



HAL
open science

Quantifying the heterogeneity of chemical structures in complex charged polymers through the dispersity of their distributions of electrophoretic mobilities or of compositions

Joel J Thevarajah, Adam T Sutton, Alison R Maniego, Elizabeth G Whitty, Simon Harrisson, Hervé Cottet, Patrice Castignolles, Marianne Gaborieau

► To cite this version:

Joel J Thevarajah, Adam T Sutton, Alison R Maniego, Elizabeth G Whitty, Simon Harrisson, et al.. Quantifying the heterogeneity of chemical structures in complex charged polymers through the dispersity of their distributions of electrophoretic mobilities or of compositions. *Analytical Chemistry*, 2016, 88 (3), pp.1674-1681. 10.1021/acs.analchem.5b03672 . hal-04036459

HAL Id: hal-04036459

<https://hal.science/hal-04036459v1>

Submitted on 19 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Quantifying the heterogeneity of chemical structures in complex charged polymers through the dispersity of their distributions of electrophoretic mobilities or of compositions

Joel J. Thevarajah^{1,2}, Adam T. Sutton^{1,2}, Alison R. Maniego^{1,2}, Elizabeth G. Whitty^{1,2}, Simon Harrisson³, Hervé Cottet⁴, Patrice Castignolles^{1*}, Marianne Gaborieau^{1,2}

1 Western Sydney University, School of Science and Health, Australian Centre for Research of Separation Science (ACROSS), Parramatta NSW 2150, Australia

2 Western Sydney University, Molecular Medicine Research Group, School of Science and Health, Parramatta NSW 2150, Australia

3 IMRCP, UMR 5623, Université de Toulouse, 118 route de Narbonne, F-31062 Toulouse Cedex 9, France

4 Institut des Biomolécules Max Mousseron (IBMM, UMR 5247 CNRS, Université de Montpellier, Ecole Nationale Supérieure de Chimie de Montpellier), Place Eugène Bataillon CC 1706, 34095 Montpellier Cedex 5, France

* Corresponding author : Patrice Castignolles, p.castignolles@westernsydney.edu.au, Fax: +61 2 9685 9915

ABSTRACT: The complexity of synthetic and natural polymers used in industrial and medical applications is expanding, thus it becomes increasingly important to improve and develop methods for their molecular characterization. Free-solution capillary electrophoresis is a robust technique for the separation and characterization of both natural and synthetic complex charged polymers. In the case of polyelectrolytes free-solution capillary electrophoresis is in the “critical conditions” (CE-CC): it allows their separation by factors other than molar mass for molar masses typically higher than 20,000 g/mol. This method is thus complementary to size-exclusion chromatography (SEC). SEC is widely used to determine molar mass distributions and their dispersities. Utilizing CE-CC, an analogous calculation of dispersity based on the distributions of electrophoretic mobilities was derived and the heterogeneity of composition or branching in different polysaccharides or synthetic polymers was obtained in a number of experimental cases. Calculations are based on a ratio of moments and could thus be compared to simulations of polymerization processes, in analogy to the work performed on molar mass distributions. Among five possible types of dispersity, the most precise values were obtained with the calculation analogous with the dispersity of molar mass distribution M_w/M_n . In addition, the dispersity value allows conclusions based on a single value: the closer the dispersity is to 1, the more homogenous the polymer is in terms of composition or branching. This approach allows the analysis of dispersity of important molecular attributes of polymers other than molar mass and aims at improving the overall molecular characterization of both synthetic and natural polymers. The dispersity can also be monitored online while performing a chemical reaction within the CE instrument.

INTRODUCTION

The accurate molecular characterization of polymers is a necessity as their production and development expands in both industry and research. This requires rigorous method development for the characterization of more complex polymers. Complex polymers can vary in a range of molecular attributes including molar mass, composition, type of copolymer, branching, charge, chain-ends. Each of these attributes exists as distribution(s) in a given sample. These distributions can vary further and be for example broad, narrow, uni- or bimodal¹. Therefore, the distributions of particular molecular attributes should be characterized.

Currently, the commonly assessed molecular attribute of polymers is molar mass. The heterogeneity of molar mass in a polymer sample can be assessed through

determination of its dispersity typically by size-exclusion chromatography (SEC, also known as GPC)². The dispersity is calculated as the weight-average molar mass divided by the number-average molar mass and most, if not all, commercial software operating SEC instruments is routinely performing this calculation for the users. The average molar masses and the dispersity are also predicted using simulations of polymerization processes by the method of the moments.³

Heterogeneity is not often quantified for the other molecular attributes of polymers such as composition (of copolymers) or branching. A number of methods for separating by composition or branching exist⁴. Following the SEC separation of copolymers of methyl methacrylate and styrene a quadruple detection was implemented which included heavy reliance on the differential UV

absorbance of each monomer unit⁵. It was further shown that a relation between sequence length heterogeneity and the molar mass distribution could be achieved, however, a number of conditions were required including: the intrachain interaction of the different monomers to be different to the monomers of the same type, appropriate solvation conditions for the chemical functionalities and the architecture of the copolymer should remain the same as a function of the molar mass⁶. SEC gradient was shown to be able to determine the chemical composition distribution of statistical copolymers.⁷ The coupling of SEC and gradient elution liquid chromatography^{8,9} was shown to be required for the analysis of both synthetic and artificial copolymers since both dimensions suffered from coelution when performed individually. The dispersity of the distribution of compositions is not determined in the literature. This may result from the absence of straightforward separation and characterization method for the composition or branching of polymers especially for hydrophilic ones, as well as from the absence of a recognized method to calculate their dispersity. SEC separates by hydrodynamic volume, which depends on molar mass as well as on composition or branching. A local dispersity at a given hydrodynamic volume can be determined using multiple-detection SEC, which assesses the accuracy of the determined molar mass or the local heterogeneity at a given hydrodynamic volume; however, it does not directly assess the heterogeneity of the branching or composition distributions.^{1,10} Various forms of liquid chromatography¹¹ as well as thermal field flow fractionation¹² have been shown to separate polymers according to composition or branching.

Capillary electrophoresis (CE) has been proven to be an appropriate technique for the separation of polyelectrolytes¹³ and specifically the analysis of copolymers (natural^{14,15} and synthetic^{16,17}) and branched polymers^{18,19}. Free-solution capillary zone electrophoresis of evenly charged polyelectrolytes (with regularly distributed charges along the polymer backbone) leads to analogous separations to liquid chromatography in the critical conditions (with different separation mechanisms) in which separation is not dependent on molar mass²⁰. The technique free-solution capillary electrophoresis (CE) is thus defined here as the method CE in the critical conditions (CE-CC). This method allows the separation and analysis of polymers according to composition or branching as will be discussed. It further allows the distributions of these attributes to be obtained. Importantly, the determination of mass-relative distributions of electrophoretic mobilities, or of any other characteristic parameter such as chemical composition, has been recently addressed by Chamieh et al.²¹. Theoretical background required to convert the electropherogram into a distribution of electrophoretic mobilities was described with an emphasis on the fact that both x and y axes should be converted. Estimation of sample dispersity was performed via the determination of the standard deviation (or RSD) of the samples chemical composition distribution.

We propose different expressions of the dispersity based on different moments of the distributions. In this work, they were used to estimate the dispersity of distri-

butions obtained through CE-CC (distributions of electrophoretic mobilities and of compositions). The different types of dispersity were then compared by applying them to various natural and synthetic (co)polymers.

THEORY

Dispersities of molar mass distributions. The expressions of dispersity obtained in this paper are analogous to expressions of dispersity of molar mass distributions. Shortt²² demonstrated that the number-, weight- and z-average molar mass, M_n , M_w and M_z , can be obtained from the ratio of moments of molar mass distributions from SEC. To calculate the dispersity of molar mass distributions, the ratio M_w/M_n is quasi-exclusively used nowadays, but the ratio M_z/M_w has also been used for example to establish relations between the radius of gyration and M_w . The dispersity calculated as M_w/M_n (eq S1) is related to the standard deviation σ through a simple expression²³:

$$D - 1 = \frac{M_w}{M_n} - 1 = \frac{\sigma^2}{M_n^2} \quad (1)$$

Dispersities of electrophoretic mobility distributions. In this work, weight distributions of electrophoretic mobilities (and of compositions) are considered. $W(\mu)$, the weight fraction of polyelectrolyte chains with electrophoretic mobility μ , can be obtained from a mass-sensitive detection such as UV detection of the monomer units. The number distribution is usually not straightforward to obtain except in the case of end-labeled polymers (derivatization may be required)²⁴. The number-average electrophoretic mobility is thus generally not accessible by CE and it is not possible to access it by single detection contrary to the case of molar mass averages in SEC where M_n and M_w are both determined with single-detection. In this work, ratios of moments were used to express the dispersity of electrophoretic mobility distributions (see Table S1 for the expression of the individual moments).

