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# Characterization of ultrahigh molar mass polyelectrolytes by capillary electrophoresis

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## ABSTRACT

High to ultrahigh molar mass (above 1 million g/mol) anionic poly(acrylic acid-co-acrylamide)s are widely used industrial polymers for water treatment and oil drilling. Their properties are strongly related to their charge density and molar mass distributions. However, due to inherent separation limits of SEC with currently available columns ( $< 5 \times 10^6$  g/mol) and possible occurrence of chain breakage, and/or adsorption leading to abnormal elution, characterization of unusually high molar masses polyelectrolytes is challenging. In this work, we investigate the use of polymer sieving capillary electrophoresis for the size-based characterization of these high to ultrahigh molar mass polyelectrolytes. By optimizing the operating conditions (electric field, ionic strength, injected polyelectrolyte concentration, nature of the polymer sieving), it has been possible to considerably reduce polyelectrolyte aggregation and to get sufficient size-based selectivity, allowing to obtain the size distribution of the polyelectrolytes over a large range of molar mass from  $10^5$  up to  $\sim 10 \times 10^6$  g/mol. The data processing of the raw electropherograms is a key step in the analytical protocol leading to the molar mass distribution. The polyelectrolyte effective mobility in sieving conditions has to be normalized to its free-draining electrophoretic mobility in free solution conditions to take into account possible variability in the charge density between the different samples.

*Keywords:* high molar mass polymer, capillary gel electrophoresis, sieving, molar mass distribution, polyacrylamide derivatives

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## 34 **1. Introduction**

35

36 High to ultrahigh molar mass ( $> 1$  MDa) anionic polyacrylamides and their analogues are  
37 polyelectrolytes which are widely used as flocculent in wastewater treatment [1], as soil  
38 conditioner [2], as drag reducer [3], and as viscosity enhancer in Enhanced Oil Recovery for  
39 flooding of trapped crude oil from the reservoir [4]. In the oil field application, however, loss  
40 of flooding efficiency often arises from shear sensitive chain scissions in porous media and  
41 rheological performance deterioration provoked by salinity and high temperature [5, 6]. It is  
42 therefore desirable to thoroughly characterize these polyelectrolytes including their chemical  
43 nature (charge density) and molar mass distributions, and to study the impacts of shear stress,  
44 salinity and temperature on stability and rheological behaviors for optimal operations.  
45 Nevertheless, the characterization of such ultra-high molar mass polyelectrolytes with broad  
46 molar mass dispersion using classical methods such as size exclusion chromatography (SEC)  
47 is challenging due to the occurrence of chain breakage in size-exclusion chromatography,  
48 possible adsorption leading to abnormal elution [7-11], and especially the limited range of the  
49 molar mass analyzable with currently available SEC columns. In-batch techniques such as  
50 multi-angle laser light scattering (MALLS) [12,13], or open-medium separation techniques  
51 such as Field-Flow Fractionation (FFF) [14-17] can be used with a certain success to extend  
52 the analysis limits.

53 Capillary Electrophoresis (CE) is also an attractive separation technique without stationary  
54 phase, which was originally mainly developed for biomacromolecules such as DNA [18],  
55 proteins [19] and polysaccharides [20]. More recently, CE has been used for the  
56 characterization of synthetic polyelectrolytes [21-26], owing to its rapidity of analysis, minor  
57 sample consumption and orthogonal separation mechanism compared to chromatography.  
58 Free solution capillary electrophoresis allows to characterize the polyelectrolytes according to  
59 their charge density since the polyelectrolyte electrophoretic mobility in absence of gel is  
60 independent of the molar mass, due to free draining behavior above a typical molar mass of  
61 about 20,000 g/mol [27]. It was demonstrated that the distribution in charge density and the  
62 associated dispersity indexes can be determined by free solution CE [24, 25]. The  
63 characterization of highly charged polyelectrolytes above the Manning condensation  
64 threshold [28] is challenging, due to the low dependence of the effective mobility against the  
65 polyelectrolyte charge density above this threshold [29-31]. Indeed, due to counter-ion  
66 condensation, the polyelectrolyte effective charge density levels off above the Manning  
67 condensation threshold. Despite the low selectivity, it is however still possible to characterize

68 the charge density distribution of highly charged polyelectrolyte by adjusting the ionic  
69 strength of the background electrolyte in counter electroosmotic mode free solution CE [32].  
70 Increasing the ionic strength lowers the electroosmotic mobility, and consequently the  
71 apparent polyelectrolyte electrophoretic mobility, resulting in a better selectivity of the  
72 separation according to the charge density.

