



# Effect of the operational parameters on the electromigration of proteins in sodium dodecyl sulfate capillary gel electrophoresis in the presence of propidium iodide fluorescent dye

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## ABSTRACT

Sodium dodecyl sulfate capillary gel electrophoresis (SDS-CGE) is a frequently used analytical technique in size-based separation of proteins, playing a vital role in the biopharmaceutical industry for the analysis and characterization of therapeutic proteins, employing both UV and fluorescent detection. Understanding the effect of the operational parameters using easily applicable *in migratio* fluorescent labeling is increasingly critical, especially because multicapillary electrophoresis systems with fluorescent detection have recently gained prominence in high-throughput biopolymer analysis. In this study, the effects of the three most important user-adjustable operational parameters (temperature, gel concentration, and electric field strength) were investigated on the electrophoretic mobility and resolution of SDS-protein complexes in the presence of propidium iodide in the gel-buffer system. In the separation temperature study, the Arrhenius equation was applied to calculate the required activation energy for the electromigration through the propidium iodide-containing separation matrix for the sample proteins. With increasing gel concentration, the presence of the fluorophore in the gel-buffer system resulted in linear Ferguson plots, suggesting more predictable and consistent sieving behavior, in contrast to the non-linear plots observed earlier without the addition of the fluorescent dye. Finally, increase in the applied electric field resulted in elevated electrophoretic mobilities, both in the presence and absence of propidium ions in the sieving matrix. The resolution between the consecutively migrating SDS-protein complexes, on the other hand, decreased above 500 V/cm, probably due to conformation changes caused by the high field strengths. Our results underline the importance of optimizing these key parameters in SDS-CGE with propidium-mediated LIF detection to obtain rapid and high-resolution separation of complex protein samples including biopharmaceuticals.

## 1. Introduction

Capillary gel electrophoresis (CGE) of biologically important macromolecules such as DNA, RNA, and proteins is a powerful separation technique that is widely used in the pharmaceutical industry, molecular biology laboratories, and other fields of bioanalytical importance [1]. CGE offers distinct advantages over traditional slab gel electrophoresis, including automation, online detection, higher resolution, faster analysis times, and lower sample/buffer consumption. Sodium dodecyl

sulfate capillary gel electrophoresis (SDS-CGE) is a special subset of capillary gel electrophoresis, developed to separate proteins based on their size [2]. In SDS-CGE, the proteins are covered by sodium dodecyl sulfate at 1.4 g surfactant/1 g protein ratio via hydrophobic interactions. Thus, most SDS-protein complexes have very similar hydrodynamic volume/charge ratios requiring a sieving polymer to enable their size-based separation [3]. Please note that this ratio is not valid for some molecules, e.g., glycoproteins. In case of monoclonal antibodies it only affects the HC fragment as in the intact form the sugar moieties are

**Abbreviations:** CGE, capillary gel electrophoresis; SDS, sodium dodecyl sulfate; mAb, intact monoclonal antibody; LC, light chain; ngHC, non-glycosylated heavy chain; HC, heavy chain; PI, propidium iodide.

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facing inward, i.e., not affecting SDS binding [4].

SDS-CGE is particularly important to analyze therapeutic proteins throughout the biopharmaceutical product development workflow, encompassing such crucial tasks as initial characterization, stability testing, and lot release [5]. As the field of process analytical technology applied to biopharmaceuticals continues to expand, the importance of high throughput SDS-CGE applications is thriving [6]. Multicapillary electrophoresis systems coupled with laser-induced fluorescence detectors offer high throughput separation capabilities to simultaneously analyze a large number of therapeutic proteins with excellent detection sensitivity [7,8]. Therefore, simple fluorescent staining approaches during the separation process are gaining increasing attention [9,10]. One of the important developments is the introduction of phenanthridin-type dyes [11], which have been predominantly recognized for their excellent DNA and RNA staining capabilities, but also ventured beyond their original scope and found some post-task utilization in SDS-PAGE [12]. These positively charged fluorophores, if part of the background electrolyte, are counter-currently migrating against the negatively charged biopolymers (nucleic acids and SDS-proteins) during the separation process, supporting instant *in migratio* labeling upon their encounter within the capillary column. The use of propidium iodide (PI) in SDS-CGE represents a significant forward step in protein analysis, enabling fluorescence detection without the time-consuming and labor-intensive pre- or post-column derivatization steps. This novel *in situ* staining approach not only saves time but also enhances the efficiency of protein separation, particularly for complex biopharmaceutical samples like protein therapeutics [10].