Let $D(W(A),b,c)$ be the dispersity of the weight distribution of the variable A (which can be either mobility or composition), as a function of the $(b-2)^{\text{th}}$, $(b-1)^{\text{th}}$ and b^{th} order moments, with respect to the reference c . The general expression of the moment, $\overline{A_c^b}$, (eq 2) and dispersity, $D(W(A),b,c)$, (eq 3) is given by:

$$\overline{A_c^b} = \frac{\int_0^\infty W(A)(A-c)^b dA}{\int_0^\infty W(A)dA} \quad (2)$$

$$D(W(A),b,c) = \frac{\overline{A_c^b} \times \overline{A_c^{b-2}}}{[\overline{A_c^{b-1}}]^2} \quad (3)$$

It is noted that the number-average mobility could be mathematically defined as the ratio of the 0th and the -1st order moments in analogy to molar mass distribution dispersity²² but this does not correspond to the definition of the number-average electrophoretic mobility based on the number distribution (see supporting information, eq S28 and S29). Two reference values were used for the calculation of the moments of the distributions: either $c = 0$ as in the Shortt equations²², or the weight-average mobility, $c = \mu_w$, as for the standard deviation (SD). μ_w is determined as the ratio of the 1st and the 0th order moments of the mobility distributions:

$$\mu_w = \frac{\overline{\mu_0^{-1}}}{\mu_0^0} = \frac{\int W(\mu)\mu d\mu}{\int W(\mu)d\mu} \quad (4)$$

In the first approach, an analogy with M_w/M_n is used, and the dispersity is calculated as the ratio of 1st and 0th order moments divided by the ratio of 0th and -1st order moments. Eq 5 shows this dispersity with the reference (c) taken as 0:

$$D(W(\mu), 1, 0) = \frac{\overline{\mu_0^{-1}} \times \overline{\mu_0^0}}{[\mu_0^0]^2} \quad (5)$$

In the second approach, an analogy with M_z/M_w is used, and the dispersity is calculated as the ratio of 2nd and 1st order moments (or z-average) divided by the ratio of 1st and 0th order moments (eq 6):

$$D(W(\mu), 2, 0) = \frac{\overline{\mu_0^2} \times \overline{\mu_0^0}}{[\mu_0^0]^2} \quad (6)$$

In the third approach, dispersity is calculated as a ratio of 3rd and 2nd order moments divided by the ratio of 2nd and 1st order moments (eq 7).

$$D(W(\mu), 3, 0) = \frac{\overline{\mu_0^3} \times \overline{\mu_0^1}}{[\mu_0^0]^2} \quad (7)$$

The SD of the weight distribution of electrophoretic mobilities, taking μ_w as reference, is represented by eq 8:

$$D_\sigma = \left[\frac{\overline{\mu_w^2}}{\overline{\mu_w}} \right]^{0.5} \quad (8)$$

The integral expression of eq 3 to eq 8 are given as eq S6 to S13.

μ_w can be used as a reference instead of 0 similarly to the calculation for SD. However, for eq S3 to S5 never gives a finite value different from 0 (see supporting information).

Dispersities of composition distributions for copolymers. The determination of distributions of compositions from electrophoretic mobility distributions first requires establishing a correlation between the electrophoretic mobility and the composition. This can be completed using the weight-average mobility of standard samples and a complementary method to measure their average composition such as NMR spectroscopy¹⁴; for synthetic polymers the composition may also be estimated from the monomer concentrations used in the synthesis. The challenge faced with establishing a correlation is the requirement of having appropriate samples to cover the whole range of compositions (as for any calibration curve). Samples with particularly broad distributions such as the natural samples studied in this paper could not be studied through composition distributions without acknowledgement of the possibility of significant error caused by the calibration curve.

The (weight-average) composition C is defined as the weight of one type of monomer unit over the weight of all monomer units. Assuming a linear correlation between the electrophoretic mobility and composition^{14,25,26}, the weight fraction $W(C)$ of the polyelectrolyte chains with the composition C is calculated as:

$$W(C) = \frac{W(\mu)}{dC/d\mu} = \frac{W(\mu)}{m} \quad (9)$$

where m is the slope of the calibration curve of the composition versus mobility. Linear correlation between mobility and composition is generally observed for evenly charged polyelectrolytes when charge densities are either below or above the Manning condensation threshold with a breaking slope at the condensation threshold^{27,28}. The weight-average composition C_w is calculated as:

$$C_w = \frac{\overline{C^1}}{C^0} = \frac{\int W(C)C dC}{\int W(C)dC} \quad (10)$$

In analogy with the dispersities of the mobility distributions, the dispersities of the composition distributions are calculated as ratios of moments using 0 as a reference (eq S20 to S23).

Note that in the calculation of dispersity values for experimental cases for both mobility and composition distributions, the discrete forms were used in the software Origin for the moments (eq S14 to S17 and S24 to S27).

Estimating the uncertainty on the dispersity values. In order to compare the calculated dispersity values on different samples it is necessary to estimate the precision of their determination. The error on the dispersity values caused by the uncertainty on the electrophoretic mobility values was calculated for each type of dispersity in eq 5 to 7 (eq S30 to S32 for the derivation) using experimental data. This resulted in eq 11 to 13 for electrophoretic mobility dispersities.

$$\frac{dD(W(\mu), 1, 0)}{D(W(\mu), 1, 0)} = \frac{d\mu}{\mu} \sqrt{\left(\frac{W(\mu)\mu^2}{\int W(\mu)\mu d\mu} \right)^2 + \left(\frac{W(\mu)}{\int W(\mu)\mu^{-1} d\mu} \right)^2 + 2 \left(\frac{W(\mu)\mu}{\int W(\mu)d\mu} \right)^2} \quad (11)$$

$$\frac{dD(W(\mu), 2, 0)}{D(W(\mu), 2, 0)} = \frac{d\mu}{\mu} \sqrt{\left(\frac{W(\mu)\mu}{\int W(\mu)d\mu} \right)^2 + \left(\frac{W(\mu)\mu^3}{\int W(\mu)\mu^2 d\mu} \right)^2 + 2 \left(\frac{W(\mu)\mu^2}{\int W(\mu)\mu d\mu} \right)^2} \quad (12)$$

$$\frac{dD(W(\mu), 3, 0)}{D(W(\mu), 3, 0)} = \frac{d\mu}{\mu} \sqrt{\left(\frac{W(\mu)\mu^2}{\int W(\mu)\mu d\mu} \right)^2 + \left(\frac{W(\mu)\mu^4}{\int W(\mu)\mu^3 d\mu} \right)^2 + 2 \left(\frac{W(\mu)\mu^3}{\int W(\mu)\mu^2 d\mu} \right)^2} \quad (13)$$

For the uncertainty of the dispersity of the distribution of compositions, the same expressions apply and only require the substitution of μ In addition, the error due to the calibration may play an important additional role.

The derivation of the uncertainty of the electrophoretic dispersity expressed as a SD (eq 8) is shown in supporting information (eq S36 to S38) and yields eq 14:

$$\frac{dD_\sigma}{D_\sigma} = \frac{d\mu}{\mu} \sqrt{0.5 \left(\frac{W(\mu)(\mu - \mu_w)^2 \mu}{\int W(\mu)(\mu - \mu_w)^2 d\mu} \right)^2 + 0.5 \left(\frac{W(\mu)\mu}{\int W(\mu)d\mu} \right)^2} \quad (14)$$

The relative uncertainty of the electrophoretic mobility $d\mu/\mu$ was taken as 1 % corresponding to the order of magnitude of the relative SD of the electrophoretic mobility for free-solution CE of polyelectrolytes¹⁸ and sugars^{29,30} (Table S2). The calculated relative uncertainties were shown to be lower than 4×10^{-6} (sometimes by orders of magnitude) which shows that the error caused

by the mobility is negligible for the dispersity values calculated (Table S3). Although there does not seem to be a correlation between the samples and the uncertainty, it is noted that $D(W(\mu),1,0)$ seems to have the lowest uncertainty. The major contributor of the uncertainty on the dispersity value is thus not the error on the determination of μ but likely other factors such as the error in setting the baselines and the start/end limits of the integration. Therefore, six significant digits (corresponding to 5 decimal places) were considered for the dispersity values. In the case of composition distributions, a higher order of magnitude (10^{-1} - 10^{-6}) for the relative uncertainty of the dispersity and of SD (10^{-6}) were calculated (Table S4). As mentioned previously, a significant error can be introduced through the imperfect correlation between mobility and composition for the dispersity on composition.

EXPERIMENTAL

Separation conditions were as previously described for chitosan¹⁴, poly(sodium acrylate)/poly(sodium acrylate-co-*N*-antipyrine acylamide)¹⁸ and poly(acrylic acid-*b*-acrylamide)¹⁶.

RESULTS AND DISCUSSION

Dispersity of electrophoretic mobility distributions.

The treatment of CE-CC data allows the calculation of dispersity values based on electrophoretic mobility distributions. As mentioned previously, CE-CC separates evenly charged polymers based on molecular attributes other than molar mass, provided the polyelectrolyte degree of polymerization is typically higher than 10 g.mol⁻¹.^{20,31} Three experimental cases are presented, two based on the heterogeneity of composition and one on the heterogeneity of branching.

Chitosan. Chitosan is a polysaccharide derived from the deacetylation of chitin. It is a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine in varying proportions (Figure S1A). Its composition is generally referred to as degree of acetylation, *DA* (the fraction of *N*-acetyl-D-glucosamine units). Being a natural product, chitosan varies in a range of molecular attributes including molar mass and composition (including the distribution of units along the chain being rather in blocks or statistical). Using CE-CC, different chitosan samples had been separated based on their *DA* and through appropriate data treatment²¹ the weight distributions of the mobilities have been obtained (Figure 1)¹⁴. The heterogeneity of *DA*, which is the distribution of the compositions of different chains within a chitosan sample, is represented by the distribution of electrophoretic mobilities.

Using eq 5 to 8 dispersity values were obtained for the different chitosan samples (Table S5). Dispersity values calculated as $D(W(\mu),1,0)$, $D(W(\mu),2,0)$, $D(W(\mu),3,0)$ and SD were in good agreement in terms of the trend observed (Figure 2). They showed an overall decreasing trend in which the chitosan samples with higher average electrophoretic mobilities (lower average *DA*s) have more narrow distributions of compositions (lower *D* values). Thus the calculation of the dispersity values provided a valid numerical demonstration of the data which was represented graphically. The lower selectivity above counter-ion condensation, at the lowest *DA*, may contribute to this trend. The trend of the dispersity with *DA*

might also be due to the rate of deacetylation varying for different parts of the chitosan sample which can lead to heterogeneity and may also explain the variation in dispersity of samples between the weight-average electrophoretic mobility of 3.3 and $3.4 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$. This effect would be weaker at low *DA*, since the *DA* cannot (physically) go below 0. It shows the importance of the chemical treatment to which many polysaccharides are subjected in their preparation. This overall trend may not extend to other families of polymers, as seen below.

