Capillary electrophoresis for aluminum ion speciation: Optimized separation conditions for complex polycation mixtures

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TITLE RUNNING HEAD. Optimizing aluminum chlorohydrate separations by CE

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ABSTRACT. Aluminum chlorohydrates (ACH) are used in numerous applications and commercial products on a global scale including water treatment, catalysis or antiperspirants. They are complex mixtures of water soluble aluminum polycations of different degrees of polymerization, that are difficult to separate and quantify due to their susceptibility to depolymerize in solution when placed out of equilibrium, which is inherent to any separation process. We recently achieved the first capillary electrophoresis separation and characterization of ACH oligomers using 4-morpholineethanesulfonic acid (MES) as background electrolyte counter-ion. MES stabilizes the separated ACH oligomers during the electrophoretic process leading to highly repeatable and fast separations. In this work, the separation of ACH oligomers was further studied and perfected by varying the ionic strength, MES concentration and pH of the background electrolyte. Complex electrophoretic behavior is reported for the separation of Al_{13} , Al_{30} and Na^+ ions according to these experimental parameters. The transformation of the electropherograms in effective mobility scale and the use of the slope-plot approach are used to better understand the observed changes in selectivity/resolution. Optimal conditions (700 mM MES at 25 mM ionic strength containing 0.1 mM didodecyldimethylammonium bromide for dynamic capillary coating, pH 4.8) obtained for the separation of ACH oligomers are used for the baseline separation of samples difficult to analyze with other methods, including different molecular, aggregated and colloidal forms of aluminum from the Al_{13} , Al_{30} and Na^+ mixture, validating the rationale of the approach.

Keywords: capillary electrophoresis, aluminum chlorohydrates, oligocations, antiperspirant ingredients, counterions, ionic strength.

1. Introduction

Aluminum chlorohydrates (ACH) are of great use in numerous fields [1] such as water treatment [2–7], clay pillaring [8,9] and antiperspirants [10,11]. They are hydrolyzed salts containing large, highly positively charged aluminum oligomers [12]. In the antiperspirant applications, aluminum salts are the active ingredients and the mechanism of action consists in the precipitation of the ACH in contact with organic material contained in sweat, such as proteins, leading to a temporary obstruction of the sweat duct [13–16]. Some antiperspirants may also contain zirconium salts mixed with aluminum salts [14,17]. Active research is now oriented towards a better understanding of the structure/activity correlation, particularly through the analysis and characterization of ACH ingredients. ACH are prepared by neutralizing aluminum chloride by metallic aluminum, leading to several oligomers [18,19] obtained from the arrangement of different number of monomeric forms present under acidic conditions, including $[\text{Al}(\text{H}_2\text{O})_6]^{3+}$, $[\text{Al}(\text{OH})(\text{H}_2\text{O})_5]^{2+}$, $[\text{Al}(\text{OH})_2(\text{H}_2\text{O})_4]^+$ and $[\text{Al}(\text{OH})_3(\text{H}_2\text{O})_3]$, which contain aluminum in octahedral coordination. $[\text{Al}(\text{OH})_4]^-$, in which aluminum is tetrahedrally coordinated, is present in alkaline solutions, but may also be involved in the formation of cage-like polycations such as the Al_{13} and Al_{30} -mer, since the structure of Al_{13} units comprises an AlO_4 tetrahedral core. In aluminum monomers and oligomers, the hydrolysis ratio h is an important parameter that is defined as the ratio of total number of OH^- ligands to the total number of aluminum atoms [20,21], and therefore strongly influences the charge and efficacy of the polycations in applications. Liquid state ^{27}Al NMR is particularly useful to access structural information about specific oligomers such as the ϵ - Al_{13} Keggin cluster and Al_{30} (containing two δ - Al_{13} Keggin clusters linked by aluminum monomers), which formulas are $[\text{Al}_{13}\text{O}_4(\text{OH})_{24}(\text{H}_2\text{O})_{12}]^{7+}$ and $[\text{Al}_{30}\text{O}_8(\text{OH})_{56}(\text{H}_2\text{O})_{26}]^{18+}$, respectively [11]. To complete the information given by NMR, separation techniques are crucial for characterizing ACH solutions which comprise, most