73 For polyelectrolytes of constant charge-to-mass ratio, Capillary Gel Electrophoresis (CGE)  
74 using entangled or diluted neutral sieving polymer solution, can be employed for molar mass-  
75 based separation of polyelectrolytes [18]. The electrophoretic migration of a polyelectrolyte  
76 through a polymer network has been elucidated via different separation mechanisms,  
77 depending on shape and size of the solute relative to the pore (or blob) size, and on the  
78 applied electric field [33, 34]. In the Ogston model [35], a small sized solute is described as a  
79 rigid sphere migrating through a porous medium with a relatively large pore size. The  
80 reptation model [36, 37] depicts a snake-like motion of a solute that is too large to pass freely  
81 through the pores of the network and must deform / unfold to pass through the matrix. The  
82 biased reptation migration (BRM) [21, 38, 39] describes the forced reptation of a  
83 polyelectrolyte under electric field, with orientation and stretching of the chain, leading to  
84 electric field- and size-dependent effective electrophoretic mobility,  $\mu$  [21, 39]:

$$85 \quad \mu : \left( \frac{1}{N} + b \left( \frac{E}{T} \right)^2 \right) \quad (1)$$

86 where  $b$  is a function related to the solute and the network, and  $N$  is the number of monomers  
87 of the polyelectrolyte. For polymer size comparable to or smaller than the pore size (or blob  
88 size) and at low electric field,  $\mu$  is a linear function of the inversed size, corresponding to pure  
89 reptation regime. For large polyelectrolytes, the size-dependence of the mobility disappears,  
90 and the electric field term becomes predominant. These regimes were both identified for DNA  
91 separation, as well as for synthetic polyelectrolytes such as polystyrene sulfonates (PSS) [33,  
92 34, 40].

93 In this work, we report the size-based characterization of high to ultra-high anionic  
94 poly(acrylic acid-co-acrylamide) (molar mass ranging between 22 kDa – 15 MDa), including  
95 standards and industrial samples with charge density comprises between 25 - 33% (expressed  
96 as a molar content in acrylic acid) by CGE using replaceable polymer solution as sieving  
97 matrix. A thorough optimization of the operating conditions including the nature of the  
98 separating polymer, the electric field strength, the ionic strength and the sample

99 concentration, was investigated to permit the molar mass characterization of the APAM  
100 samples.

101

## 102 **2. Experimental**

103

### 104 *2.1. Chemicals*

105 Tris(hydroxymethyl)aminomethane (Tris), anthraquinone-2-sulfonic acid sodium salt  
106 monohydrate 97%, hydroxyethylcellulose (HEC,  $M_w$   $1.3 \times 10^6$  g/mol) and polyethylene oxide  
107 (PEO, average  $M_w$   $2 \times 10^6$  g/mol,  $5 \times 10^6$  g/mol and  $8 \times 10^6$  g/mol) were purchased from  
108 Aldrich (Saint Quentin Fallavier, France), pullulan from *Aureobasidium pullulans* ( $M_w$   $575 \times$   
109  $10^3$  g/mol) from Chem Cruz (Heidelberg, Germany), dextran from *Leuconostoc mesenteroids*  
110 ( $M_w$   $1.5 - 2.8 \times 10^6$  g/mol) from Sigma (Saint Quentin Fallavier, France), dextran SGO ( $M_w$   
111  $50 \times 10^6$  g/mol) kindly provided by SNF (Andrézieux, France), PEO ( $M_w$   $1 \times 10^6$  g/mol) from  
112 Alfa Aesar (Illkirch, France), hydrochloric acid 37% and potassium chloride 99.6% from  
113 VWR (Fontenay-sous-Bois, France), sodium chloride 99.5% from Fluka (Illkirch, France),  
114 lithium chloride anhydrous 99% and lithium hydroxide 98% from Sigma-Aldrich (Saint  
115 Quentin Fallavier, France) and boric acid from Fluka (Illkirch, France).

116

### 117 *2.2. Samples*

118 All samples of anionic poly(acrylic acid-co-acrylamide) (APAM) in white powder were  
119 provided by SNF. Standard APAM samples were synthesized from monomers of sodium  
120 acrylate and acrylamide by Controlled Radical Polymerization (CRP) leading to narrow  
121 distributed molar mass and anionicity [41]. Industrial samples of APAM (labelled as IPAM)  
122 were obtained by hydrolysis of polyacrylamide in a NaOH medium to reach expected  
123 anionicity. The molar mass and PDI were determined by Size Exclusion Chromatography  
124 with a Multi Angle Laser Light Scattering detector (SEC-MALS) [11] for the APAM  
125 standards, and by batch Static Light Scattering (SLS) for the industrial samples (IPAM). PDI  
126 and weight-average molar masses are gathered in Table 1.

127 For capillary electrophoresis, all samples were used as received and non-filtered. Stock  
128 polyelectrolyte solutions were prepared at 5 g/L as following: 1 g sample was carefully added  
129 into the vortex formed by a pitched-blade impeller in 200 mL buffer and agitated at 500 rpm  
130 for 6 h. As buffer system 20 mM Tris/Cl buffer containing 35.5 mM LiCl, pH 8.0 was  
131 selected, and as comparison, a borate buffer (50 mM boric acid /Li buffer containing 7 mM  
132 LiCl, pH 8.0) was also tested. Before injection, the stock solutions were diluted with the

133 background electrolyte (*i.e.* the sieving medium excluding the sieving polymer) to the desired  
134 injected concentration and homogenized on an orbital mixer for 3 min. Stock solutions kept at  
135 5°C were warmed up to room temperature before use.

