Small-Angle X-Ray Scattering as a Useful Supplementary Technique to Determine Molecular Masses of Polyelectrolytes in Solution

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ABSTRACT: Determination of molecular masses of charged polymers is often nontrivial and most methods have their drawbacks. For polyelectrolytes, a new possibility for the determination of number-average molecular masses is represented by small-angle X-ray scattering (SAXS) which allows fast determinations with a 10% accuracy. This is done by relating the mass to the position of a characteristic peak feature which arises in SAXS due to the local ordering caused by charge-repulsions between polyelectrolytes. Advantages of the technique are the simplicity of data analysis, the independency from polymer architecture, and the low sample and time consumption. The method was tested on polyelectrolytes of various structures and chemical compositions, and the results were compared with those obtained from more conventional techniques, such as asymmetric flow field-flow fractionation, gel permeation chromatography, and classical SAXS data analysis, showing that the accuracy of the suggested method is similar to that of the other techniques. © 2016 The Authors. Journal of Polymer Science Part B: Polymer Physics Published by Wiley Periodicals, Inc. J. Polym. Sci., Part B: Polym. Phys. **2016**, *54*, 1913–1917

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INTRODUCTION

Determination of molecular masses is a crucial step in polymer science and has to be done before the polymer is used in any experiment or application. Obtaining the number (M_n) and mass-average (M_w) molecular masses is frequently a challenging task and this is especially true for charged polymers, as the strong intermolecular interactions may influence the results. Some of the most employed methods to obtain absolute masses are mass spectroscopy, multiangle laser light scattering, membrane osmometry, and NMR, as well as size exclusion techniques such as size exclusion chromatograph and asymmetric flow field-flow fractionation (AF4) which, combined with multidetector and refractive index analysis, can give information on masses and polydispersity indexes (PDIs).[1–5] However, most of those methods are time consuming (especially that based on osmometry), sample consuming or may not be applied to all types of polymers. As an example, end-group analysis using NMR is probably the most straightforward and quick analysis method to determine M_n, but it requires the presence of a clearly recognizable, nonoverlapping end-group signal with a good signal-to-noise ratio, which is not always present especially for high-molecular mass polymers. Here, we suggest the use of small-angle X-ray scattering (SAXS) as a reliable supplementary technique to be employed for a fast estimation of molecular mass of charged polymers using only small amounts of sample.

In SAXS the samples are irradiated with X-rays, which interact with the electrons in the molecules and generate scattered waves. Interactions between scattered waves give rise to scattering patterns, which can be recorded on a detector and analyzed. As stated by the name, SAXS focuses on small angles, analyzing the radiation scattered very close to the direct beam, usually below 5°. The intensity of x-rays is usually displayed as a function of the length of the scattering vector q, so that the data variation is wavelength independent. The parameter is related to the scattering angle as

Additional Supporting Information may be found in the online version of this article.
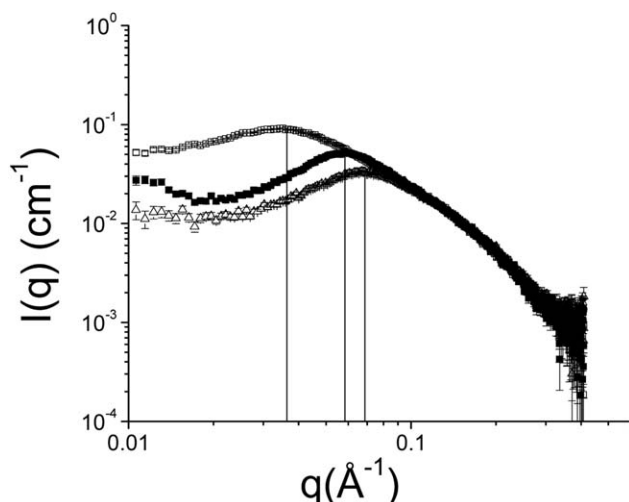
FIGURE 1 Example of SAXS data obtained from polyelectrolytes of different molecular weights, showing the presence of a peak-like structure arising from interparticle interactions. Data shown are for 1 wt % solutions of Poly(sodium styrene sulfonate) with molecular masses of approximately 32,000 $gmol^{-1}$ (empty squares), 6500 $gmol^{-1}$ (full squares), and 4200 $gmol^{-1}$ (empty triangles) as given by the supplier.

$$q = \frac{4\pi}{\lambda}\sin\theta \tag{1}$$

where θ is half the scattering angle and λ is the wavelength of the incoming x-ray beam.