generally, mixtures of oligomers. Size exclusion chromatography (SEC) which elutes oligomers (or polymers) in the decreasing order of their molar mass, has been extensively studied for the separation of ACH solutions [11,22,23]. Using SEC on SephadexTM 6-25 column, ACH samples have been separated into five peaks (noted 1, 2a, 2, 2b, 3 [11] also noted 1 to 5 [24]) in their increasing order of detection (decreasing order of molar mass from 8000 g/mol for the first peak down to a few hundreds for the last peak). The weight average molar mass in ACH solution ranged from 4000 to 6000 g/mol, while Al₁₃ displays a molar mass close to 1000 g/mol [11]. Antiperspirant activity was found to increase with the amount of peaks 3 and 4 oligomers [24]. However, the eluent used for SEC analysis is generally highly acidic (diluted hydrochloric acid, pH 3 [25] or nitric acid, pH ~2 [26]), in analytical conditions that are far from the conditions of the sample and the conditions of applications.

More recently, we have developed a CE method allowing the separation of Al₁₃ and Al₃₀ oligomers in electrophoretic conditions with a pH ~4.8, which is closer to the conditions of antiperspirant applications / formulations [27]. It was shown that the choice of the counter-ion, chromophore, capillary I.D. and capillary coating were crucial to get highly repeatable separations. In this new study, the impacts of ionic strength and 4-morpholineethanesulfonic acid (MES) concentration in the background electrolyte are studied, discussed and optimized with respect to the separation of different ACH oligomers. The influence of these parameters on the relative proportions of Al₁₃ and Al₃₀ oligomers in ACH samples is also reported. Application of the optimized conditions to the separation of Keggin Al₁₃, Al₃₀ and a range of larger size Al-based colloids is demonstrated.

2. Experimental section

2.1. Chemicals

Imidazole (Im), hydrochloric acid (HCl) and 4-morpholineethanesulfonic acid (MES) were purchased from Acros Organics (Morris Plains, New Jersey, USA). Di-dodecyldimethylammonium bromide (DDAB) was purchased from Aldrich (Milwaukee, WI, USA). Aluminum chloride hexahydrate and Amberlite IRA67 (15-60 mesh in free base form) used for AH1-2 synthesis were purchased from Sigma-Aldrich (Overijse, Belgium).

Ultrapure water was prepared with a Milli-Q system from Millipore (Molsheim, France). All aluminum standards and ingredients were kindly provided by L'Oréal laboratories (Aulnay-sous-bois, France): Al_{13} and Al_{30} were synthesized using the protocol of Chen *et al.* [28]; aluminum chlorohydrate (ACH) was provided in a 50% m/v water solution. AH1 was prepared by neutralization of an 0.2 M acidic aluminum chloride solution to a theoretical hydrolysis ratio of 3.0 using a strong anion exchange resin (Amberlite IRA67, Aldrich), followed by 6 months aging at room temperature. AH2 Was prepared by neutralization of an $\epsilon\text{-Al}_{13}$ solution (0.4 M total $[\text{Al}^{3+}]$) to a theoretical hydrolysis ratio of 3.0 using the same anion exchanger, leading to a pH 6.5 suspension [29,30] . For AH1 and AH2, the particle size distribution was obtained by dynamic light scattering, DLS, (Malvern Zetasizer Nano ZS), and the morphological analysis was performed using atomic force microscopy (Agilent 5500 AFM, MSNL-F cantilevers, AC mode) (Figs. SI5-6).

2.2. Capillary electrophoresis

CE experiments were carried out on a G7100A CE Agilent technologies system (Waldbronn, Germany) equipped with a diode array detector. Bare fused silica capillary was purchased from Polymicro Technologies (Phoenix, Ar., USA). Capillary dimensions were 38.5 cm (30 cm to the UV detector) $\times$ 25 μm I.D. (375 μm O.D.). The temperature of the capillary cassette was set at 25°C. Background electrolyte (BGE) was prepared by adding the desired amount of imidazole (used as a chromophore in the indirect UV detection mode) and the calculated amount of MES to reach the desired pH and ionic strength. 0.1 mM DDAB was added to the BGE to

