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Research Article

In-capillary approach to eliminate SDS interferences in antibody analysis by capillary electrophoresis coupled to mass spectrometry

Capillary electrophoresis is an important technique for the characterization of monoclonal antibodies (mAbs), especially in the pharmaceutical context. However, identification is difficult as upscaling and hyphenation of used methods directly to mass spectrometry is often not possible due to separation medium components that are incompatible with MS detection. Here a CE-MS method for the analysis of mAbs is presented analyzing SDS-complexed samples. To obtain narrow and intensive peaks of SDS-treated antibodies, an in-capillary strategy was developed based on the co-injection of positively charged surfactants and methanol as organic solvent. For samples containing 0.2% (v/v) of SDS, recovered MS peak intensities up to 97 and 95% were achieved using cetyltrimethylammonium bromide or benzalkonium chloride, respectively. Successful removal of SDS was shown in neutral coated capillaries but also in a capillary with a positively charged coating applying reversed polarity. The usefulness of this in-capillary strategy was demonstrated also for other proteins and for antibodies dissolved in up to 10% v/v SDS solution, and in other SDS-containing matrices, including the sieving matrix used in a standard CE-SDS method and gel-buffers applied in SDS-PAGE methods. The developed CE-MS approaches enable fast and reproducible characterization of SDS-complexed antibodies.

Keywords:
Cationic surfactant / In-capillary / Mass spectrometry / Monoclonal antibody / Sodium dodecyl sulfate
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1 Introduction

Advances in gene technology have greatly facilitated the production of new protein biopharmaceuticals. Due to the importance of these drugs in current health care, there is a great demand on knowledge about their structural or size variants, variants impurities, and degradation products. Therefore, in order to enable the characterization of these species, the development of fast, selective, and sensitive analytical methodologies is necessary. SDS-PAGE has been widely used in most biological research laboratories [1, 2]. However, complementary CE-SDS non-gel sieving has emerged as a popular choice for the fast, highly resolving and quantitative separation of therapeutic proteins and their fragments. CE coupling with MS is a useful tool for obtaining essential information about protein pharmaceuticals as glycosylation [3–6], structural characterization [7], or post-translational modifications [8]. Nevertheless, to date, only few CE-MS applications have been developed for the study of therapeutic proteins, summarized in recent reviews [9–12].

CIEF [13], CZE [14] and CE-SDS [15] are the modes widely used for CE separation of protein pharmaceuticals. Most of the background electrolytes, matrices, or additions employed in these modes are incompatible with MS. One well-known example is SDS, which is an important additive for size-based separation of proteins [1]. SDS binds to the protein in a ratio of 1.4 g SDS per g of protein and, although several modes have been described for the interaction of SDS-protein, both hydrophobic and ionic interactions contribute to the complex formation [16,17]. The impediment of MS analysis by the SDS is well known. This effect is related to several factors, such as low surface tension, low volatility, high signal background,

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and formation of protein-SDS adducts [18]. Therefore, pre-treatment procedures for SDS removal are required for optimal MS analysis. For this purpose, several off-line methods, such as precipitation, chromatographic purification, in-gel digestion, or centrifugal filtration have been successfully applied [19–21]. However, available clean-up methods are often impeded by protein loss, time effort, costs and low sensitivity. Therefore, methodologies for in-line removal of SDS prior to MS analysis are desirable. Recently, some interesting in-line approaches with microfluidic devices were based on free-flow zone electrophoresis [22], on semipermeable membranes in a cross-flow filtration process [21], or on membranes to collect CGE resolved peaks [23]. The latter approach made use of additional membrane washing steps for SDS removal. Subsequently, it allowed direct on-membrane MALDI-TOF-MS protein analysis. In the case of MEKC-MS where SDS is included in the BGE, partial filling strategies or anodically migrating micelles were the most employed strategies to avoid SDS entering the MS source [22].

An interesting approach for removal of SDS and Tris buffer from a protein solution was developed by Shieh et al. [24]. In order to form dodecyl sulfate-cetyltrimethylammonium ion pairs equal moles of positively charged cetyltrimethylammonium bromide (CTAB) were added to the SDS sample solution. Afterwards, the sample was analyzed by fused-droplet electrospray ionization mass spectrometry (FD-ESI-MS) with methanol as a electrospraying solution. The low solubility of CTAB-SDS complexes in methanol excluded them from the droplets, which prevented suppression of the analytes of interest.

The objective of this study was to develop a simple in-capillary CE-ESI-MS strategy for the analysis of protein samples in the presence of SDS. In order to demonstrate its suitability and versatility, the strategy was then evaluated for different applications.