The electromigration properties of proteins in SDS-CGE are influenced by several user-adjustable operational parameters such as temperature, gel concentration, and applied electric field strength [13]. Corresponding theoretical considerations have respectively addressed the i) effects of temperature by the activation energy concept [14,15], ii) gel concentration utilizing the Ferguson equation [3,16], and iii) the electric field, possibly altering the conformation of the electromigrating charged polyions [17]. The activation energy, in this case, represents the energy required for the solute molecules to transit the barriers represented by the sieving matrix. In other words, each sample component has a distinct activation energy requirement to migrate through the reticulations. The gel concentration, on the other hand, defines the sieving capability, which is especially important in the case of the generally used transiently cross-linked dextran matrices in SDS-CGE. Finally, the high electric field may change the conformation (e.g., by orientation or stretching) of the electromigrating biopolymers as was discussed earlier in the case of dsDNA analysis by slab [17] and capillary [18] gel electrophoresis.

In this study, the impact of three important operational parameters: i) capillary temperature, ii) gel concentration, and iii) electric field strength are thoroughly investigated concerning the electromigration properties of SDS-protein complexes in the presence of the positively charged fluorophore ligand of propidium iodide in the gel-buffer system. Understanding the effects of these parameters is crucial during the optimization of the separation conditions to enhance resolution and shorten the analysis time for the sample components of interest.

## 2. Materials and methods

### 2.1. Chemicals

Boric acid, Tris, EDTA.Na<sub>2</sub>, glycerol, sodium dodecyl sulfate (SDS), 2-mercaptoethanol, hydrochloric acid, and sodium hydroxide were obtained from VWR (Radnor, PA, USA). Propidium iodide was from Thermo Fisher (Waltham, MA, USA). Iodoacetamide, lysozyme, and the 1.8–2.5 MDa molecular mass dextran polymer were from Sigma-Aldrich (St. Louis, MO, USA). Daratumumab (marketed as Darzalex®), a targeted therapeutic monoclonal antibody to treat myeloma multiplex, was sourced from Janssen Biotech (Horsham, PA, USA). The PNGase F

enzyme used in the study was procured in-house as described in Ref. [19].

### 2.2. Sample and gel-buffer preparation

Preparation of the non-reduced (intact) monoclonal antibody (mAb) sample: 5  $\mu$ L of a 20 mg/mL solution of therapeutic monoclonal antibody was mixed with 5  $\mu$ L of 250 mM iodoacetamide, and 85  $\mu$ L sample buffer containing 100 mM TRIS-HCl and 1 % (w/v) SDS (pH 9). Preparation of the reduced mAb sample: 5  $\mu$ L of a 20 mg/mL solution of the therapeutic monoclonal antibody was mixed with 5  $\mu$ L of 10 mg/mL non-glycosylated lysozyme (internal standard), 5  $\mu$ L of 2-mercaptoethanol and 80  $\mu$ L of sample buffer. All samples (non-reduced intact, and reduced subunits) were subject to incubation for 15 min at 70° and cooled in ice-water before injection. The asparagine-linked carbohydrates were released from the reduced/denatured daratumumab (5  $\mu$ L) by digesting with 2  $\mu$ L endoglycosidase (PNGase F, 200 mU/mL) at 37° for 1 h. In all capillary electrophoresis separations, the Borate-TRIS-EDTA-Glycerol-PI buffer was prepared as follows: 0.4 g of boric acid was dissolved in 5 mL of HPLC-grade water and the pH was adjusted to 8 by the addition of solid TRIS-base followed by the addition of 1 mL of 20 mM EDTA.Na<sub>2</sub>, 1 mL of glycerol, 1 mL of 2 % (w/v) SDS (w/v) and 1 mL of 1 mg/mL propidium iodide. Depending of the experiment, 0.2–1.0 g of dextran (1.8–2.5 MDa) was added and the volume was supplemented by HPLC grade water to 10 mL.

