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Research Article

Rapid analysis of charge variants of monoclonal antibodies with capillary zone electrophoresis in dynamically coated fused-silica capillary

A capillary zone electrophoresis (CZE) method was developed for the rapid analysis of charge heterogeneity of immunoglobulin G (IgG) monoclonal antibodies (mAbs). The separation was carried out in a short, dynamically coated fused-silica capillary. A number of separation parameters were investigated and optimized, including pH, concentration of the separation buffer (ε-amino caproic acid), concentration of the triethylenetetramine (TETA) dynamic coating, the capillary internal diameter and the field strength used for the separation. The effects of between-run flushing of the capillary and the data acquisition rate were also evaluated. Under the optimized conditions, a fast (< 5 min), selective and reproducible separation of mAb charge variants was achieved under a very high electric field strength (1000 V/cm). This method also requires only a short conditioning of the capillary, with between-run conditioning completed within 2 min. The method was evaluated for specificity, sensitivity, linearity, accuracy and precision. The same separation conditions were applied to the rapid separation (2–5 min) of charge variants of multiple monoclonal antibodies with p*I* in the range of 7.0–9.5. Compared with other existing methods for charge variants analysis, this method has several advantages including a short run time, rapid capillary conditioning and simple sample preparation.

Keywords: Charge variants / CZE / mAbs
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1 Introduction

In the past decade, there has been rapid progress in the development of therapeutic proteins produced using recombinant DNA technology. Among them, monoclonal antibodies (mAbs) have become the most rapidly growing class of biotherapeutics in the development for many different disease indications [1–4]. Novel mAb molecules are entering clinical studies at a rate of nearly 40 per year, and the research pipeline includes about 250 therapeutic mAbs in clinical studies [4]. Concomitantly, there is steadily increasing need for an analytical method that can be used for high-throughput analysis of a large number of samples to support bioprocess and formulation development. Like other biotherapeutics, mAbs are complex molecules and a variety of methods are required to monitor the heterogeneities associated with the mAb to ensure product quality and consistency. One measure of product heterogeneity includes quantification of charge variants; these charge variants may be present at the time of manufacture or originate on stability and may include C-terminal lysine variants [5, 6], N-terminal pyroglutamate formation [7], deamidation [8, 9], glycation [10] and glycosylation [11, 12].

One traditional method for charge heterogeneity of proteins is slab gel isoelectric focusing (IEF), introduced by Svensson and Vesterberg in the 1960's [13, 14]. While IEF provides high-resolution separation of very similar charge variants of proteins [13–15], it is a time-consuming and labor-intensive method that often results in high assay variability in quantitation. Capillary isoelectric focusing (CIEF) was developed by Hjerten and Zhu in 1985 [16]. The method involves the use of a coated capillary to eliminate the electroosmotic flow (EOF) and to prevent undesirable adsorption of proteins to active sites on the capillary wall. Direct, on-line UV detection for enhanced assay precision is feasible without the requirement for staining of the focused protein zone. CIEF can be performed automatically, allowing unattended analysis of multiple samples. One limitation of CIEF is that the commonly used two-step separation process (focusing and mobilization) is relatively slow, usually taking about 0.5 to 1 h [17–19]. In recent years, imaged capillary electrophoresis (iCE) has been increasingly

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Abbreviation: **EACA**, ε-amino caproic acid; **HPMC**, hydroxypropyl methyl cellulose; **iCE**, imaged capillary electrophoresis; **IgG**, immunoglobulin G; **TETA**, triethylenetetramine

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used to monitor charge heterogeneity of therapeutic mAbs [20, 21]. Similar to CIEF, iCE requires the use of a coated capillary to reduce both protein adsorption and EOF to prevent the mobilization of bulk solution during the period of focusing. Though it has drastically reduced the separation time (usually about 6–9 min), iCE requires approximately 7–10 min for column flushing time, sample transfer and equilibration. Sample preparation for iCE separation also requires multiple dilution and mixing steps. Both CIEF and iCE utilize analyte detection at 280 nm due to the high-background absorption of ampholytes at 214 nm. Detection at 280 nm may cause over- or underestimation of charge variants, since charge variants may have different UV absorption at 280 nm. The use of capillary zone electrophoresis (CZE) as a tool for the analysis of charge heterogeneity of therapeutic mAbs was investigated by several groups [22, 23]. Detection at universal wavelength of 200–220 nm was used. The separation was mostly performed in permanently coated capillary to minimize protein adsorption to the inner wall of the capillary.