2 Materials and methods

2.1 Chemicals

All solutions were prepared using ultrapure water (18 MΩ cm at 25°C, SG Ultra Clear UV from Siemens Water Technologies, USA) and all reagents were of analytical grade. Methanol, 2-Propanol, (each LC-MS grade), acetic acid, formic acid, sodium hydroxide, hydrochloric acid, and aqueous ammonia (30% p.a.) were obtained from Carl Roth GmbH und Co. KG (Karlsruhe, Germany). Ammonium acetate, ammonium carbonate, citric acid monohydrate, calcium chloride, acrylamide/bis-acrylamide, dextran, glycerol, DDT, SDS (10% in water), PVA, glutaraldehyde solution 50% (v/v), Polybrene (hexadimethrine bromide), 5-carboxytetramethylrhodamine succinimidyl ester (5-TAMRA.SE), CTAB, benzalkonium chloride (ADBAC), tetrabutylammonium bromide (TBAB), tetramethylammonium chloride (TMAC), benzyltrimethylammonium chloride (BTAC), tricaprylylmethylammonium chloride (Aliquat), tetraethylammonium bromide (TEAB), methyl-β-cyclodextrin, myoglobin (from horse skeletal muscle, 95–100%, molecular weight approx. 17.0 kDa), cytochrome c (from horse heart, ≥ 95%, molecular weight approx. 12.4 kDa), RNase A (from bovine pancreas, ≥ 80%, molecular weight approx. 13.7 kDa) and β-lactoglobulin A (from bovine milk, ≥ 90%, molecular weight approx. 18.4 kDa) were obtained from Sigma-Aldrich (Steinheim, Germany). Tris and boric acid were obtained from Merck (Darmstadt, Germany). Dynamic UltraTrol™ coatings were from Target discovery (Palo Alto, CA, USA). "ES tuning mix" solution was obtained from Agilent Technologies (Waldbronn, Germany). SDS gel buffer was obtained from Beckman Coulter, Inc. (Fullerton, CA).

Sample solution of a monoclonal antibody IgG1 was employed (25 mg/mL mAb, molecular weight approx. 150 kDa).

2.2 Preparation of solutions and samples

Sample preparation was carried out diluting the mAb samples (25 mg/mL) directly in water or SDS solution (at the desired percentage v/v) and adding 1 M DDT in order to obtain the reduced mAb. After heating the sample for 5 min at 70°C in a Thermomixer (Eppendorf, Wesseling-Berzdorf, Germany), it was centrifuged at 14 100 *g* during 45 s in a minicentrifuge (eppendorf, Wesseling-Berzdorf, Germany). Protein samples were prepared at 0.5 mg/mL in water or 0.2% (v/v) SDS solution.

SDS-PAGE buffer consisted in a mixture of 2.5 mL of 40% acrylamide/bisacrylamide (29:1), 3.1 mL of 1.5 M Tris-HCl buffer pH 8.8, 4.2 mL of water and 0.1 mL SDS solution 10% v/v. In eppendorfs, the mixture was added to the antibody to obtain a final concentration of 2.5 mg/mL and then, it was reduced and incubated during 5 min at 70°C.

SDS gel buffer was made in the laboratory following the patent by Liu et al. [25] and prepared with and without contents of SDS to study the surfactant influence in the MS analysis.

2.3 Capillary coatings

Dynamic coatings UltraTrol™ low normal EOF (neutral, LN), high normal EOF (anionic, HN) and high reverse EOF (cationic, HR) were carried out after a preconditioning of the capillaries by flushing (1000 mbar) with water (5 min), 1 M NaOH (20 min), and water (5 min). The coating procedure consisted of: 1 M HCl for 5 min, water for 5 min, coating solution for 5 min, water for 2 min, and BGE for 2 min. Recoating needed to be done every five runs. To avoid contamination of the electrospray ionization source and the mass spectrometer, the capillary was removed from the CE-ESI-MS interface during the recoating procedure.

A homemade cross-linked permanent coating by PVA was carried out according to Belder et al. [26] procedure with some modifications. The coating was performed by treating

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the inner capillary surface with a solution of glutaraldehyde as cross-linking agent (3.25 pg/mL in 1.21 M HCl) followed by a solution of PVA (45 mg/mL in 0.6 M HCl), which resulted in an immobilization of the polymer on the capillary surface. The procedure consisted of: (i) the capillary was dried with air during 15 min at 5 bar after the first conditioning, (ii) the capillary was 35% filled with the glutaraldehyde solution at 5 bar, (iii) immediately afterwards an acidified PVA solution was pressed at 5 bar into the column (70% of the capillary), (iv) the capillary was then emptied and predried under nitrogen flow for 15 min,(v) thereafter, the capillary was incorporated in a gas chromatography oven heated from 40 to 160°C with 6°C/min while the columns were flushed in a nitrogen flow. After cooling down the capillary to room temperature, 5 cm of both ends were cut.

Polybrene was used as a noncovalent polycationic coating according to the procedure by Sanchez-Hernandez et al. [27]. Briefly, a new capillary was flushed with 1 M NaOH for 20 min, with water for 5 min, with Polybrene solution at 1% (w/v) in water for 15 min, wait for 15 min, and finally with buffer for 20 min. The coating procedure was performed outside the MS source.

2.4 Labeling mAb

First, 500 μL of the antibody at 2 mg/mL were buffer exchanged using NAP-5 columns (GE Healthcare, Buckinghamshire, UK) and eluted into 1 mL labeling buffer [15]. Then, it was labeled by 5-TAMRA.SE. For the removal of excess dye, the antibody-dye conjugate was loaded onto a second NAP-5 column and eluted by citrate-phosphate buffer.