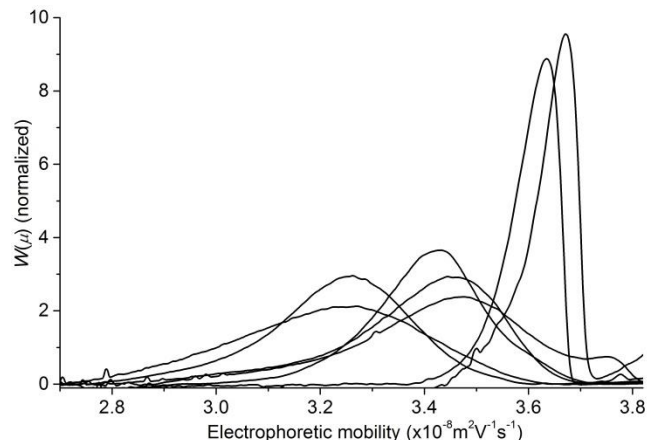


Figure 1. Mobility weight distributions obtained by CE-CC for chitosan samples with varying degrees of acetylation.

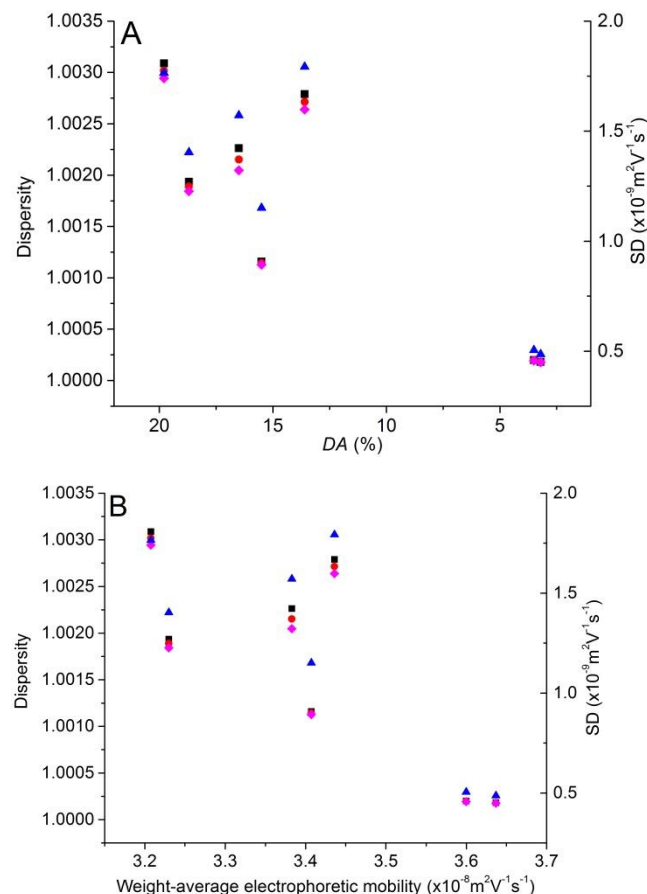


Figure 2. (A) Dispersity values shown for chitosan samples as $D(W(\mu),1,0)$ (red circles, eq 5), $D(W(\mu),2,0)$ (black squares, eq 6), $D(W(\mu),3,0)$ (magenta diamonds, eq 7) and SD (blue triangles, eq 8) against (A) their number average degree of acetylation, or (B) against their weight-average electrophoretic mobility.

Despite the relatively low selectivity of the separation, dispersity values calculated on the mobility distributions still allows information regarding the sample heterogeneity to be obtained.

Poly(sodium acrylate-co-N-antipyrine acrylamide), P(NaA-co-APA). This copolymer is synthesized using a known coupling reaction³² between the amine of the 4-aminoantipyrine, AAP, and the carboxylate of the PNaA (Figure S1B). The grafting reaction was performed within a CE vial and monitored in real time using capillary electrophoresis in previously established conditions^{33,34} (Figure 3). The increase of the copolymer peak area with reaction time is consistent with the much larger UV absorption (Beer-Lambert coefficient) for AAP than PNaA which was calculated to be greater by a factor of almost 20 (see supporting information). The decrease of the electrophoretic mobility with grafting is consistent with both the reduced charge (loss of carboxylate units) and the increased hydrodynamic friction (increased size). As the reaction time increases the P(NaA-co-APA) peak becomes broader, indicating a larger heterogeneity of composition.

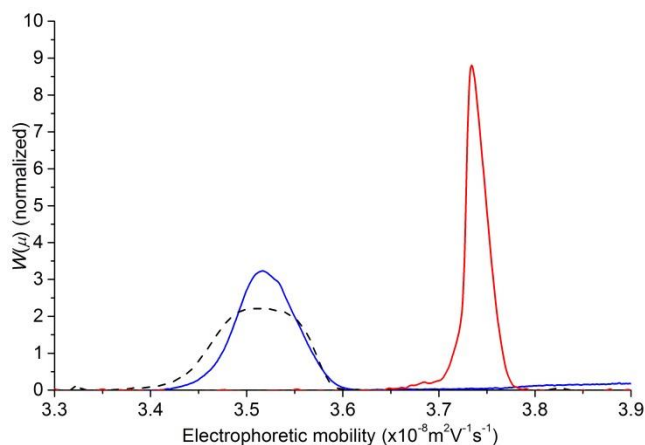


Figure 3. Electrophoretic mobility distributions of P(NaA-co-APA) samples at time 0 (red), 5 min (blue) and 4 h (black dash).

The dispersity of electrophoretic mobility of the copolymer was obtained from eq 5 to 8 (Table S6). The linear PNaA homopolymer was used as a reference and its dispersity subtracted from the P(NaA-co-APA) dispersity values. This enabled to monitor the grafting reaction and assess the heterogeneity of composition of the P(NaA-co-APA). The values of $D(W(\mu),1,0)$, $D(W(\mu),2,0)$, $D(W(\mu),3,0)$ and SD are in good agreement in terms of the trend observed (Figure 4). They increase reaching a maximum at approximately 4 h. The dispersity allows a numerical representation of the change in composition. The heterogeneity of the copolymer increases during the first 4 h, as most of the grafting proceeds. The reaction

then slows down and it might lead to a more homogeneous copolymer.

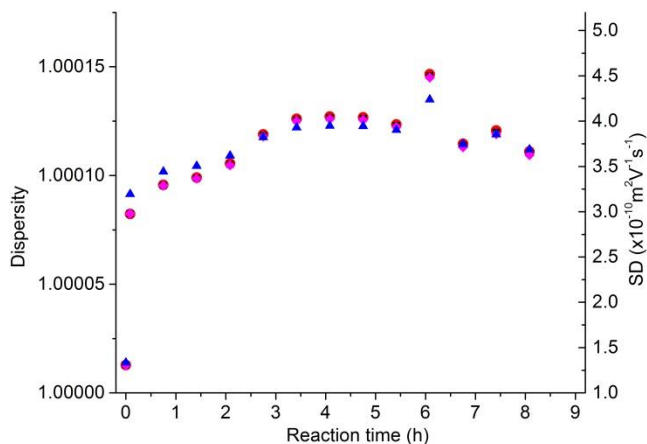


Figure 4. Dispersity values shown for P(NaA-co-APA) samples as $D(W(\mu),1,0)$ (black square, eq 5), $D(W(\mu),2,0)$ (red circle, eq 6), $D(W(\mu),3,0)$ (magenta diamonds, eq7) and SD (blue triangle, eq 8) against reaction time.

Branched poly(sodium acrylate), PNaA. This synthetic polymer (Figure S1C) is industrially produced using conventional polymerization; in this study it was synthesized using a controlled radical polymerization: nitroxide mediated polymerization (NMP)³⁵. The occurrence of branching in PNaA has been noted³⁶ and it is an important factor which can be overlooked in PNaA characterization. The branching of PNaA can be used to tailor polymers for specific applications through the choice of an appropriate polymerization technique. CE-CC was shown to separate different PNaA samples based on their branching (Figure 5)¹⁸.

Dispersity values were obtained for the 3 samples (Figure 6, Table S7). It is to be noted that the linear sample does not have any branching since it has been obtained by anionic polymerization¹⁸ and thus the broadness of the peak results from intrinsic experimental factors. Considering the dispersity values are representative of the heterogeneity of branching, the linear (which had the lowest dispersity value and the highest mobility) and the hyperbranched (at a weight-average mobility of $3.53 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$) samples have dispersity values which are quite close (and low). As expected due to its branching architecture, 3-arm star had the highest dispersity and the lowest mobility. This is because although the hyperbranched sample is more branched than 3-arm star it was designed to have a homogenous branching through the use of an inimer for its synthesis.