Recently, we have demonstrated the analysis of charge variants of immunoglobulin G (IgG) mAbs by CZE in an uncoated capillary [24]. The use of uncoated capillaries provides a simple, low-cost option for CZE. However, the time (ca. 30 min) required for separation and capillary conditioning was relatively long, which may limit its application to high-throughput analyses. In this work, we systematically investigated and optimized a number of parameters to improve separation and significantly reduce the separation time and conditioning time. The method was applied to charge variants analysis of multiple humanized IgG mAbs and high-throughput analysis of stability samples.

2 Theory

A number of parameters were tested and optimized with a goal of improving separation resolution and reducing the separation time. The following equations were used to guide the method development.

Separation resolution (R_s) is regulated by the equation [25]:

$$R_s = 0.25\,N^{1/2}\frac{\mu_1-\mu_2}{\bar{\mu}_{ep}+\mu_{eof}} = 0.25\,N^{1/2}\frac{\Delta\mu}{\bar{\mu}_{ep}+\mu_{eof}} \quad (1)$$

where N is the number of theoretical plates, μ_1 is the electrophoretic mobility of the analyte one, μ_2 is the electrophoretic mobility of the analyte two, $\Delta\mu$ is the mobility difference of the two analytes, $\bar{\mu}_{ep}$ is the average mobility of the two analytes, and μ_{eof} is the electroosmotic mobility of bulk solution. If EOF is entirely suppressed and μ_{eof} is much smaller than $\bar{\mu}_{ep}$, then Eq. (1) gives

$$R_s \approx 0.25\,N^{1/2}\frac{\Delta\mu}{\bar{\mu}_{ep}} \quad (2)$$

The mobility of an analyte is given by [26, 27]:

$$\mu_{ep} = \frac{kq}{M_r^{2/3}} \quad (3)$$

where k is an empirical constant, q is effective charge and M_r is relative molecular mass. We assume charge variants of IgG mAb have similar M_r. Hence, a combination of Eqs. (2) and (3) gives:

$$R_s = 0.25\,N^{1/2}\frac{\Delta\mu}{\bar{\mu}_{ep}} \approx 0.25\,N^{1/2}\frac{\Delta q}{\bar{q}} \quad (4)$$

Accordingly, to improve separation resolution of charge variants with similar size, N and $\Delta q/\bar{q}$ (relative charge difference between charge variants) need to be optimized.

In addition, optimum migration time (t_{opt}) is governed by the equation [28]:

$$t_{opt} = \frac{1}{2.3^{1/5}}\left(\frac{1}{D}\right)^{1/5}\left(\frac{B\kappa}{\lambda}\right)^{2/5}\left(\frac{R}{\mu T_0}\right)^{4/5}L^{6/5} \quad (5)$$

where D is the diffusion coefficient, B a constant, κ the electrical conductivity of the buffer, λ the thermal conductivity of the buffer, μ the electrophoretic mobility of the analyte, R and L the radius and effective length of the capillary, respectively. To get a short optimum separation time, electric conductivity κ, capillary length L, and diameter R should be as low as possible.

3 Materials and methods

3.1 Chemicals

ε-Amino-caproic acid (EACA) was purchased from MP Biomedical (Solon, OH, USA). Acetic acid, hydroxypropyl methyl cellulose (HPMC), and triethylenetetramine (TETA) were purchased from Sigma-Aldrich (Saint Louis, MO, USA), 0.1 M HCl was purchased from Beckman Coulter (Fullerton, CA, USA). Commercially marketed humanized mAbs were purchased from Amerisourcebergen (Chesterbrook, PA, USA). Other mAbs were produced in-house (Pfizer).

3.2 Instruments

CZE analysis was performed with a Beckman Coulter (Brea, CA, USA) CE-MDQ instrument equipped with a UV detector. Bare fused-silica capillaries of 370 μm od 50 and 40 μm id were obtained from Polymicro Technologies (Phoenix, AZ, USA). iCE analysis was carried out on iCE 280 analyzer (Convergent Bioscience, Toronto, Canada) with a whole column imaging system.