For reduced SDS-antibody complexes, 5 μL of the labeled mAb (25 mg/mL) was mixed with 45 μL of SDS solution (0.2% v/v) and 5 μL of 1 M DTT. Finally, reduced samples were incubated in a Thermomixer at 70°C for 5 min.

2.5 CE-ESI-MS analysis

A HP^{3D}CE instrument (Agilent Technologies, Palo Alto, CA, USA) was coupled through an orthogonal electrospray interface (ESI, model G1607A from Agilent Technologies) to a micrOTOF-Q (Bruker Daltonics, Bremen, Germany) for MS detection. CE experiments were performed using coated capillaries of 50 μm id with a total length of 55 cm (Polymicro Technologies, AZ, USA) at +30 kV (inlet) and at 25°C. The sample was injected hydrodynamically by pressure (50 mbar for 15 s) followed by dipping the capillary into a water vial in order to prevent contamination of the BGE (1 M acetic acid). Between injections, the capillary was rinsed with water for 2 min and the BGE for 2 min.

MS control and data analysis were carried out using Bruker Daltonics micrOTOF control version 2.3 (Bruker, Bremen, Germany). Electrospray ionization was used in positive ion mode applying 4.5 kV on the MS inlet (sprayer on ground). Electrical contact at the electrospray needle tip was established via a sheath liquid which consisting of isopropanol/water (50:50, v/v) containing 0.2% (v/v) formic acid and delivered at a flow rate of 3.3 μL/min by a syringe pump (model 100, Holliston, USA) with Hamilton syringe of 5 mL from Supelco (Bellefonte, PA, USA). The flow rate of dry gas (nitrogen) was 4.0 L/min at a temperature of 170°C. The nebulizer gas pressure was set to 0.2 bar. Spectra were acquired in a mass range of m/z 700–3500. The ion transfer was optimized in this mass range. Relative high fields are applied for efficient ion transfer of proteins (ion energy: 5 V, collision energy: 10 V). An external calibration was performed by infusing tuning mix solution every morning.

3 Results and discussion

3.1 CE-MS method for the analysis of reduced mAb

First, a CZE-MS was adapted for the analysis of mAbs based on previous results from the working group using LN coating [28]. From the different buffers tested (1 M acetic acid, 1 M formic acid, 50 mM ammonium formate and 50 mM ammonium bicarbonate buffers), 1 M acetic acid presented the best separation results. At this point, different dynamic coatings than LN (anionic HN and cationic HR) and a permanent neutral coating (PVA) were tested. LN coating revealed higher peak efficacy and intensity in comparison with the other dynamic coatings showing the light chain (LC) and heavy chain (HC) peaks partially resolved in the 5.5–6.5 min region. In principle, baseline separation is not required in this method because the final motivation will be to use the procedure to peaks previously separated in an SDS-based system. Similar results in migration times, peak efficacy, and intensity were obtained for LC and HC comparing PVA and LN coating. Better capillary-to-capillary reproducibility for the area and migration time (RSDs up to 7.4 and 2.7%, respectively) was achieved in three different PVA capillaries (10 runs in each PVA capillary) (cp. Supporting Information Table S1). Thus, PVA was selected as the optimal coating.

Different mixtures of water with methanol and isopropanol were tested as sheath liquid using low percentages of acetic acid or formic acid. Among them, a sheath liquid of isopropanol:water (50:50, v/v) with 0.2% (v/v) formic acid at 3 μL/min was selected. Supporting Information Fig. S1 shows the EIEs of LC peak and HC peak and the deconvoluted mass spectrum of HC and LC at optimal conditions. The LOD obtained was estimated from the ion traces of the LC and HC individually and from the intensity of the deconvoluted spectra. Thus, LC and HC could be detected down to 0.05 mg/mL (2×10^{-6} M) and 0.1 mg/mL (2×10^{-6} M) from the reduced mAb, respectively.

3.2 Study of strategies to remove SDS

By the developed CE-MS methodology, the reduced mAb in presence of 0.2% (v/v) SDS (7 mM) was analyzed. The signal