### 2.3. SDS-capillary gel electrophoresis (SDS-CGE)

All samples were separated using a PA800 Plus capillary electrophoresis instrument (Beckman Coulter, Brea, CA, USA) with laser-induced fluorescence (LIF) detection (488 nm excitation laser and 600 nm emission filter). The system was controlled by the 32 Karat software (version 10.1) that was also used for resolution calculation with the USP Rs option. The figures were generated by GraphPad Prism (San Diego, CA, USA). The bare fused-silica capillary had an effective length of 20 cm (total length 30 cm, 50  $\mu$ m ID, and 375  $\mu$ m OD). The temperature of the separation capillary was closely controlled with  $\pm 0.1^\circ\text{C}$  precision. Prior to the separations, the capillary underwent a washing/regeneration process with 0.5 M NaOH, 0.5 M HCl, and HPLC-grade water, followed by a rinse with the actual gel-buffer system, each lasting for 2 min by applying 80 psi forward pressure. The injection process involved introducing first the intact denatured mAb at 5 kV for 20 s, followed by the reduced and denatured sample mixture (lysozyme internal standard as well as the light chain, non-glycosylated heavy chain, and heavy chain fragments of daratumumab) under the same conditions. Please note that the sample buffer of the intact protein did not contain the reducing agent (2-ME), while the other sample components were reduced, therefore, mixing them would have caused reduction of the intact form, falsifying the results. The electric field strength for the separations was 500 V/cm with the cathode at the injection end (reversed polarity), with no pressure applied. All runs were made in triplicates and the average migration times were used for the downstream calculations to increase the reliability of the electromigration behavior, therefore, to obtain a better understanding of the experimental results.

## 3. Theory

As suggested earlier [20], the following equation describes the mobility ( $\mu$ ) of biopolymers in capillary electrophoresis:

$$\mu = \text{const} \cdot \frac{Q}{Mw^k} \cdot e^{-E_a/RT} \quad (1)$$

where *const* is a pre-exponential factor, Q is the net charge of the migrating analyte molecule with a molecular weight of Mw, k = 0.167

for SDS–protein complexes [15],  $E_a$  is the activation energy,  $R$  is the universal gas constant, and  $T$  is the absolute temperature.

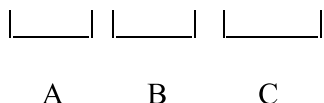
In capillary gel electrophoresis of biopolymers, size separation of the sample components is achieved in a polymer matrix with a molecular size sieving function. Therefore, the electromigration rate is also affected by the concentration of the sieving matrix ( $T\%$ ) and the retardation coefficient  $K_R$ , as defined by the Ferguson equation [16]:

$$\mu = \mu_0 \cdot e^{-K_R \cdot T\%} \quad (2)$$

where,  $\mu_0$  is the electrophoretic mobility in gel-free solution.

Based on the above, as a first approximation, we consider that  $\mu_0$  can be defined by equation (1), consequently, combining equations (1) and (2) delineates the effective electrophoretic mobility of the solute molecules, accounting for the gel concentration effect.

$$\mu = const \cdot \frac{Q}{Mw^{0.167}} \cdot e^{-E_a/RT} \cdot e^{-K_R \cdot T\%} \quad (3)$$



Terms A, B, and C represent the effects of the molecular characteristics of the electromigrating species, the separation temperature, and the gel concentration, respectively.