3.3 Procedures

The optimal CZE separation was performed with a bare fused-silica capillary (total length of 30 cm, effective length

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of 10 cm, id of 40 μm) at 25°C. The running buffer for CZE contained 400 mM EACA–acetic acid, 0.05% m/v HPMC, 2 mM TETA, pH 5.7. Samples were diluted with deionized water to 1 mg/mL, kept at a temperature of 15°C and injected at 2.1 Pa for 5 s. The separation voltage was 30 kV and detection was by UV at 214 nm. Data acquisition rate was set at 8 Hz. New capillaries were pretreated with 0.1 M HCl at 413 Pa for 5 min, separation buffer at 413 Pa for 10 min, followed by the application of 30 kV for 10 min. Between injections, the capillary was flushed with 0.1 M HCl at 413 Pa for 1 min followed by separation buffer at 413 Pa for 1 min. The capillary was filled with 10 mM H_3PO_4 and two ends of the capillary were immersed in water for overnight and long-term storage.

The separation cartridge for iCE analysis contains a 5 cm long, 100 μm id × 200 μm od separation capillary that has its inner wall pre-coated with fluorocarbon. The anolyte was 80 mM phosphoric acid and the catholyte 0.1 M NaOH. IgG samples were diluted to 2 mg/mL with deionized water. The working sample mixture was prepared by mixing 30 μL of 2 mg/mL IgG, 1 μL of 5.3 p*I* marker, 1 μL of 9.5 p*I* marker, 70 μL of 1% m/v methylcellulose and 8 μL of pharmalytes (pH 3–10), and 90 μL deionized water. The mixture was mixed vigorously by vortexing, and centrifuged for 2 min to remove bubbles. The prepared sample mixture was introduced to the capillary by an autosampler and focused and separated under the following conditions: pre-focusing for 1 min at 1500 V, focusing for 5–8 min at 3000 V. The focused proteins were detected by a whole-column imaging detector monitoring absorbance at 280 nm.

Data were collected and analyzed utilizing the 32 Karat software, Waters Empower Chromatography data collection system and the iCE 280 software. Protein Calculator version 3.3 from Scripps Research Institute (CA, USA) was used to calculate the charge and p*I* of mAbs.

4 Results and discussion

4.1 Optimization of electrophoretic parameters

Development of the CZE method for rapid separation and detection of charge variants required the selection and optimization of the following parameters: (i) pH of EACA buffer; (ii) concentration of EACA and TETA; (iii) capillary internal diameter (id) and field strength; (iv) sampling rate and (v) flushing and equilibration conditions.

Separation of proteins by CZE is strongly affected by the pH of background electrolytes. As charge variants of IgG mAbs have similar Stokes' radii but different acid–base dissociation constants, the buffer pH has to be chosen such that [29–31] the analytes exhibit significant relative difference in charge ($\Delta q/\bar{q}_{ep}$ in Eq. 4), maximizing the relative difference in electrophoretic mobility ($\Delta\mu/\bar{\mu}_{ep}$ in Eq. 2). The main isoform of the model mAb used in this work has p*I* of 7.3. Modifications such as deamidation and sialylation would introduce additional negative charges to the molecule and cause a decrease in p*I*; these peaks are labeled as acidic species. Conversely, modifications such as C-terminal unprocessed lysine variants or cyclic imide formation would introduce additional positive charge to the molecule and cause an increase in p*I*; these peaks are labeled as basic species. For simplicity in modeling the separation, it will be assumed that acidic species only arise from deamidation of an asparagine (N) residue to generate aspartic acid (D) residue [8, 9], and that basic species are only due to the addition of a lysine (K) to the heavy chain C-terminus [5, 6]. Figure 1 shows the change in calculated charge of basic, acid and main species with pH in the range of 4.5–9.5, and Fig. 2 shows the change in relative charge difference of charge variants with pH in the range of 4.0–7.3. Protein Calculator was used to calculate the p*I* and charge at different pH conditions. The calculation is based on the amino acid sequence of the mAb (not shown). Sequences of two heavy chains and two light chains of the native IgG were entered as one sequence to Protein Calculator to generate plot of charge versus pH of main species. In addition, sequences modified with a C-terminal lysine variation or

