study has been performed. Different concentrations of 2,5-dihydroxybenzoic acid (DHB) or SA have been used. In addition, the concentration of ACN in the matrix solvent has also been varied from 30 to 70%. Figure 5 compares some of the different conditions that have been tried. As also shown in Fig. 5, the highest sensitivity was reached when 2 mg/mL of SA in 0.1% TFA:ACN (30:70) has been used as the MALDI matrix.

To couple the developed FESI-CE strategy with MALDI-MS, an automated iontophoretic fraction collection interface previously developed in our laboratory has been used. This fraction collection approach is based on electromigration and diffusion and it is demonstrated that separation resolution is independent of spotting process [31].

Figure 6 shows the electropherogram obtained by the FESI-CE analysis of a standard protein mixture and the MS spectra of the different fractions collected. Here, while the sample contained eight different proteins, only lactoferrin (76 kDa) could not be detected by MALDI-MS. It could be explained by the lower electrophoretic mobility of this protein that causes a less effective preconcentration and potentially also by its high molecular weight which induces a lower detection sensitivity in MALDI-MS.

Moreover, to evaluate the LOD enabled by the developed FESI-CE-MALDI-MS platform, a standard protein sample containing 5–15 nM of the different proteins has further been analyzed under optimized conditions. To be able to isolate each protein on different MALDI plate spots, the sampling rate of the fractionation has been increased to 10–-15 s per spot. In this case, while the total amount of each protein in the CE sample vial was ranging from 1.5 to 5 pmol (3–14 nM in 600 μL) and in spite of the presence of ammonium acetate in the CE BGE, the detection of each protein by MALDI-MS was possible thanks to the efficiency of the FESI preconcentration step. Figure 7 shows the MS spectra of different proteins after FESI-CE-MALDI-MS. It should be noted that the obtained LOD values attained by FESI-CE-MALDI-MS are comparable to those achieved by the direct analysis of proteins by MALDI-MS in the presence of ammonium acetate, which is limited to 0.2–1 pmol depending on the considered protein.

Considering the results discussed above, we demonstrate, as in the case of CE-UV, limits of detection in the low nanomolar range by FESI-CE-MALDI-MS, which are comparable with detection limits of proteins achieved by state-of-art sheathless CE-ESI-MS [37].