136

### 137 *2.3. Free solution and Capillary Gel Electrophoresis*

138 Free solution and CGE were performed on a Beckman P/ACE MDQ apparatus (Sciex,  
139 Villebon sur Yvette, France). Capillaries were prepared from a fused silica tubing (Composite  
140 Metal Services, Photon Lines, France). Unless otherwise stated, the capillary dimensions were  
141 40 cm (30 cm to the detector) × 50 μm ID. The capillary was first activated by successive  
142 flushing (at 20 psi) with 1 M NaOH for 30 min, 0.1 M NaOH 10 min, deionized water 10  
143 min, and finally with the background electrolyte for 10 min. Between runs, capillaries were  
144 successively flushed at 20 psi with 1 M NaOH for 15 min, 0.1 M NaOH for 5 min, deionized  
145 water for 5 min and BGE for 10 min. The BGE for free solution CE consisted in a 20 mM  
146 Tris/Cl buffer solution of pH 8.0 containing varying LiCl concentration ( $n$  mM) providing a  
147 total ionic strength of  $(n + 11)$  mM. A 100 mM borate/Li buffer pH 8.0 containing varying  
148 LiCl concentration ( $n$  mM) was also used, the total ionic strength being  $(7 + n)$  mM. For  
149 CGE, the sieving polymers (pullulan, dextran, PEO) were used as received except HEC that  
150 was purified by dialysis at a cutoff of 50,000 against deionized water overnight and then  
151 lyophilized. The BGE was made up by dissolving the sieving polymer in the buffer of  
152 selected ionic strength under gentle magnetic agitation overnight.

153 Before injection, 1 mL of APAM sample solution was obtained by dilution of the stock 5  
154 g/L APAM solution in the BGE (without sieving polymer) to the desired injected  
155 concentration (comprised between 0.5 and 2 g/L), mixed with 25 μL of DMF (at 0.2 % in  
156 water, electro-osmotic flow marker) and 20 μL of anthraquinone-2-sulfonic acid sodium salt  
157 (2 % in water, mobility marker) on an orbital mixer. Samples were injected at 0.5 psi for 5 s.  
158 Separation voltage of +2 kV (unless otherwise stated) was applied in normal mode (*i.e.*  
159 positive polarity with counter-electroosmotic mode). UV absorption was monitored at 200  
160 nm. Temperature of the sample chamber and of the capillary cassette was set at 25°C.

161

### 162 *2.4. Data processing of the electropherograms*

163 The electropherograms were transformed into effective mobility scale. The effective  
164 mobility was calculated as:

165 
$$\mu = \left( \frac{1}{t} - \frac{1}{t_{eof}} \right) \frac{lL}{V} \quad (2)$$

166 where  $t_{eof}$  is the elution time of the neutral marker (DMF),  $t$  the migration time of the solute,  
 167  $l$  the effective capillary length to the detector,  $L$  the total capillary length,  $V$  the separation  
 168 electric voltage. In this transformation, the y-axis was transformed to a mobility mass-  
 169 weighted distribution function  $P(\mu)$  using the following equation:

170 
$$P(\mu) = \left( \frac{A(t)/t}{\left| \frac{d\mu}{dt} \right|} \right) \approx \left( \frac{A(t)/t}{1/t^2} \right) = A(t) \times t \quad (3)$$

171 with  $A(t)$  the UV absorbance of the detector [24]. The average effective mobility of the  
 172 polymer calculated on the whole polymer distribution was determined according to:

173 
$$\bar{\mu} = \frac{\int P(\mu) \mu d\mu}{\int P(\mu) d\mu} = \frac{\int A(t) t \mu d\mu}{\int A(t) t d\mu} \quad (4a)$$

174 The detector signal is recorded in digitalized form of  $N_{data}$  data points ( $A(t_i); t_i$ ) varying from  
 175 the initial ( $i = 1$ ) to the end time point ( $i = N_{data}$ ) of the polymer signal, eq (4a) can be  
 176 rewritten in its discretized form (4b):

177 
$$\bar{\mu} = \frac{\sum_{i=1}^{N_{data}} A(t_i) t_i \mu_i (\mu_{i+1} - \mu_i)}{\sum_{i=1}^{N_{data}} A(t_i) t_i (\mu_{i+1} - \mu_i)} \quad (4b)$$

178 To get the molar mass distribution, it is first required to normalize the effective mobility  $\mu$   
 179 obtained in sieving conditions to the average free-solution effective mobility  $\bar{\mu}_0$  obtained in  
 180 the same electrophoretic conditions but in the absence of sieving matrix. This normalization is  
 181 important to take into account to correct from the possible differences in polyelectrolyte  
 182 charge density from one sample to the other. For that, the effective mobility distributions were  
 183 transformed in distribution of  $\mu/\bar{\mu}_0$  using the following equation:

184 
$$P\left(\frac{\mu}{\bar{\mu}_0}\right) = \frac{P(\mu)}{d\left(\frac{\mu}{\bar{\mu}_0}\right)} \approx A(t) \times t \times \bar{\mu}_0 \quad (5)$$

