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Received February 4, 2013
Revised June 12, 2013
Accepted June 12, 2013

Research Article

Static coatings for the analysis of intact monoclonal antibody drugs by capillary zone electrophoresis

This work reports on the investigation of the optimal conditions associated with good isoform resolution, minimized adsorption, and acceptable repeatability for the analysis of intact monoclonal antibodies. To this end, various static capillary coatings were evaluated, and major trends were observed. For a positively charged coating, a low EOF mobility value was the main criterion for satisfactory resolution. For neutral coatings, adsorption was the main concern. If the coating does not fully cover the surface silanols, electrostatic interactions tend to decrease protein recovery through irreversible adsorption. The influence of the BGE pH was also investigated, as the resolution of monoclonal antibody isoforms is driven by their charge difference. The nature and concentration of the BGE were proven to be important, as an ampholytic compound such as ε-aminocaproic acid was found to decrease adsorption compared with ammonium acetate. With the optimal conditions determined during method development, a complete investigation according to four criteria, i.e. migration time, separation efficiency, EOF mobility, and protein recovery, did not present evidence of any adsorption. Finally, the repeatability and intermediate precision were assessed, and low variability was demonstrated with an RSD less than 1.3% for the main peak migration time.

Keywords:
Adsorption / Coating / CZE / Intact protein / Monoclonal antibody
DOI 10.1002/elps.201300070

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1 Introduction

The development of mAb pharmaceuticals has been constantly growing since the approval of Muromonab-CD3 in 1986. These molecules are particularly interesting because they have specific targets and lead to fewer adverse effects than their smaller counterparts [1]. However, because they exhibit high molecular complexity, small changes in production conditions, storage, or exposure to light or chemicals might induce micro-heterogeneity, possibly affecting the antibody charge, size, and/or glycosylation pattern [2]. Common modifications encountered are deamidation, isomerization, aggregation, and the incomplete formation of disulfide bonds [3]. These modifications may alter the therapeutic activity and/or induce adverse side effects. Therefore, a wide range of analytical methods is necessary to evaluate micro-heterogeneity and assess the overall quality of mAb products by comparisons among batches [4, 5].

CE is particularly well suited for mAb analysis due to its high resolution and low sample consumption. Among the various CE modes available, CGE (or CE-SDS) allows the assessment of product size heterogeneity and is one of the most important techniques for mAb characterization in pharmaceutical industry. CIEF is also commonly used within companies to highlight charge heterogeneity [4]. CZE presents a separation selectivity based on both charge and size. It has been frequently used to analyze intact proteins [6, 7] such as insulin [8, 9], hGH [10, 11], or the different glycoforms of EPO [12–14], but mAbs remain relatively unstudied, although glycosylation patterns are often obtained with CZE [15, 16]. To the best of our knowledge, only four publications reported methods for the CZE analysis of intact mAbs. He

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Abbreviations: DS, dextran sulfate; **EACA**, ε-aminocaproic acid; **FS**, fused silica; **HPC**, hydroxypropylcellulose; **MT**, migration time; **PB**, polybrene

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et al. used a bare fused-silica capillary to resolve the patterns of IgG1 and IgG2 with a peak area RSD less than 8% [17]. This method was further modified to include a dynamic coating for comparison with imaging-CIEF (iCIEF). The CZE approach achieved separation within 2–5 min, whereas iCIEF required 6–9 min to obtain a similar profile [18]. More recently, another group developed a CZE method with a higher resolution than the previous work using a dynamic coating composed of polyethylene oxide and triethylenetetramine [19]. In addition, microchip zone electrophoresis enabled very fast separations (80 s) with resolutions suitable for high-throughput profiling during manufacturing or development [20].

A major issue in the analysis of biomolecules using CZE concerns adsorption. Indeed, due to their high molecular complexity, proteins can interact with their environment through various interactions (e.g. ionic and/or hydrophobic), thus leading to their adsorption onto the negatively charged capillary walls of conventional fused-silica capillaries. This phenomenon, extensively reviewed in CE [21, 22], distinguishes between reversible and irreversible adsorption, both of which degrade the separation performance. Reversible adsorption can be monitored by the repeatability of migration times as well as separation efficiency, whereas irreversible adsorption measurements include protein recovery and EOF mobility changes [21]. Adsorption can be minimized through strategies such as appropriate rinsing of the capillary, the choice of BGE, the use of additives, and especially the use of capillary coatings. The coatings can be distinguished according to their fabrication process: dynamic coatings, with the coating agent being part of the BGE, or static coatings, which are prepared before the experiments. Static coatings are made either by physical adsorption with a simple capillary rinsing protocol or by permanent surface modification (chemical reaction or crystallization) [23].