#### 4. Results and discussion

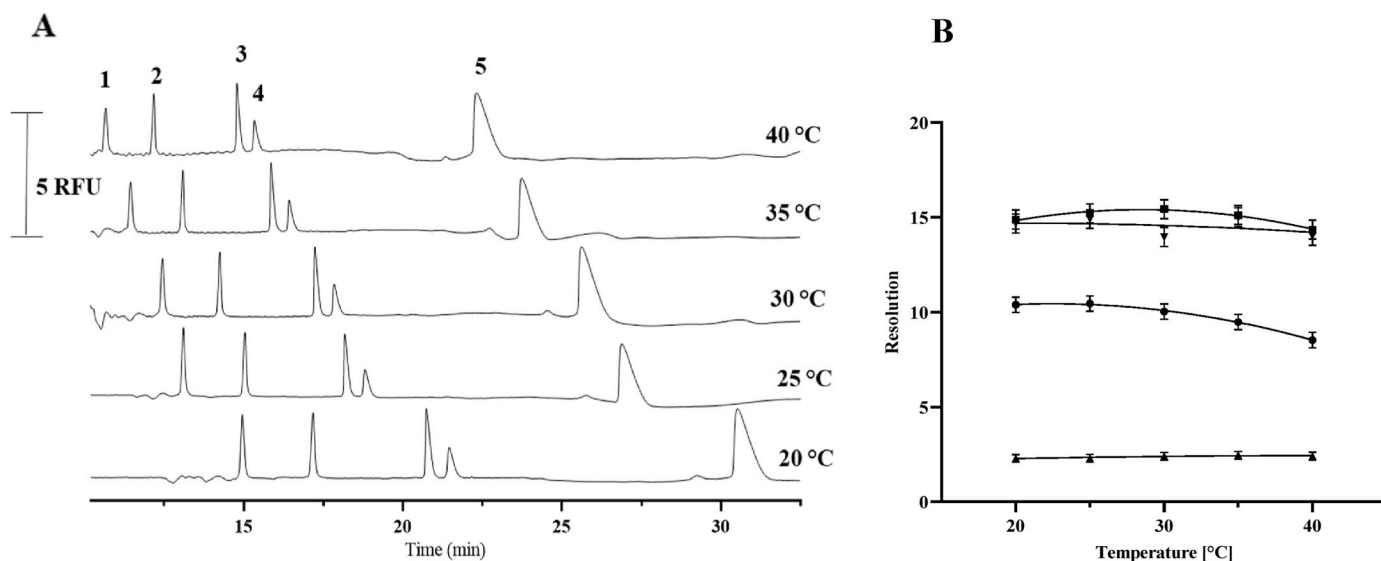
In this work, the effect of the primary operational parameters in capillary gel electrophoresis including the separation temperature (using the Arrhenius plot), the gel concentration (using the Ferguson plot), and the applied electric field strength (considering the molecular conformation) were investigated on the electrophoretic mobility of SDS-proteins in the presence of propidium iodide (PI) fluorescent ligand,

considering the respective terms of A, B, and C in equation 3. Albeit the electrophoretic mobility is not an apparent function of the applied electric field strength in the equation, its influence on the electromigration and resolution between the different molecular weight sample components was also scrutinized.

##### 4.1. Effect of the separation temperature

To understand the thermal sensitivity of the electromigration of SDS-protein complexes in PI-containing borate cross-linked dextran sieving matrices, the effect of capillary temperature was evaluated on the separation of daratumumab and its subunits at every 5°C between 20°C and 40°C using lysozyme as internal standard. As one can observe in Fig. 1A, the mobility of all sample components increased with elevating temperature, assumable due to the decreasing viscosity of the gel-buffer system. The %RSD values for the migration times during the SDS-CGE-PI measurements were 0.92, 0.94, 0.95, 0.98, and 0.98 for the 20, 25, 30, 35, and 40°C separations, respectively. The resolution ( $R_s$ ) between the analyte molecules was also assessed as the function of the separation temperature (Fig. 1B). Considering the error bars on the curves derived from the standard deviation of three parallel runs the  $R_s$  values did not significantly change with elevating temperature between the higher molecular weight sample components of the intact mAb and the heavy chain (HC) as well as the non-glycosylated heavy chain (ngHC) and the HC fragments. On the other hand, regarding the resolution among the lower Mw species, an apparent local maximum was observed between the ngHC and the light chain (LC) at 30°C, as well as an  $R_s$  decline of  $17.9 \pm 1.98\%$  between the LC and lysozyme between 25 and 40°C. These results suggested that the separation temperature should be optimized to achieve the best separation performance between the sample components of interest considering appropriate resolution with short migration time.