185 Assuming the charge density of samples was evenly distributed according to the molar mass  
 186 distribution, the molar mass distribution  $P(M_w)$  is obtained using a calibration curve obtained  
 187 with APAM standards according to equations (5) and (6) [24]:

$$189 \quad \frac{\mu}{\bar{\mu}_0} = \alpha M_w^\beta \quad (6)$$

$$190 \quad P(M) = \frac{P\left(\frac{\mu}{\bar{\mu}_0}\right)}{\left|\frac{dM}{d\frac{\mu}{\bar{\mu}_0}}\right|} = \alpha^{1/\beta} \beta \left(\frac{\mu}{\bar{\mu}_0}\right)^{\beta-1} \times A(t) \times t \times \bar{\mu}_0 \quad (7)$$

191 The calculation of the weight- and the number-average molar mass leading to the dispersity  
 192 index can finally be calculated for each sample using equations (8-10):

$$193 \quad M_w = \frac{\int P(M)M dM}{\int P(M) dM} \quad (8)$$

$$194 \quad M_n = \frac{\int P(M) dM}{\int \frac{P(M)}{M} dM} \quad (9)$$

$$195 \quad PDI = \frac{M_w}{M_n} \quad (10)$$

196

### 197 2.5. Size-Exclusion Chromatography (SEC)

198 Weight and number average molecular mass,  $PDI$ , and radius-of-gyration of APAM standards  
 199 were collected by a SEC-MALS system. The SEC system used consisted of an on-line  
 200 degasser, a high-pressure pump (Agilent 1260 Infinity I), an automatic sampler (Agilent 1260  
 201 Infinity I) a  $8 \times 300$  mm SEC column packed with polyhydroxymethacrylate-based gel  
 202 (OHpak columns, Shodex, Japan), a MALS detector (Dawn Heleos II, Wyatt Technologies)  
 203 and a refractive index detector (Optilab, Wyatt Technologies). Experimental conditions of the  
 204 analysis have been realized according to Jouenne et al. study [11]. A 0.5M sodium nitrate  
 205 solution and 55 mM HEPES sodium buffer (pH 8) was used as the eluent. The eluent was  
 206 filtered through a 0.1  $\mu\text{m}$  cellulose membrane before use. The flow rate was set at 0.3 mL/min



207 mL/min. Sample concentration of 0.02% (w/V) in the eluent. Before injection, the sample  
208 solutions were filtered through a 1.2µm cellulose disposable membrane. The injection volume  
209 was 100 µL. The detector cells of MALS and RI were kept at ambient temperature. The value  
210 of the refractive index increment ( $dn/dc$ ) of each sample was measured by using the Optilab  
211 detector (Wyatt Technology). Data acquisition and processing were carried out using the  
212 ASTRA software (version 6.1, Wyatt Technologies).

213

## 214 2.6. Batch Static Light Scattering (SLS)

215 SLS batch analysis was carried out on a Dawn HELEOS II equipment (Wyatt Technology,  
216 Toulouse, France). Polymer solutions were prepared in an aqueous mobile phase (0.5 M  
217 NaNO<sub>3</sub>) at 0.5 wt % concentration of polymer and mixed with a mechanical stirrer (at 400  
218 rpm), at room temperature for 2 h, to obtain dissolution. The 0.5 wt % polymer solutions were  
219 further diluted to 0.01 wt % and stirred with a stir bar at 200 rpm for 1 h. The diluted  
220 solutions were then filtered through 1.2 µm syringe filters to remove dust and any other large  
221 particle contaminants. The filtered 100 ppm solutions were diluted sequentially to 4-5  
222 different concentrations with a set of two syringe pumps, and then directly injected into a the  
223 MALS detector. The MALS cell temperature was set at 30°C. The flow rate was 0.3 mL/min.  
224 The scattering data were collected at 17 different angles with an incident laser wavelength of  
225 664 nm. Calibration of the MALS detectors was done using HPLC grade toluene. The data  
226 analysis was conducted with Astra 6 software provided by Wyatt Technology (Toulouse,  
227 France). In a batch MALS measurement, the angular and concentration dependent light  
228 scattering data are fitted with Ornstein-Zernick formalism:

$$229 \quad \frac{R}{KC} = M_w \left( 1 + \frac{2q^2 R_g^2}{3D_f} \right)^{\frac{D_f}{2}} \quad (11)$$

230 where  $R$  is the excess Rayleigh ratio,  $K$  the optical constant,  $C$  the polymer concentration,  $M_w$   
231 the weight-average molar mass,  $D_f$  the fractional size,  $q$  the scattering factor and  $R_g$  the radius  
232 of gyration.

