Determination of ultrahigh molar mass of polyelectrolytes by Taylor dispersion analysis

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ABSTRACT

Taylor dispersion analysis (TDA) was successfully applied to obtain broadly distributed, ultrahigh molar masses of industrial anionic polyacrylamides (IPAMs) up to 25 $\times 10^6$ g/mol, far beyond the detection limit of SEC (about 7.3×10^6 g/mol for anionic polyacrylamides standards). Two protocols of TDA differing in capillary surface and rinsing procedure were employed: *(i)* bare fused silica capillaries under intensive between-run rinsing with 1M NaOH, and (*ii*) capillaries coated with polyelectrolyte multilayers composed of polydiallyldimethylammonium chloride polycation and sodium polystyrenesulfonate polyanion under simple rinsing with background electrolyte. Both cases led to similar results and in agreement with those obtained by static light scattering, the rinsing capillary step being much shorter in the second case (8 min instead of 30 min). The data processing of the obtained taylorgrams was realized using multiple-Gaussian fitting of the overall taylorgrams, by separating the contribution of low molar mass impurities from the polymeric profiles, and by determining the mean hydrodynamic radii and diffusion coefficients of the polymers. The molar masses of ultra-high molar mass industrial anionic polyacrylamides were derived from the hydrodynamic radii according to $\log R_h$ *versus* $\log M_w$ linear correlation established with APAM standards. Compared to capillary gel electrophoresis for which the size separation was only feasible up to $M_w \sim 10 \times 10^6$ g/mol due to field induced polymer aggregation, TDA largely extended the range of accessible molar mass with easy-to-run and time saving assays.

Keywords: Taylor dispersion analysis, size-based separations, ultrahigh molar mass determination, polyacrylamides

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Highlights

- Linear logR_h *vs* logM_w correlation obtained by TDA on a broad range of molar masses
- Analysis of ultrahigh molar mass polyacrylamides up to 25×10^6 g/mol by TDA
- TDA results were consistent with those obtained by static light scattering
- TDA offers a straightforward, absolute, easy-to-run and rapid method for size-based characterization

1. Introduction

In numerous industrial applications such as wastewater treatment and enhanced oil recovery, high to ultrahigh molar mass (above 1 $\times 10^6$ g/mol) anionic poly(acrylic acid-co-acrylamide)s have been playing an important role as media thickener for decades [1-3]. However, characterization of unusually high molar mass polyelectrolytes remains challenging and laborious. Due to inherent separation limits of conventional SEC, such as abnormal elution due to polymer adsorption or chain breakage in the column packing, polyacrylamide copolymers with molar masses higher than 7.5 $\times 10^6$ g/mol cannot be analyzed by SEC [4-7]. It was found that higher molar mass polyelectrolytes, when passing through a porous medium, are sensible to mechanical degradation based on coil-stretch transition of polymer chains above a critical extension rate, leading to central scissions of polymers in diluted regime and random scissions in semi-diluted regime [5]. Moreover, largely polydisperse polymers usually elute in abnormal overlapped peaks that cannot be resolved by SEC. Other techniques are pursued with certain success including in-batch multi-angle laser light scattering (MALLS) [9, 10] and asymmetric field-flow fractionation [11, 12]. In a previous study [13], we reported the performance of capillary gel electrophoresis (CGE) with 2-hydroxyethyl cellulose (HEC) as sieving polymer for the characterization of ultrahigh molar mass industrial polyacrylamides (IPAMs) up to 10 $\times 10^6$ g/mol, in agreement with the results obtained by static light scattering (SLS). However, the limit of CGE lied in the problem of aggregation of the IPAMs under action of electric fields [14], as well as field-dependent biased reptation of the polyelectrolyte chains.

To alleviate the problem of aggregation, Taylor dispersion analysis (TDA) is an interesting alternative that applies a mobilizing pressure instead of an electric field as driving force in the capillary. TDA is based on the dispersion of sample plug injected in narrow capillary (typically 50 µM i.d.) under a Poiseuille-like flow. The Taylor dispersion is due to the combination of the convective parabolic velocity profile with molecular diffusion. TDA is an absolute method (no calibration is required) allowing to determine the determine diffusion coefficients (D) from the dispersion (temporal variance) of the elution profile. D and hydrodynamic radii (R_h) of large varieties of solutes were determined by TDA including macromolecules [15-18], nanoparticles [19-21], proteins [22, 23] and more recently, vaccine antigens and/or adjuvants [24]. Regarding the limits of analysis of TDA, it is predicted that R_h should be smaller than 210 nm on a 50 µm i.d. capillary to keep the relative error ε of diffusion coefficient D below 5%. For higher solute sizes, convective and hydrodynamic

chromatography (HDC) regimes would occur, leading to non-Gaussian elution profiles (see e.g. Fig. SI1 to visualize the different regimes) [22].