To shed light on the possible causes of the observed temperature-dependent differential electromigration of the sample molecules and the consequent resolution alterations, the activation energy concept was



**Fig. 1. Panel A:** Effect of the capillary temperature in SDS-CGE on the separation of the intact and subunit forms of daratumumab in the presence of PI in the gel-buffer system. Peaks: 1 – lysozyme (internal standard, ~14 kDa), 2 – light chain (LC, ~24 kDa), 3 – non-glycosylated heavy chain (ngHC, ~49 kDa), 4 – heavy chain (HC, ~50 kDa), and 5 – intact monoclonal antibody (mAb, ~148 kDa). Conditions: Gel-buffer system: 4 % (w/v) aqueous boric acid solution (adjusted to pH 8 by TRIS-base), 10 % (w/v) dextran (1.8–2.5 MDa), 2 mM EDTA.NA<sub>2</sub>, 10 % (v/v) glycerol, 0.2 % (w/v) SDS and 100 µg/mL PI; 20 cm effective length (30 cm total, 50 µm ID/375 µm OD) bare fused-silica capillary; detection (LIF): excitation 488 nm/emission 600 nm; applied electric potential: 15 kV with the cathode at the injection end; Electrokinetic injection: 5 kV/20 s for the denatured intact mAb, followed by 5 kV/20 s for the reduced and denatured mixture of lysozyme as well as the LC, ngHC, and HC subunits of daratumumab. The separation temperatures are given on the traces. **Panel B:** Separation temperature vs resolution plots between the consecutively migrating sample components in Panel A. Symbols representing the  $R_s$  between LC/lysozyme (●), ngHC/LC (■), HC/ngHC (▲), and intact mAb/HC (▼). The symbols and error bars represent the average and standard deviations from triplicate measurements.

applied. As mentioned in the introduction, the electrophoretic migration of the sample molecules in a sieving medium is an activated process [21–23]. To elucidate the activation energy ( $E_a$ ) requirement of the analyte molecules, the Arrhenius plots were generated by extracting the corresponding data from Fig. 1A. The resulting relationship between the logarithmic electrophoretic mobility values (corrected by 4 % for every 5 °C temperature increase [24]) and the reciprocal absolute temperatures are shown in Fig. 2A, considering term B in equation (3). Please note that in this case all capillary electrophoresis runs were accomplished using the same gel concentration (constant T%, isocratic), so term C of equation (3) did not influence the results. To understand the possible effect of temperature-mediated changes in the shape of the electromigrating sample molecules (term A), e.g., due to temperature-dependent pKa changes and the associated PI complexation, the mobility vs  $Mw^{-0.167}$  graphs were plotted (not shown), and found to be linear ( $r^2 > 0.999$ ) at all investigated temperatures. This suggested retainment of the earlier reported cylindrical molecular shapes of the SDS-proteins during their electromigration in borate cross-linked dextran sieving matrices [15], even in the presence of propidium iodide. Thus, it is reasonable to consider thermal independence of the molecular characteristics term (A in equation (3)) as well.

The slopes in Fig. 2A were used to calculate the activation energy requirement for the electromigration of the lysozyme internal standard as well as the intact and subunit forms of the mAb. The resulting  $E_a$  values were plotted against the molecular weights of the sample components, shown in Fig. 2B. The dotted line is to guide the eye on the apparent association between the  $E_a$  and the Mw of the mAb-related, i.e., similar amino acid sequence holding sample components, also emphasizing the outlier position of lysozyme as a non-mAb-related protein. Please note that the pI of lysozyme is 11.3, while the mAb and its subunits are ~8–9, probably influencing the SDS and PI complexation as well as the associated activation energy requirement. The distribution of the data points representing the mAb related molecules clearly shows the lower activation energy requirement for the higher Mw, consequently highly charged molecules (Mw: mAb > HC > ngHC > LC) capable of pushing through the transitional reticulations of the sieving matrix by their larger electric force (the product of charge and electric field), while the lower Mw fragments were only capable to do so at the decreasing extent, resulting the following order of  $E_a$ : mAb < HC < ngHC < LC.