The x-ray flux of modern laboratory-based SAXS instrumentation[6,7] is high enough to alllow measurements on relatively dilute solutions of singly dissolved polymer molecules with reasonable acquisition times.

The SAXS signal is proportional to the square of the electron density difference between polymer and solvent, and the q dependence is given by the size and shape of the polymers in solution (the form factor) and by the interactions between different polymers (described by the structure factor).[8] In the case of charged polymers, the structure factor is important because of the electrostatic repulsions between the polymers. For scattering data for salt-free solutions, this leads to a suppression of the signal at low q values, creating a characteristic peak-like feature at intermediate q values (Fig. 1). Data are shown on a logarithmic scale, as usual for SAXS data. Note however, that to determine the exact peak position it is helpful to plot the data on linear scale.

The "peak" position is related to the distance between two polymer coils (d) as

$$d = \frac{2\pi}{q} \tag{2}$$

To minimize the electrostatic repulsion, the polymers have to be as far from each other as possible for a given concentration and we therefore assume that they create a locally ordered and close-packed structure, similar to that of Wigner crystals.[9,10] From this, it is possible to calculate the density of

particles in the unit cell (Fig. 2) and, knowing the amount of polymer solubilized, to obtain the number average molar mass. It should be noted that the values obtained by this method are approximate, as defects in the local ordering are ignored.

For our calculations, we consider a face centered cubic (FCC) structure, as it is a close packed structure:

Noticing that a face diagonal is nothing else than $2d$, we can easily calculate the volume of the unit cell V_{cube} and then obtain the number density of particles, N, as

$$N = \frac{4}{V_{cube}} \tag{3}$$

where 4 is the number of polymeric units present in a single cell (note that the polymers present on the edges are shared between eight different FCC cells and therefore their contribution should be counted as $\frac{1}{8}$, while those on the faces are shared by two cells only and thus should be counted as $\frac{1}{2}$).

From the number density of polymers, we can obtain the number average molecular mass of the polymer, M_n, as,

$$M_n = \frac{C_w N_A}{N} \tag{4}$$

where C_w is the mass concentration of polymer in the sample and N_A is.

Combining eqs 2 to 4 and writing the volume of the cube as function of the q value, q^*, for the peak, we obtain

$$M_n = \frac{C_w N_A \left(\frac{4\pi}{q^* \sqrt{2}}\right)^3}{4} \tag{5}$$

where the only variables are C_w and q^*. Note that even though the method can only be applied to charged species,
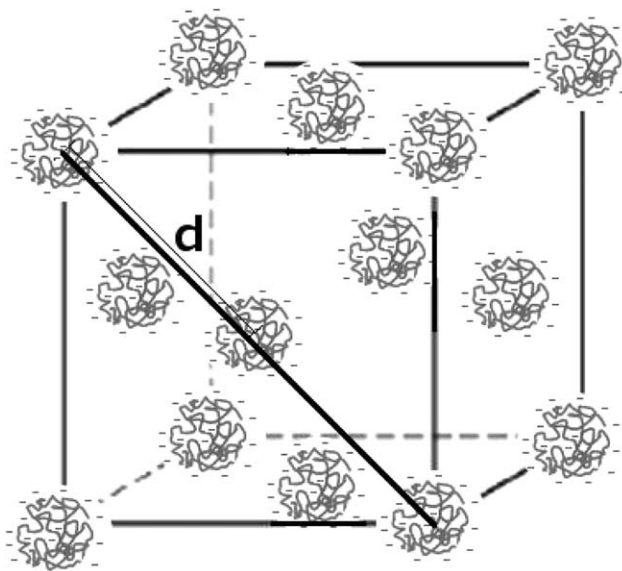

FIGURE 2 Schematic representation of the local ordering of the polyelectrolytes. For a FCC structure the distance between close neighbors is equal to half the face diagonal ($2d$ in the figure).

the applicability is still vast, as it is virtually independent of the chemical nature and structure of the polymer.