In this work, we investigated the potential of TDA for the size (hydrodynamic radius) and molar mass characterization of high to ultrahigh molar mass industrial anionic poly(acrylic acid-co-acrylamide)s (IPAMs). Due to the concern of maintaining the ultrahigh molar mass IPAMs within Taylor regime, the increase of the background electrolyte ionic strength has been investigated to get more compact polymer conformations. To check the impact of the capillary wall surface on the TDA results, two experimental protocols using *(i)* bare capillaries combined with rigorous between-run rinsing with 1M NaOH and *(ii)* SMIL (Successive Multiple Ionic Layers)-coated capillaries based on polydiallyldimethylammonium chloride as a polycation and sodium polystyrene sulfonate as a polyanion with simple rinsing with background electrolyte.

2. Experimental

2.1. Chemicals

Tris(hydroxymethyl)aminomethane (TRIS) and polydiallyldimethylammonium chloride (PDADMAC) 20%, M_w = 400,000-500,000 g/mol, 4-(2-Hydroxyethyl)piperazine-1-ethane sulfonic acid (HEPES), tris(hydroxyméthyl) aminométhane (Tris), sodium hydroxide, lithium chloride anhydrous, and lithium hydroxide were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France), sodium polystyrenesulfonate (PSS), M_w=70,000 g/mol, from Acros (Illkirch, France), hydrochloric acid and potassium chloride from VWR (Fontenay-sous-Bois, France), and finally sodium chloride from Fluka (Illkirch, France).

2.2. Samples

The anionic poly(acrylic acid-co-acrylamide)s industrial samples (IPAMs) and standard samples (APAMs) were provided by SNF Floerger (Andrézieux, France). APAMs were synthesized from monomers of sodium acrylate and acrylamide by controlled radical polymerization (CRP) with narrowly distributed molar mass and anionicity [26]. IPAMs were obtained by hydrolysis of polyacrylamide in a NaOH medium to the defined anionicity. The molar mass and polydispersity index (PDI) of the APAMs were determined by SEC-MALS [7], and the molar mass of the IPAMs by batch-SLS (see Table 1).

Stock solutions of APAMs and IPAMs were prepared in small scale by dissolving 50 mg sample in 10 mL background electrolyte (BGE) under magnetic stirring at 500 rpm for 6 h. The BGE was composed of 20 mM Tris/HCl buffer at pH 8.0 containing 35.5 mM LiCl (total ionic strength I = 46.5 mM). For the study of the impact of ionic strength, BGE of the same Tris/Cl buffer containing 1M NaCl or 1M KCl were used. The stock solutions were kept at 5°C before and after use. The test solutions were then prepared by dilution of the stock solutions with BGE to the injected concentration of 2 g/L and homogenized using an orbital mixer for 3 min. All samples were used non-filtered.

2.3. Size-Exclusion Chromatography (SEC-MALS)

Weight and number average molar mass, PDI, and radius of gyration of APAMs were obtained by a SEC-MALS Agilent 1260 Infinity I system (Agilent Technologies, Les Ulis, France). It consisted of an on-line degasser, a high-pressure pump, an automatic sampler, a 8 × 300 mm SEC column packed with polyhydroxymethacrylate-based gel (OHpak Shodex columns, Showa Denko, Munich, Germany), a multi-angle light scattering (MALS) detector (Dawn Heleos II, Wyatt Technology, Toulouse, France) and a refractive index (RI) detector (Optilab, Wyatt Technology, Toulouse, France). Experimental conditions were given in [7]. Buffer containing 0.5 M sodium nitrate and 55 mM HEPES (pH 8) was used as the eluent that was filtered through a 0.1 µm cellulose membrane before use. The flow rate was set at 0.3 mL/min. Samples of 0.02% (w/V) concentration in the eluent were filtered through a 1.2 µm cellulose membrane before injection. The injection volume was 100 µL. The detectors of MALS and RI were kept at room temperature. The refractive index increment (dn/dc) of the samples were measured by using the Optilab detector. Data acquisition and processing were achieved by the ASTRA software (version 6.1, Wyatt Technologies).

2.4. SLS

SLS batch analysis was carried out on the Dawn Heleos II equipment (see 2.3). Polymer solutions were prepared in 0.5 M sodium nitrate at 0.5 wt % and mixed under mechanical stirring (at 400 rpm) at room temperature for 2 h till dissolution. The 0.5 wt % polymer solutions were further diluted to 0.01 wt % under stirring with a magnetic stirrer at 200 rpm for 1 h, then filtered through 1.2 µm syringe filters to remove dusts and other large particle contaminants. The filtered solutions were diluted sequentially to 4-5 different concentrations by two syringe pumps before injection into the MALS detector. The MALS cell temperature

was set at 30°C, the flow rate at 0.3 mL/min. The scattering data were collected at 17 different angles with an incident laser wavelength of 664 nm. Calibration of the MALS detector was performed using HPLC grade toluene. The data analysis was conducted using the Astra 6.1 software (see 2.3). In a batch MALS measurement, the angular and concentration dependent light scattering data were fitted with Ornstein-Zernick equation:

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

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

2.5. Taylor dispersion analysis (TDA)

TDA experiments were performed on a Beckman P/ACE MDQ apparatus (AB Sciex Life Sciences Holding, Villebon-sur-Yvette, France). Capillaries were prepared from a fused silica tubing (Photonlines, St Grégoire, France) with dimensions of 60 cm (50 cm to the detector) × 50 µm ID. Capillaries were first activated by successive flushing at 20 psi with 1 M NaOH for 30 min, 0.1 M NaOH for 10 min, Milli-Q water for 10 min, and finally background electrolyte (BGE) for 10 min. BGE was 20 mM Tris/HCl with 35.5 mM LiCl, I = 46.5mM, pH 8.0. For the study of the impact of ionic strength on hydrodynamic radii, BGE was also prepared by adding 1M NaCl or 1M KCl instead of 35.5 mM LiCl.

Protocol of intensive between-run rinsing consisted of successively flushing the bare capillary with 1 M NaOH at 35 psi for 10 min, 0.1 M NaOH at 20 psi for 5 min, Milli-Q water at 20 psi for 5 min and BGE at 20 psi for 10 min, leading to total rinsing time of 30 min.

In a second approach, capillaries were coated as following: *(i)* activation with 1 M NaOH for 20 min at 20 psi, *(ii)* flushing with a solution of PDADMAC polycation at 3 g/L in 20 mM HEPES pH 7.4 at 20 psi for 7 min, *(iii)* rinsing with 20 mM HEPES pH 7.4 at 20 psi for 3 min, *(iv)* flushing with a solution of PSS polyanion at 3 g/L in 20 mM HEPES pH 7.4 at 20 psi for 7 min, *(v)* rinsing with 20 mM HEPES pH 7.4 at 20 psi for 3 min, and *(vi)* repetition of the *(ii)* to *(v)* steps. $(PDADMAC/PSS)_2$ SMIL coated capillary was finally rinsed with BGE for 8 min before sample injection. Between-run rinsing was carried out with BGE at 20 psi for 8 min.

Samples were injected hydrodynamically at 0.5 psi for 7 s. The mobilizing pressure was 0.4 psi. UV absorption was detected at 200 nm. Temperature was set at 25°C.

2.6. Theoretical aspects

In TDA [27-29], when a solute plug is injected into a fluid flowing under laminar Poiseuille profile through a capillary, it spreads out under the combined action of molecular diffusion and the variation of velocity over the cross-section. The distribution of concentration thus generated is centred on a point which moves with the mean speed of flow. The elution profile is recorded at a certain distance from the injection point, via UV absorbance of the polymer solute through the capillary tube. For a single size population, the elution profile is normally distributed (Gaussian), as far as two conditions are satisfied: *(i)* the axial diffusion should be negligible compared to convection (*i.e.* the Péclet number $P_e = R_c u/D > 40$) where R_c is the capillary radius, u is the linear velocity and D is the molecular diffusion coefficient, and *(ii)* the average elution time t_0 should be longer than the characteristic diffusion time τ of the solute (*i.e.* $\tau = t_0 D/R_c^2 > 1.25$) [25].

For polydisperse samples, the taylorgram is a sum of Gaussians [17]:

$$S(t) = \sum_{i=1}^{n} S_i(t) = \sum_{i=1}^{n} \frac{A_i}{\sigma_i\sqrt{2\pi}} exp\left[\frac{-(t-t_0)^2}{2{\sigma_i}^2}\right] \quad (2)$$

where $S(t)$ is the elution signal at time t, $S_i(t)$ the Gaussian of the component i and σ_t is the temporal variance of the component i. The temporal variance σ^2 of the overall taylorgram is calculated by integration of the taylorgram using Eq. 3:

$$\sigma^2 = \frac{\int S(t)(t-t_0)^2 dt}{\int S(t) dt} \quad (3)$$

From the overall temporal variance, an average diffusion coefficient $\langle D \rangle$ can be deduced from Eq. 4:

$$\langle D \rangle = \frac{{R_c}^2 t_0}{24\sigma^2} = \frac{{R_c}^2 t_0}{24} \frac{\int_0^\infty S(t) dt}{\int_0^\infty S(t)(t-t_0)^2 dt} \quad (4)$$

Finally, the average hydrodynamic radius $\langle R_h \rangle$ (a weight-average value for mass concentration sensitive detector) is derived from Stokes-Einstein equation (Eq. 5):

$$\langle R_h \rangle = \frac{k_B T}{6\pi\eta\langle D \rangle} \quad (5)$$

In this work, data processing of taylorgrams including Gaussian fitting (up to 3 Gaussians using Excel solver), calculation temporal variances, diffusion coefficients and hydrodynamic radii of the polymers were achieved with a lab-made Excel spreadsheet.