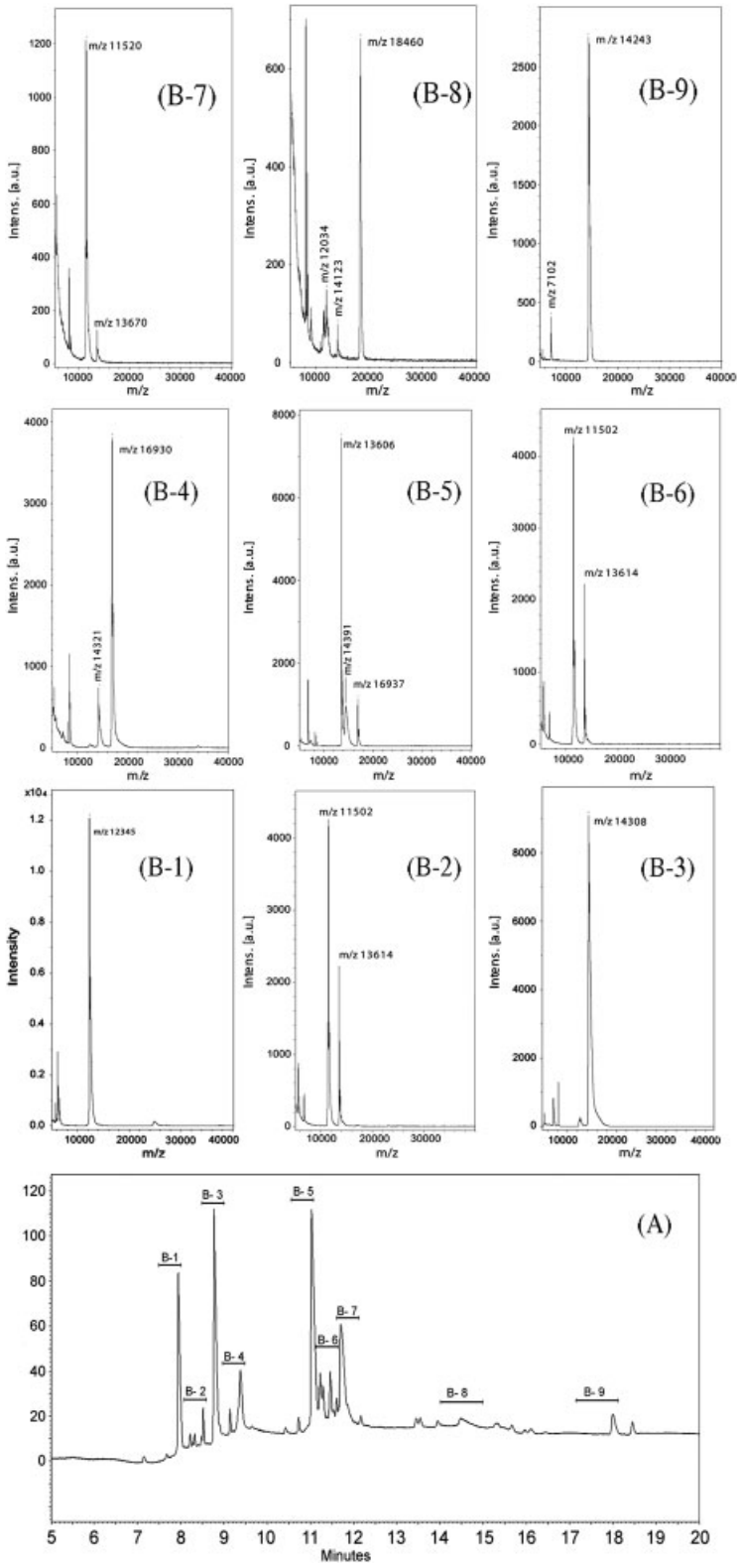

Figure 6. Electropherogram and corresponding MS spectra of the analysis of protein sample by FESI-CE-MALDI-MS. Sample: 35 nM Cyt. *c* (12.3 kDa), 50 nM lysozyme (14.3 kDa), 53 nM RNase A (13.6 kDa), 90 nM α-lac (14.2 kDa), 90 nM S-RNase (11.5 kDa), 70 nM β-lac (18.3 kDa), 20 nM lactoferrin (76 kDa) and 25 nM myoglobin (16.9 kDa). Other conditions are as described in Section 2. Electropherogram obtained in similar conditions by FESI-CE-UV. (B1) Sampling time: 7:30–8:00 min, Cyt. *c* (B2) sampling time: 8:00–8:30 min, Cyt. *c*, lysozyme. (B3) Sampling time: 8:30–9:00 min, lysozyme. (B4) Sampling time: 9:00–9:30 min, myoglobin, lysozyme. (B5) Sampling time: 10:30–11:00 min, S-RNase, myoglobin, lysozyme. (B6) Sampling time: 11:00–11:30 min, S-RNase, RNase A. (B7) Sampling time: 11:30–12:00 min, S-RNase, RNase A. (B8) Sampling time: 14:00–15:00 min, S-RNase, RNase A, β-lac. (B9) Sampling time: 17:00–18:00 min, α-lac.