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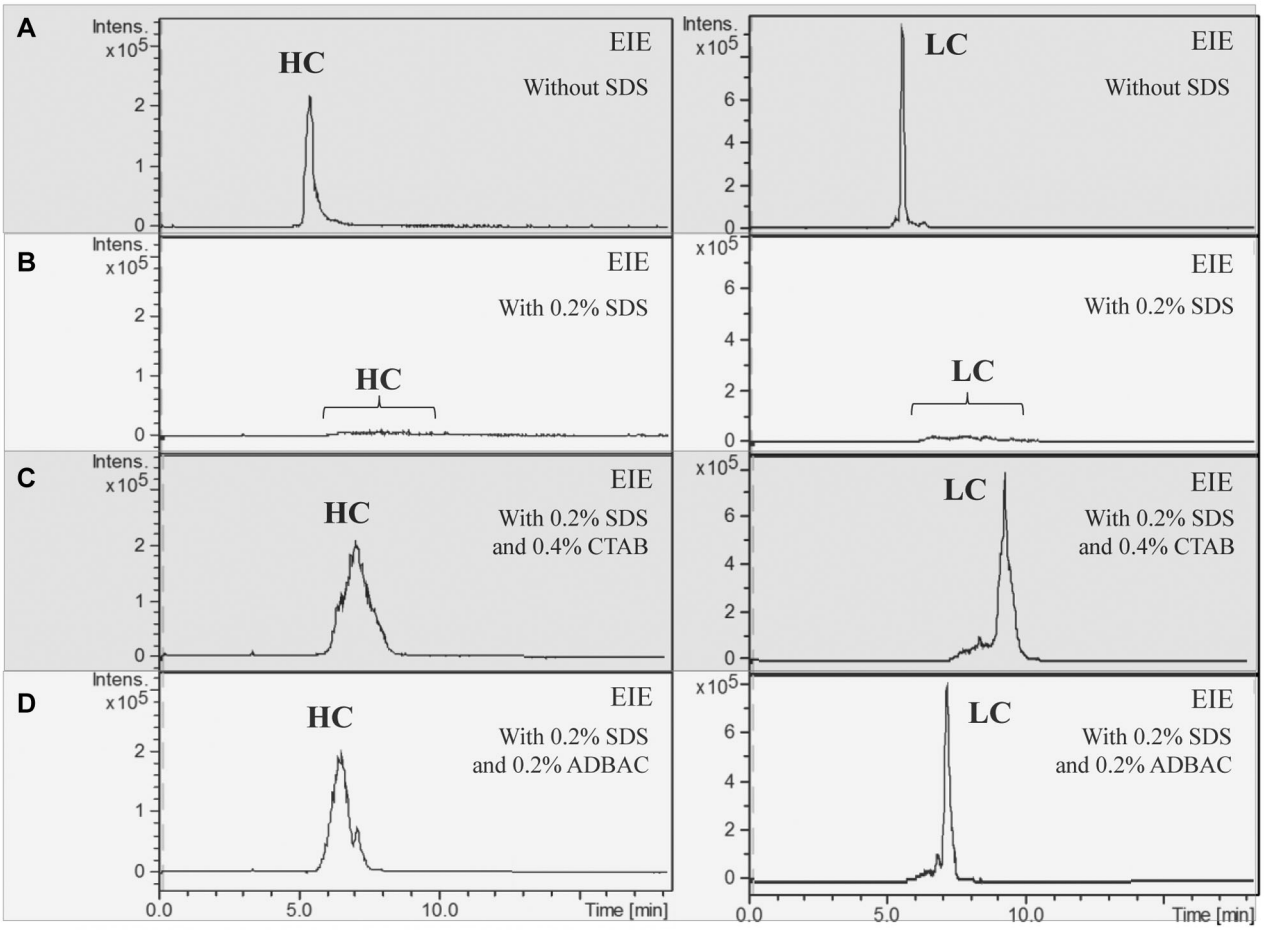


Figure 1. CE-ESI-MS EIEs in optimal conditions of (A) HC and LC of mAb without SDS, (B) HC and LC of mAb with 0.2% (v/v) SDS, (C) HC and LC of mAb with 0.2% (v/v) SDS after in-capillary strategy with 0.4% (w/v) CTAB, (D) HC and LC of mAb with 0.2% (v/v) SDS after in-capillary strategy with 0.2% (w/v) ADBAC.

decreased significantly (reduction of 97% in MS intensity) compared with the reduced non-SDS mAb sample (Fig. 1A and B). Only weak and broad signals were observed for HC and LC. This is most likely due to different migration directions of the SDS-mAb complex (negatively charged) and the non-SDS mAb (positively charged), and the slow dissociation of the SDS-mAb complex due to the strong hydrophobic and ionic interactions existing [17]. This yields a very broad zone of non-SDS mAb towards the MS. In addition, the quality of the mass spectra is potentially also degraded by the presence of SDS and the high SDS background. Thus, a study of an in-capillary removal of SDS from the antibody solutions was carried out for MS detection.

Transient ITP was tested for zone sharpening for the analysis of the reduced mAb with 0.2% (v/v) SDS. Several pre- and post-plugs volumes (from 5 till 20 s with 50 mbar) of 1 M acetic acid or ammonium acetate as leading electrolyte and 1 M ammonium acetate as terminating electrolyte were investigated. By a 20 s pre and post plug with ammonium acetate an improvement of signal intensity around 13% was obtained with less peak broadening in comparison with the SDS-mAb sample without any ITP strategy. However, it was not possible to further improve this result.

Pre-plugs and post plugs of solvents (methanol (MeOH), 2-propanol, and ACN) with different water content (between 5 and 20% v/v) and volume (from 5 to 20 s with 50 mbar) were tested for SDS removal. Best results were obtained with 50 mbar × 5 s MeOH as pre-plug and 50 mbar × 20 s MeOH:water (50:50, v/v) as post-plug. In this case, significant increase in signal intensity resulted for the SDS-mAb sample (36% relative to the sample without SDS). Although this was still not appropriate, the idea was included in the upcoming strategy design.