In this work, various static capillary coatings and BGEs were investigated to find the optimal conditions associated with good resolution between isoforms, minimized adsorption, and acceptable repeatability. The strategy for method development was first to find a suitable static coating and then optimize the BGE. To monitor the adsorption of commercial intact mAbs, four criteria, namely separation efficiency, migration time repeatability, EOF change, and protein recovery, were evaluated. Finally, the repeatability and intermediate precision obtained with the selected operating conditions were assessed from the mAb patterns.

2 Materials and methods

2.1 Chemicals and samples

Ortho-phosphoric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, and acetone were of analytical grade from Fluka (Buchs, Switzerland). Acetic acid, ammonia, sodium hydroxide, ε-aminocaproic acid (EACA), and *L*-histidine were obtained from Sigma Aldrich (Buchs, Switzerland). Hydroxypropylcellulose (HPC) was purchased from Acros (Geel, Belgium). Ultrapure water was supplied by a Milli Q purification unit from Millipore (Bedford, USA).

Commercially PVA-coated capillary was from Agilent (Waldbronn, Germany) and FunCap®-type A and -type D from GL Sciences (Torrance, USA). UltraTrol™ LN and HR coating agents were obtained from Target Discovery (Palo Alto, USA). Hexadimethrin bromide (polybrene) (PB) and dextran sulfate (DS) were purchased from Sigma Aldrich (Buchs, Switzerland). Polyethylenimine 50% m/v in isopropanol was from Gelest (Morrisville, USA). All coating protocols are described in Supporting Information.

Monoclonal antibodies infliximab (Remicade, p*I* 9.2 [19]) and rituximab (MabThera, pI 7.3 [19]) were purchased from Merck Sharp & Dohme (Luzern, Switzerland) and Roche (Basel, Switzerland), respectively. Bevacizumab (Avastin) and tocilizumab (Actemra) were purchased from Roche (Basel, Switzerland), cetuximab (Erbitux) from Merck (Darmstadt, Germany). Monoclonal antibodies were first dissolved at 5 mg/mL in 50 mM phosphate buffer pH 7.4 and then further diluted with water to the desired concentration.

All buffers were prepared by dissolving the main component in Milli Q purified water prior to pH adjustment by addition of a titrant. EACA solutions (50–400 mM ε-aminocaproate) were titrated by acetic acid until the desired pH was reached. Ammonium acetate solutions (25 mM acetate) were titrated with ammonia until the desired pH was reached. The pH of the sodium acetate solution (44 mM acetate) was adjusted to pH 4.4 with sodium hydroxide. The phosphate buffer used for the dissolution of mAbs was prepared from ortho-phosphoric acid (50 mM phosphate) and pH was adjusted to pH 7.4 with sodium hydroxide. Finally, the phosphate buffer used for EOF measurements was prepared by titrating a solution of sodium dihydrogen phosphate (25 mM phosphate) with di-sodium hydrogen phosphate (25 mM phosphate) until pH 7.0 is reached.

2.2 CE-UV instrumentation

CE experiments were performed with an HP^{3D}CE system (Agilent, Waldbronn, Germany) equipped with a diode array detector, an autosampler, and a power supply able to deliver up to 30 kV. Fused silica (FS) capillaries (BGB Analytik, Böckten, Switzerland) that were 50 μm id, 64.5 cm in total length, and 56 cm in effective length were used for separations. Samples were hydrodynamically injected for 15 s at 50 mbar (1.8% of the capillary effective length). Prior to each sample injection, the capillary was rinsed at 1 bar for 5 min with fresh BGE. A constant positive voltage of +30 kV for the neutral coatings and a constant negative voltage of –30 kV for the positive coatings, both with a 5000 V/s initial ramp (6 s), were applied during the experiments. The capillary temperature was kept at 25°C, and UV detection was performed at 200 nm.