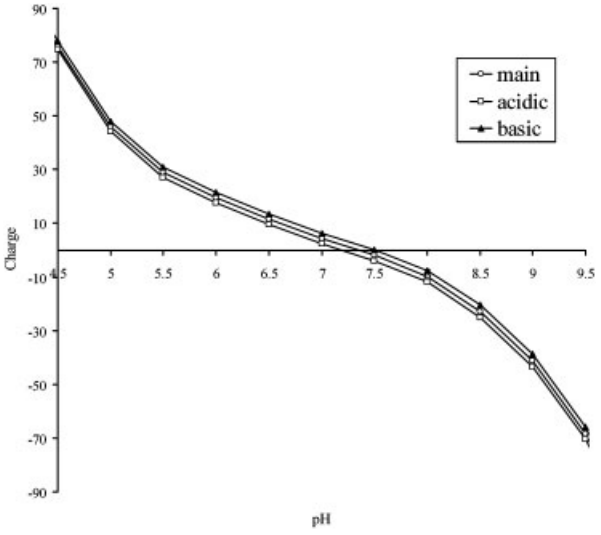


Figure 1. Calculated charge versus pH profile.

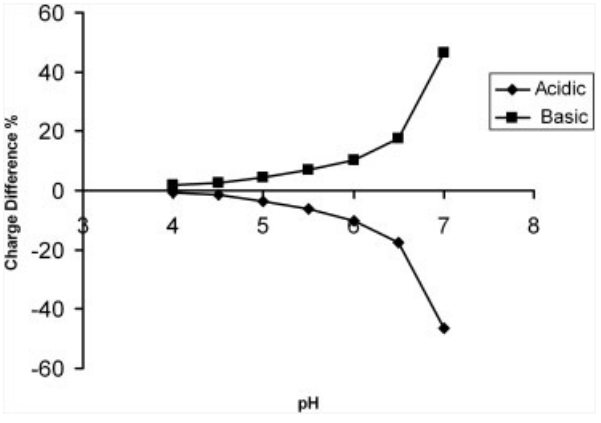


Figure 2. Calculated relative charge difference versus pH profile.

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deamidation of an asparagine were entered to generate plots of charge versus pH of basic and acidic species respectively. The p*I* and charge estimate assumes all residues have pK_a values that are equivalent to the isolated residues. The calculated p*I* for this mAb of 7.3 is comparable to the experimental p*I* of 7.3 as measured by iCE. As shown in Fig. 1, all charge variants have a similar charge profile in the entire pH range. However, the total charge of all charge variants decreases quickly whereas the relative charge differences among charge variants increases significantly when the pH increases from 4.0 to 7.3. Hence, it is expected that the separation of charge variants should increase when pH increases from 4.0 to 7.3. However, in general, the pH should not be too close to the p*I* of the proteins since the solubility of proteins may be limited close to their p*I*. Furthermore, the tendency of proteins to adsorb to the capillary wall is most pronounced close to their p*I* [29], which will lead to an increase in peak broadening and a decrease in separation efficiency (*N* in Eq. 4). In addition, Figs. 1 and 2 show that the relative charge difference of the charge variants (e.g. 4% relative charge difference between basic and main species at pH 5.7) is much higher than the relative difference of M_r of the charge variants (e.g. ca. 0.1% relative difference of M_r between basic and main species). This means that separation of the charge variants in the tested pH range is predominantly based on their charge difference.

With the concentration of EACA buffer fixed at 400 mM, the effect of pH on the separation was evaluated in the range of 4.7–6.0 (Fig. 3). Increasing pH from 4.7 to 5.2 resulted in a significant improvement in the separation of both acidic and basic species with a slight increase in migration time (Fig. 3A and B). Further increase in pH from 5.2 to 5.7 resulted in additional improvement in separation of acidic and main species, but deteriorated the separation of basic and main species (Fig. 3B and C). Moreover, peaks become broadened, especially for the more basic species. Peak broadening became more significant at pH 6.0, bringing about a total loss in separation of basic species (Fig. 3D). The loss of efficiency and resolution observed from pH 5.7 to 6.0 is likely due to increased adsorption at the pH closer to the mAb's p*I*. The increased adsorption of protein brought about more peak broadening and significant reduction of separation efficiency (*N* in Eq. 4).