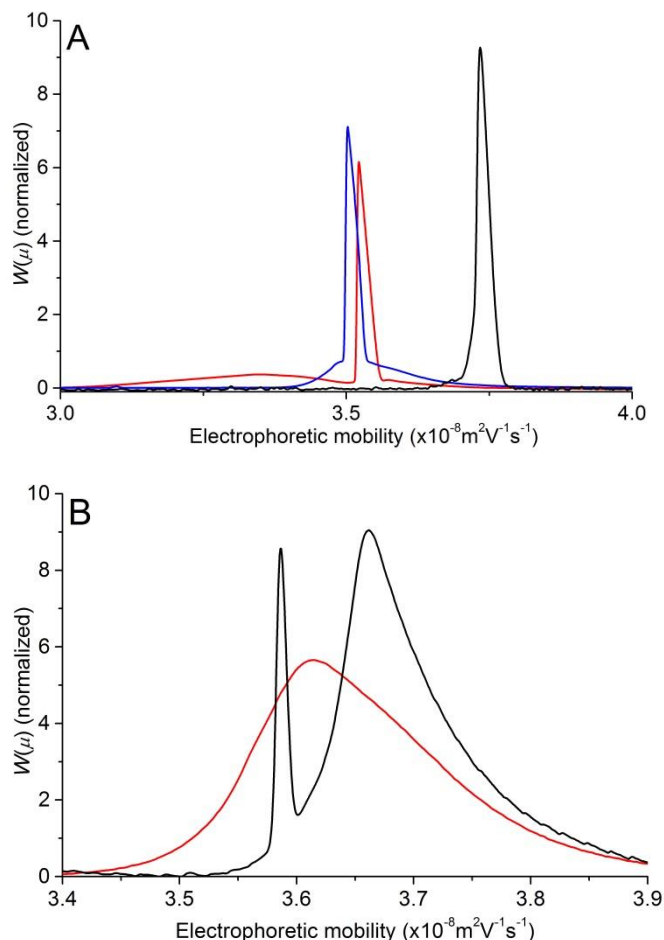


Figure 5. Electrophoretic mobility distributions of PNaA samples. (A) PNaAs with different topologies: linear injected at a lower concentration than previously¹⁸ (black), 3-arm star (red) and hyperbranched (blue). (B) PNaAs synthesized by NMP of acrylic acid (black) and *tert*-butyl acrylate (red).

Similar samples synthesized from different monomers were analyzed with the same method (Figure 5b). PNaA samples synthesized by the NMP of acrylic acid and of *tert*-butyl acrylate (*t*BA) had different distributions and dispersities. It is to be noted that different end-groups¹⁸ can also contribute to the heterogeneity of the electrophoretic mobility distribution.

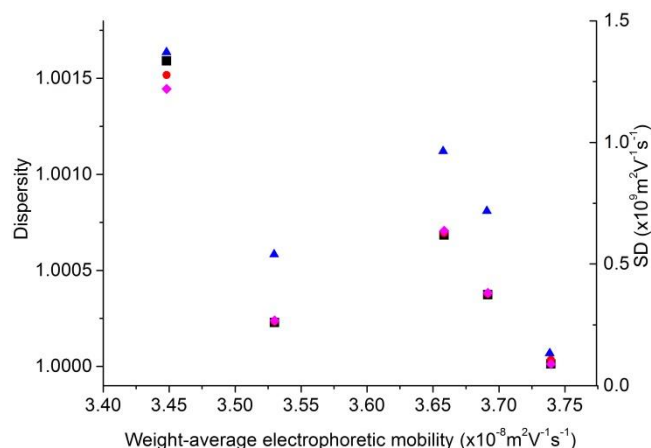
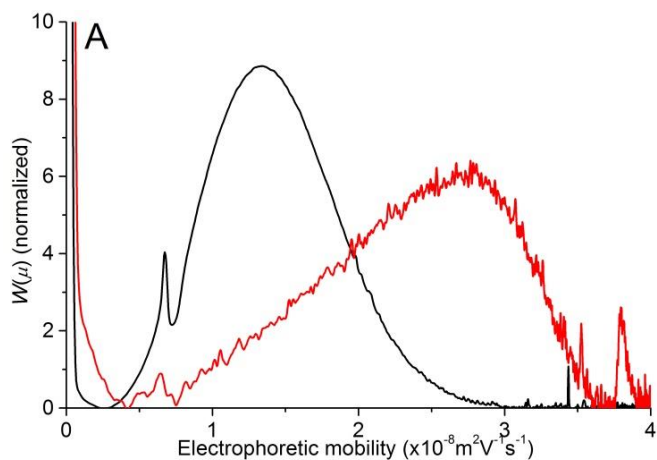


Figure 6: Dispersivity values shown for PNaA samples as $D(W(\mu),1,0)$ (black square, eq 5), $D(W(\mu),2,0)$ (red circle, eq 6), $D(W(\mu),3,0)$ (magenta diamonds, eq 7) and SD (blue triangle, eq 8) against their weight-average electrophoretic mobility. The samples in increasing order of average electrophoretic mobility are 3-arm star ($3.45 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$), hyperbranched ($3.53 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$), NMP of *t*-BA ($3.66 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$), NMP of AA ($3.69 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$) and linear ($3.74 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$).

Dispersivity of composition distributions

Poly(acrylic acid-*b*-acrylamide), P(AA-*b*-AM) block copolymers were synthesized by Reversible-Addition Fragmentation chain Transfer/Macromolecular Design via Interchange of Xantates (RAFT/MADIX) polymerization. Using CE-CC the block copolymers could be separated (Figure 7)¹⁶. The P(AA-*b*-AM) was synthesized with different ratios of the monomers (2K and 10K in the sample name refer to 2,000 and 10,000 $\text{g} \cdot \text{mol}^{-1}$ theoretical molar mass of the corresponding block). The electrophoretic mobility distributions were simple to obtain as shown previously, extra treatment was required to obtain composition distributions.

The separation of P(NaA-*b*-AM) using CE-CC is dependent on both the charge of the PNaA monomer units and the hydrodynamic friction of the charged PNaA and uncharged PAM blocks. Therefore, to obtain meaningful composition distributions, both the charged and uncharged blocks of the block copolymer need to be taken into account (eq S38, S39). However, the necessary rescaling factor α is challenging to obtain (see supporting information). The weight average composition of both samples was determined from the theoretical values of the uncharged and charged block lengths. Dispersivity values were obtained for the distributions of both mobilities and compositions (Table S8).



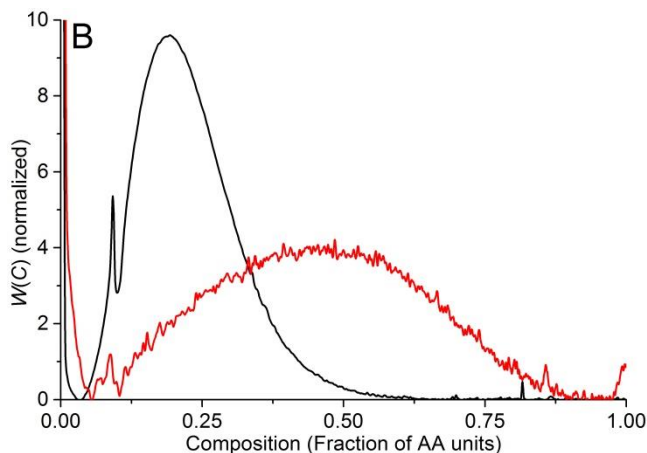


Figure 7. (A) Electrophoretic mobility distribution and (B) composition distribution of P(NaA-*b*-AM) samples: PAA2kPAM10k (black) and PAA10kPAM10k (red). The theoretical M_n values were listed for each sample previously¹⁶.

The accuracy of the composition distributions of P(AA-*b*-AM) and of their dispersity depends on the accuracy of α ¹⁶ (eq S39) and of the correlation between electrophoretic mobility and composition. In the case of statistical copolymers α would not need to be calculated and therefore the accuracy of the composition distribution would depend only on the correlation mobility and composition. The larger uncertainty obtained for dispersities of composition distributions (Table S4) compared to that of electrophoretic mobility distributions (Table S3) is likely due to a weak correlation between mobility and composition (despite the high selectivity in this case). In some cases a complementary method such as NMR spectroscopy could be used to obtain the average composition of specific samples, thus allowing a more accurate α to be obtained.

CONCLUSIONS

In this study both synthetic and natural polymers were characterized. The distributions of compositions and the heterogeneity of branching were analyzed using the dispersity of the distributions. Using the correct treatment of CE-CC data, the dispersity was quantified, including the monitoring of grafting on polymers. Further, using the dispersity values a numerical representation of the mobility and composition distributions were calculated and allowed comparisons between samples. Dispersity values using 0 as a reference were in good agreement with SD calculations in terms of trends observed. With further research dispersity values obtained through CE-CC can be used as commonly as molar mass dispersity values. Dispersity values calculated using D_σ and $D(W(\mu),1,0)$ were in good agreement. The dispersity values obtained in this work are closer to unity than typical values obtained on molar mass dispersity by SEC. It is however, to be noted that SEC overestimates \bar{D} and this largely due to band broadening^{37,38}. Temperature gradient interaction chromatography³⁹ and molecular radius analysis with multi-angle light scattering combined with SEC⁴⁰ were shown to reduce this effect. In analogy to SEC characterization of molar mass distributions of polymers, we recommend $D(W(\mu),1,0)$ to quantify the heterogeneity

due to either composition (copolymers) or branching. However, the three different types of quantification of the dispersity should be further compared, especially in the cases of non-symmetric distributions (with a tail) or bimodal distributions. It would also be very interesting to compare the CE method established in obtaining composition distributions with other methods, typically based on liquid chromatography⁴¹; however, this has been noted as tedious⁴² or was not attempted yet for hydrophilic polymers. Field-flow fractionation, especially thermal¹² may provide very interesting comparisons. In addition, simulation of polymerization processes should allow the prediction of $D(W(\mu),1,0)$ and the results can be compared with the values determined by CE-CC.

ACKNOWLEDGEMENTS

The authors would like to thank Matthew Van Leeuwen, Michelle Toutounji (Western Sydney University) and Aidan Grosas (Australian National University) for insightful discussions. JJT thanks the Australian Government for the Endeavour Research Fellowship to visit the University of Montpellier.

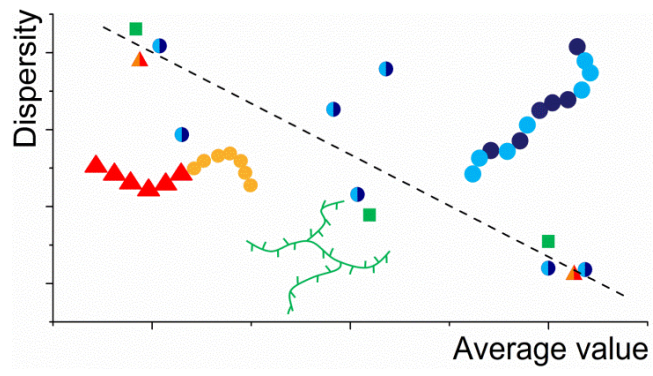
Supporting information. Derivation and expressions of moments, dispersity, and uncertainty; dispersity values for experimental; molar absorptivity and rescaling factor determination.