3. Results and discussion

The objective of this work was to develop and apply a TDA methodology to characterize the size (hydrodynamic radius) and molar mass of ultrahigh molar mass industrial samples (IPAM). All samples possess an anionicity between 27 and 33%. Since TDA gives access to the diffusion coefficient (and hydrodynamic radius) instead of molar masses, $\log R_h$ *vs* $\log M_w$ correlation was experimentally determined by TDA using 6 standard samples (APAMs) of molar mass ranging between 2.2 kDa and 7.3 MDa. These APAM standards were fully characterized by SEC with relatively narrow PDI ranging between 1.1 and 1.5 (see Fig. SI2 for the molar mass distributions). The molar masses of 5 IPAM samples were obtained by SLS and found between 6.5 MDa and 25 MDa (see Fig. SI3 for the molar mass determination by SLS). All APAM and IPAM predetermined characteristics are provided in Table 1.

Efforts to improve the robustness of TDA data was emphasized on minimizing the adsorption of anionic polyacrylamides onto the capillary surface that is governed by an equilibrium between attractive interactions (H-bonding between silanol groups present at the capillary wall surface and amide groups of the polymer) and electrostatic repulsion, depending on several parameters including pH, molar mass and dispersity of the polymer and ionic strength of BGE [31-33]. In this study, pH of the background electrolyte (BGE) was set at 8.0, at which the capillary surface was negatively charged and the anionic polyacrylamides remained stable without remarkable hydrolysis. To keep the capillary wall under 'steady-state conditions' to achieve good intra-capillary repeatability, two different protocols were employed for comparison. In the first case, an intensive between-run rinsing process using 1 M NaOH at 35 mbar for 10 min followed by 0.1 M NaOH at 20 psi for 5 min, Milli-Q water at 20 psi for 5 min and BGE at 20 psi for 10 min, was undertaken in order to eliminate previous history of the fused silica capillary before a new run, especially when ultrahigh molar mass polymers were assayed, with the expense of re-equilibration time (for a total rinsing time of 30 min). In the second case, the capillary surface was coated with SMIL [34, 35], namely two PADMAC/PSS bilayers, to enhance electrostatic repulsion between the capillary wall and the analytes, using simple between-run rinsing with BGE for 8 min only. The reproducibility of TDA in R_h determination was checked by intra-capillary repeatability by repeating 3 assays per sample on the same capillary and inter-capillary reproducibility by running all samples successively on 3 different capillaries.

3.1. TDA on bare fused silica capillary

In TDA, the original taylorgrams (Fig. 1A for APAMs and Fig. 1B for IPAMs) were bimodal and contained an additional sharp peak that is due to the residual monomers and dimers as well as UV absorbing impurities such as urea, according to the provider. To extract hydrodynamic information of the polymer, each taylorgram was deconvoluted into 2 to 3 Gaussian profiles using Excel Solver according to eq. (2). After subtraction of the small molecule contribution that corresponded to sizes between 0.38 nm and 0.60 nm, the remaining polymer profile was obtained and shown in Fig. 1C (for APAMs) and Fig. 1D (for IPAMs) with normalization at the maximum intensity for better interpretation. Narrowly distributed APAMs appeared relatively symmetric, and peak widths broadened systematically with increasing the molar mass, as expected according to Taylor dispersion. Slight distortion of the elution profile could be observed for the IPAM samples and was likely due to some polymer adsorption on the capillary wall that was not observed with APAMs. To avoid any bias in the average size determination, Gaussian fitting and calculation of the peak variance σ^2 were only operated on the left half part of the taylorgram relative to the peak apex (t_0).

All the hydrodynamic radius values obtained by TDA are presented in Table 1. To get a better insight about the repetability / reproducibility of the TDA measurements, n = 3 repetitions on m=3 fused silica capillaries were performed. RSD on the average R_h determination were always below 10% for both repeatability and reproducibility. Fig. 2 shows the logR_h – logM_w correlations lines obtained for the APAM standards (see plain blue dots and triangles for 20 mM Tris/HCl pH 8.0 with 35.5 mM LiCl eluent (I = 46.5 mM)). Inter-capillary mean hydrodynamic radii are presented in this Figure with inter-capillary RSD as error bars. The molar masses used for this representation were obtained by SEC-MALS for APAM samples and by SLS for IPAM samples (see sections 2.3 and 2.4 for experimental conditions and see Table 1 for the data). The least squared regression displays good correlation ($\langle R_h \rangle = 31.020 \times M_w^{0.5346}$, $R^2 = 0.9926$, with M_w in 10^6 g/mol and R_h in nm) and extended into the data range of IPAMs with very good agreement, on a range of R_h reaching up to 183.7 nm for IPAM5. This result proves that the experimental TDA conditions allows a true (non-biased) determination of large polymer sizes, even at moderate ionic strength (46.5 mM). It is worth noting that the conditions of validity of the Taylor regime were always fulfilled (i.e. $P_e = R_c u/D > 40$ and $\tau = t_0 D/R_c^2 > 1.25$) for all polymer samples, even for the largest IPAM5 ($P_e \sim 4400$; $\tau \sim 4.5$). This was ensured by relatively low mobilization pressure to let the time to the large polymer solutes to average the parabolic velocity profile during the analysis time. Using the logR_h – logM_w correlation obtained with the APAM samples, it was