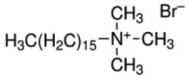
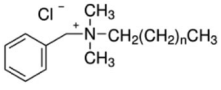
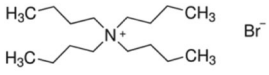
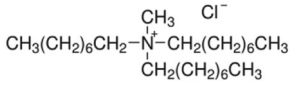
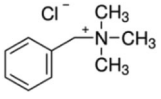
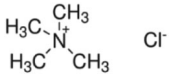
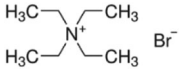
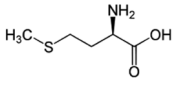
The possibility of usingcertain molecules as stripping agents to remove SDS from the mAb solution was already tested elsewhere. Rozema et al. demonstrated how cyclodextrins could bind a detergent to remove it from a protein by a chaperone system [29]. In this study, methyl-β-cyclodextrin at 7 mM (same molar concentration as SDS) was selected to bind the hydrophobic region of the SDS. Different plugs before and after the reduced SDS-mAb sample were

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evaluated [30]. However, no improvements were observed by using cyclodextrin.

For SDS removal, Shieh et al. [24] added CTAB into the sample solution to form ion pairs with SDS. In our acidic BGE using a neutral coated capillary (low EOF), the negatively charged SDS-mAb complex will migrate towards the anode (capillary inlet). Thus, the cationic CTAB was injected as a post-plug to interact with the SDS-mAb complex enabling the CTAB-SDS complex formation. mAb should become positively charged after SDS removal and migrate towards the cathode at the capillary outlet for MS detection. Different concentrations (0.2–1%, w/v) and volumes (2.5–20 s with 50 mbar) of CTAB post-plugs were tested for a plug of SDS-mAb sample of 50 mbar × 12 s. Lowest peak broadening was obtained with 10 s injection of 0.4% (w/v) CTAB at 50 mbar. In addition to the CTAB plug, MeOH plugs were tested (different pre-plug and post-plug volumes from 5 to 20 s with 50 mbar) since MeOH has a strong impact on complex formation and solubility (both CTAB-SDS and SDS-mAb complex). The best results were obtained with a 100% (v/v) MeOH pre-plug (5 s at 50 mbar) and 0.4% (w/v) CTAB post-plug (10 s at 50 mbar) dissolved in MeOH:water (50:50 v/v). In comparison to the non-SDS mAb, signal intensity in MS was now in the range of 96.7% (HC) and 94.7% (LC) (Fig. 1A and C). Additionally, the mass spectra did not show any interference of SDS or CTAB. Thus, the combination of an organic solvent and a cationic surfactant is well suited for in-capillary SDS removal in CE-MS.

Table 1. Results by the in-capillary strategy with other cationic compounds

	Cationic surfactant Structure	Electrophoretic mobility x 10^{-4} (cm^2/sV) [a]	Signal Intensity (%)[b]		Migration time (min)	
			HC	LC	HC	LC
Antibody	–	2.9	–	–	5.3	5.6
CTAB		2.1	96.7	94.7	7.0	7.7
ADBAC		1.8	94.0	95.2	6.5	7.1
TBAB		2.0	88.1	96.4	6.6	7.3
Aliquat		1.2	85.4	90.5	6.3	7.0
BTAC		3.2	36.9	66.6	7.2	8.1
TMAC		4.9	33.5	50.5	7.0	7.7
TEAB		4.0	42.8	56.0	7.1	7.8
NH_4^+	NH_4^+	7.2	–	–	–	–
Methionine		0.9	70.7	53.9	7.1	8.2 9.1
$CaCl_2$	$CaCl_2$	2.1	39.4	52.5	11.0	11.4 12.3

a) Electrophoretic mobility = (length effective (m) x length total (m))/(Tm (s) x voltage (V)).
b) Signal intensity respect the signal of antibody without SDS.

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These results can be explained by reduction of destacking effects, complex formation between the cationic surfactant and the SDS, by dissociation of the SDS-mAb complex in MeOH, and by solubility of both complexes in the MeOH:water system. Furthermore, stacking effect of the non-SDS mAb in MeOH or transient-ITP effect might play a role.

In order to better understand the strategy, other cations were tested as alternative to CTAB (Table 1). The same method (50 mbar × 5 s pre-plug of MeOH and 50 mbar × 20 s post-plug of the cation diluted in MeOH:water (50:50, v/v)) was used just replacing CTAB by one of the other cations at the same molar concentration than SDS. Reasonable spectra quality was observed for several cations, with the best results for CTAB and ADBAC (Table 1). Since the cationic surfactants (TBAB, ADBAC, and Aliquat) with long alkyl chains and lower electrophoretic mobility than the mAb ($2.9\ 10^{-8}$ m^2/sV) provided the best results, different effects might be responsible for the results obtained in the strategy: (i) a transient-ITP effect produced by the lower electrophoretic mobility of the cation, and (ii) a (hydrophobic) interaction effect between the SDS and the cation when the cation contains alkyl chains.