For the protein recovery experiments, an ActiPix™ D100 UV Area Imaging System (Paraytec, York, UK) was coupled to CE, enabling measurements of the peak area for two

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consecutive detection windows. Homemade HPC-coated capillaries with a 50 μm id had a 115 cm total length, and the two Actipix detection windows were placed 32 and 69 cm from the inlet. Samples were hydrodynamically injected at 50 mbar for 20 s (2.4% and 1.1% of the capillary effective length to the first and second ActiPix windows, respectively). Prior to each sample injection, the capillary was rinsed at 1 bar for 5 min with fresh BGE. UV detection was performed at 200 nm.

2.3 Measurements and calculations

2.3.1 EOF

EOF mobilities were calculated immediately after capillary coating, according to the methodology of Williams and Vigh [24]. Briefly, three acetone 5% v/v zones were injected with 60 s mobilization and 180 s voltage application (+30 kV for neutral coatings and –30 kV for positive coatings). A 25 mM phosphate buffer at pH 7 was used for all EOF experiments, except for the FunCap®-type A, where 44 mM sodium acetate at pH 5.5 was used, as suggested by the manufacturer.

2.3.2 Protein recovery

The procedure proposed by Staub et al. was applied to the protein recovery measurements [25], using the Actipix system. With two consecutive detection windows, this system enables the monitoring of the peak area at each window. The ratio of the peak area at the second window to the peak area at the first window is defined as the protein recovery. This value provides a measure of irreversible adsorption onto the capillary walls and is given as a percentage.

3 Results and discussion

3.1 Method development

First, a simple bare FS capillary was unsuccessfully used to analyze rituximab. No mAb peak was detected with any tested BGE (from pH 2.2 to 6.0), even with the addition of up to 20% organic solvent (ACN, MeOH) to the BGE. As mAbs generally possess high pIs, they are globally positively charged at the pH range investigated (pH < pI), thus showing the need for a capillary coating to avoid the attractive electrostatic interactions between the deprotonated silanols and the mAbs in the pH range of interest. Three types of coatings are available: negatively charged, positively charged, and neutral. Negatively charged coatings were excluded to avoid the attractive electrostatic interactions experienced with the bare FS capillary. The strategy for method development was first to find a suitable static coating and then optimize the BGE. The coatings were evaluated according to different criteria: EOF mobility measurements ($n = 3$), isoform resolution, and adsorption.

Table 1. EOF mobilities measured for positive coatings and neutral coatings ($n = 3$)

Coating name	Surface charge	$\lvert\mu_{EOF}\rvert$ $m^2V^{-1}s^{-1}$ (RSD)
FunCap® type A[a)]	+	$5.3 \cdot 10^{-8}$ (1.5%)
PB-DS-PB	+	$2.9 \cdot 10^{-8}$ (1.9%)
PEI	+	$1.2 \cdot 10^{-8}$ (3.3%)
UltraTrol™ HR	+	$1.0 \cdot 10^{-8}$ (3.1%)
FunCap® type D	Neutral	$8.9 \cdot 10^{-9}$ (4.0%)
UltraTrol™ LN	Neutral	$1.8 \cdot 10^{-9}$ (1.5%)
HPC	Neutral	~0
PVA	Neutral	~0

PEI, polyethylenimine.
BGE: 25 mM phosphate buffer, pH 7.0.
a) 44 mM sodium acetate, pH 5.5.

3.1.1 Positive coatings

With positively charged coatings, both rituximab and the coating present positive charges (at pH < pI), leading to repulsive electrostatic interactions. Four coatings were investigated, and the calculated EOF mobilities are presented in Table 1. All values are in the same order of magnitude with an EOF absolute value five times higher for the FunCap®-type A than for UltraTrol™ HR. As illustrated in Fig. 1A for PB-DS-PB, coatings with the highest absolute EOF values (PB-DS-PB and FunCap®-type A) provided related electropherograms with a single peak, whereas a pattern presenting a main peak