Two approaches were examined to eliminate the adsorption of protein at high pH to improve the separation efficiency (*N* in Eq. 4). First, the use of more concentrated EACA buffer was evaluated. As shown in Fig. 4, the separation of charge variants was continuously improved when the concentration of EACA buffer increased from 300 to 600 mM. The improvement in the separation of basic species was more obvious than that of acidic species. Next, the addition of triethylenetetraamine (TETA) to the separation buffer to form a dynamic coating on the capillary inner wall was investigated. TETA has been demonstrated to be useful in improving the peak shape in peptide mapping with CZE in uncoated capillary [32]. It was observed that 2 mM TETA was sufficient to reduce the adsorption of basic species of the mAb on the capillary inner wall, resulting in significant improvement of peak shape (e.g. Peak 1 in Fig. 5) and resolution. The beneficial effect of TETA on reducing the adsorption of the protein was more clearly illustrated in separation at higher pH 6.0 (Fig. 6). The use of low level of TETA was preferred over the large increase in EACA concentration as it caused little increase in conductivity of the separation buffer (κ in Eq. 5) and allowed for the use of higher field strength for rapid analysis.

In our previous work [24], a 50 μm id capillary and electric field strength of 480 V/cm were used for separation of IgG charge variants. The separation time was about 12 min. In order to reduce separation time, 1000 V/cm electric field strength was tested. The use of 1000 V/cm electric field strength along the 50 μm id capillary caused a decrease in separation resolution due to the increased peak

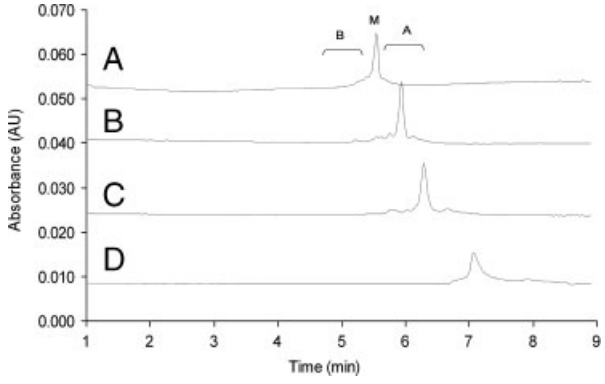


Figure 3. CZE of mAb charge variants in EACA buffer at different pH. Conditions: bare fused-silica capillary: total length 30 cm, effective length 10 cm, id 40 μm; capillary temperature, 25°C; voltage, 20 kV; injection, 2.1 Pa × 5 s; detection, 214 nm; separation buffer: 400 mM EACA–acetic acid, 0.05% m/v HPMC; pH (A) 4.7, (B) 5.2, (C) 5.7, (D) 6.0; Protein concentration, 1 mg/mL; Peak identity: A, acidic; M, main; B, basic.

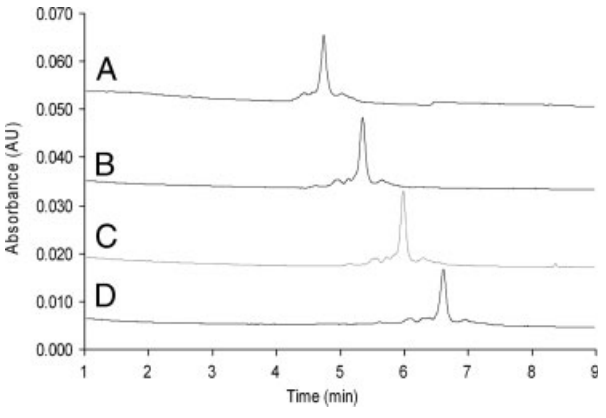


Figure 4. CZE of mAb charge variants in EACA buffer at different concentrations. Separation buffer: EACA concentration (A) 300 mM, (B) 400 mM, (C) 500 mM, (D) 600 mM, pH 5.7, 0.05% m/v HPMC. For other experimental conditions, see Fig. 3.