possible to determine the TDA molar masses ($M_{w1,\,TDA}$) by using the reciprocal of the $R_h - M_w$ correlation (M_w = 0.00162 × $\langle R_h \rangle^{1.8706}$, for bare fused silica capillary (case 1) and M_w = 0.00195 × $\langle R_h \rangle^{1.7986}$, for PDADMAC/PSS SMIL-coated capillary (case 2), with M_w in 10^6 g/mol and R_h in nm). Regarding the limit in size that can be determined by TDA due to occurrence of the hydrodynamic chromatography regime that can affect the accuracy of the R_h determination, a maximum R_h value of 212 nm is expected as the upper limit in size if we accept a maximum relative error of 5% (as calculated by $R_h \leq 0.17 \times R_c \times \varepsilon$, where ε is the relative error [22]).

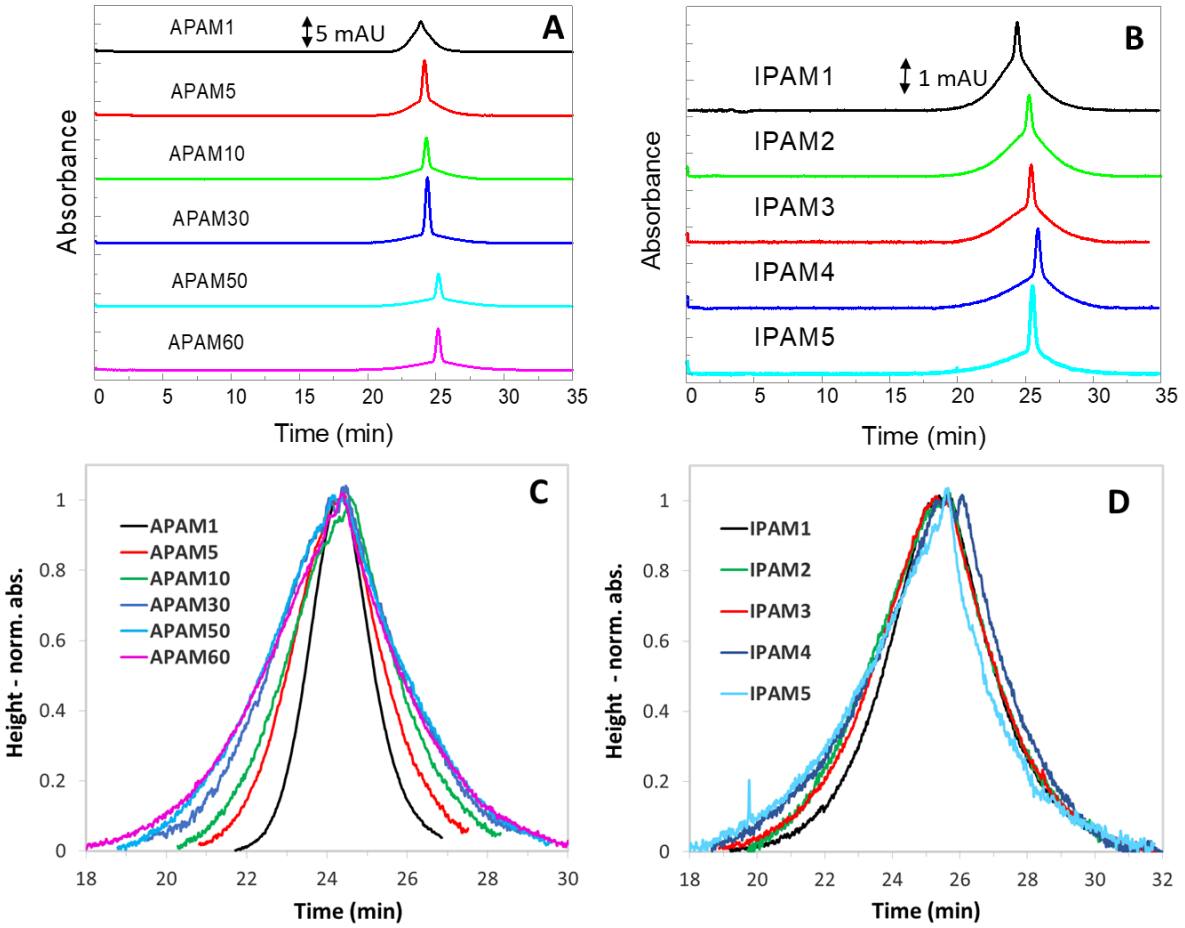
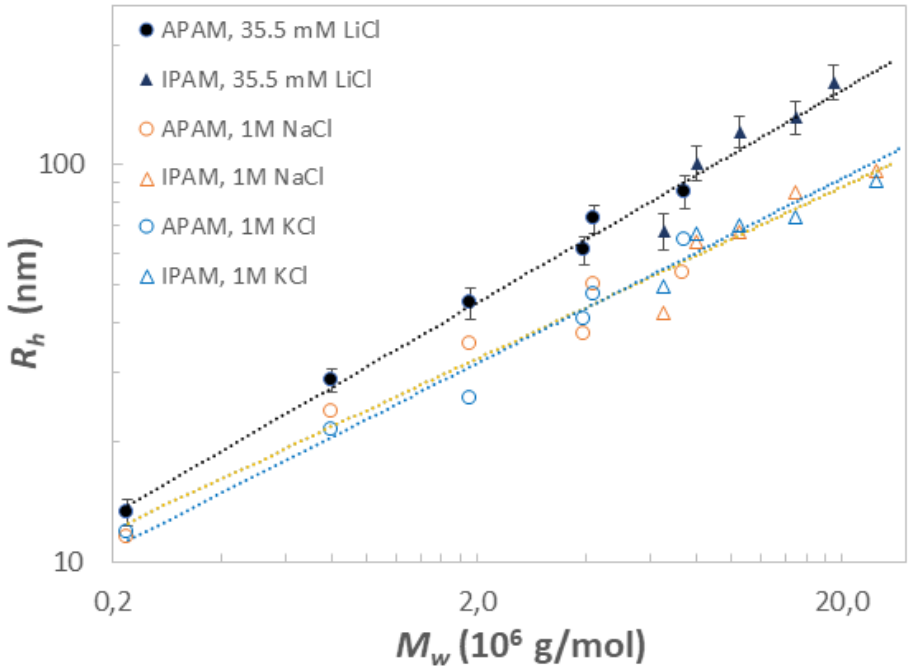