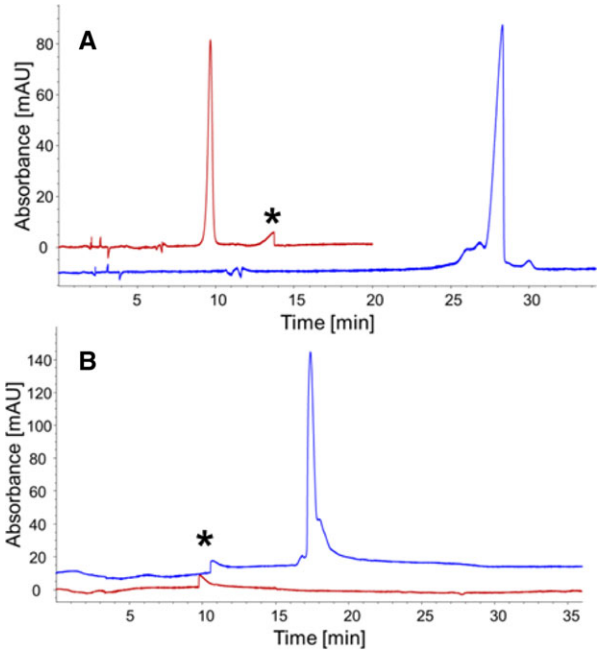


Figure 1. (A) Comparison of positive coatings for rituximab analysis (0.5 mg/mL): PB-DS-PB (upper trace) and UltraTrol™ HR (lower trace). (B) Comparison of neutral coatings for rituximab analysis (0.5 mg/mL): HPC (upper trace) and UltraTrol™ LN (lower trace). Capillary: 50 μm id and 64.5 cm long. BGE: 25 mM ammonium acetate, pH 5.7. * control standard: *L*-histidine.

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and several smaller isoforms was obtained with capillaries experiencing a low absolute EOF value (UltraTrol™ HR). As defined in Eq. (1), the resolution *Rs* is inversely proportional to the EOF mobility, μ_{EOF} [26]:

$$Rs = 0.18 \cdot \Delta\mu_e \cdot \sqrt{\frac{|U|}{D \cdot |\mu_{EOF} + \overline{\mu_e}|}} \quad (1)$$

where $\Delta\mu_e$ is the effective mobility difference between two isoforms, U is the applied voltage, D is the diffusion coefficient, and $\overline{\mu_e}$ is the mean mobility of the isoforms. With a positive coating at pH 5.7, the EOF and mAb effective mobility possess opposite signs. Consequently, the best resolution would be obtained when $|\mu_{EOF}| \approx |\overline{\mu_e}|$. Here, the absolute effective mobility of the rituximab isoforms was very low, close to $1.0 \cdot 10^{-8}$ m^2 $V^{-1}s^{-1}$. The resolution improved as $|\mu_{EOF}|$ decreased but was not lower than $|\overline{\mu_e}|$, thus explaining why UltraTrol™ HR, of the four positive coatings investigated, produced the best results. Polyethylenimine, despite its low EOF value at pH 7.0, did not provide any resolution between isoforms. Unlike the other coatings, its EOF mobility was found to be pH-dependent and had a significantly higher EOF absolute value at pH 5.7. Therefore, the choice of a positive coating was determined by the resolution of the mAb isoform pattern, which was inversely proportional to the EOF mobility. Considering this criterion, UltraTrol™ HR was the most suitable coating for rituximab analysis. However, the coating performance was not repeatable (RSD = 8.9% for five successive recoating steps in the course of mAb analysis) and was eventually discarded. The total analysis time criterion, addressed in [27] for peptide analysis, was not considered as an issue in this study.

3.1.2 Neutral coatings

Neutral coatings were then studied. Because they are supposed to eliminate the EOF, the maximum resolution should be obtained when μ_{EOF} is equal to zero. As the coating is neutral, no electrostatic interactions should theoretically occur between rituximab and the capillary walls. If adsorption takes place, it should proceed through hydrophobic interactions only. However, if the surface charges are not thoroughly covered by the coating agent, electrostatic interactions with the residual silanols may still take place. Table 1 summarizes the EOF mobilities of the four neutral coatings evaluated. For the FunCap®-type D and UltraTrol™ LN coatings, an EOF was measurable, indicating that the capillary wall was not fully covered by the coating. In the case of HPC and PVA, no EOF was observed. Figure 1B illustrates the results obtained for HPC and UltraTrol™ LN. For HPC, a peak with two shoulders was clearly visible, whereas with the UltraTrol™ coating, no peak was detected due to strong irreversible adsorption, like for FunCap®-type D (not shown). The peak shape obtained for PVA (not shown) was similar to that of HPC. In conclusion, as with the positive coatings, the choice of the coating was determined by the EOF value, but for different reasons. For the neutral coatings, a measurable EOF indicates incomplete charge masking, leading to potential adsorption through electrostatic interactions between the negatively charged silanols and the positively charged mAb. The HPC and PVA coatings presented similar properties and isoform resolutions. HPC was selected for further experiments because it is easily homemade with relatively basic materials. Infliximab was then analyzed using the same conditions as rituximab to verify whether the HPC coating was adaptable to analyzing other mAbs. Three isoforms were revealed (Fig. 2C), and due to this feature, the BGE investigation was carried out with infliximab.