For verification of the influence of the complexation effect two non-surfactant cationic molecules with lower electrophoretic mobility than the mAb were also tested: methionine ($0.9\ 10^{-8}$ m^2/ sV) and the calcium ion ($2.06\ 10^{-8}$ m^2/ sV). With these two molecules, a SDS removal effect was observed but lower MS signal intensities were obtained in comparison to the use of alkyl chain cationic surfactants (see Table 1). This result confirms that the strategy is based on the transient-ITP effect, however surfactant properties of the cation improve the removal of SDS.

Since both cationic surfactants, CTAB (cp. Fig. 1C) and ADBAC (cp. Fig. 1D) were adequate, the precision of the method using both CTAB and ADBAC was studied (Table 2). Percentage values of RSDs, for both surfactants were similar and acceptable (<13.8% for peak areas, <8.6% for MT and <11.9% for peak intensities, cp. Table 2). However, at the beginning, the lifetime of the capillary was reduced to 25 analyses possibly due to the precipitation of the complex SDS-CTAB or SDS-ADBAC [31] or their adsorption on to the capillary wall. This issue was solved by extensive flushing with separation buffer or by washing between analyses with 20 mM phosphate buffer pH 7.0, which has been described to be useful to solubilize these complexes [32].

Table 2. Repeatability and intermediate precision of the in-capillary strategy to remove SDS comparing CTAB and ADBAC as cationic surfactants

	CTAB		ADBAC	
	HC	LC	HC	LC
Repeatability				
Time RSD (%)	3.1	3.3	2.7	2.5
Area RSD (%)	6.1	7.0	6.8	6.1
Peak intensity RSD (%)	5.3	6.0	4.7	5.4
Intermediate precision				
Time RSD (%)	8.3	8.6	7.1	7.4
Area RSD (%)	12.3	13.8	11.5	13.2
Peak intensity RSD (%)	11.9	11.1	10.6	10.9

At this point, a cationic capillary coating was used instead of the neutral PVA to test its potential to remove SDS using the plugs strategy. Using reversed polarity (−30 kV), the SDS-mAb complex migrates in the same direction of the EOF toward the anode (in this case the MS side). Under these conditions, the cationic surfactant plug can be injected as a pre-plug step. Good results were accomplished using a pre-plug of 50 mbar × 20 s 0.4% (w/v) ADBAC in MeOH:water 50:50 (v/v) without the requirement of a plug of MeOH. Values of RSD of 12.1 and 10.3% in area and 10.5 and 13.9% in signal intensity were obtained for HC and LC after six consecutive injections in three different capillaries. With this Polybrene strategy, the MS signal intensities were about 60% for both HC and LC of SDS-mAb sample using the plug strategy in comparison with the non-SDS mAb. Although the efficacy to remove SDS was lower in comparison with the results for PVA capillaries, the simplicity of this one-plug strategy is promising. Thus, this made us check its efficacy to remove SDS from a sample diluted in a commercial CE-SDS gel buffer (as described later for PVA coated capillary). However, components from the gel buffer were interfering in the MS ionization which resulted in poor signal intensities (data not shown). In this way, this one-plug strategy was discarded for further experimental work.

3.3 Versatility of the developed in-capillary SDS removal strategy

In order to demonstrate the versatility of the in-capillary SDS removal strategy, different applications based on real problems related with SDS in protein analysis were tested.

3.3.1 Other standard proteins

In parallel to the experiments carried out using the mAb, the strategy was tested also for different standard proteins as β-lactoglobulin, myoglobin, RNAse A, and cytochrome C. The proteins were studied individually by diluting in water or in 0.2% SDS (v/v). Due to the different molecular weight of these proteins compared to mAb, experiments on sample volume (50 mbar for 5–15 s), CTAB or ADBAC concentration (0.1–0.4%, w/v) and post-plugs injection volumes (50 mbar for 5–15 s) were carried out. The same optimum conditions were achieved for all the proteins studied (5s of sample injection and 10s of 0.1% (w/v) CTAB) obtaining recoveries in peak intensities of 98.0% for β-lactoglobulin, 78.6% for RNAse A, 88.3% for cytochrome C, and 53.0% for myoglobin compared to the sample without SDS in each case (see Supporting Information Fig. S2). These results justify the expectation that the presented method is applicable to SDS-complexed proteins in general.