Figure 1. Original taylorgrams of poly(acrylic acid-co-acrylamide) standards (APAMs) (A) and industrial samples (IPAMs) (B), and the corresponding height normalized taylorgrams subtracted from small molecules contribution (C and D, respectively). Experimental conditions: fused silica capillary 50 µm ID × 60 cm (50 cm to the detector). Eluent: 20 mM Tris/HCl with 35.5 mM LiCl, I = 46.5 mM, pH 8.0. Mobilization pressure: 0.4 psi. Injection: 0.5 psi, 7 s. Injected sample concentration: 2 g/L in eluent. UV detection at 200 nm. Temperature: 25°C. Between-run rinsing: 1 M NaOH at 35 psi for 10 min, 0.1 M NaOH at 20 psi for 5 min, Milli-Q water at 20 psi for 5 min, and eluent at 20 psi for 10 min.



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290 **Figure 2.** logR_h – logM_w correlations obtained for poly(acrylic acid-co-acrylamide)s standards (APAMs) and
291 industrial samples (IPAMs) using 20 mM Tris/HCl pH 8.0 eluents containing 35.5 mM LiCl, 1 M NaCl or 1 M
292 KCl. Least-squared regressions are, with M_w in 10^6 g/mol and R_h in nm: $\langle R_h \rangle = 31.020 \times M_w^{0.5346}$, $R^2 = 0.9926$ for
293 35.5 mM LiCl, error bar = ±1 RSD (n = 3); $R_h = 24.398 \times M_w^{0.4546}$, $R^2 = 0.9609$ for 1 M NaCl and R_h =
294 $18.328 \times M_w^{0.5109}$, $R^2 = 0.9251$ for 1 M KCl, n = 1. Injected sample concentration in the eluent: 1 g/L. Other
295 experimental conditions: see Fig. 1.

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Table 1. Hydrodynamic radius (R_h) and molar mass (M_w) determination by TDA using bare fused silica capillary (case 1) or PDADMAC/PSS SMIL-coated capillary (case 2) with eluents of various ionic strengths. Eluent as indicated on the Table. Other TDA experimental conditions as in Fig. 1 or Fig. SI4 (case 1) or Fig. SI5 (case 2). RSD (%) or error bars are calculated based ± 1 SD. Intra-capillary values based on n = 3 determinations; inter-capillary values based on m = 3 capillaries and n = 3 repetitions per capillary. Eluant: 20 mM Tris/HCl with 35.5 mM LiCl, pH 8. [a] Data given by the provider: anionicity by ^{1}H NMR, M_w and PDI by SEC-MALS for APAMs, M_w by SLS for IPAMs. [b] TDA molar masses were obtained using the reciprocal of the least-squared regressions given in Fig. 2 (with M_w in 10^6 g/mol and R_h in nm) $M_w = 0.00162 \times \langle R_h \rangle^{1.8706}$ (case 1) and $M_w = 0.00195 \times \langle R_h \rangle^{1.7986}$ (case 2).