ensure better coating and electroosmotic flow stabilities of the double layer DDAB coating. The pH of the BGE was controlled using a Mettler Toledo Seven Compact pH meter (Columbus, Ohio, USA) before use. To ensure a proper equilibrium of the sample, the samples were prepared at least three days before injection, by diluting the ingredient into ultrapure water. 0.1 mM Tris(hydroxymethyl)aminomethane (TRIS) was added to all samples as internal standard and electrophoretic mobility marker. The injection volume was set to approximately 1% v/v of the capillary effective volume, i.e. 50 mbar, 8 s. Indirect UV detection was performed at 205 nm $\pm$ 3 nm with a reference at 300 nm $\pm$ 5 nm.

2.3. Capillary coating

Didodecyl dimethylammonium bromide (DDAB) capillary coating is a cationic surfactant that comprises two long alkyl chains. It was previously used for the separation of proteins by capillary electrophoresis [31]. The coating procedure was performed as described by Melanson et al. [31] following the successive flushes (at 3 bars): 1 M NaOH for 10 min, water for 5 min, 2 mM DDAB in BGE for 30 min, 0.1 mM DDAB in BGE for 4 min. Then, between two analyses, the capillary is flushed with BGE containing 0.1 mM DDAB for 4 min at 1 bar.

2.4. Time-correction on electropherograms and mobility scaling

Since solutes have their own mobility, their velocity into the electric field is also different from one another. Therefore, low mobility solutes have a lower velocity and they will pass through the UV detector slower than those of a higher mobility. Consequently, low mobility solutes spend more time in front of the detection window and the peak area will therefore be overestimated. To take into account the differences in apparent velocity, a time correction is necessary to normalize the absorbance response of the detector $S(t)$ according to eq. (1) [32]:

$$h(t) = \frac{S(t)}{t} \quad (1)$$

Using this approach, the time-corrected electropherograms (i.e. $h(t)$ signal) are mass-weighted distributions of migration time. This means that two distinct peaks having the same area in the $h(t)$ representation correspond to the same amount of solutes (as far as the response factors are the same) [32]. Furthermore, electropherograms are transformed to the mobility scale in abscissa using eq. (2), so they can be compared to one another:

$$\mu(t) = \frac{L \times l}{t \times V} \quad (2)$$

where L is the total length of the capillary, l is the distance from injection to detection window and V is the separation voltage. The y-axis of the electropherograms in the effective mobility scale, $f(\mu)$, is given by [32]:

$$f(\mu) = \frac{h(t)}{\frac{d\mu}{dt}} \sim h(t) \times t^2 \quad (3)$$

3. Results and discussion

In a previous study, MES was found as a suitable counter-ion for the separation of ACH oligomers since it provides a stabilization of the oligomeric forms in the time-range of the electrophoretic migration (i.e. within a few min). As for all separation processes, the separated solutes are inherently put out of equilibrium and should thus be stable enough (no depolymerization) during the separation process to allow for a correct separation and quantification. In this work, the influence of ionic strength, pH and MES concentration in the BGE on ACH oligomers separation was investigated. However, the three aforementioned parameters are not conjointly independent. pH is mainly controlled by the imidazole (Im)/ MES ratio. At a given pH, the ionic strength is directly proportional to the Im concentration. All the BGE compositions that were used in this study are presented in Table SI1 (as supporting information) with theoretical pH (given by peak master 5.1 [33]) and the corresponding measured pH.

3.1. Influence of the BGE ionic strength at constant pH

Fig.1 shows the changes in the separation of a 0.5 g/L ACH sample with increased ionic strength at constant pH (experimentally measured at 4.8 (see Table SI1) that matches the pH of the aluminum samples). The ionic strength I is set by the imidazole concentration and varied from 3 mM (at 3 mM Im) to 25 mM (at 25 mM Im). Concomitantly, the analysis time increased from about 2 to 4 min, due to the decrease in the electroosmotic mobility (from $-86 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ at $I = 3 \text{ mM}$, to $-56 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ at $I = 25 \text{ mM}$). The selectivity of the separation was also highly affected by ionic strength, with some inversions in migration order between sodium ion and the ACH oligomers. For that reason, the peak identification should be carefully performed with systematic co-injection of the standards. To better monitor the impact of ionic strength on the separation, the time-scale electropherograms (Fig. 1A) were converted into effective mobility scale (Fig. 1B) corrected from the impact of the viscosity change (due to high MES concentrations at high I). For that correction, the viscosity-corrected effective mobility $\mu_{ep,corr}$ was determined according to eq. (4):