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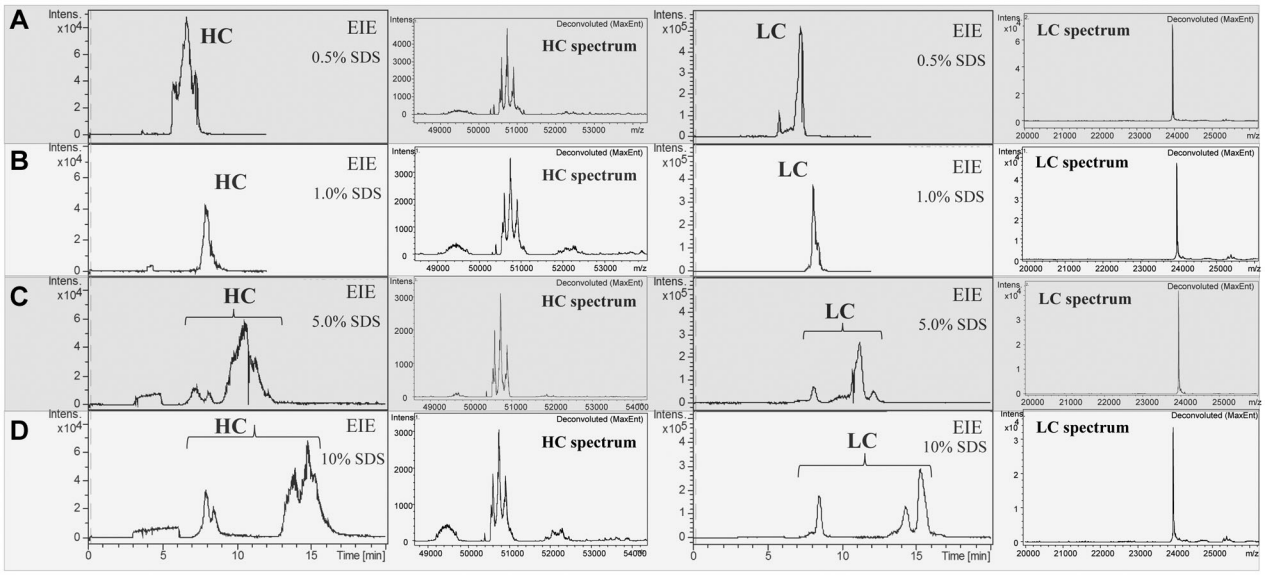


Figure 2. CE-ESI-MS EIEs of HC and LC of the antibody at different SDS concentrations in the sample solution: (A) 0.5% (v/v) SDS, (B) 1.0% (v/v) SDS, (C) 5.0% (v/v) SDS, (D) 10.0% (v/v) SDS, using the developed in-capillary strategy with 0.4% (w/v) CTAB.

3.3.2 High SDS concentrations

To test the capacity of the developed in-capillary strategy by CE-MS to remove SDS, the reduced mAb was dissolved in different SDS concentrations from 0.5 to 10% (v/v). Maintaining the optimized conditions for the use of ADBAC and CTAB, longer times with broader peaks and lower intensity for the HC and LC were observed when the SDS percentage was increased (Fig. 2A–D). Also, note that from 5% (v/v) of SDS, the peaks of the mAb were splitted. However, although the MS signal intensity was from 50.6 to 92.5% for LC and from 27.5 to 58.3% for HC, favorable and suitable results to remove high content of SDS were achieved even without adapting the removal parameter. Finally, it is necessary to emphasize that CTAB worked more effective than ADBAC at higher SDS concentrations where ADBAC system was unstable.

3.3.3 SDS-PAGE buffer

The use of MS as a detection system in SDS-PAGE for protein separations is usually associated to tedious and time-consuming sample treatments and, sometimes, it can be not reproducible due to inconsistency in staining/detaining steps used for analyte detection. The in-capillary strategy was tested in samples dissolved in conventional SDS-PAGE buffer containing acrylamide and SDS (10%, v/v). The results showed promising outcomes achieving signal intensity in MS up to 40% using CTAB as cationic surfactant. The lower intensity and SDS removal capacity obtained in this matrix was attributed to the acrylamide presence, which also reduced the lifetime of the PVA capillaries. Therefore, in this case different capillary washing procedures and other in-depth studies would be necessary.

3.3.4 CE-SDS gel buffer

As the CE-SDS approach has arisen as an important alternative to classical SDS-PAGE methods, the application of the developed strategy for the removal of SDS from a CE-SDS sample is of particular interest. In this case, the reduced mAb sample was dissolved both in a commercial gel buffer and in the same gel buffer without SDS. Sharper peaks and lower signal responses were obtained in comparison with those samples dissolved only in 0.2% (v/v) SDS (Fig. 3A in comparison to Fig. 1C and D). This was attributed to additionally interfering substances, which may not be separated completely. MS signal intensity of 39 and 100% were achieved for HC and LC, respectively, referring to the sample diluted in the same gel buffer without SDS. Although the SDS removal efficiency was lower for the HC, MS sensitivities were good. Thus, this approach allows direct CE-MS analysis of fractions from CE-SDS separations without any previous treatment. Recently, we developed a new two-dimensional CE system, which allows peak transfer from a first CE dimension to a second CE dimension [33]. The second dimension could be used for our in capillary SDS-removal approach. That will be part of further investigations.