To summarize, there are different criteria that need to be considered when choosing an appropriate coating for mAb analysis. Isoform resolution is influenced by both the EOF mobility and adsorption. The resolution is inversely proportional to the EOF mobility, whereas adsorption is a dispersive effect that affects the peaks' width and shape and, consequently, the resolution. For a positive coating and at a pH inferior to the mAb pI, the first criterion is an EOF mobility and mAb effective mobility that must be relatively close to each other to obtain the best isoform resolution and detection. Adsorption should only be evaluated when this criterion is fulfilled. For neutral coatings, the EOF mobility is directly linked to adsorption, and as consequence, resolution. Indeed, if the coating does not cover the surface charges completely, implying an EOF mobility greater than zero, electrostatic interactions will decrease or eliminate protein recovery through adsorption. With both types of coating, repeatability, and cost criteria are investigated once the first criterion is fulfilled.

3.1.3 BGEs

Several BGEs were evaluated in conjunction with the HPC coating for infliximab analysis: acetic acid, ammonium acetate, and EACA. Various pH values between 2.2 and 6.0 were tested, and major tendencies are described in this section. As illustrated in Fig. 2A–C, the pH had a large influence on isoform resolution. With 10% acetic acid (pH 2.2), a single peak with a slight peak tailing indicating little adsorption was observed (Fig. 2A). As mentioned by He et al. [11], the pH has to be chosen to produce the highest charge difference between isoforms to resolve them. By increasing the pH from 2.2 to 4.5 by adding ammonia to the BGE, the resolution was slightly improved with the presence of shoulders, but strong peak tailing appeared due to adsorption (Fig. 2B). A further increase to pH 5.7 enabled the separation of three major peaks, the two peaks on the left corresponding to incomplete posttranslational cleavage of C-terminal lysine [19], but with a strong peak tailing (Fig. 2C). Further increasing the pH did not significantly improve the separation and did not decrease adsorption (not shown).

Changing the buffering species from ammonium acetate (Fig. 2C) to EACA (Fig. 2F) at the same pH and similar ionic strength remarkably improved the isoform resolution and reduced the peak tailing. Isoelectric buffers are well known

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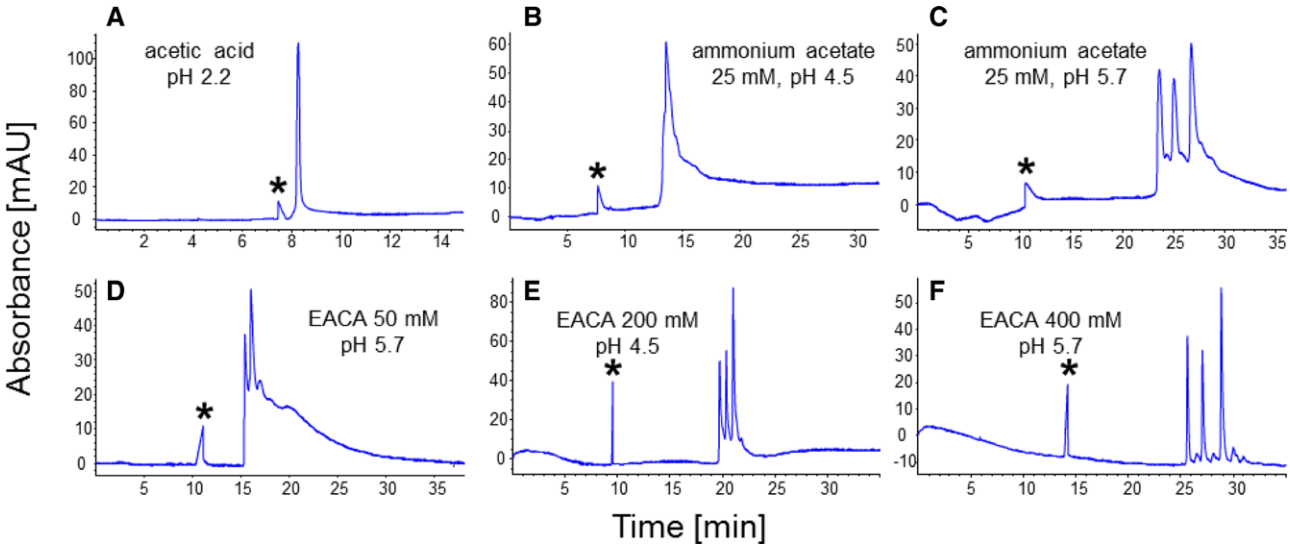