#### 4.2. Effect of the gel concentration

Our group has reported earlier the non-classical sieving behavior (concave Ferguson plots) with the use of borate cross-linked dextran matrices in SDS-CGE of proteins [25]. Therefore, it was important to investigate the influence of PI on the sieving properties as a function of the gel concentration, especially by considering Term C in eq (3). In this part of the study 2.0, 5.0, 7.5, and 10.0 % (w/v) dextran (Mw 1.8–2.5 MDa) concentrations were used with 4 % (w/v) boric acid cross-linker, and the Ferguson diagrams were plotted (Fig. 3A) considering unchanged Terms A and B under the isotherm and constant electric field based separation conditions. In contrast to the non-linear plots reported earlier without PI in the background electrolyte [26], in this instance linear plots were obtained, suggesting more predictable sieving characteristics with the presence of the positively charged fluorophore in the gel-buffer system. Bearing in mind the slightly negative charge of the borate cross-linked dextran gel [25], one could assume that possible ionic interactions with the positively charged propidium ions might cause some changes in the ratio of the intrachain as well as the reticulation forming interchain borate-dextran adducts and consequently the sieving capability. As Fig. 3B delineates, the resolution values between the majority of the consecutively migrating sample component pairs (LC/lysozyme, ngHC/LC and mAb/HC) revealed increasing tendencies with molecular weight-dependent convex curvatures, i.e., higher Mw, steeper bending. The HC/ngHC pair, on the other hand, did not show significant changes with the increase in dextran concentration. Please note that the resolution of this sample component pair can be increased by changing the boric acid concentration utilizing the resulting borate-glycosylation interaction as reported earlier [4].

#### 4.3. Effect of the applied electric field strength

According to equation (3), the applied electric field strength should not play a role in influencing the resulting electrophoretic mobility of the sample components. However, we observed that similar to what was reported earlier in DNA electrophoresis [18], high electric fields caused field-dependent mobility changes as shown in Fig. 4A. In the case of nucleic acid analysis, this effect was reportedly associated with the orientation/stretching of the coiled DNA molecules due to the high electric field. In SDS-CGE of proteins, both in the presence (dashed lines) and absence (solid line) of PI in the gel-buffer system, linearly increasing electric field vs mobility relationships were found in the entire