REFERENCES

- (1) Berek, D. *J. Sep. Sci.* **2010**, *33*, 315-335.
- (2) Gilbert, R. G.; Hess, M.; Jenkins, A. D.; Jones, R. G.; Kratochvil, R.; Stepto, R. F. T. *Pure Appl. Chem.* **2009**, *81*, 779-779.
- (3) Zabisky, R. C. M.; Chan, W. M.; Gloor, P. E.; Hamielec, A. E. *Polymer* **1992**, *33*, 2243-2262.
- (4) Philipson, H. J. A. *J. Chromatogr. A* **2004**, *1037*, 329-350.
- (5) Haidar Ahmad, I. A.; Striegel, A. M. *Anal. Bioanal. Chem.* **2010**, *396*, 1589-1598.
- (6) Haidar Ahmad, I. A.; Striegel, D. A.; Striegel, A. M. *Polymer* **2011**, *52*, 1268-1277.
- (7) Maier, H.; Malz, F.; Radke, W. *Macromol. Chem. Phys.* **2015**, *216*, 228-234.
- (8) Reingruber, E. M.; Chojnacka, A.; Jellema, E.; de Bruin, B.; Buchberger, W.; Schoenmakers, P. J. *J. Chromatogr. A* **2012**, *1255*, 259-266.
- (9) Shakun, M.; Heinze, T.; Radke, W. *Carbohydr. Polym.* **2015**, *130*, 77-86.
- (10) Gaborieau, M.; Castignolles, P. *Anal. Bioanal. Chem.* **2011**, *399*, 1413-1423.
- (11) Radke, W. *J. Chromatogr. A* **2014**, *1335*, 62-79.
- (12) Ponyik, C. A.; Wu, D. T.; Williams, S. K. R. *Anal. Bioanal. Chem.* **2013**, *405*, 9033-9040.
- (13) Cottet, H.; Gareil, P. In *Capillary Electrophoresis*, Schmitt-Kopplin, P., Ed.; Humana Press Inc.: Totowa, NJ, United States, 2008, pp 541-567.
- (14) Mnatsakanyan, M.; Thevarajah, J. J.; Roi, R. S.; Lauto, A.; Gaborieau, M.; Castignolles, P. *Anal. Bioanal. Chem.* **2013**, *405*, 6873-6877.
- (15) Taylor, D. L.; Ferris, C. J.; Maniego, A. R.; Castignolles, P.; in het Panhuis, M.; Gaborieau, M. *Aust. J. Chem.* **2012**, *65*, 1156-1164.
- (16) Sutton, A. T.; Read, E.; Maniego, A. R.; Thevarajah, J. J.; Marty, J. D.; Destarac, M.; Gaborieau,

- M.; Castignolles, P. *J. Chromatogr. A* **2014**, *1372*, 187-195.
- (17) Oukacine, F.; Bernard, S.; Bobe, I.; Cottet, H. *J. Control. Release* **2014**, *196*, 139-145.
- (18) Maniego, A. R.; Ang, D.; Guillaneuf, Y.; Lefay, C.; Gignes, D.; Aldrich-Wright, J. R.; Gaborieau, M.; Castignolles, P. *Anal. Bioanal. Chem.* **2013**, *405*, 9009-9020.
- (19) Sisavath, N.; Le Saux, T.; Leclercq, L.; Cottet, H. *Langmuir* **2014**, *30*, 4450-4457.
- (20) Thevarajah, J. J.; Gaborieau, M.; Castignolles, P. *Adv. Chem.* **2014**, *2014*, ArticleID 798503.
- (21) Chamieh, J.; Martin, M.; Cottet, H. *Anal. Chem.* **2015**, *87*, 1050-1057.
- (22) Shortt, D. W. *J. Liq. Chromatogr.* **1993**, *16*, 3371-3391.
- (23) Burchard, W. *Adv. Polym. Sci.* **1999**, *143*, 113-194.
- (24) Yao, Y.; Guiltinan, M. J.; Thompson, D. B. *Carbohydr. Res.* **2005**, *340*, 701-710.
- (25) Zhong, H. J.; Williams, M. A. K.; Keenan, R. D.; Goodall, D. M.; Rolin, C. *Carbohydr. Polym.* **1997**, *32*, 27-32.
- (26) Guillotin, S. E.; Bakx, E. J.; Boulenguer, P.; Schols, H. A.; Voragen, A. G. J. *Food Hydrocoll.* **2007**, *21*, 444-451.
- (27) Hoagland, D. A.; Smisek, D. L.; Chen, D. Y. *Electrophoresis* **1996**, *17*, 1151-1160.
- (28) Cottet, H.; Biron, J. P. *Macromol. Chem. Phys.* **2005**, *206*, 628-634.
- (29) Oliver, J. D.; Gaborieau, M.; Hilder, E. F.; Castignolles, P. *J. Chromatogr. A* **2013**, *1291*, 179-186.
- (30) Oliver, J. D.; Sutton, A. T.; Karu, N.; Phillips, M.; Markham, J.; Peiris, P.; Hilder, E. F.; Castignolles, P. *Biotechnol. Appl. Biochem.* **2015**, *62*, 329-342.
- (31) Cottet, H.; Gareil, P.; Theodoly, O.; Williams, C. E. *Electrophoresis* **2000**, *21*, 3529-3540.
- (32) Taylor, D. L.; Thevarajah, J. J.; Narayan, D. K.; Murphy, P.; Mangala, M. M.; Lim, S.; Wuhrer, R.; Lefay, C.; O'Connor, M. D.; Gaborieau, M.; Castignolles, P. *Anal. Bioanal. Chem.* **2015**, *407*, 2543-2555.
- (33) Castignolles, P.; Gaborieau, M.; Hilder, E. F.; Sprong, E.; Ferguson, C. J.; Gilbert, R. G. *Macromol. Rapid Commun.* **2006**, *27*, 42-46.
- (34) Gaborieau, M.; Causon, T.; Guillaneuf, Y.; Hilder, E. F.; Castignolles, P. *Aust. J. Chem.* **2010**, *63*, 1219-1226.
- (35) Couvreur, L.; Lefay, C.; Belleney, J.; Charleux, B.; Guerret, O.; Magnet, S. *Macromolecules* **2003**, *36*, 8260-8267.
- (36) Lacik, I.; Stach, M.; Kasak, P.; Semak, V.; Uhelska, L.; Chovancova, A.; Reinhold, G.; Kilz, P.; Delaittre, G.; Charleux, B.; Chaduc, I.; D'Agosto, F.; Lansalot, M.; Gaborieau, M.; Castignolles, P.; Gilbert, R. G.; Szablan, Z.; Barner-Kowollik, C.; Hesse, P.; Buback, M. *Macromol. Chem. Phys.* **2015**, *216*, 23-37.
- (37) Vander Heyden, Y.; Popovici, S. T.; Staal, B. B. P.; Schoenmakers, P. J. *J. Chromatogr. A* **2003**, *986*, 1-15.
- (38) Popovici, S. T.; Kok, W. T.; Schoenmakers, P. J. *J. Chromatogr. A* **2004**, *1060*, 237-252.
- (39) Park, S.; Chang, T. *Macromolecules* **2006**, *39*, 3466-3468.
- (40) Shortt, D. W. *J. Chromatogr. A* **1994**, *686*, 11-20.
- (41) Rollet, M.; Pelletier, B.; Altounian, A.; Berek, D.; Maria, S.; Beaudoin, E.; Gignes, D. *Anal. Chem.* **2014**, *86*, 2694-2702.
- (42) Beaudoin, E.; Favier, A.; Galindo, C.; Lapp, A.; Petit, C.; Gignes, D.; Marque, S.; Bertin, D. *European Polymer Journal* **2008**, *44*, 514-522.

for TOC only



Supporting Information

for

Quantifying the heterogeneity of chemical structures in complex charged polymers through the dispersity of their distributions of electrophoretic mobilities or of compositions

Joel J. Thevarajah^{1,2}, Adam T. Sutton^{1,2}, Alison R. Maniego^{1,2}, Elizabeth G. Whitty^{1,2}, Simon Harrison³, Hervé Cottet⁴, Patrice Castignolles^{1*}, Marianne Gaborieau^{1,2}

1 Western Sydney University, School of Science and Health, Australian Centre for Research of Separation Science (ACROSS), Parramatta NSW 2150, Australia

2 Western Sydney University, Molecular Medicine Research Group, School of Science and Health, Parramatta NSW 2150, Australia

3 IMRCP, UMR 5623, Université de Toulouse, 118 route de Narbonne, F-31062 Toulouse Cedex 9, France

4 Institut des Biomolécules Max Mousseron (IBMM, UMR 5247 CNRS, Université de Montpellier, Ecole Nationale Supérieure de Chimie de Montpellier), Place Eugène Bataillon CC 1706, 34095 Montpellier Cedex France

* Corresponding author : Patrice Castignolles, p.castignolles@westernsydney.edu.au, Fax: +61 2 9685 9915

Dispersity calculated through M_w and M_n

Integral expression of the dispersity using M_w and M_n

$$\frac{M_w}{M_n} = \frac{[\int W(M)M dM][\int W(M)M^{-1} dM]}{[\int W(M) dM]^2} \quad S1$$

Moments and dispersity of electrophoretic mobility distributions

The moments are defined as in Table S1¹.