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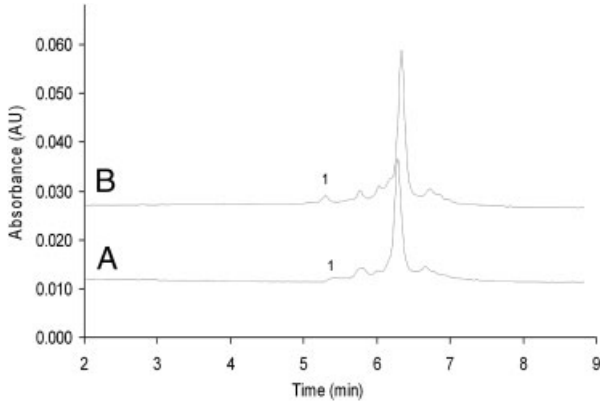


Figure 5. Effect of TETA concentration on CZE separation of the mAb charge variants at pH 5.7. Separation buffer: 400 mM EACA–acetic acid, 0.05% m/v HPMC, TETA concentration (A) 0, (B) 2 mM. For other experimental conditions, see Fig. 3.

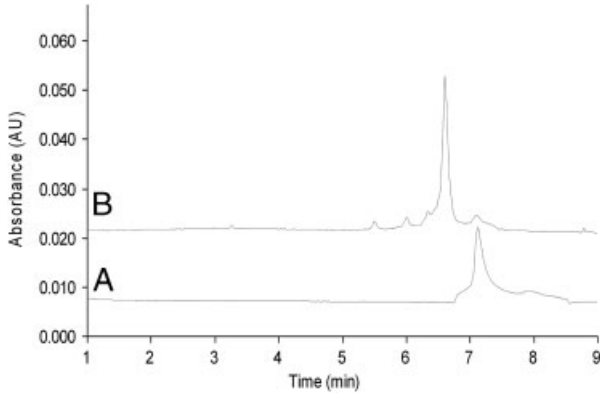


Figure 6. Effect of TETA concentration on CZE separation at pH 6.0. Separation buffer: 400 mM EACA–acetic acid, 0.05% m/v HPMC, TETA concentration (A) 0, (B) 2 mM. Other experimental conditions are the same as in Fig. 3.

broadening caused by higher current induced joule heating. Equation (5) shows that the use of narrower capillary is helpful to enhance heat dissipation and increase separation speed. The separation with 40 and 50 μm id capillaries under an electric field strength of 1000 V/cm was compared. The separation with 40 μm id capillary was improved compared to that of the 50 μm id capillary. The baseline was also smoother with the capillary with 40 μm id, which might be related to the lower and more stable current profile. Interestingly, the peak height obtained with 40 μm id capillary was comparable to that seen with 50 μm id capillary, which was largely due to the narrower peak width with 40 μm id capillary. It should be noted that 1000 V/cm electric field strength was achieved by applying 30 kV across 30-cm-long capillary. Using the existing commercial CE instruments, 30 cm is the minimal length of capillary that can be used and 30 kV is the maximum voltage that can be generated. It is expected that the separation speed should be further enhanced by using ultrahigh voltage CE developed by Hutterer and Jorgenson [25]. This will be investigated in the near future.

As the peak width is very narrow under high field strength, a high data acquisition rate is needed to avoid peak deformation and loss of resolution. The peak width of the main species was about 7 s. Sampling rate in the range of 1–8 Hz was examined. The peak was obviously deformed and separation was totally lost when 1 Hz sampling rate was used. Increase in sampling rate from 1 to 8 Hz led to continuous improvement of separation resolution. With a sample rate at 8 Hz, the best separation was achieved with only a slight increase in baseline noise. Hence, 8 Hz was selected as the optimal balance between observed resolution and detection sensitivity.