					Case 1 Bare-fused silica capillary			**Case 2 SMIL-coated capillary**		
	Anionicity (by H^1 NMR)[a]	**M_w (by SEC-MALS)[a]** $\times 10^6$ g/mol)	**PDI (by SEC-MALS)[a]**	**M_w (by SLS)[a]** $\times 10^6$ g/mol	Intra-cap $\langle R_h \rangle_{intra}$ (nm)	Inter-cap $\langle R_h \rangle_{inter}$ (nm)	$M_{w,\,TDA}$[b] (10^6 g/mol)	Intra-cap $\langle R_h \rangle_{intra}$ (nm)	Inter-cap $\langle R_h \rangle_{inter'}$ (nm)	$M_{w,\,TDA}$[b] (10^6 g/mol)
APAM1	26%	0.22±0.02	1.1±0.1		14.0 (3.0%)	13.4 (3.7%)		13.4 (3.0%)	13.5 (3.7%)	
APAM5	25%	0.80±0.1	1.2±0.2		30.0 (3.3%)	28.4 (7.0%)		28.0 (3.1%)	29.0 (3.2%)	
APAM10	29%	1.92±0.3	2.5±0.1		45.0 (4.0%)	43.0 (7.7%)		48.7 (5.4%)	47.0 (6.8%)	
APAM30	25%	3.93±0.5	1.4±0.2		55.0 (8.0%)	61.8 (8.9%)		68.7 (11%)	64.0 (7.8%)	
APAM50	30%	4.18±0.4	3.0±0.2		70.2 (12%)	80.6 (9.7%)		78.0 (7.3%)	80.0 (8.8%)	
APAM60	26%	7.34±0.7	1.5±0.2		104.0 (11%)	92.6 (8.9%)		103.0 (5.5%)	92.0 (8.5%)	
IPAM1	27%	4.3±0.4		6.5±0.7	70.0 (8.0%)	70.5 (3.5%)	4.3±0.4	75.5 (6.6%)	74.5 (4.8%)	4.7±0.2
IPAM2	28%			8±0.8	100.1 (9.1%)	91.2 (7.9%)	8.4±0.6	96.3 (2.4%)	93.7 (5.9%)	7.2±0.4
IPAM3	29%			10.5±1.1	114.2 (7.0%)	109.2 (8.7%)	12.3±1.1	106.6 (3.0%)	110.5 (6.8%)	9.7±0.7
IPAM4	33%			15±1.5	127.9 (8.1%)	131.9 (9.9%)	14.1±1.4	136.8 (2.9%)	138.4 (7.2%)	15.0±1.1
IPAM5	27%			25±2.5	167.0 (9.7%)	159.0 (9.5%)	20.8±2.0	191.3 (6.0%)	183.7 (7.0%)	25.2±1.8

3.2. Impact of BGE ionic strength using fused silica capillary

To possibly extend the range of molar masses that can be analyzed by TDA; higher eluent ionic strengths were used to get more compact polyelectrolyte conformations by screening of the electrostatic repulsions between charged monomers in the polyelectrolyte chain. Accordingly, the BGE (20 mM Tris/HCl) was completed by adding 1 M NaCl or 1 M KCl (instead of 35.5 mM LiCl) in order to investigate the impact of high ionic strength on the R_h determined by TDA for APAM and IPAM samples. Open symbols in Fig. 2 shows the logR_h – logM_w correlations obtained at 1 M NaCl and 1 M KCl on bare fused silica capillaries. As expected lower R_h values were obtained at 1 M NaCl (or KCl) with a decrease by a factor ~ 1.5 compared to 35.5 mM LiCl, while the correlation was slightly lower at high ionic strength (R^2 = 0.9926 at 35.5 mM LiCl compared to R^2 = 0.9613 at 1M NaCl, and R^2 = 0.9686 at 1 M KCl). The expected decrease in hydrodynamic radii of the polymers at high ionic strengths was similar in 1 M KCl and in 1 M NaCl.

3.3. SMIL-coated capillary

Fig. 3 compares the logR_h – logM_w correlations achieved with APAMs and IPAMs using a bare-fused silica capillary and a SMIL coated capillary (20 mM Tris/HCl with 35.5 mM LiCl eluant). Two PADMAC/PSS bilayers were deposited on the silica capillary by simple flushes (see section 2.5 for experimental protocol), to enhance electrostatic repulsion between the capillary wall and the polyelectrolyte analytes. The purpose of this SMIL coting was to decrease the consuming rinsing time between runs (30 min on fused silica capillary *vs* 8 min on SMIL coted capillary), and possibly to get better reproducibility in the taylorgrams, and consequently lower RSD in the R_h determination. The correlation lines were very close to each other on the two type of capillaries with similar scaling laws and correlation coefficients: $\langle R_h \rangle = 32.145 \times M_w^{0.5560}$, R^2 = 0.9910 (SMIL-coating) and $\langle R_h \rangle = 31.020 \times M_w^{0.5346}$, R^2 = 0.9926 (bare fused silica capillary), with M_w in 10^6 g/mol and R_h in nm. The molar masses of IPAMs were then derived from $\langle R_h \rangle$ using the reciprocal of the scaling laws and reported in Table 1. The TDA results with RSD in the range of 3-12% are in good agreement with the SLS-ones (~ 10% discrepancies). Comparing the two protocols, SMIL-coating method is advantageous over fused silica capillary for time-saving (8 min rinsing time between runs instead of 30 min) and more symmetric taylorgrams (see Fig. SI4/SI6 and Fig. SI5/SI7). SMIL coting also provided better intra and inter capillary RSD for the polydisperse IPAM samples. It is worth noting that the stability of SMIL coated capillaries sustained throughout the repeatability assay of all the samples. Compared to the upper limit in molar mass obtained

in CGE (M_w < 10 MDa [13]), TDA extended the accessible molar mass range up to 25 MDa for IPAM copolymers.