Figure 2. Comparison of various BGEs for infliximab analysis (0.5 mg/mL). (A) 10% acetic acid, pH 2.2, (B) 25 mM ammonium acetate, pH 4.5, (C) 25 mM ammonium acetate, pH 5.7, (D) 50 mM EACA, pH 5.7, (E) 200 mM EACA, pH 4.5, and (F) 400 mM EACA, pH 5.7. Capillary: 50 μm id and 64.5 cm long, HPC coating. *control standard: *L*-histidine.

for their capacity to suppress protein wall interactions and improve glycoform resolution [28]. This result may have occurred because EACA is ampholytic and is mostly in the uncharged form at pH 5.7. Therefore, the EACA concentration can be higher than that of ammonium acetate, which helps minimize adsorption without producing high Joule heating [29]. This concentration effect was confirmed by an experiment using a 50 mM EACA BGE at the same pH (Fig. 2D), which resulted in strongly reduced resolution and increased peak tailing relative to the initial 400 mM BGE. The pH of EACA was also varied from 4.5 to 6.0, and the best separation was obtained at pH 5.7. Lower pH values resulted in reduced resolution (Fig. 2E), and a further pH increase did not significantly improve the method performance (not shown).

3.2 Method performance

3.2.1 Adsorption evaluation

A complete evaluation of adsorption was performed with the conditions selected in the method development section (HPC coating and 400 mM EACA at pH 5.7). As mentioned above, some indicators enable adsorption monitoring. For reversible adsorption, a low separation efficiency and/or peak tailing can be observed as well as a high RSD for migration times. Irreversible adsorption can be evidenced by changes in EOF or by measuring protein recovery using a consecutive double window detection setup.

The two criteria for reversible adsorption were investigated. As presented in Fig. 2F by the infliximab electropherogram for 400 mM EACA at pH 5.7, no significant peak tailing was observed and acceptable separation efficiency ($N > 100{,}000$) was obtained for the main peaks. However, as shown in Table 2, the migration time (MT) repeatability value

Table 2. Migration time repeatability ($n = 5$) for reversible adsorption study

BGE	MT (min)	RSD (%)
Infliximab		
EACA	30.59	7.2
EACA/Tween 20	28.93	0.2
Rituximab		
EACA	18.54	0.3
EACA/Tween 20	18.65	0.4

BGE: 400 mM EACA, pH 5.7.

of 7.2% was not satisfactory ($n = 5$). The MT increased and resolution became worse, whereas the RSD for the control standard was only 1.6%, indicating reversible adsorption that only affected the mAb migration. The addition of Tween 20 into the EACA solution reduced the variability with an RSD decrease to approximately 0.2% and a constant resolution over the set of experiments. Tween 20 is a surfactant capable of reducing nonspecific interactions such as hydrophobic interactions and complete EACA that acts as an electrostatic interaction shield. Nevertheless, each mAb behaved differently, as shown by the MT repeatability for rituximab. In this case, the addition of Tween 20 did not produce a significant improvement. Both RSD values were similar and already acceptable with the original conditions. In conclusion, the addition of Tween 20 to the BGE may improve MT repeatability in some cases.

Irreversible adsorption can be monitored by changes in EOF mobilities. In the case of an HPC-coated capillary, the EOF was not measurable. Consequently, if any change in the EOF occurred, it would directly affect the mAb MT. As shown above, low MT RSDs were obtained for both infliximab and rituximab, indicating that there was probably no irreversible

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Table 3. Protein recovery measured for infliximab with the Actipix system ($n = 5$) for irreversible adsorption study

BGE	Protein recovery (RSD)	
	Without flow	With 40 mbar flow
EACA	94% (3%)	98% (2%)
EACA/Tween 20	100% (2%)	100% (2%)

BGE: 400 mM EACA, pH 5.7.

adsorption with either EACA or EACA with 0.1% Tween 20. To confirm this hypothesis, the protein recovery was also measured using the ActiPix set-up. A decrease in peak area is a sign of irreversible adsorption, since proteins are definitely lost when they adsorb to the capillary walls. Only infliximab was studied because its MT RSD values with and without Tween 20 were different. As the electrophoretic mobility was extremely low at pH 5.7 with an intrinsic long apparent MT (75 min), due to the long capillary (115 cm total length), and large peak widths, a 40 mbar pressure was applied along with the voltage to speed up the analysis time and allow for easier peak integration. The results are presented in Table 3 and show that independently of an applied flow, the protein recovery was close to 100%, confirming that no irreversible adsorption occurred.