As for every technique, there are some issues to consider when employing the suggested SAXS-based method for mass determinations. First of all samples have to be suitable for SAXS analysis, meaning that there should be an electron density difference between solvent and polymer. Second, the polymer samples should be sufficiently dilute so that polymer species are molecularly dispersed and separated. This means that the polymer concentration must be much lower than the overlap concentration c^*, where the polymer entities start to overlap and the semidilute concentration regime is approached. The value of c^* can be estimated from[11] $c^*=1/[\eta]$, where $[\eta]$ is the intrinsic viscosity and can be determined from a capillary viscometer. The criterion is that $c/c^* \ll 1$.

Note that if the radius of gyration of the chains is determined in the scattering experiment, it is also possible to calculate the concentration relative to the overlap concentration of polymer coils[12] as

$$\frac{c}{c^*}=1/\left(N\pi\frac{4}{3}R_g^3\right) \tag{6}$$

It is also possible to check whether one is in the correct concentration regime by measuring the samples at several concentrations and determining the scaling of the peak position q^* with concentration cw. In the dilute regime the scaling is $q^* \propto C_w^{1/3}$ as the polymers are organized in a 3D lattice-like structure, as displayed in Figure 2. If the scaling is instead $q^* \propto C_w^{1/2}$ we are in a semidilute regime where the polymer chains tend to locally organize into a parallel arrangement with a 2D hexagonal structure[13] and the samples should be diluted further before proceeding to mass determinations.

The precision of the measurement can be calculated as

$$\frac{\delta M}{|M|}=\sqrt{\left(\frac{\delta C_w}{C_w}\right)^2+\left(\frac{3\delta q^*}{q^*}\right)^2} \tag{7}$$

The uncertainty on the concentration is usually very low, while it is more significant for q^*, where it is connected to the bin sizes for the scattering vector. For data acquired with the same bin size, and therefore the same δq^*, the precision decreases with decreasing q^*, and consequently with increasing masses.

Concerning the accuracy, assuming no defects (vacancies or interstitials) leads to an over-estimation of the number density and therefore of the mass value. The relative accuracy will then depend on the density of the defects. Comparing the results obtained from our method to more classical analysis (see below), we saw that the discrepancy is approximately 10% of the mass value.

Another possible contribution to the accuracy is if the thermal energy of the system is stronger than the electrostatic repulsions. In that case, the polymer peak position will be related to the minimum separation between polymer chains rather than to the average separation, giving again an over-

estimation of the number density and therefore again an underestimation of the mass value. This latter problem may be solved by measuring the sample at different temperatures and seeing when the peak position is constant.

To verify its applicability, the method was tested on a series of charged polymers of various chemical natures and chain lengths, including homopolymers and diblock copolymers with a charged and an uncharged block. The polymers were Poly(Sodium 4-Styrene Sulfonate) (PSSS) and Poly(2-vinyl N-methylpyridinium iodide) (P2V) purchased, respectively, from Sigma Aldrich and Polymersource and used as received, and Poly(N-isopropylacrylamide)-block-Poly(sodium styrene sulfonate) with 43/9 and 117/36 PNIPAm/PSSS repeat units, respectively, (PNIPAm-b−1 and PNIPAm-b−2) synthesized at Oslo University. The synthesis of the latter polymers was done using atom transfer radical polymerization via a simple "one-pot" procedure in a H_2O/DMF mixture solvent at room temperature. The copolymers were purified by dialysis and characterized using NMR and AF4, using similar conditions as described in detail previously.[14] The chemical structures of the polymers and their NMR characterization can be found in the Supporting Information, Sections S1 and S2, while their masses and PDI can be found in Section S3.