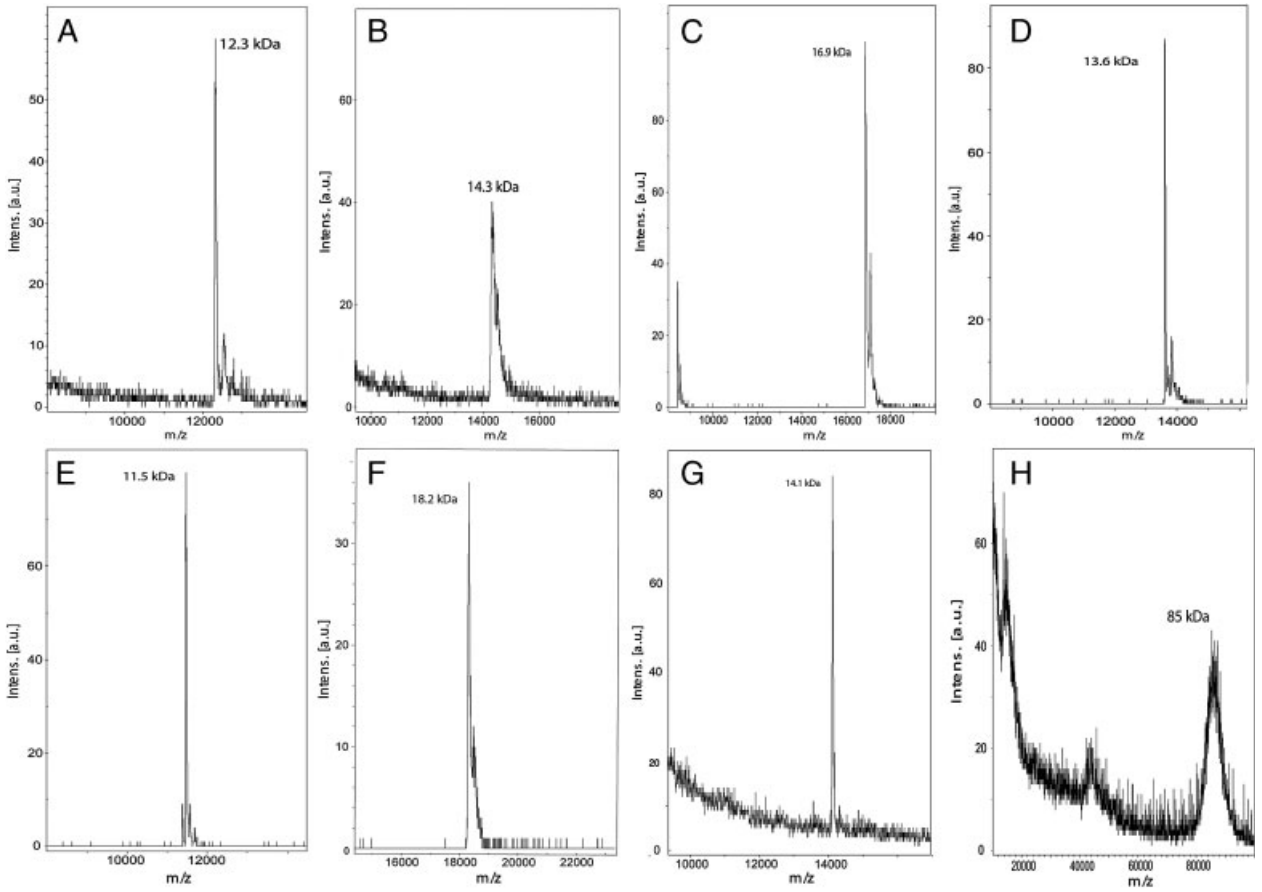

Figure 7. MS spectra of analysis of protein sample by FESI-CE-MALDI-MS. (A) 4.5 nM Cyt. *c* (12.3 kDa), (B) 3.8 nM lysozyme (14.3 kDa), (C) 6 nM myoglobin (16.9 kDa), (D) 3 nM RNase A (13.6 kDa), (E) 3.5 nM S-RNase (11.5 kDa), (F) 10 nM β-lac (18.3 kDa), (G) 14.5 nM α-lac (14.2 kDa) and (H) 13.5 nM lactoferrin (76 kDa).

4 Concluding remarks

Based on the FESI principle, a practical preconcentration methodology has been developed. It can be simply applied to concentrate low amount of protein dissolved in relatively large volume of sample solutions prior to CE analysis. Under optimized conditions, detection limits of about 1 nM have been achieved with UV detection. Estimation of SEF values showed that average values of 3200 and 4800 could be obtained based on peak height or peak area, respectively. Furthermore, we confirm that in spite of the use of a high conductivity buffer, required to perform successful FESI preconcentration, the developed preconcentration methodology could also be efficiently used in conjunction with MALDI-MS for the achievement of low nanomolar detection limits. The high-resolution separation and sensitive detection/identification of low nanomolar concentrations of different proteins by UV/MALDI-MS demonstrate the great potential of the developed methodology for proteomic and/or biopharmaceutical applications.

The authors have declared no conflict of interest.

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