233

## 234 3. Results and discussion

235

### 236 3.1. Free solution capillary electrophoresis

237 The electropherograms of the APAM standards in free solution CE obtained in a 20 mM  
238 Tris-HCl buffer containing 85.5 mM LiCl, pH 8 (96.5 mM ionic strength) are presented in  
239 Fig. 1, both in time (Fig. 1A) and in effective electrophoretic mobility scales (Fig. 1B). LiCl  
240 was used instead of NaCl to reduce the conductivity, since  $\text{Li}^+$  has a lower ionic mobility than  
241  $\text{Na}^+$ . The separation is performed under counter-electroosmotic mode on fused silica capillary,  
242 using positive polarity. The transformation of the electropherograms in effective  
243 electrophoretic mobility scale allows to get rid of the EOF fluctuations, as it can be observed  
244 by the perfect alignment of the acrylic acid peak (peak 3) in Fig. 1B. As observed on the  
245 distributions, the effective mobilities of the APAM standards vary between -20 TU and -25  
246 TU (where TU stands for Tiselius Unit,  $10^{-9} \text{ m}^2\text{s}^{-1}\text{V}^{-1}$ ), depending on their polymer charge  
247 density distributions. The mobility distributions of the APAM standards largely overlap one  
248 each other, with slightly faster average mobility for APAM50 and APAM10, in good  
249 agreement with their higher anionicity (see Table 1). The average free solution electrophoretic  
250 effective mobility  $\bar{\mu}_0$  were calculated for each APAM sample according to equation (4b) and  
251 are gathered in Table 1.

252

### 253 3.2. Evaluation of the nature of the sieving polymer

254 As a sieving media, several neutral linear polysaccharides or synthetic polymers were  
255 considered: pullulan (575 KDa), dextran (1.6-2.8 MDa), dextran SGO (50 MDa),  
256 hydroxyethyl cellulose (1.3 MDa) and four polyethylene oxides (PEO) of molar mass varying  
257 between 1.5-8 MDa. Polymers with relatively high molar masses were used in order to reach  
258 large mesh size or blob size  $\xi_b$  (i.e. low polymer concentration) in entangled conditions ( $C >$   
259  $C^*$ ). The entanglement threshold  $C^*$  depends on the polymer molar mass, and can be derived  
260 from the intrinsic viscosity using equation (12) [33]:

$$261 \quad C^* = \frac{1.5}{[\eta]} \quad (12)$$

262

263 The  $C^*$  values of the polymers used in this work are given in Table 2. The 'blob size'  $\xi_b$  is a  
264 decreasing function of the polymer concentration, and does not depend on the polymer molar  
265 mass (as far as the solution is entangled, otherwise it is not defined) according to equation  
266 (13) [40]:

$$\xi_b = \left( \frac{4\pi N_a}{3} \right)^{-\frac{1+a}{3a}} = \left( \frac{K}{6^{\frac{3}{2}}\Phi} \right)^{-\frac{1}{3a}} C^{\frac{1+a}{3a}} \quad (13)$$

268 where  $a$  and  $K$  are the Mark-Houwink coefficients,  $N_a$  the Avogadro constant and  $\Phi$  the  
 269 modified Flory viscosity constant. The characteristics of the polymers including the molar  
 270 mass  $M_w$ , the entangled threshold  $C^*$ , the  $[\eta] - M_w$  relationship, the used concentrations  $C$ ,  
 271 and the blob sizes  $\xi_b$  at the used concentrations are gathered in Table 2. For HEC, PEO and  
 272 pullulan, the used concentrations were chosen close to  $C^*$  to get the largest blob size (164-174  
 273 nm for HEC and PEO) and to limit the viscosity of the entangled polymer solution.

274 The separation of the 6 APAM standards were investigated using pullulan, dextran, dextran  
 275 SGO or HEC at 46.5 mM ionic strength and at a constant electric voltage of +2 kV (50 V/cm)  
 276 (except + 8 kV, i.e. 200 V/cm, for pullulan). The best results were obtained with HEC  
 277 entangled solution, where the 4 first APAM standards with molar mass between  $0.22 \times 10^6$  and  
 278  $3.93 \times 10^6$  g/mol were partially separated (see Fig. 2). However, the analysis of APAM50  
 279  $4.18 \times 10^6$  g/mol and APAM60  $7.34 \times 10^6$  g/mol was perturbed by the aggregation phenomenon  
 280 during the CGE (discussed in the next section), that generates spikes during the  
 281 electrophoretic separation. Compared to the decreasing mobility with molar mass observed in  
 282 the presence of HEC, lower (or even zero) selectivity according to the APAM molar mass was  
 283 recorded in pullulan, dextran or dextran SGO solutions (see Fig. SI1 to SI3), whatever the  
 284 concentration of the separating polymer tested (see Table 2). The lacking sieving ability of  
 285 these polymers might be related to a too low molar mass of the polymer for pullulan or by the  
 286 presence of ramifications for dextrans, which may lead to short mesh lifetime [40]. It can also  
 287 be explained by differences in chain stiffness (pullulan and dextran, 0.4 nm persistence  
 288 length, being much more flexible than rigid HEC 26-40 nm [42]). As for PEO, un-entangled  
 289 1.5 g/L solutions (PEO 1.5, 2 or 5 MDa) at 96.5 mM ionic strength provided good molar mass  
 290 selectivity up to about  $3-4 \times 10^6$  g/mol (i.e. up to APAM30, see Fig. SI4 A and B), however,  
 291 the detection sensitivity was overall lower than in the HEC media. The least sensitivity of  
 292 detection was observed in entangled PEO 8 MDa solution, together with lower baseline  
 293 stability and aggregation of APAM60 (see Fig. SI4 C and D).