The overall cycle time of sample analysis is dependent on not only separation time, but also time for between-run capillary conditioning. Adequate between-run conditioning is essential for reproducible separation from injection to injection. In our previous work [24], the capillary was cleaned with 0.1 M HCl for 5 min, and then conditioned by separation buffer for additional 10 min prior to the injection of the next sample. A number of combinations of HCl cleaning and buffer conditioning were evaluated to reduce the between-run conditioning time. To our surprise, 1 min cleaning with 0.1 M HCl followed by 1 min conditioning with separation buffer was found to be sufficient to generate reproducible separation from injection to injection. Excellent repeatability ($n = 6$) in separation of sample (1.0 mg/mL) was attained with RSD of migration time less than 0.1%. It should be noted that the observation was opposite to most of the existing reports on CZE separation in the pH range of 4–8 [28, 32, 33]. It has been suggested that separation at intermediate pH took a long time for conditioning and equilibration due to the pH hysteresis effect and slow ionization of silanol groups on capillary inner surface [33]. Fresh capillaries are often pretreated with concentrated sodium hydroxide to fully ionize the capillary inner surface [28, 33, 34]. The capillaries used here were only treated by hydrochloric acid, but never treated with sodium hydroxide. Hence, the number of residual ionized silanol should be low and could be quickly titrated by the concentrated EACA buffer and diluted TETA. In fact, the injected neutral EOF marker was not detected within 1 h, indicating that the EOF was extremely slow (data not shown).

4.2 Method validation

The performance of the optimized method was evaluated in terms of specificity, linearity, LOQ, accuracy and precision. The specificity of the method was examined for the potential interference from any components (polysorbate 80, EDTA, acetate and trehalose) in the sample matrix. The sample matrix did not show interfering peaks (data not shown).

The linearity was assessed by varying the protein concentration from 0.5 to 2.5 mg/mL. The absolute areas of acidic, main and basic peaks were plotted against the total

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concentration of protein. The linear regression equations for acidic, main and basic species were $y = 2111.5x - 284.8$, $y = 13\,891x + 256.2$ and $y = 1904.7x - 234.9$, respectively. The correlation coefficients for acidic, main and basic were 0.979, 0.993 and 0.984, respectively. The LOQ was established as the minimum concentration at which the main species can be reliably measured with signal-to-noise ratio of 10:1. The LOQ was determined to be 20 μg/mL, which corresponds to 2% of the total concentration of protein at 1 mg/mL.

Due to the complex heterogeneity of mAbs and the unavailability of purified impurities for spiking recovery, accuracy was studied by sample dilution. Samples were serially diluted to obtain final concentration representing 50–150% of target protein concentration. These samples were injected in triplicate. The mean recovery was calculated by comparing the mean experimental peak area to the theoretical value. As shown in Table 1, the mean recoveries ranged from 99 to 117% for basic, main and acidic species.

Intermediate precision was assessed by two analysts. Separation buffer was prepared independently by each analyst. Six individual preparations of samples (1.0 mg/mL) were analyzed using one injection per sample on each day. The precision of migration time and area percent are shown in Table 2. The RSD values were in the range of 0.14–0.19% and 2.4–7.3% for migration time and area percent, respectively.

4.3 Method applications to stability sample and multiple mAbs

The ability of the method to monitor changes in sample during storage under different conditions was evaluated.

Table 1. Recovery by sample dilution

mAb Con (mg/mL)	Mean recovery% ($n = 3$)		
	Basic	Main	Acidic
0.5	101.9	114.1	117.5
1 (target)	100.0	100.0	100.0
1.5	103.0	99.3	110.5

Table 2. Precision of migration time and area percent of charge variants ($n = 12$)

	Basic	Main	Acidic
Migration time (min)			
Mean	3.50	3.80	4.07
RSD%	0.14	0.19	0.18
Area%			
Mean	20.8	67.6	11.6
RSD%	7.3	2.4	3.4

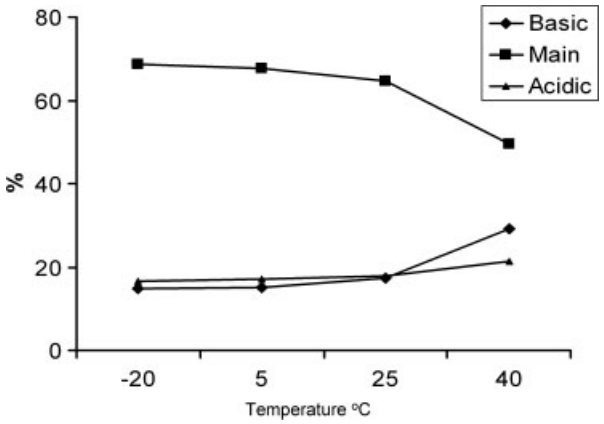


Figure 7. IgG mAb stability during storage at different temperatures.