Table S1. Summary of the integrals and discrete expressions of the moments relevant to this work

Moment order	Integral form	Discrete form
-1	$\int W(\mu) \mu^{-1} d\mu$	$\sum_z W(\mu_z) \mu_z^{-1} (\mu_{z+1} - \mu_z)$
0	$\int W(\mu) d\mu$	$\sum_z W(\mu_z) (\mu_{z+1} - \mu_z)$
1	$\int W(\mu) \mu d\mu$	$\sum_z W(\mu_z) \mu_z (\mu_{z+1} - \mu_z)$
2	$\int W(\mu) \mu^2 d\mu$	$\sum_z W(\mu_z) \mu_z^2 (\mu_{z+1} - \mu_z)$
3	$\int W(\mu) \mu^3 d\mu$	$\sum_z W(\mu_z) \mu_z^3 (\mu_{z+1} - \mu_z)$

$$\mu_w = \frac{[\sum_z W(\mu_z)\mu_z(\mu_{z+1}-\mu_z)]}{[\sum_z W(\mu_z)(\mu_{z+1}-\mu_z)]} \quad S2$$

The general expressions for the equations taking μ_w as a reference are as follows:

$$D(W(\mu), 1, \mu_w) = \frac{\overline{\mu_{\mu_w}^1} \times \overline{\mu_{\mu_w}^{-1}}}{[\overline{\mu_{\mu_w}^0}]^2} \quad S3$$

$$D(W(\mu), 2, \mu_w) = \frac{\overline{\mu_{\mu_w}^2} \times \overline{\mu_{\mu_w}^0}}{[\overline{\mu_{\mu_w}^1}]^2} \quad S4$$

$$D(W(\mu), 3, \mu_w) = \frac{\overline{\mu_{\mu_w}^3} \times \overline{\mu_{\mu_w}^1}}{[\overline{\mu_{\mu_w}^2}]^2} \quad S5$$

The integral forms of the general equations (eq 3, 5 to 8 and S3 to S4) are defined as:

$$D(W(A), b, c) = \frac{[\int W(A)(A-c)^b dA][\int W(A)(A-c)^{b-2} dA]}{[\int W(A)(A-c)^{b-1} dA]^2} \quad S6$$

$$D(W(\mu), 1, 0) = \frac{[\int W(\mu)\mu d\mu][\int W(\mu)\mu^{-1} d\mu]}{[\int W(\mu)d\mu]^2} \quad S7$$

$$D(W(\mu), 1, \mu_w) = \frac{[\int W(\mu)(\mu-\mu_w)d\mu][\int W(\mu)(\mu-\mu_w)^{-1} d\mu]}{[\int W(\mu)d\mu]^2} \quad S8$$

$$D(W(\mu), 2, 0) = \frac{[\int W(\mu)\mu^2 d\mu][\int W(\mu)d\mu]}{[\int W(\mu)\mu d\mu]^2} \quad S9$$

$$D(W(\mu), 2, \mu_w) = \frac{[\int W(\mu)(\mu-\mu_w)^2 d\mu][\int W(\mu)d\mu]}{[\int W(\mu)(\mu-\mu_w)d\mu]^2} \quad S10$$

$$D(W(\mu), 3, 0) = \frac{[\int W(\mu)\mu^3 d\mu][\int W(\mu)\mu d\mu]}{[\int W(\mu)\mu^2 d\mu]^2} \quad S11$$

$$D(W(\mu), 3, \mu_w) = \frac{[\int W(\mu)(\mu-\mu_w)^2 d\mu][\int W(\mu)(\mu-\mu_w)d\mu]}{[\int W(\mu)(\mu-\mu_w)^2 d\mu]^2} \quad S12$$

$$D_\sigma = \left[\frac{[\int W(\mu)(\mu-\mu_w)^2 d\mu]}{[\int W(\mu)d\mu]} \right]^{0.5} \quad S13$$

In the calculation of dispersity values for the experimental cases in the article, the discrete forms were used for the moments. Eq S5, S7 and S9 thus became:

$$D(W(\mu), 1, 0) = \frac{[\sum_z W(\mu_z)\mu_z(\mu_{z+1}-\mu_z)][\sum_z W(\mu_z)\mu_z^{-1}(\mu_{z+1}-\mu_z)]}{[\sum_z W(\mu_z)(\mu_{z+1}-\mu_z)]^2} \quad S14$$

$$D(W(\mu), 2, 0) = \frac{[\sum_z W(\mu_z) \mu_z^2 (\mu_{z+1} - \mu_z)] [\sum_z W(\mu_z) (\mu_{z+1} - \mu_z)]}{[\sum_z W(\mu_z) \mu_z (\mu_{z+1} - \mu_z)]^2} \quad S15$$

$$D(W(\mu), 3, 0) = \frac{[\sum_z W(\mu_z) \mu_z^3 (\mu_{z+1} - \mu_z)] [\sum_z W(\mu_z) \mu_z (\mu_{z+1} - \mu_z)]}{[\sum_z W(\mu_z) \mu_z^2 (\mu_{z+1} - \mu_z)]^2} \quad S16$$

$$D_\sigma = \left[\frac{[\sum_z W(\mu_z) (\mu_z - \mu_w)^2 (\mu_{z+1} - \mu_z)]}{[\sum_z W(\mu_z) (\mu_{z+1} - \mu_z)]} \right]^{0.5} \quad S17$$

μ_w as a reference

In eq S7, S9 and S11 the term $W(\mu)(\mu - \mu_w)d\mu$, can be split. According to eq 4 the 2 resulting terms in eq S18 are equal and therefore the 1st order moment remains undefined. As this term is undefined it explains why the results using μ_w as a reference do not produce the same trend as $D(W(\mu), 1, 0)$, $D(W(\mu), 2, 0)$ and $D(W(\mu), 3, 0)$.

$$\int W(\mu)(\mu - \mu_w)d\mu = \int W(\mu)\mu d\mu - \mu_w \int W(\mu)d\mu = 0 \quad S18$$

Dispersivity of composition distributions

The discrete form used in the calculation derived from eq 10 is defined as:

$$C_w = \frac{[\sum_z W(c_z) c_z (c_{z+1} - c_z)]}{[\sum_z W(c_z) (c_{z+1} - c_z)]} \quad S19$$

The integral forms of the general equations (eq 5 to 8) for composition are defined as:

$$D(W(C), 1, 0) = \frac{[\int W(c) c dc] [\int W(c) c^{-1} dc]}{[\int W(c) dc]^2} \quad S20$$

$$D(W(C), 2, 0) = \frac{[\int W(c) c^2 dc] [\int W(c) dc]}{[\int W(c) c dc]^2} \quad S21$$

$$D(W(C), 3, 0) = \frac{[\int W(c) c^3 dc] [\int W(c) c dc]}{[\int W(c) c^2 dc]^2} \quad S22$$

$$D(W(C), \sigma, C_w) = \left[\frac{[\int W(c) (c - C_w)^2 dc]}{[\int W(c) dc]} \right]^{0.5} \quad S23$$

In the calculation of dispersity values for the experimental cases in the article, the discrete forms were used for the moments. Eq S20 to S23 thus became:

$$D(W(C)1,0) = \frac{[\sum_z W(C_z) C_z (C_{z+1}-C_z)][\sum_z W(C_z) C_z^{-1} (C_{z+1}-C_z)]}{[\sum_z W(C_z) (C_{z+1}-C_z)]^2} \quad S24$$

$$D(W(C), 2,0) = \frac{[\sum_z W(C_z) C_z^2 (C_{z+1}-C_z)][\sum_z W(C_z) (C_{z+1}-C_z)]}{[\sum_z W(C_z) C_z (C_{z+1}-C_z)]^2} \quad S25$$

$$D(W(C), 3,0) = \frac{[\sum_z W(C_z) C_z^3 (C_{z+1}-C_z)][\sum_z W(C_z) C_z (C_{z+1}-C_z)]}{[\sum_z W(C_z) C_z^2 (C_{z+1}-C_z)]^2} \quad S26$$

$$D(W(C), \sigma, C_W) = \left[\frac{[\sum_z W(C_z) (C_z - C_W)^2 (C_{z+1} - C_z)]}{[\sum_z W(C_z) (C_{z+1} - C_z)]} \right]^{0.5} \quad S27$$

Number average electrophoretic mobility

By definition the number-average electrophoretic mobility can be expressed as:

$$\mu_n = \frac{\int N(\mu)\mu d\mu}{\int N(\mu)d\mu} \quad S28$$

Where $N(\mu)$ is the number distribution of electrophoretic mobility (which could be obtained by the detection of end-groups of the polymer after derivatization and use of a fluorescence detector). The ratio between the weight distribution and the number distribution of chains at a given electrophoretic mobility is the number-average molar mass of all polymer chains having the same electrophoretic mobility μ , $M_n(\mu)$. $M_n(\mu)$ has never been determined experimentally. Its determination by coupling the CE separation to a SEC second dimension is highly unlikely due to the injected volume in the CE being several orders of magnitudes smaller than in SEC. The number distribution of electrophoretic mobility is thus not considered further in this work.