$$\mu_{ep,corr} = \frac{\eta}{\eta_0} \times \mu_{ep} \quad (4)$$

where η is the viscosity of the BGE given in Table SI1 and η_0 is the water viscosity, at 25°C. In Fig. 1B, it can be observed that the effective mobility of all solutes decreases with increasing ionic strength. However, this decrease is much more important for Al₁₃ and Al₃₀ oligomers than for mono-charged sodium and TRIS ions. This behavior can explain the inversion in migration order between ACH oligomers and sodium. The impact of ionic strength on ACH oligomers migration will be discussed in more detail in a subsequent section using the recently developed slope plot approach [34,35]. It can be also noticed from Fig. 1A, that the ACH oligomer peaks are much less dispersed, and much less asymmetrical, at higher ionic strength (20-25 mM) than at low values. This effect is mainly due to lower electromigration dispersion when the effective

mobility of ACH oligomers tends to reach the I_m effective mobility (about 46.8 TU at $I=20$ mM; TU stands for Tiselius Units, 1 TU being equivalent to $10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$).

The proportion of Al_{13} in the ACH sample was estimated as a function of ionic strength by determining the time corrected peak area proportion relative to the total $\text{Al}_{13}+\text{Al}_{30}$ content, as given in Fig. 2. The relative time-corrected peak area proportion of Al_{13} oligomer was stable at $\sim 6 \pm 0.3 \%$ for $I \leq 7$ mM and stable at $\sim 13 \pm 0.4 \%$ ($\pm$ one standard deviation on $n = 3$ repeats) for $I \geq 12$ mM. However, since MES concentration was varied concomitantly with I to keep a constant pH of 4.8, the proportion of Al_{13} in ACH sample may rather be linked to MES concentration than to the ionic strength of the BGE.

3.2. Influence of MES concentration and pH of the BGE at a constant ionic strength

Fig.3 displays the time-scale electropherograms (Fig. 3A) and the viscosity corrected effective mobility-scale electropherograms (Fig. 3B) obtained for ACH sample at a constant 12 mM I_m concentration ($I=12$ mM) and for different MES concentrations and, thus, different pH from pH 5.7 at 50 mM MES down to pH 4.29 at 700 mM MES (see Table SI1). From Fig. 3, we can observe that the detection time was higher at low MES concentration, due to lower electroosmotic mobility at higher pH. DDAB coatings are known to be more stable and to generate stronger electroosmotic flow at lower pH [31]. From the viscosity corrected effective mobility scale electropherograms (Fig. 3B), it appears that the mobility of the solutes was not much affected by changes in both pH and MES concentrations, as compared to the impact of ionic strength. Nevertheless, there is an inversion of migration order between sodium and Al_{30} between pH 4.8 and 5.0. Overall, pH of the BGE has a much lower impact on the ACH separation, as compared to ionic strength.

3.3. Influence of ionic strength of the BGE at constant MES concentration and investigation of the electrophoretic behavior using the slope plot approach

After changing the ionic strength at constant pH, and changing the pH at constant ionic strength, this study was completed by varying the ionic strength at constant MES concentration. This study was performed by varying the Im concentration from 1.5 to 25 mM, at three different levels of MES concentration (90 mM, 320 mM and 700 mM). Fig. 4 displays the electropherograms obtained at 700 mM MES in time-scale (Fig. 4A) or in effective mobility scale (Fig. 4B). The electropherograms corresponding to 90 mM and 320 mM MES levels are available in Fig. SI1 and Fig. SI2, respectively. The electrophoretic behavior observed in Fig. 4 is relatively similar to that observed in Fig. 1 with a strong impact of ionic strength on the effective mobility of the solutes, and with the inversion of the migration orders between sodium and ACH oligocations. Some differences in the impact of ionic strength on the resolutions between solutes are observed and will be discussed below.