SAXS data were recorded using the two instruments available at Aarhus University. For both instruments, the range of modulus of the scattering vectors q spans between 0.01 and 0.35 Å^{-1}. One instrument uses a copper rotating source generating a wavelength of 1.54 Å. The other uses a new generation metal-jet x-ray source (Excillum) with a liquid gallium anode, generating a wavelength of 1.34 Å. Both instruments are flux- and background optimized cameras from Bruker AXS[6,7] that employ homebuilt scatterless slits.[15]

Samples were measured in a homebuilt holder for quartz capillaries of 2 mm diameter, holding approximately 30 μL. The acquisition time for the data was of 900 s for the instrument with a Cu source and 300 s for the instrument with a Ga source. Data were measured at a temperature of 20 °C. Polymer scattering was obtained by subtracting from the scattering of the sample a background obtained from the scattering of the solvent. The initial data treatment was performed using Milli-Q water as an absolute scale standard[16] for the scattering intensity. All samples were solubilized in Milli-Q water, at a concentration of 1 % wt.

To assess accuracy of the proposed method, the results were compared with values obtained from gel permeation chromatography (GPC) analysis, from AF4 and from SAXS data modeling on absolute scale. NMR was also used to determine the monomer ratio between the two components of the copolymers; it was, however, not possible to determine masses through NMR endgroup analysis due to the poor definition of the endgroup peak (see Section S2 of the Supporting Information for the NMR spectra). Data from GPC analyses were obtained from the supplier, while the other analyses were done at the universities of Aarhus (SAXS) and Oslo (NMR, AF4). Details on the analysis are provided in the following paragraphs.

AF4[17] was used to measure M_n, M_w, and PDI (M_w/M_n, PDI), with an estimated precision in mass determination between 5% and 10%. The AF4 experiments were conducted on an AF2000 FOCUS system (Postnova Analytics, Landsberg, Germany) equipped with an RI detector (PN3140, Postnova) and a multiangle (seven detectors in the range 35°−145°) light scattering detector (PN3070, $\lambda = 635$ nm, Postnova). The field-flow fractionation channel was installed with a 350 μm spacer and a regenerated cellulose membrane with a cut-off of 10,000 gmol^{-1} (Z-MEM-AQU-427N, Postnova) or 1000 gmol^{-1} (Z-MEM-AQU-425N, Postnova). A 20 μL sample was injected through an injection loop. The refractive index increment dn/dC$_w$ was obtained using the RI detector in a separate run employing only the tip flow, hence excluding separation, possible interactions with the membrane, salt gradients, and polymer escaping through the membrane. Assuming 100% mass recovery, the values of dn/dC$_w$ for the polymers were ascertained. Molar mass measurements were conducted with a constant detector flow rate of 1 mL/min. The focusing time was 5 min at a cross-flow of 3 mL/min. After the focus step, the cross-flow was linearly decreased to 0 mL/min over 12 min, followed by 8 min of elution using only the tip flow at 1 mL/min. To hinder aggregation of the PNIPAm containing polymers, the measurements were carried out with a channel temperature of 10 °C. The data were analyzed using the Postnova software (AF2000 Control, version 1.1.025). Values of M_w of the samples were determined using the Zimm-type fit. For PNIPAm-b-PSSS-1 and PNIPAm-b-PSSS-2 86 and 83% mass recovery was obtained, respectively.

Lastly, we employed a more traditional way to calculate the masses by SAXS which uses the values obtained from modeling on absolute scale. The intensity scattered at zero q, $I(0)$, is dependent on the number density of particles in the sample (N), volume of one polymer chain (V), the scattering length density difference between polymer and solvent ($\Delta\rho$), and the static structure factor $S(0)$ as