In this work, the dispersity of the electrophoretic mobility (or composition) distributions are calculated using the ratio of the 0th to the -1st order moments of the relevant distribution. In the case of M_n , the ratio of the 0th to the -1st order moments of the molar mass distribution leads to a different but corresponding expression to the definition of $M_n^{2,3}$. In the case of μ_n the ratio of the 0th to the -1st order moments of the electrophoretic mobility distribution does not correspond to the definition above:

$$\frac{\int W(\mu)d\mu}{\int \frac{W(\mu)}{\mu} d\mu} = \frac{\int \frac{N(\mu)}{M_n(\mu)} d\mu}{\int \frac{N(\mu)}{\mu M_n(\mu)} d\mu} \neq \mu_n \quad S29$$

Estimation of the uncertainty on the electrophoretic mobility dispersity

To estimate the uncertainty of the dispersity due to the experimental error of the electrophoretic mobility, each of the dispersity equations was derived as follows. Each of the electrophoretic mobility dispersities expressed in eq 5 to 7 as a function of μ (except the standard deviation) can be expressed as a combination of other functions of μ : f , g and h (each of them being a moment). The fractional uncertainties add in quadrature.

$$D = \frac{f \cdot g}{h^2} \quad \text{S30}$$

The differentiation of eq S30 results in:

$$\frac{dD}{D} = \sqrt{\left(\frac{df}{f}\right)^2 + \left(\frac{dg}{g}\right)^2 + 2\left(\frac{dh}{h}\right)^2} \quad \text{S31}$$

Eq S31 can be rearranged in:

$$\frac{dD}{D} = \frac{d\mu}{\mu} \sqrt{\left(\frac{\mu \cdot f'}{f}\right)^2 + \left(\frac{\mu \cdot g'}{g}\right)^2 + 2\left(\frac{\mu \cdot h'}{h}\right)^2} \quad \text{S32}$$

For example for eq 5 with $D(W(\mu),1,0)$, $f = \int W(\mu)\mu d\mu$; $g = \int W(\mu)\mu^{-1}d\mu$; and $h = \int W(\mu) d\mu$

Therefore $f' = W(\mu)\mu$; $g' = W(\mu)\mu^{-1}$; and $h' = W(\mu)\mu$

The expression of eq S32 in the case of the derivation of eq 5 to 7 are given in eq 11 to 13.

To calculate the relative uncertainty of the dispersity values based on eq 11 to 13, the discrete forms below were used (eq S33 to S35). Peak areas of the appropriate functions were used for sums, and peak heights for μ_z values outside of sums.

$$\frac{dD(W(\mu),1,0)}{D(W(\mu),1,0)} = \frac{d\mu}{\mu} \sqrt{\left(\frac{W(\mu)\mu^2}{\sum_z W(\mu)\mu_z(\mu_{z+1}-\mu_z)}\right)^2 + \left(\frac{W(\mu)}{\sum_z W(\mu)\mu_z^{-1}(\mu_{z+1}-\mu_z)}\right)^2 + 2\left(\frac{W(\mu)\mu_z}{\sum_z W(\mu)(\mu_{z+1}-\mu_z)}\right)^2} \quad \text{S33}$$

$$\frac{dD(W(\mu),2,0)}{D(W(\mu),2,0)} = \frac{d\mu}{\mu} \sqrt{\left(\frac{W(\mu)\mu^3}{\sum_z W(\mu)\mu_z^2(\mu_{z+1}-\mu_z)}\right)^2 + \left(\frac{W(\mu)\mu}{\sum_z W(\mu)(\mu_{z+1}-\mu_z)}\right)^2 + 2\left(\frac{W(\mu)\mu^2}{\sum_z W(\mu)\mu_z(\mu_{z+1}-\mu_z)}\right)^2} \quad \text{S34}$$

$$\frac{dD(W(\mu), 3,0)}{D(W(\mu), 3,0)} = \frac{d\mu}{\mu} \sqrt{\left(\frac{W(\mu)\mu^4}{\sum_z W(\mu)\mu_z^3(\mu_{z+1} - \mu_z)}\right)^2 + \left(\frac{W(\mu)\mu^2}{\sum_z W(\mu)\mu(\mu_{z+1} - \mu_z)}\right)^2 + 2\left(\frac{W(\mu)\mu^3}{\sum_z W(\mu)\mu_z^2(\mu_{z+1} - \mu_z)}\right)^2}$$

S35

The differentiation of the electrophoretic mobility dispersity expressed in eq 8 as a standard deviation follows a different path, as this dispersity can be expressed as a combination of functions f and g of μ (each of them being a moment):

$$D = \left(\frac{f}{g}\right)^{0.5}$$

S36

The differentiation of eq S36 results in:

$$\frac{dD}{d\mu} = 0.5 \sqrt{\left(\frac{df'}{f}\right)^2 + 0.5 \left(\frac{\mu g'}{g}\right)^2}$$

S37

Eq S37 can be rearranged in:

$$\frac{dD}{d\mu} = \frac{d\mu}{\mu} \sqrt{0.5 \left(\frac{\mu f'}{f}\right)^2 + 0.5 \left(\frac{\mu g'}{g}\right)^2}$$

S38

In eq 8 with D_σ , $f = \int W(\mu)(\mu - \mu_w)^2 d\mu$ and $g = \int W(\mu) d\mu$,

Therefore $f' = W(\mu)(\mu - \mu_w)^2$ and $g' = W(\mu)$

Substituting f, g, f', g' in eq S38 yields eq 14. To calculate the uncertainty of the dispersity values based on eq 8, the discrete form below was used (eq S39).

Peak areas of the appropriate functions were used for sums, and peak heights for μ values outside of sums.

$$\frac{dD_\sigma}{D_\sigma} = \frac{d\mu}{\mu} \sqrt{\left(\frac{W(\mu)(\mu - \mu_w)^2 \mu}{2 \sum_z W(\mu)(\mu_z - \mu_w)^2 (\mu_{z+1} - \mu_z)}\right)^2 + \left(\frac{W(\mu)\mu}{2 \sum_z W(\mu)(\mu_{z+1} - \mu_z)}\right)^2}$$

S39

The value of the dispersity uncertainty was calculated for a sample of each of the experimental cases through eq S33 to S35 and S39, using 1 % for $\frac{d\mu}{\mu}$ as an estimate of the published RSDs of the electrophoretic mobilities (Table S2). Results are listed in Table S3.

Table S2. RSD of the electrophoretic mobilities of polyelectrolytes⁴ and sugars^{5,6} in the literature.

Sample	RSD (%)		
Linear PNaA	1.63		
3-arm star PNaA	1.34		
Hyperbranched PNaA	1.15		
Cellobiose	0.39		
Galactose	0.41	0.53	0.17
Glucose	0.45	0.63	0.06
Rhamnose	0.63	0.76	
Mannose	0.40	0.77	0.71
Arabinose	0.57	0.60	0.46
Xylose	0.40	0.54	0.29
Arabitol	0.74	1.33	

Table S3. Relative uncertainty values of the dispersity of the electrophoretic mobility distribution, calculated for a sample of each of the experimental cases using eq S33 to S35 and S39. The chitosan sample with a DA of 19.8, P(NaA-co-APA) at 5 minutes reaction time, PNaA 3 arm star and PAAkPAM10k were chosen. The uncertainty is given in $m^2V^{-1}s^{-1}$.

Sample	$\frac{dD(W(\mu), 1,0)}{D(W(\mu), 1,0)}$	$\frac{dD(W(\mu), 2,0)}{D(W(\mu), 2,0)}$	$\frac{dD(W(\mu), 3,0)}{D(W(\mu), 3,0)}$	$\frac{dD(W(\mu), \sigma, \mu_w)}{D(W(\mu), \sigma, \mu_w)}$
Chitosan	3.6×10^{-17}	1.3×10^{-12}	4.3×10^{-9}	1.3×10^{-12}
P(NaA-co-APA)	4.4×10^{-17}	1.1×10^{-12}	4.8×10^{-10}	1.3×10^{-12}
PNaA	9.3×10^{-18}	2.8×10^{-14}	1.4×10^{-13}	9.5×10^{-14}
PAA2kPAM10k	8.0×10^{-18}	5.5×10^{-14}	2.3×10^{-12}	2.0×10^{-13}

Estimation of the uncertainty on the composition dispersity

The differentiation of the composition dispersity follows the same approach as detailed above for the electrophoretic mobility dispersity. The resulting equations are similar to eq S33 to S35 and S39, except that C must be substituted for μ . The values of the dispersity uncertainty were calculated for block copolymer samples (Table S4).

Table S4. Relative uncertainty values of the dispersity of the composition distribution, for block copolymer samples (calculated from equations analogous to S33 to S35 and S39, with C substituted for μ)

Sample	$\frac{dD(W(C), 1,0)}{D(W(C), 1,0)}$	$\frac{dD(W(C), 2,0)}{D(W(C), 2,0)}$	$\frac{dD(W(C), 3,0)}{D(W(C), 3,0)}$	$\frac{dD(W(C), \sigma, C)}{D(W(C), \sigma, C)}$
PAA2kPAM10k	2.3×10^{-3}	5.4×10^{-7}	1.2×10^{-6}	3.1×10^{-6}
PAA10kPAM10k	5.6×10^{-3}	2.2×10^{-6}	2.7×10^{-6}	2.8×10^{-6}

Experimental samples

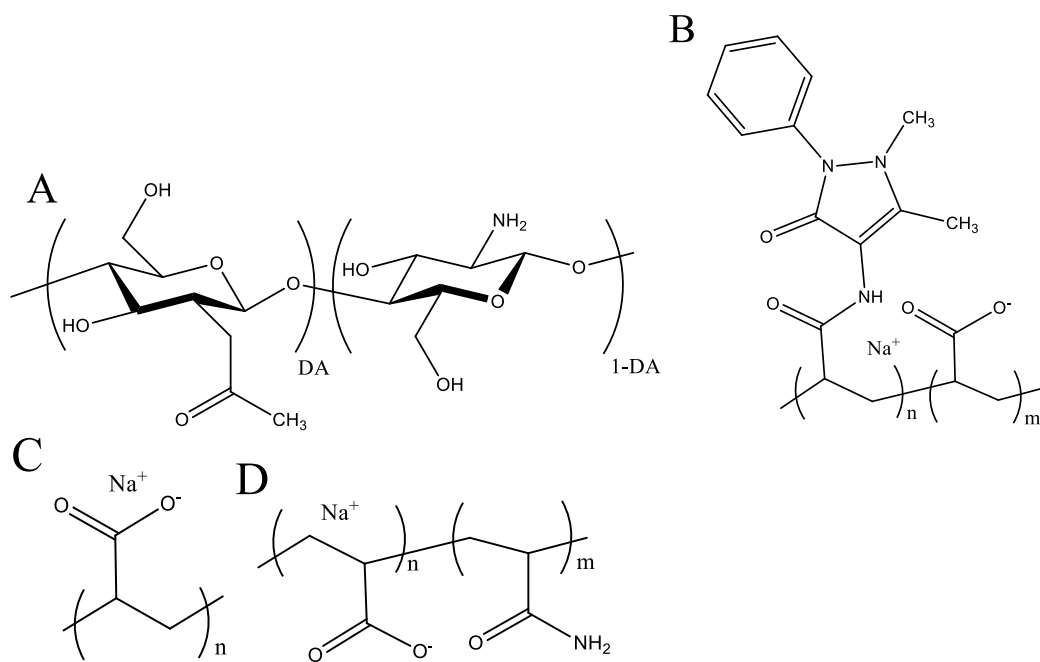


Figure S1. The molecular structure of (A) chitosan with *N*-acetyl-D-glucosamine and D-glucosamine units⁷ (B) P(NaA-co-APA), (C) PNaA and (D) P(NaA-*b*-AM).