294

### 295 3.3. Reduction of aggregation phenomenon

296 The phenomenon of electrohydrodynamically-induced aggregation was already  
 297 encountered in CE of long-chain DNA ( $> 30$  kbp) [43, 44] or polysaccharides [45]. It was

298 explained as the electroneutrality breakdown between the polyanions and the co- and  
299 counterions of the BGE under action of electric field, that extended on a scale of a few  
300 microns far beyond the Debye layer (typically, 1 to 2 nm) [43]. The resulted non-linear  
301 electric force is capable to produce an electrohydrodynamic flow and in turn concentration  
302 fluctuation that leads to the aggregation of the polyanions. The electric field threshold  $E_a$  at  
303 which aggregation occurs is a function of the polymer concentration  $C_p$  and the electrolyte  
304 concentration  $C_s$  according to the following scaling law:

$$305 \quad E_a \sim \sqrt{\frac{C_s \eta}{C_p}} \quad (14)$$

306 where  $\eta$  is the solution viscosity. Equation (14) predicts that aggregation occurs at higher  
307 electric field strength by increasing the electrolyte concentration and/or by decreasing the  
308 polymer concentration.

309

### 310 *3.3.1. Impact of ionic strength and sample concentration on aggregation reduction*

311 In addition to its beneficial effect on aggregation phenomenon, it is known that higher  
312 ionic strength decreases the electrophoretic mobility of solutes and electroosmotic mobility  
313 [46], and also leads to more compact conformations of polyelectrolytes [47]. The impact of  
314 ionic strength on aggregation was significant: by increasing ionic strength from 46.5 mM to  
315 96.5 mM at 2 kV (50 V/cm) in the HEC solution, APAM50 and APAM60 aggregated much  
316 less, as demonstrated in Fig. 3 with better peak shape for the APAM signals. Further increase  
317 of the background electrolyte ionic strength from 96.5 mM to 136.5 mM (or even 196.5 mM)  
318 did not lead to improvement of the separation, while the total run time was severely increased  
319 due to decreased apparent electrophoretic mobility, both in sieving and free solution  
320 conditions. Thus, 96.5 mM ionic strength was considered as a good balance between  
321 separation performance and analysis time. As expected [43], lower injected concentration (1.5  
322 g/L vs 2 g/L) also tends to reduce the adverse effect of aggregation. However, due to limited  
323 sensitivity of detection of APAM, it was hardly possible to reduce the injected concentration  
324 below 0.5 g/L.

325

### 326 *3.3.2. Impact of the electric field strength on aggregation reduction*

327 Since aggregation was induced by action of the electric field, decreasing the electric field  
328 strength was an effective counteraction. In the HEC solution at the optimized ionic strength of  
329 96.5 mM, decreasing the applied separation voltage from +2 kV (50 V/cm) to +1 kV (25

330 V/cm) effectively suppressed the aggregation of high polyelectrolytes (see Fig. SI5), but the  
331 peak broadening due to late migration times generated low signal to noise ratio. Therefore, the  
332 separation voltage was retained at + 2 kV in the following sections.

333

#### 334 *3.4. Effect of the nature of the buffering species on the size-based separation*

335

336 Surprisingly, changing the Tris/Li/Cl buffer by a Li/borate/Cl buffer, and keeping constant  
337 the other conditions (ionic strength at ~100 mM, pH 8, and 0.8 g/L HEC), led to much lower  
338 size-based selectivity of the APMA standards (see Fig. SI6), indicating Tris/Li/Cl buffer was  
339 better suited for HEC sizing of APAMs. One possible explanation of this effect could be that  
340 the selectivity obtained in HEC/Tris sieving medium is partly due to a transient interaction  
341 between the APAM polyelectrolyte and the neutral separating HEC chain (as already  
342 observed for DNA separation [48]), and not only due to a sieving mechanism. In the context  
343 of such prevailing separation mechanism, the nature of the buffering ions could change the  
344 strength of the APAM / HEC interaction, which would be higher in the case of Tris/Li/Cl  
345 buffer compared to Li/borate/Cl buffer.

346

#### 347 *3.5. Reduced electrophoretic mobility distributions*

348 The effective mobility distributions presented in Fig. 3D were transformed in distribution  
349 of  $\mu/\bar{\mu}_0$  according to equation (5), as presented in Fig. 4A. This transformation aimed at a  
350 correction of the impact of slightly higher charge density values for samples APAM 50 and  
351 APAM 10, leading to a smoother ‘non-biased’ variation of the reduced effective mobility  
352 according to the molar mass.

353 Under the optimized conditions established with APAM standards, CGE was performed on  
354 4 industrial samples (IPAM) of molar mass up to 15 MDa (as estimated by SLS, see Table 1)  
355 and an anionicity between 27 and 33%. The results were presented in Fig. 4B.  
356 Electropherograms obtained in free solution for the IPAM samples under similar  
357 electrophoretic conditions can be found in Fig. SI7.