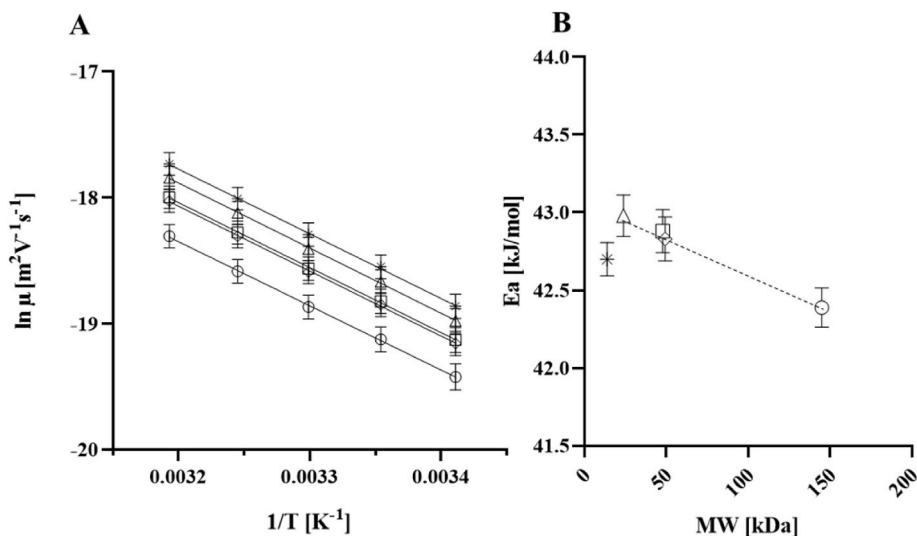
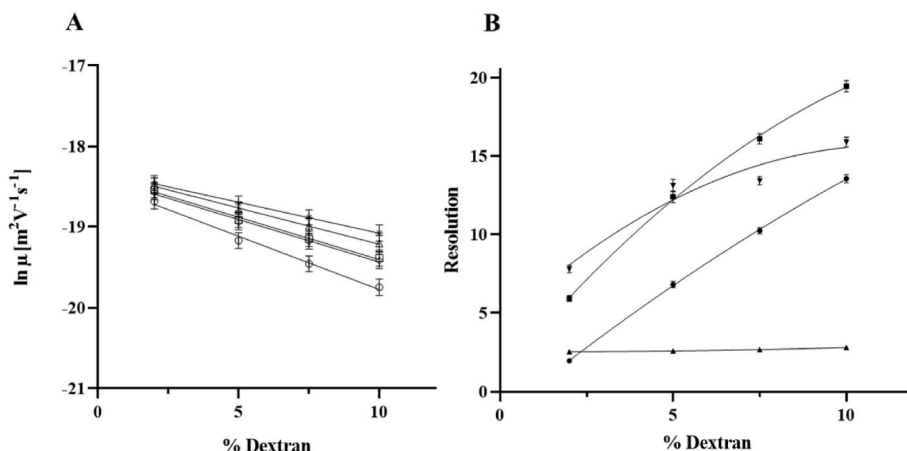
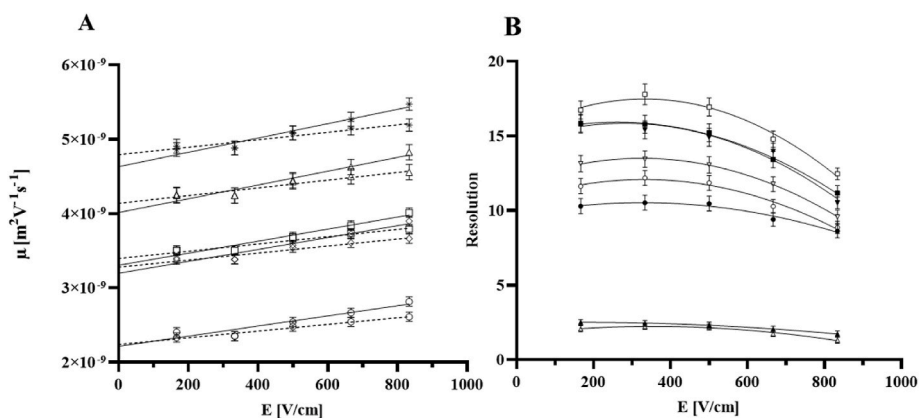


Fig. 2. Arrhenius (Panel A) and activation energy (Panel B) plots of the SDS-protein-propidium complexes in the test mixture, based on the data extracted from Fig. 1. Symbols: lysozyme (internal standard) (\*), light chain (Δ), non-glycosylated heavy chain (□), heavy chain (◇) subunits, and the intact mAb (○). The symbols and error bars represent the average and standard deviations from triplicate measurements.



**Fig. 3.** Panel A: Ferguson plots of the logarithmic electrophoretic mobilities of the sample mixture components as a function of dextran concentration in a 4 % (w/v) boric acid cross-linker containing sieving matrix with 100  $\mu\text{g}/\text{mL}$  PI. Symbols: lysozyme internal standard (\*), light chain ( $\Delta$ ), non-glycosylated heavy chain ( $\square$ ), heavy chain ( $\diamond$ ) subunits, and intact mAb ( $\circ$ ); Panel B: The associated resolution plots. Symbols representing the  $R_s$  between LC/lysozyme ( $\bullet$ ), ngHC/LC ( $\blacksquare$ ), HC/ngHC ( $\blacktriangle$ ), and intact mAb/HC ( $\blacktriangledown$ ). The symbols and error bars represent the average and standard deviations from triplicate measurements.