$$I(0) = N(\Delta\rho V)^2 S(0) \tag{8}$$

Rewriting N and V as function of the mass-average molar mass (M_w) we obtain

$$I(0) = \frac{C_w M_w}{N_A}(\Delta\rho v)^2 S(0) \tag{9}$$

where v is the specific volume of the polymer. The mass is then obtained as

$$M_w = \frac{I(0)N_A}{C_w(\Delta\rho v)^2 S(0)} \tag{10}$$

The volume v can be determined from density measurements and the scattering length density, $\Delta\rho$, is straightforwardly calculated for homopolymers.[18] For block copolymers, the calculation requires that the monomer ratio between the two blocks is known from NMR or from other techniques.
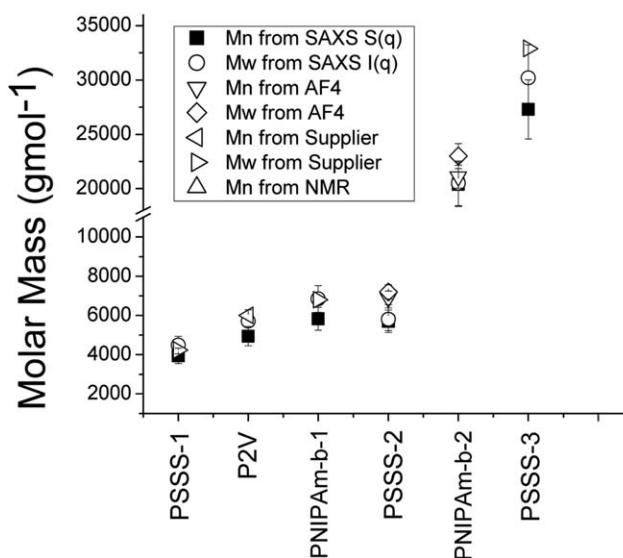

FIGURE 3 Molar masses obtained for seven different polyelectrolytes varying in length and chemical nature. Values obtained from the proposed method using the peak position are shown as black squares and are in good agreement with those obtained from the suppliers and by other techniques. Please notice the presence of a break in y axis from 11,000 to 18,000 gmol^{-1}.

To determine the forward scattering, it is possible to fit the full q range of the data using a chain form factor and an effective hard-sphere structure factor describing the interactions.[14] Note that in this way the obtained forward scattering is already corrected for structure factor effects, and should be used as $I(0)/S(0)$. As an alternative, the effect of $S(0)$ can be eliminated by adding small amounts of salt and/or by diluting the sample. Small amounts of salt will screen the electrostatic interactions, but not change significantly the scattering length density of the solvent, so that the contrast is unaltered. In this case the equation for mass determination should be modified to exclude structure factor contributions and will be

$$M_w = \frac{I(0)N_A}{C_w(\Delta\rho v)^2} \tag{11}$$

The estimated precision of determinations from the forward scattering is about 10%. Contributions to the errors are mainly from the absolute scale determination of $I(0)$ and from uncertainties in the calculations of the scattering length densities. Although the density measurements are very precise (better than 0.5%), its contribution to the contrast ($\Delta\rho$) is based on differences of typically 10% between the scattering density of the polymer and the solvent thus increasing the error. Also, there is some degree of uncertainty also in the calculation of the scattering length density due to the dissociation of counterions. In fact fully dissociated counterions will not contribute to the scattering from the polymer, but will give a small but significant change of the solvent scattering length density (for dilute polymer solutions), however, the effect is not trivial to take into account.

Additionally, even though it is possible to obtain both M_n and M_w from SAXS, it is not advisable to calculate PDI values from those, as they are approximate values only and as the two determinations are affected by very different error sources. In contrast, separation techniques such as AF4 give access to the entire distribution, and M_w, M_n are thus influenced in the same way by systematic errors so that the PDI as the ratio between them is more reliable.

The values obtained from the different determinations are shown in Figure 3 for both homopolymers and partially charged block copolymers with masses in the range from 4000 to 30,000 gmol^{-1}. Tables with the numerical mass values can be found in the Supporting Information, Section S3.

The figure shows the masses estimated using the proposed method agrees well with the results obtained by other techniques, thus giving an accurate estimate of the polymer masses. Comparing the results, we see that the discrepancy for all peak-position estimates is always within 10% of the mass value. Interestingly, this always corresponds to an underestimation of the mass value, possibly suggesting that the structural defects are mostly vacancies.

It is interesting to note that for the block copolymers less than one fourth of the repeat units were actually charged. This combined with the fact that the determination is virtually independent of the polymer architecture, extends the applicability of the technique to a wide range of complex linear or branched polyelectrolytes as well as to small charged particles (of a size within the q range of the instrument, in most cases between 1 and 100 nm). Even though we limited our work to strongly charged polyelectrolytes, the proposed method could also be applied to weak polyelectrolytes, at least as long as they are in a highly charged state and thus show a repulsion peak.

Another important aspect is that using this method the masses were determined within 20–30 min from when the sample was prepared. The results demonstrate that the proposed method based on SAXS peak positions analysis constitutes an accurate, simple and useful way for estimating the molecular masses of polyelectrolytes, proving that it as a good complement to the existing methods.

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