358

#### 359 *3.6. Electrophoretic mobility – molar mass calibration curve*

360 The size-based selectivity obtained for the APAM (standards) in 0.8 g/L HEC at +2 kV  
361 and 96.5 mM ionic strength is quantitatively represented on a Ferguson-like plot (see the red  
362 dots in Fig. 5), where the log of the average reduced electrophoretic mobility ( $\bar{\mu}/\bar{\mu}_0$ ) is

363 plotted against  $\log M_w$ . The weight-average molar mass  $M_w$  of the APAM standards was  
364 obtained by SEC-MALS and varied between  $0.22 \times 10^6$  and  $7.34 \times 10^6$  g/mol (chromatograms  
365 are presented in Figure SI8). The average effective mobility of the polyelectrolyte migrating  
366 in the presence of HEC solution  $\bar{\mu}$ , was calculated according to equation (4) to the average  
367 effective mobility in free solution ( $\bar{\mu}_0$ ). The latter was separately determined in the absence of  
368 the sieving solution under the same experimental conditions using equation (4b). A linear log-  
369 log correlation calculated on the APAM standards (red dots) was obtained leading to the  
370 following scaling law by least-square regression on the APAM standards:  $\log(\bar{\mu}/\bar{\mu}_0) = (-$   
371  $0.113 \pm 0.007) \times \log M_w - (0.109 \pm 0.004)$  ( $M_w$  in  $10^6$  g/mol),  $R^2 = 0.9864$  or  $\bar{\mu}/\bar{\mu}_0 =$   
372  $(0.778 \pm 0.007) \times M_w^{(-0.113 \pm 0.007)}$ . As expected, the slope was far from -1 value predicted for a  
373 pure reptation regime, suggesting that the migration was strongly electric field-dependent  
374 (biased reptation with orientation), and possibly mixed with a transient polyelectrolyte / HEC  
375 interaction, as discussed in section 3.4.

376

### 377 *3.7. Calculation of molar mass distribution and polydispersity of industrial samples*

378 The molar mass distributions  $P(M)$  of the standard and industrial samples were calculated  
379 from the electropherogram in the reduced electrophoretic mobility scale according to equation  
380 (6) and (7), where the calibration equation is derived from Fig. 5 as  $\bar{\mu}/\bar{\mu}_0 =$   
381  $(0.778 \pm 0.007) \times M_w^{(-0.113 \pm 0.007)}$ . Fig. 6 displays the area-normalized molar mass distributions  
382  $P(\log M) \sim P(M) \times M$  as a function of  $\log M$  for the standard and industrial samples. In these  
383 optimized conditions, the molar mass distribution can be obtained up to 10 MDa without  
384 significant effect of the aggregation. Above 10 MDa, the molar mass distribution can be  
385 impacted by the aggregation effect as visualized by the multiple spikes for the IPAM 3 and 4  
386 samples (see Fig. 6B). A comparison on the Ferguson-like plot using  $M_w$  from SLS is  
387 displayed in Fig. 5 for the IPAM samples, showing a reasonable extrapolation of the  $\log(\bar{\mu}/$   
388  $\bar{\mu}_0)$  vs  $\log M_w$  correlation for molar mass higher than the largest APAM standard (i.e.  $M_w >$   
389 7.34 MDa). The average molar mass  $M_w$  (CGE), of the industrial samples calculated by  
390 integration of the molar mass distributions according to equation (8), as well as the  
391 polydispersity of all samples using equation (10) are gathered in Table 1. The  $M_w$  values  
392 obtained by CGE for most the IPAM industrial samples: 6.5 MDa (IPAM2), 10.1 MDa  
393 (IPAM3) and 14.9 MDa (IPAM4), are similar to those obtained by SLS (8, 10.5 and 15 MDa,  
394 respectively) within the confidence interval (calculated as  $\pm$  one SD on  $n=3$  repetitions). The

395  $M_w$  CGE values of IPAM1 industrial sample (4.3 MDa) is in better agreement with the SEC  
396 value (4.3 MDa), but lower than the SLS one (6.5 MDa).

397 As for the polydispersity, and as expected, APAM standards have relatively low  
398 polydispersity indexes ranging between 1.2 and 1.5 in CGE; which are in reasonably good  
399 agreement with those obtained by SEC for most of the APAM samples. Significantly smaller  
400 PDI were obtained by CGE for APAM 10 and APAM 50 samples compared to SEC, which  
401 may be related to higher peak broadening in SEC compared to CGE, as observed in previous  
402 studies [25,49,50]. As for IPAM industrial samples, polydispersity indexes are obviously  
403 much higher than for APAM samples, and are comprised between 2.4 and 2.9 for IPAM 1 and  
404 IPAM 2 samples. It is worth noting that IPAM samples cannot be analyzed by SEC because  
405 of shear degradation occurring through the columns, adsorption phenomena and lack of  
406 separation due to actual SEC column technology. The main limitation is due to the exclusion  
407 limit of the gel matrix used in SEC [11]. IPAM 3 and IPAM 4 polydispersities could not be  
408 accurately determined by CGE due to the presence of spikes above 10 MDa (aggregation  
409 phenomenon).

410 On the whole, one can conclude that the  $M_w$  values obtained by CGE are quite consistent with  
411 those obtained from other methods within the experimental confidence intervals. CGE can be  
412 used to determine average molar mass, molar mass distribution and polydispersity index of  
413 APAM/IPAM samples up to about 10 MDa.