3.2.2 Repeatability

With the optimal conditions determined above, the repeatability and intermediate precision were assessed for infliximab and rituximab. Repeatability was calculated for ten consecutive runs with a BGE change after five runs. Intermediate precision was evaluated using three different coated capillaries with ten consecutive runs and a BGE change every five runs. Analysis of variance was used to decompose intra- and intercapillary variability. Migration time and peak area RSDs (for "acidic", main, and "basic" species) are reported in Table 4. MT RSDs for intermediate precision were less than 1.5% for both mAbs, showing good repeatability. For the peak areas, RSDs below 5% were obtained, except for the acidic species of infliximab. The higher variability was explained because acidic species corresponded to less than 10% of the total area and were detected close to their limit of detection. To evaluate the applicability of the method to other mAbs, the MT repeatability ($n = 10$) was assessed for bevacizumab (0.4%), cetuximab (1.8%), and tocilizumab (1.1%). The low RSDs confirmed the conditions chosen during development. In conclusion, the HPC coating was obtained in a repeatable manner and is suitable for mAb analysis with appropriate BGE conditions, namely 400 mM EACA at pH 5.7 containing 0.1% of Tween 20.

4 Concluding remarks

This work concerns the investigation of optimal conditions associated with good resolution, minimized adsorption, and acceptable repeatability for analyzing intact mAbs. To this end, various static capillary coatings were studied, and major trends were observed. For a positive coating and at pH < pI, the first criterion is that the EOF mobility and the mAb effective mobility have to be relatively close to each other to obtain the best isoform resolution. Adsorption should be investigated only if this criterion is fulfilled. For neutral coatings, the EOF mobility is directly linked to adsorption and, as a consequence, resolution. Indeed, if the coating does not cover the surface charges completely, i.e. implying an EOF mobility greater than zero, electrostatic interactions will decrease or eliminate protein recovery through adsorption. The importance of the BGE pH was also demonstrated since the resolution of mAb isoforms was driven by their charge difference, which is optimal at pH 5.7. The nature and concentration of the BGE were proven to be important, and an ampholyte such as EACA was found to decrease adsorption compared with ammonium acetate. A full adsorption investigation according to four criteria did not evidence any adsorption with the optimal conditions determined during method development. Finally, the repeatability and intermediate precision were assessed, and low variability was demonstrated with RSDs lower than 1.3% for main peak MTs. With a view of identifying the various isoforms detected in UV, the coupling of CZE with MS should be considered. Because no bleeding of the static capillary coating used in this study should occur in the ESI source, it could be implemented to decrease mAb adsorption and improve isoforms resolution, while enabling direct CZE-ESI-MS coupling together with the use of a MS-friendly BGE.

Table 4. Repeatability ($n = 10$) and intermediate precision ($n = 30$) for migration time and peak area

	Repeatability (%)	Intermediate precision (%)
Infliximab		
MT	0.66	1.3
Peak area		
"Basic" isoforms	1.2	1.2
Main isoform	1.2	1.2
"Acidic" isoforms	6.8	7.4
Rituximab		
MT	0.50	0.96
Peak area		
"Basic" isoforms	3.6	3.9
Main isoform	0.50	0.61
"Acidic" isoforms	1.8	2.0

BGE: 400 mM EACA, pH 5.7 with 0.1% Tween 20.

The authors wish to thank GL Sciences Inc. for providing FunCap®-type A and -type D capillaries. The authors are grateful to G. Rozing from Agilent Technologies for the loan of the Actipix system. Prof. Goodall and Dr. Chapman from Paraytec are also thanked for technical support and fruitful discussions. Sandrine Fleury from the University Hospitals of Geneva is also

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acknowledged for the kind gift of bevacizumab, cetuximab, and tocilizumab.

The authors have declared no conflict of interest.

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