Table S5. Dispersity of chitosan samples

DA^a	μ_w^b	SD^c (10^{-10})	$D(W(\mu),1,0)-1$ (10^{-4})	$D(W(\mu),2,0)-1$ (10^{-4})	$D(W(\mu),3,0)-1$ (10^{-4})
19.8	3.21	17.6	30.9	30.2	29.4
13.6	3.44	17.9	27.9	27.1	26.4
3.5	3.60	5.05	2.00	1.97	1.94
18.7	3.23	14.0	19.4	18.9	18.4
16.5	3.38	15.7	22.6	21.5	20.5
3.2	3.64	4.86	1.81	17.9	1.77
15.5	3.41	11.5	11.6	11.4	11.3

^a number-average DA measured using quantitative 1H NMR spectroscopy at $90^\circ C^8$

^b the weight-average electrophoretic mobility is given in $10^{-8} \cdot m^2 V^{-1} s^{-1}$

^c the standard deviation is given in $m^2 V^{-1} s^{-1}$

Molar absorptivity P(NaA-co-APA)

The UV absorbance of PNaA and AAP were calculated using the absorbance obtained experimentally in the conditions of the separation (195 nm wavelength, sodium borate buffer 110 mM at 25 °C).

Linear PNaA: $1.7 \cdot 10^{-6}$ a.u.·V·s·m⁻²

AAP: $3.4 \cdot 10^{-5}$ a.u.·V·s·m⁻²

Table S6. Dispersity values of P(NaA-co-APA) samples. The values are first given as determined from eq 5 to 8; the value obtained for the linear PNaA (sample at time 0) was then subtracted from these values.

Time (min)					After subtracting value for Linear PNaA			
	$D(W(\mu),1,0) -1$ (10^{-4})	$D(W(\mu),2,0) -1$ (10^{-4})	$D(W(\mu),3,0) -1$ (10^{-4})	SD ^a (10^{-10})	$D(W(\mu),1,0) -1$ (10^{-4})	$D(W(\mu),2,0) -1$ (10^{-4})	$D(W(\mu),3,0) -1$ (10^{-4})	SD ^a (10^{-10})
0	0.13	0.13	0.13	1.33	0	0	0	0
5	0.82	0.82	0.825	3.20	0.696	0.696	0.697	1.86
45	0.96	0.96	0.954	3.44	0.830	0.828	0.826	2.11
85	0.99	0.99	0.986	3.51	0.863	0.861	0.859	2.17
125	1.05	1.05	1.05	3.62	0.927	0.923	0.92	2.28
165	1.19	1.18	1.18	3.82	1.06	1.06	1.05	2.49
205	1.26	1.26	1.25	3.93	1.13	1.13	1.12	2.60
245	1.27	1.27	1.26	3.95	1.14	1.14	1.13	2.61
285	1.27	1.26	1.26	3.94	1.14	1.13	1.13	2.61
325	1.24	1.23	1.22	3.90	1.11	1.10	1.1	2.57
365	1.47	1.46	1.45	4.24	1.34	1.33	1.32	2.90
405	1.15	1.14	1.13	3.75	1.02	1.01	1	2.41
445	1.21	1.2	1.19	3.85	1.08	1.07	1.06	2.52
485	1.11	1.1	1.09	3.69	0.983	0.97	0.967	2.35

^a the standard deviation is given in $m^2V^{-1}s^{-1}$

Branched PNaA

Table S7. Summary of PNaA dispersity values representing the heterogeneity of samples with different branching topologies and samples produced from different monomers

Sample	μ_w $10^{-8} \cdot \text{m}^2 \text{V}^{-1} \text{s}^{-1}$	SD (10^{-10}) $\text{m}^2 \text{V}^{-1} \text{s}^{-1}$	$D(W(\mu), 1, 0) - 1$ (10^{-4})	$D(W(\mu), 2, 0) - 1$ (10^{-4})	$D(W(\mu), 3, 0) - 1$ (10^{-4})
3-Arm star	3.45	13.7	15.9	15.2	14.4
Hyper- branched	3.53	5.4	2.28	2.34	2.39
Linear	3.74	1.33	0.10	0.10	0.13
PNaA-AA	3.69	7.18	3.73	3.78	3.83
PNaA-tBA	3.66	9.64	6.84	6.95	7.06

P(AA-*b*-AM) composition calculation

The composition C taken as the molar fraction of acrylic acid monomer units in the copolymer is linked to the electrophoretic mobility μ of the copolymer through eq S40⁹:

$$C = \frac{\alpha\mu}{\mu(\alpha-1) + \mu_0} \quad \text{S40}$$

where α is a rescaling factor, and μ_0 is the electrophoretic mobility of the charged homopolymer. α depends on the chemical nature of both homopolymers, on the background electrolyte, and on the temperature¹⁰. Combining eq S40 with eq 9 leads to:

$$W(C) = W(\mu) \frac{[\mu(\alpha-1) + \mu_0]^2}{\alpha\mu_0} \quad \text{S41}$$

Any error in the determination of the rescaling factor α would thus result in an error in the calculated composition distribution, $W(C)$.

Determining alpha

For a block copolymer consisting of one charged block and one neutral block the number of effective monomer units can be related to its electrophoretic mobility in the following equation¹¹:

$$\mu = \mu_0 \frac{n_c}{n_c + \alpha n_u} \quad \text{S42}$$

Where n_c is the number of charged monomer units in the copolymer and n_u is the number of uncharged monomer units in the copolymer.

Eq S42 can be rearranged into eq S43 so that a plot of $\mu_0/\mu - 1$ vs n_u/n_c will yield α as the slope.

$$\frac{\mu_0}{\mu} - 1 = \alpha \frac{n_u}{n_c} \quad \text{S43}$$

Since synthetic block copolymers contain a distribution of n_u and n_c values, which then produce multiple μ values, careful selection of n_u and n_c values is required to accurately represent the sample. The values for n_u and n_c were calculated from the theoretical M_n of the block copolymer (previously listed in ¹² as well as in the caption of Figure 7).

Table S8. Dispersity of P(AA-*b*-AM) samples

Sample	PAA2KPAM10K		PAA2KPAM10K	
	A is μ	A is C	A is μ	A is C
$D(W(A),1,0)$	1.12	1.19	1.12	1.25
$D(W(A),2,0)$	1.10	1.16	1.08	1.15
$D(W(A),3,0)$	1.08	1.36	1.05	1.10
SD ($\times 10^{-9}$)	4.46 ^a	0.090	6.56 ^a	0.178

^a the standard deviation is given in $\text{m}^2\text{V}^{-1}\text{s}^{-1}$

- (1) Zabisky, R. C. M.; Chan, W. M.; Gloor, P. E.; Hamielec, A. E. *Polymer* **1992**, *33*, 2243-2262.
- (2) Hamielec, A. E.; Ouano, A. C. *J. Liq. Chromatogr.* **1978**, *1*, 111-120.
- (3) Gaborieau, M.; Gilbert, R. G.; Gray-Weale, A.; Hernandez, J. M.; Castignolles, P. *Macromol. Theory Simul.* **2007**, *16*, 13-28.
- (4) Maniego, A. R.; Ang, D.; Guillaneuf, Y.; Lefay, C.; Gigmes, D.; Aldrich-Wright, J. R.; Gaborieau, M.; Castignolles, P. *Anal. Bioanal. Chem.* **2013**, *405*, 9009-9020.
- (5) Oliver, J. D.; Gaborieau, M.; Hilder, E. F.; Castignolles, P. *J. Chromatogr. A* **2013**, *1291*, 179-186.
- (6) Oliver, J. D.; Sutton, A. T.; Karu, N.; Phillips, M.; Markham, J.; Peiris, P.; Hilder, E. F.; Castignolles, P. *Biotechnol. Appl. Biochem.* **2015**, *62*, 329-342.
- (7) Domard, A. *Carbohydr. Polym.* **2011**, *84*, 696-703.
- (8) Mnatsakanyan, M.; Thevarajah, J. J.; Roi, R. S.; Lauto, A.; Gaborieau, M.; Castignolles, P. *Anal. Bioanal. Chem.* **2013**, *405*, 6873-6877.
- (9) Vreeland, W. N.; Desruisseaux, C.; Karger, A. E.; Drouin, G.; Slater, G. W.; Barron, A. E. *Anal. Chem.* **2001**, *73*, 1795-1803.
- (10) Nedelcu, S.; Slater, G. W. *Electrophoresis* **2005**, *26*, 4003-4015.
- (11) Vreeland, W. N.; Desruisseaux, C.; Karger, A. E.; Drouin, G.; Slater, G. W.; Barron, A. E. *Anal. Chem.* **2001**, *73*, 1795-1803.
- (12) Sutton, A. T.; Read, E.; Maniego, A. R.; Thevarajah, J. J.; Marty, J. D.; Destarac, M.; Gaborieau, M.; Castignolles, P. *J. Chromatogr. A* **2014**, *1372*, 187-195.