414

#### 415 **4. Conclusion**

416 The molar mass-based characterization of anionic polyacrylamides by CGE using  
417 entangled HEC solutions as sieving media was successfully achieved up to about 10 MDa by  
418 unifying two prerequisites: analysis of high polymers was made possible by aggregation  
419 reduction and HEC was capable to size the whole range of the molar masses studied. The  
420 sample aggregation was reduced by optimization of the operating parameters: low electric  
421 field strength (50 V/cm), relatively high ionic strength of 96.5 mM, low sample concentration  
422 ( $\sim 1$  g/L). The buffer nature (TRIS) was also found crucial to get appropriate selectivity that is  
423 both depending on sieving mechanism and possible polyelectrolyte / HEC transient  
424 interaction. Aggregation was also promoted by the presence of separating polymer, while no  
425 detectable aggregation was observed in free solution under the same conditions.

426 Based on the  $\log(\bar{\mu} / \bar{\mu}_0) - \log M_w$  relationship established with the APAM standards, the  
427 molar mass distribution and the polydispersity index of the APAM and IPAM samples were

428 obtained as far as the molar mass did not exceed 10 MDa. The molar mass values were  
429 similar to those determined by SLS, proving the potential of CGE in molar mass based  
430 characterization of high ultra-high molar mass polyelectrolytes up to about 10 MDa. Further  
431 or future developments may focus on the limitation of the aggregation above this threshold.

432

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436

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580 **FIGURE CAPTIONS**

581 **Figure 1.** Free solution electropherograms of standards (APAM) obtained at 96.5 mM ionic strength in time  
582 scale (A) and in effective electrophoretic mobility scale (B). Experimental conditions: Fused silica capillary 40  
583 cm (30 cm to the detector) × 50 μm ID. BGE: 20 mM Tris/Cl buffer containing 85.5 mM LiCl, pH 8. Separation  
584 voltage: + 2 kV. Injection: 0.5 psi, 3 s. Sample concentration: 1 g/L APAM + 0.05% DMF in BGE. UV detection  
585 at 200 nm. Temperature: 25°C. Identification: DMF (1), APAM (2) and acrylic acid (3). Acrylic acid was  
586 originally present in the polymer samples.

587 **Figure 2.** Electropherograms obtained for the size-based separation of APAM standards in HEC solution at  
588 46.5mM ionic strength and +2 kV in time scale (A) and effective mobility scale (B). Experimental conditions:  
589 Fused silica capillary 40 cm (30 cm to the detector) × 50 μm ID. BGE: HEC: 0.8 g/L (blob size: 164 nm) in 20  
590 mM Tris/Cl buffer containing 35.5 mM LiCl, pH 8. Electric voltage: +2 kV. Injection: 0.5 psi, 3 s. Sample  
591 concentration: 1 g/L APAM + 0.05 % DMF. UV detection at 200 nm. Temperature: 25°C. Identification: DMF  
592 (1), APAM (2), acrylic acid (3).

593 **Figure 3.** Electropherograms obtained for the size-based separation of APAM standards in HEC solution at 96.5  
594 mM ionic strength in time scale (A, C) and effective mobility scale (B, D) at different injected concentrations (A,  
595 B: 2 g/L; and C, D: 1.5 g/L). Experimental conditions: Fused silica capillary 40 cm (30 cm to the detector) × 50  
596 μm ID. BGE: HEC: 0.8 g/L (blob size 164 nm) in 20 mM Tris/Cl buffer containing 85.5 mM LiCl, pH 8.  
597 Electric voltage: + 2 kV (50 V/cm). Injection: 0.5 psi, 3 s. Sample concentration: 1.5 g/L (C, D) or 2 g/L (A, B)  
598 APAM + 0.05% DMF. UV detection at 200 nm. Temperature: 25°C. Identification: DMF (1), APAM (2),  
599 mobility marker: anthraquinone sulfonic acid (3).

600 **Figure 4.** Reduced electrophoretic mobility distributions  $P(\mu/\bar{\mu}_0)$  of APAM standards (A) and IPAM  
601 industrial samples (B) in 0.8 g/L HEC Tris/Cl electrolyte at 96 mM ionic strength. Experimental conditions as in  
602 Fig. 3. Injected concentrations at 1.5 g/L (A) and 1 g/L (B).

603 **Figure 5.**  $\log(\bar{\mu}/\bar{\mu}_0)$  vs  $\log M_w$  plot of APAM standard (●) in 0.8 g/L HEC solution and for IPAM industrial  
604 samples with  $M_w$  from SLS (▼). The calibration curve obtained on the APAM samples reads:  $\log(\bar{\mu}/\bar{\mu}_0) = -$   
605  $(0.1129 \pm 0.0069) \log M_w - (0.109 \pm 0.0040)$  ( $M_w$  in g/mol),  $R^2 = 0.9864$ . Expected molar masses for IPAM  
606 industrial samples (●) obtained by CGE by integration of the polymer peak of Fig. 6B using equation (8).  
607 Experimental conditions as in Fig. 3.

608 **Figure 6.** Area-normalized molar mass distribution ( $P(\log M) \sim P(M) \times M$ ) of APAM standards (A) and IPAM  
609 industrial samples (B) in HEC solution. Experimental conditions as in Fig. 3.

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611