3.3.1. Ionic strength dependence and slope plot approach

To shed more light on the ionic strength dependence of the effective mobility of the different ions, the viscosity corrected mobility of each ions identified in the ACH mixture was plotted against $\log I$ according to the following eq. (5):

$$\mu_{ep,corr} = -P \log(I) + \mu_{ep,corr}(1M) \quad (5)$$

where P is the effective mobility decrease per ionic strength decade and $\mu_{ep,corr}(1M)$ is the viscosity-corrected effective mobility at 1M ionic strength. This plot is displayed in Fig. 5A for MES 700 mM (resp. Fig. SI3A and SI3B, for MES 90 and 320 mM). Whatever the nature of the solute (small ion, oligomers, polyelectrolyte, nanoparticle), it was shown that eq. (5) is verified (within a 5% error margin) between typically 1 and 100 mM ionic strength [34]. Such plots have the advantage to quantify the dependence on ionic strength and to possibly interpolate the effective mobility values at any ionic strength. Fig. 5A clearly shows that sodium and

TRIS ions have a low dependence ($P \sim 3.4$ and 4.7 TU, respectively), while the ACH oligocations have about 5 times higher dependence ($P \sim 16.3$ and 17.5 TU for Al_{13} and Al_{30} , respectively). This representation also clearly points out the ionic strengths for which the order of migration is reversed between sodium and Al_{13} ($I \sim 13.3$ mM) or sodium and Al_{30} ($I \sim 15.4$ mM). It can be also observed in Fig. 5A that the analysis time increases for the highest ionic strengths because the difference in mobility between the fastest solute and the electroosmotic mobility (which represents the lowest apparent mobility) drops, notably above 15.4 mM ionic strength, when sodium becomes the solute detected last.

Another way to represent the dependence on ionic strength is to plot the relative decrease of the effective mobility compared to the mobility value at 5 mM ionic strength, which is arbitrarily chosen as a reference, as a function of $\log I$:

$$\frac{\mu_{ep,corr}}{\mu_{ep,corr}(5\text{ mM})} = -S \log(I) + \frac{\mu_{ep,corr}(1M)}{\mu_{ep,corr}(5\text{ mM})} \quad (6)$$

In this representation (not shown), the slope S derived from eq. (6) is a dimensionless parameter that represents the relative decrease in the effective mobility per ionic strength decade, taking 5 mM ionic strength as a reference. The value of $S = 0.20$, obtained in this work for the mono-charged TRIS ions, means that its effective mobility decreases by 20% of its initial value at 5 mM ionic strength per ionic strength decade. The so-called slope plot is given in Fig. 5B, where the S value is plotted as a function of $\mu_{ep,corr}(5\text{ mM})$ [34]. Data represented as plain lines are from reference [35], covering a data set of 5000 values that were numerically obtained for different small ions and nanoparticles of various charge and size, using the modified Yoon and Kim modelling [35]. On the same plot, the experimental data (S_{exp} , $\mu_{ep,corr}(5\text{ mM})$) obtained in this work for Al_{13} , Al_{30} , Na^+ and TRIS at 700 mM MES were added as single points (plain symbols). The corresponding numerical values of S_{exp} and $\mu_{ep,corr}(5\text{ mM})$ are presented in Table

1 [27,35–37]. The experimental points are close to the plain lines corresponding to their respective sizes. For a better comparison, the theoretical values for S (noted S_{th}) derived from the modified Yoon and Kim modelling [35] taking R_h and $\mu_{ep,corr}(5 \text{ mM})$ as input data (see Table 1) were also plotted in Fig. 5B (see open symbols in Figure 5B) for the four solutes. A close agreement is observed showing the consistency of the experimental data with the electrophoretic mobility modelling. The corresponding theoretical effective charge numbers z_{th} also derived from the modified Yoon and Kim modelling, are reported in Table 1 and are in very close agreement with the expectations ($z = 1$ for monocharged ions, Na^+ and TRIS) or with the effective charge calculated independently by indirect UV detection for Al_{13} and Al_{30} (3.5 and 6.7, respectively, see Table 1) [27]. As for the estimation of solute size, the hydrodynamic radius of Al_{13} has been reported by Akitt [38] as about 0.5 nm and the hydrodynamic radius for Al_{30} was calculated from the two characteristic dimensions of the oligomer using eq. (7) for the hydrodynamic radius of a cylinder [39]:

$$R_h = \frac{L}{2 \left(\ln \left(\frac{L}{2R} \right) + 0.312 + 1.13 \frac{R}{L} + 0.4 \frac{R^2}{L^2} \right)} \quad (7)$$

where L denotes the length of the cylinder and R is the radius of the cylinder. For Al_{30} , $R = 0.5$ nm and $L = 2$ nm [40,41] leading to $R_h = 0.762$ nm. With the slope plot representation given in Fig. 5B, the reason for Al_{13} and Al_{30} having similar S values despite their different charge and size characteristics appears more clearly. Al_{30} being larger than Al_{13} should have a higher S value, due to higher electrophoretic effect. This phenomenon is clearly observed in Fig. 5B, where the S value increases with the size at low effective mobility (i.e. at low charge). However, in the case of Al_{30} , the electrophoretic effect is counter-balanced by the non-linear electrostatic and relaxation effects that tend to decrease the S value with increasing charge, as for any solute

larger than 1 nm [35]. As for sodium, the data point is below the 0.2 nm line since the effective ionic radius of Na^+ is about 0.154 nm [37].

3.3.2. Relative Al_{13} proportion at different pH according to MES concentration

Since the MES counter-ion was found to be crucial to stabilize Al_{13} and Al_{30} forms during the electrophoretic separation, it was important to investigate whether the $\text{Al}_{13} / (\text{Al}_{13} + \text{Al}_{30})$ relative proportion depended or not on MES concentration, and also on the pH of the electrolyte. Fig.6 represents the time-corrected peak area proportion of Al_{13} in the ACH sample as a function of pH at the three investigated levels of MES concentration (90 mM / 320 mM / 700 mM), based on 3 repeats. At a 90 mM concentration of MES, the proportion of Al_{13} is stable with the pH with an average value for the time corrected peak area of $9 \pm 0.12 \%$ ($\pm$ one standard deviation on $n = 3$ repeats). At 320 mM and 700 mM concentrations of MES, the proportion of Al_{13} remains with the pH of the BGE but with a significantly higher proportion of $12 \pm 0.22 \%$ in average ($\pm$ one standard deviation on $n = 3$ repeats). These results confirm the stabilizing role of MES on the oligomer distribution of the ACH sample with a threshold for 300 mM MES above which the relative amount of $\text{Al}_{13} / (\text{Al}_{13} + \text{Al}_{30})$ remains constant in the BGE. Apart from the $\text{Al}_{13} / (\text{Al}_{13} + \text{Al}_{30})$ proportion, it is interesting to mention that the quantity of monomeric Al^{3+} in the ACH samples were very low (not detectable in most samples) and lower than in the previous work [27]. In fact, this quantity can vary from one sample to the other depending on the sample concentration and on the kinetics of dissolution (time between dissolution and analysis). Low ACH concentration can favor the occurrence of Al^{3+} monomers in the speciation of ACH polycations. Higher ACH concentrations (0.5 g/L) were used in this work compared to [27] (0.1 g/L), which can explain lower Al^{3+} content.

3.3.3. Optimization of the resolution between ions present in the ACH samples

Fig.7 shows the influence of the ionic strength on the resolution of the separation between Al_{13} , Al_{30} and sodium ion at 700 mM MES concentration. The same representations obtained at 90

mM and 320 mM MES are shown in Fig. SI4A and 4B, respectively. As discussed in section 3.1., there are some inversions in the order of migration between ACH oligomers and sodium ion, when increasing the ionic strength. The ionic strength at which inversion occurs corresponds to the value for which the resolution is nil (at 9.2 mM and 11.5 mM ionic strength for $\text{Al}_{13} / \text{Na}^+$ and $\text{Al}_{30} / \text{Na}^+$, respectively). These critical ionic strengths increase with the concentration of MES, reaching 12.5 mM and 15 mM, respectively, above 300 mM MES. Overall, high ionic strength is required to maximize the resolution between Al_{13} (or Al_{30}) and Na^+ . If one considers the pH value as being close to that of the sample (4.8), the optimal BGE was obtained for 700 mM MES concentration and 25 mM ionic strength.