The mAb was stored at different temperatures for 4 wk. As shown in Fig. 7, the main species decreased slightly when temperature increased from −20 to 25°C, but dropped significantly when temperature changed from 25 to 40°C. Both acidic and basic species increased with temperature supporting that this method is useful for stability indicating analysis of mAbs. The increase in acidic species is consistent with protein deamidation, whereas increase in basic species is likely due to the formation of cyclic imide. These were further demonstrated by HPLC/MS analysis of tryptic digest of stability samples (data not shown).

In order to demonstrate the general applicability of the method to different mAbs, the method was tested in the analysis of about ten different therapeutic mAb candidates and compared with standard iCE method. The p*I* values of these mAbs are in the range of 7.0–9.5. Figure 8 shows the head-to-head comparison of CZE and iCE separations of five mAbs. As can be seen, the separation profile of CZE was similar to that of iCE. Overall, the iCE method resulted in a better separation of acidic species whereas the CZE method resulted in a better separation of basic species. The rapid separation by CZE was achieved within 2–5 min, shorter than the separation (6–9 min) by iCE. The more basic mAb (higher p*I* in iCE) had a shorter separation time by CZE, indicating that the separation of IgG charge variants is predominantly based on their charge difference. In addition, CZE required only 2 min between-run conditioning, compared with 7–10 min for iCE.

5 Concluding remarks

Major parameters and operation conditions of the CZE method have been tested and optimized for the rapid analysis of charge variants for IgG mAb. Quick separation was complete within 3–5 min, and between-run equilibration done within 2 min. The pH of the buffer had a major influence on separation selectivity. The use of TETA was helpful in reducing protein adsorption, and the combination

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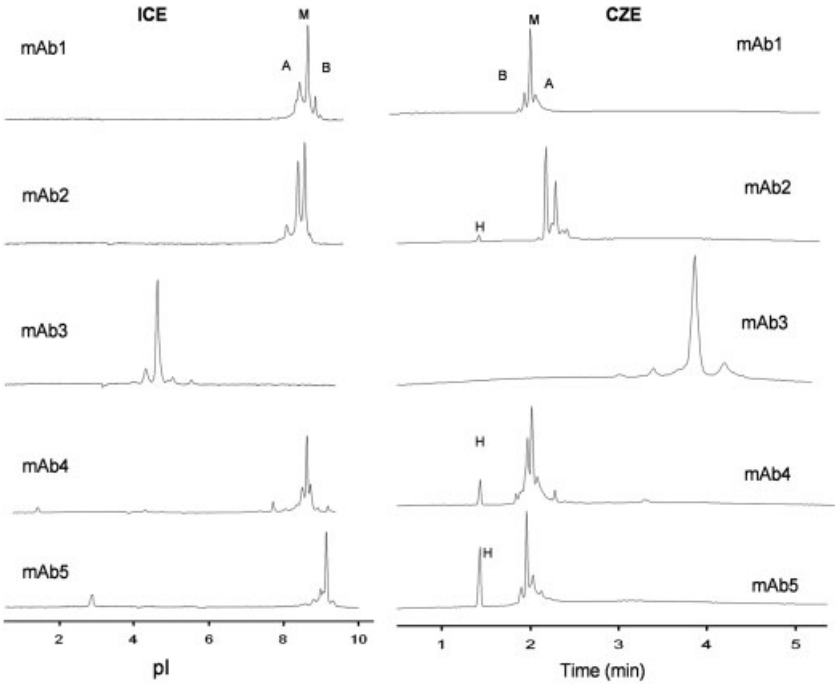


Figure 8. CZE versus iCE separation of charge variants of multiple mAbs. A, Acidic; M, main; B, basic; H, histidine in sample matrix. For iCE and CZE conditions, see Section 3.3.

of high field strength and a narrower capillary resulted in a rapid separation. The method was qualified for specificity, accuracy and sensitivity, and showed linearity of the response and data repeatability. Analysis of stability samples showed the method's suitability as a stability indicating assay. These CZE method conditions were also demonstrated to be generally applicable for the rapid analysis of charge variants of multiple different mAbs.

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The authors have declared no conflict of interest.

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