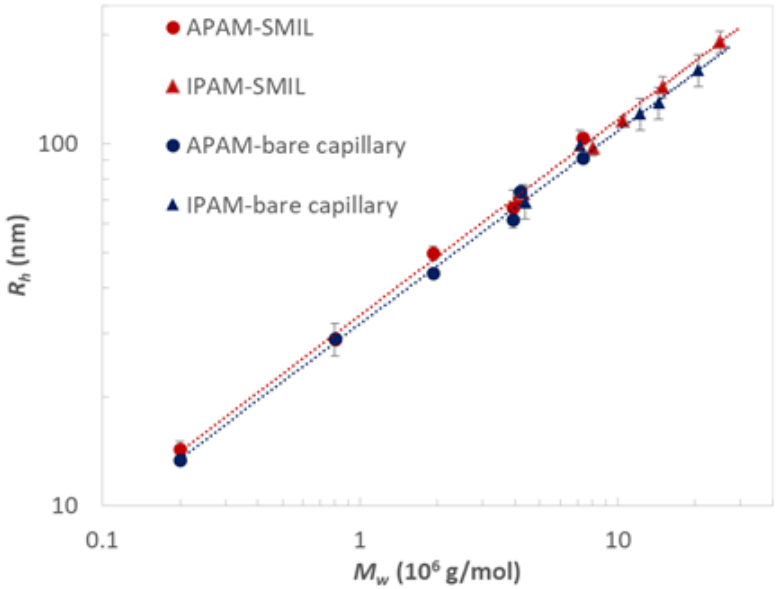

Figure 3. logR_h – logM_w plots for poly(acrylic acid-co-acrylamide)s standards (APAMs) and industrial samples (IPAMs) obtained using a bare fused silica capillary (blue) or a SMIL-coated capillary (red). Experimental conditions: capillary dimensions, 50 µm ID × 60 cm (50 cm to the detector). Eluent: 20 mM Tris/HCl with 35.5 mM LiCl, I = 46.5 mM, pH 8.0. Mobilization pressure: 0.4 psi. Injection: 0.5 psi, 7 s. Injected sample concentration: 2 g/L in eluent. SMIL consisted of two bilayers of PDADMAC/PSS at a polymer concentration of 3 g/L (see experimental part for more details). Rinsing between runs: fused silica capillary, 1 M NaOH at 35 psi for 10 min, 0.1 M NaOH at 20 psi for 5 min, Milli-Q water at 20 psi for 5 min and BGE at 20 psi for 10 min. SMIL coated capillary: BGE at 20 psi for 8 min. UV detection at 200 nm. Temperature: 25°C. The calibration lines established with APAMs read with M_w in 10^6 g/mol and R_h in nm: $\langle R_h \rangle = 32.145 \times M_w^{0.5560}$, $R^2 = 0.9910$ (SMIL coated capillary); $\langle R_h \rangle = 31.020 \times M_w^{0.5346}$, $R^2 = 0.9926$ (bare fused silica capillary). Error bars are ± one SD. Other experimental conditions: see Fig. 1.

4. Conclusion

TDA was proven straightforward and powerful for size determination of a wide range of anionic polyacrylamides of hydrodynamic radii from 10 to 190 nm. The molar mass of the industrial samples were deduced from these hydrodynamic radii and found between 4.3 MDa and 25 MDa. The data processing of the taylorgrams comprised Gaussian fitting with subtraction of the contribution from small impurities, determination of hydrodynamic radii of the polymers, calibration using logR_h – logM_w correlation with standards characterized by SEC-MALS, and thereafter calculation of molar masses of industrial samples. Two protocols were employed and compared, aiming at improving R_h-M_w correlation and repeatability / reproducibility. A first protocol was based on bare fused silica capillaries and intensive

between-run rinsing (30 min), the other was based on SMIL-coated capillaries and simple rinsing (8 min). Both cases led to consistent results that are in agreement with those determined by SLS within relative error deviation. So far, TDA provided a new, reliable methodology of sized-based molar mass determination far beyond the detection limits of SEC (< 7.3 MDa) and also a convenient alternative to SLS. Last but not least, the concern of high polymer aggregation under action of electric fields encountered in CGE was fully alleviated in TDA due to orthogonal techniques based on completely different principles. As a perspective, it would be interesting to investigate the polyelectrolyte polydispersity by TDA using constraint regularized linear inversion approach for the fitting of the taylorgrams. To that respect, improving the signal to noise ratio could be of interest.

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