3.4. Application of the optimal conditions to the separation of Al-based colloids

In addition to SEC peaks 3 and 4 species attributable to Al_{13} and Al_{30} , commercial aluminum chlorohydrates contain a variable proportion of larger oligomers eluting under SEC peaks 1 and 2. Peaks 1 and 2 species may be attributed to aggregates of Al_{13} and/or Al_{30} forming at elevated aluminum concentrations, small size aluminum hydroxide nanoparticles, or flat Anderson-type isomers larger than flat- Al_{13} . Peak 1 and 2 species convert to Al_{13} and Al_{30} upon dilution and heating of ACH used in antiperspirant ingredient activation treatments. To demonstrate the applicability of CE to the separation of colloidal Al hydroxide, and aggregates of Al_{13} , we prepared an Al hydroxide nanoparticle suspension (AH1) and a neutralized Al_{13} sample in which Al_{13} units are controllably discharged and assembled into nanoparticles by ion exchange (AH2). The results of the dynamic light scattering (DLS) analysis of the two systems is presented in supplementary information (Figure SI5), with number-based diameter distributions respectively centered at 70 and 7 nm for AH1 and AH2.

Fig.8 displays the mobility-scale electropherograms of Al_{13} , Al_{30} , AH2 and AH1 using the optimized BGE previously described. The superposition of the electropherograms in viscosity-corrected effective mobility scale was adjusted on the theoretical value of Na^+ ($46.4 \times 10^{-9} \text{ m}^2\text{V}^{-1}$).

¹s⁻¹ as given by peakmaster) and a good baseline separation of all constituents was observed, showing the high discrimination power of CE. Fig. 8 also clearly shows that the optimized BGE can be used for the quantification of all polycations and colloids potentially present in ACH samples.

The electropherograms of AH1 and AH2 display a number of additional or different features as compared to that of the reference polycations (Al₁₃ and Al₃₀). The broad peak observed for AH2 near $30 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ may originate from aggregated (colloidal) species. Interestingly, the peak at $42 \times 10^{-9} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ is also visible in the electropherogram of AH1, which may indicate that it corresponds to a new molecular form, as opposed to distributions of colloidal objects. Finally, the broadest peak observed in AH1 electropherogram can be attributed to the aluminum hydroxide platelets distribution (70 nm average diameter).

4. Conclusion

In this work, a careful optimization of ACH oligomers analysis by CE has been investigated based on systematic variation of pH, ionic strength and MES concentration in the BGE. It was found that ionic strength is the main parameter influencing resolution, with migration inversions between monocharged (sodium) and multicharged chlorohydrate oligomers due to higher dependence of the effective mobility on ionic strength for the highest charged solutes (oligomers). The relative proportion of Al₁₃ / (Al₁₃ + Al₃₀) in the ACH sample was almost independent of pH and MES concentration (as far as MES ≥ 300 mM). The optimal conditions for the oligomer resolution (700 mM MES at 25 mM ionic strength, pH 4.8) were used successfully for the baseline separation of Al₁₃, Al₃₀ and Na⁺ together with Al hydroxide colloids and aggregated forms of the polycations potentially present in applications. Using appropriate and optimized BGE, CE could become a method of choice for the characterization of ACH samples.

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348 The manuscript was written through contributions of all authors. All authors have given ap-
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Figure 1: Influence of BGE ionic strength at pH 4.8 on the separation of ACH. Time-scale (A) and mobility-scale (B) electropherograms. Electrophoretic conditions: semi-permanent DDAB capillary coating, 25 μm I.D. $\times$ 38.5 cm (effective length, 30 cm). Electrolyte: X mM imidazole (Im), Y mM MES as indicated on the graph, 0.1 mM DDAB, pH 4.8. Separation voltage: -30 kV. Hydrodynamic injection: 50 mbar, 8 s. Indirect UV detection at 205 nm. Sample: 0.5 g/L

462 ACH, 0.1 mM TRIS in H₂O. Peak identifications: (1) TRIS, (2) Al₁₃, (3) Al₃₀, (4) Na⁺. See
 463 Table 1 in supporting information for the detailed characteristics of the BGE.