3.3.5 TAMRA-labeled antibody

Pre-column labeling of mAb with 5-TAMRA.SE has been used as strategy for the quantitative analysis of antibodies, their fragments, and related impurities by CE-SDS with laser-induced fluorescence detection [15, 34]. Thus, the analysis of the TAMRA-labeled mAb containing 0.2% (v/v) SDS was also tested for the in-capillary strategy developed. Using CTAB, 90.7% for HC and 96.2% for LC in MS signal intensity were

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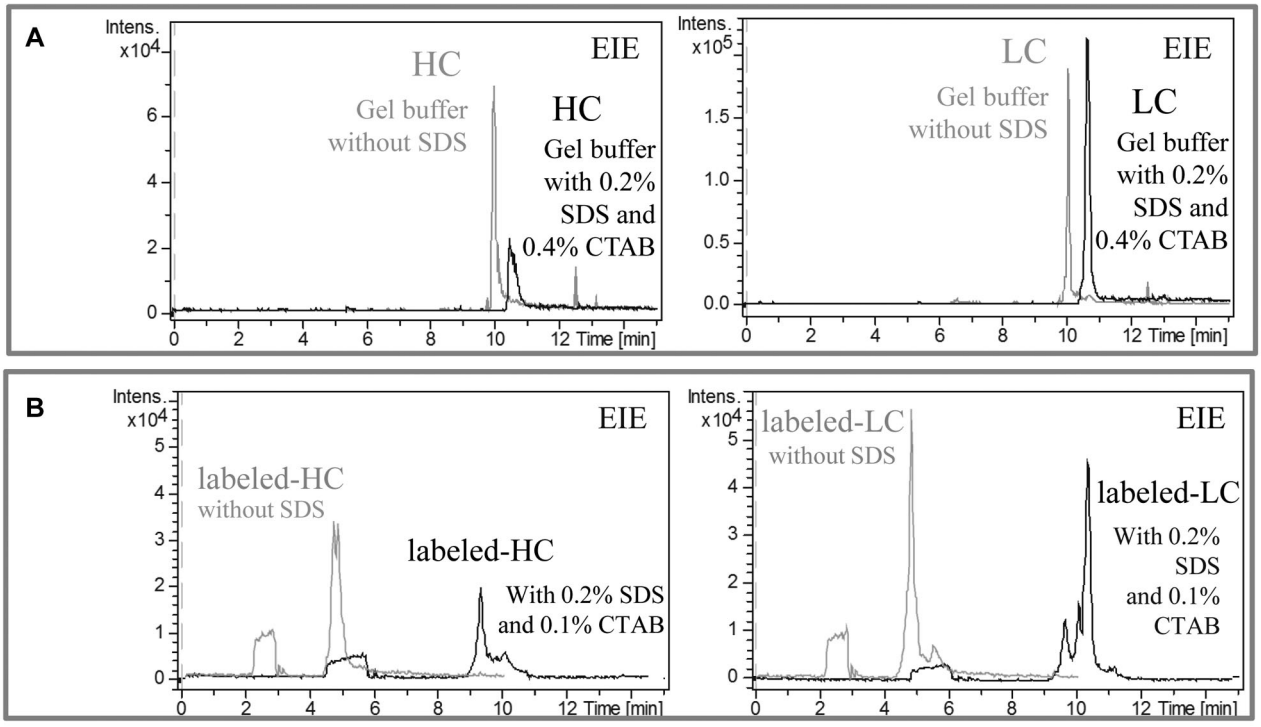


Figure 3. CE-ESI-MS EIEs of HC and LC after in-capillary strategy with cationic surfactant for other applications: (A) gel buffer solution without SDS (in grey) and with 0.2% (v/v) SDS after in-capillary strategy with 5 s pre-plug of MeOH and 10 s post-plug of 0.4% (w/v) CTAB in MeOH:water (50:50, v/v) (in black); (B) TAMRA-labeled antibody without SDS (in grey) and with 0.2% (v/v) SDS using 5 s pre-plug of MeOH and 10 s post-plug of 0.1% (w/v) CTAB (in black).

obtained compared with the analysis of SDS labeled-sample without the strategy. However, poor repeatability was observed. A decrease of in the cationic surfactant content (from 0.4 to 0.1% (w/v)) resulted in the same signal response with better separation system stability. Figure 3B shows the EIEs of HC and LC of TAMRA-labeled antibody with 0.2% (v/v) SDS concentration in the sample solution after in-capillary strategy with 0.1% (w/v) CTAB. Thus, the SDS-removal strategy also works for TAMRA-labeled antibodies.

4 Concluding remarks

A simple and novel CE-MS method for in-capillary removal of SDS from SDS denatured antibody samples that does not require sample pre-treatment has been developed. The strategy is based on the removal of SDS from denatured protein samples by cationic surfactants (CTAB or ADBAC) in the presence of methanol. Under optimized conditions, this strategy has demonstrated simplicity and efficiency in the elimination of SDS from mAb samples. In comparison to non-SDS samples, MS signal recoveries greater than 94% could be achieved. The versatility of the strategy was also demonstrated by assessing other standard proteins and testing different applications based on real problems related with SDS in protein analysis. Typical concentrations of up to 10% (v/v) SDS could be also removed by this approach. Moreover, after sample dilution in CE-SDS gel buffer, MS signal recoveries of 39% and 100% were obtained for the HC and LC, respectively.

This method will allow direct MS analysis of SDS denatured antibody and protein samples from, e.g. CE-SDS peaks without considerable sample pre-treatment. It opens possibilities for online CE-SDS peak characterization by a heart-cut two-dimensional separation (CE-(SDS)-CZE-MS).

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