**Fig. 4.** Panel A: Observed electrophoretic mobility values of the protein test mixture components. Dotted and solid lines depict the presence or absence of 100  $\mu\text{g}/\text{mL}$  PI in the gel-buffer system, respectively. Symbols: lysozyme internal standard (\*), light chain ( $\Delta$ ), non-glycosylated heavy chain ( $\square$ ), heavy chain ( $\diamond$ ) subunits, and intact mAb ( $\circ$ ); Panel B: The associated resolution plots. Filled and empty symbols correspond to the presence and absence of PI, respectively. Symbols representing the  $R_s$  between LC/lysozyme ( $\bullet$ ,  $\circ$ ), ngHC/LC ( $\blacksquare$ ,  $\square$ ), HC/ngHC ( $\blacktriangle$ ,  $\Delta$ ), and intact mAb/HC ( $\blacktriangledown$ ,  $\triangledown$ ). The symbols and error bars represent the average and standard deviations from triplicate measurements.

molecular weight range of the sample mixture (15–148 kDa). In Fig. 4A, one can readily observe the shallower and steeper slopes with and without the presence of PI in the sieving matrix, respectively. The shallower slopes obtained with the use of the fluorophore-containing gel-buffer system were probably caused by propidium complexation-mediated conformation changes in the sample molecules. As a first approximation, we consider that the smaller-scale electric field-induced mobility increase was caused by the increasing persistence length of the cylindrical shape SDS-protein-propidium complexes, similar to nucleic acid electrophoresis in the presence of ethidium bromide [27]. The associated higher rigidity most likely decreased the high voltage-mediated mobility dependence of the sample components, in contrast to the absence of the dye showing greater electric field dependence of the mobilities. Extrapolation of the mobilities of the sample components to zero electric field strength revealed the “intrinsic” electrophoretic mobility rates of the sample components, as was shown earlier in agarose gel electrophoresis of large DNA molecules [17]. In our case, the “intrinsic” electrophoretic mobility differences of the SDS-protein complexes in the presence and absence of the fluorophore decreased with increasing molecular weight, and were apparently equal at the intact mAb level ( $M_w \sim 148$  kDa).

Fig. 4B depicts the resolution plots of the consecutively migrating

sample components with increasing electric field strengths, all showing a significant decline above 500  $\text{V}/\text{cm}$ , regardless of the presence or absence of PI in the separation gel. However, with PI in the sieving matrix, the resolution values of the lower molecular weight LC/lysozyme and ngHC/LC pairs were lower, while the  $R_s$  of the higher molecular weight HC/ngHC and intact mAb/HC pairs were greater than that of without the fluorophore in the background electrolyte. This suggests Mw-dependent resolution changes in the presence and absence of the fluorophore ligand as a function of the electric field strength.

## 5. Conclusions

Changes in the three important user-adjustable operational parameters of capillary temperature, sieving gel concentration, and applied electric field strength all affected the electromigration properties of SDS-protein-propidium adducts. Selecting the appropriate capillary temperature is crucial to obtain optimal separation of SDS-protein complexes due to the different activation energy requirements of the solute molecules. Higher  $M_w$ , therefore highly charged, SDS-proteins were apparently capable of pushing through the transitional sieving network by their larger electric force, while the lower  $M_w$  fragments were not capable at the same level, but the molecular sieving effect was still

maintained. The gel concentration, as expected, is a key factor in achieving high-Rs separations, except for the borate concentration-dependent ngHC/HC pair. Unlike with no PI-containing gel-buffer systems, the Ferguson plots were linear. Most interestingly, the applied electric field strength caused some increase in the electrophoretic mobility of the solute molecules both with and without having PI in the sieving matrix. This phenomenon was probably due to electric field-induced conformation change (increased persistence length) differences of the SDS-proteins in the presence and absence of propidium ions in the sieving matrix. Mw-dependent Rs changes were also observed as a function of the applied electric field with a significant decline above 500 V/cm.

Based on our results reported in this paper, the following operational parameters offered the best performance in terms of adequate Rs and short analysis time for the separation of the intact and subunit forms of daratumumab: i) temperature: 30°C, ii) gel concentration: 10 % (w/v) dextran (1.8–2.5 MDa) with 4 % (w/v) boric acid, and iii) applied electric field strength: 500 V/cm. However, it is important to note that other protein samples, including biotherapeutics, may require different operational parameters to obtain the best performance and, therefore, should be optimized accordingly.

### CRedit authorship contribution statement

**Felicia Auer:** Writing – original draft, Methodology, Investigation, Formal analysis. **Andras Guttman:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Data availability

Data will be made available on request.

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