464
 465 Figure 2: Proportion of the time-corrected peak area of Al₁₃ in ACH as a function of BGE ionic
 466 strength at pH 4.8. See Figure 1 for electrophoretic conditions. Sample: 0.5 g/L ACH, 0.1 mM
 467 TRIS in H₂O. Error bars are $\pm$ standard deviation ($n = 3$).

Figure 3: Influence of the MES concentration and the pH of BGE at constant ionic strength on the ACH separation. Time-scale (A) and mobility-scale (B) electropherograms. Electrophoretic conditions: semi-permanent DDAB capillary coating, 25 μm I.D. $\times$ 38.5 cm (effective length, 30 cm). Electrolyte: X mM imidazole (Im), Y mM MES as indicated on the graph, 0.1 mM DDAB, pH 4.8. Separation voltage: -30 kV. Hydrodynamic injection 50 mbar, 8 s. Indirect UV

476 detection at 205 nm. Sample: 0.5 g/L ACH, 0.1 mM TRIS in H₂O. Peak identifications: (1)
 477 TRIS, (2) Al₁₃, (3) Al₃₀, (4) Na⁺. See Table 1 in supporting information for the detailed charac-
 478 terization of the BGE.

482 Figure 4: Influence of pH and ionic strength at constant 700 mM MES concentration on the
483 separation of ACH. Time-scale (A) and mobility-scale (B) electropherograms. Electrophoretic
484 conditions: semi-permanent DDAB capillary coating. 25 μ m I.D. $\times$ 38.5 cm (effective length,
485 30 cm). Electrolyte: X mM imidazole as indicated of the graph, 700 mM MES, 0.1 mM DDAB.
486 Separation voltage: -30 kV. Hydrodynamic injection: 50 mbar, 8 s. Indirect UV detection at
487 205 nm. Sample: 0.5 g/L ACH, 0.1 mM TRIS in H₂O. Peak identifications: (1) TRIS, (2) Al₁₃,
488 (3) Al₃₀, (4) Na⁺. See Table 1 in supporting information for the detailed characteristics of the
489 BGE.

Figure 5: Viscosity-corrected effective electrophoretic mobility at 700 mM MES concentration as a function of the ionic strength (A) and the corresponding slope plot representation (B) displaying S values as a function of $\mu_{ep, \text{corr}}$ (5 mM). Electrophoretic conditions as in Figure 4. Data represented as solid lines are from [27]. In Figure 5B, Al_{30} theoretical and experimental data points are overlaid.

499
 500 Figure 6: Proportion of the time-corrected peak area of Al_{13} relative to $\text{Al}_{13} + \text{Al}_{30}$ in ACH as a
 501 function of pH at three levels of MES concentration (90 mM, 320 mM & 700 mM). Electro-
 502 phoretic conditions as in Figure 4. Error bars are $\pm$ standard deviation ($n = 3$).

Figure 7: Influence of ionic strength on the resolution of the separation between Al₁₃, Al₃₀ and sodium ions in ACH at a constant 700 mM MES concentration. Electrophoretic conditions: semi-permanent DDAB capillary coating, 25 μm I.D. $\times$ 38.5 cm (effective length, 30 cm). Electrolyte as described in Table 1 in supporting information. Separation voltage: -30 kV. Hydrodynamic injection: 50 mbar, 8 s. Indirect UV detection at 205 nm. Sample: 0.5 g/L ACH, 0.1 mM TRIS in H₂O.

Figure 8: Viscosity-corrected mobility scale electropherograms of aluminum chlorohydrate oligomers. Electrophoretic conditions: semi-permanent DDAB capillary coating, 25 μm I.D. $\times$ 38.5 cm (effective length, 30 cm). Electrolyte: 25 mM imidazole, 700 mM MES, 0.1 mM DDAB, pH 4.8. Separation voltage: -30 kV. Hydrodynamic injection: 50 mbar, 8 s. Indirect UV detection at 205 nm. Samples: 1 g/L Al_{13} , 0.1 mM TRIS in H_2O ; 1 g/L Al_{30} , 0.1 mM TRIS in H_2O ; AH2 39 mM of total aluminum, 0.1 mM TRIS in H_2O ; AH1 17 mM of total aluminum, 0.1 mM TRIS in H_2O .