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Direct Molar Mass Determination of Self-Assembled Amphiphilic Block Copolymer Nanoobjects Using Electrospray-Charge Detection Mass Spectrometry

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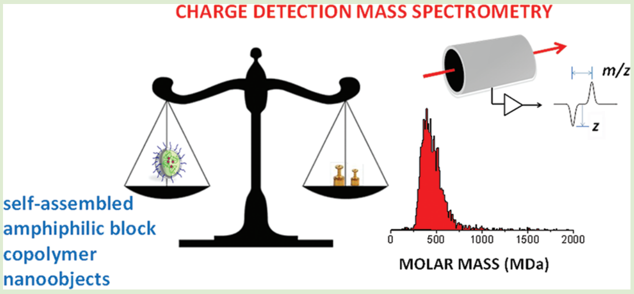
S *Supporting Information*

ABSTRACT: Charge detection mass spectrometry (CD-MS) combined with electrospray ionization was used to determine, in a direct way and for the first time, the molar mass of self-assembled amphiphilic block copolymer nanoobjects prepared via living radical emulsion polymerization. CD-MS supplies enough data for calculating statistically significant measurements of the mass of particles in the megadalton to gigadalton range and their resulting mass distribution.



With the development of living/controlled polymerization methods such as controlled radical polymerization (CRP) leading to well-defined copolymers, a huge amount of studies are currently devoted to their organization either in bulk or in a solvent.[1−8] As a typical example, the particular domain of amphiphilic block copolymer self-assemblies in a solvent selective for one of the blocks is currently highly active as the formed colloidal suspensions may find numerous applications in the materials and biomedical fields.[7−12] Such supramolecular organizations result in complex objects of nanometric dimensions with a variety of shapes and morphologies, based on a wide range of chemical structures. Accurate characterization techniques are thus particularly required to allow a better understanding of the final nanostructures and their formation process.

Two of these key data are the average molar masses of the assemblies along with their molar mass distribution. From the first value, both the number of particles per unit volume of dispersion and the average number of block copolymer chains constitutive of the nanostructures can be calculated, which are essential parameters of the self-assembly process. To date, there is however no direct way to determine these values. Most of the existing characterization techniques give a measurement of the particle dimensions from which the volume can be estimated, and the average molar mass has to be derived with the knowledge of the density of the components. When complex structures are regarded, this parameter cannot be determined with accuracy. So far, the method of choice to measure the molar mass of colloidal particles (namely, the weight average molar mass) is static light scattering technique. This technique is time-consuming and cannot provide information on the distribution. Consequently a method able to give a direct measurement of the molar mass and the molar mass distribution of nanoobjects in the megadalton to the gigadalton range, free of model assumptions and with a short response time, is highly desirable.

Two decades after the introduction of soft ionization techniques (matrix-assisted laser desorption/ionization (MALDI)[13,14] and electrospray ionization (ESI)[15]), mass spectrometry (MS) has become the method of choice for the measurement of molar mass (MM) and molar mass distribution (MMD) of individual polymer chains of kilodalton molar mass range.[16−20] Although both MALDI-MS[21,22] and ESI-MS[23,24] are able to "weigh" ions up to around 1 megadalton, a routine use of such traditional mass spectrometry techniques has shown severe limitations to determine MM around and above this value. One solution to overcome this limitation is to simultaneously measure m/z and z (the charge) for individual ions by charge detection mass spectrometry (CD-MS),[25−27] as presented in Scheme 1. Highly charged ions, produced by ESI, pass one at a time through a small metal flight tube attached to a charge-sensitive preamplifier that captures their image current (see Scheme 1). The time between the two pulses corresponds

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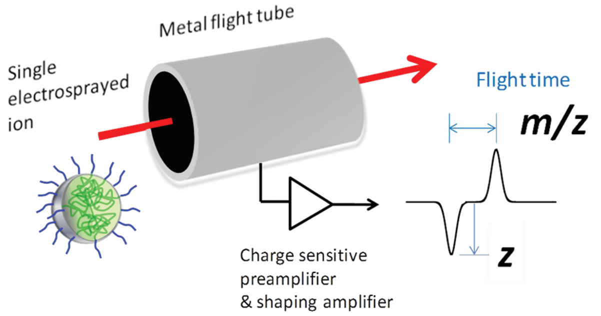
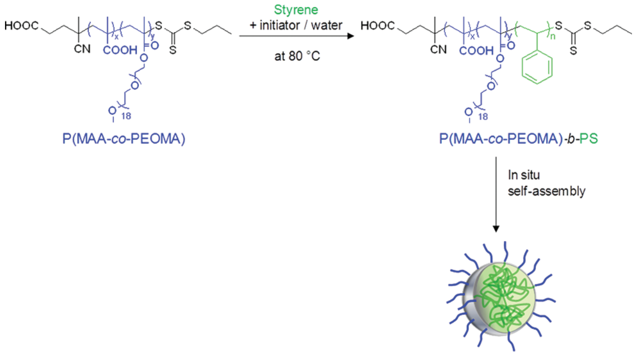
Scheme 1. Principle of Charge Detection Mass Spectrometry Applied to Weighing Electrosprayed Self-Assembled Amphiphilic Block Copolymer Nanoobjects



Scheme 2. Schematic Representation of the One-Pot Synthesis of Amphiphilic Diblock Copolymer Assemblies by RAFT-Mediated Aqueous Emulsion Polymerization of Styrene in the Presence of Poly(methacrylic acid-*co*-poly(ethylene oxide) methyl ether methacrylate) MacroRAFT Agents



to the flight time required for the ion to traverse the length of the pick-up tube. The amplitude of the image-charge signal is proportional to *z*. A primary advantage of the CD-MS measurement approach is the rate of highly charged individual ions that are analyzed. In such conditions, several thousands of ions can be analyzed individually in less than one minute.

So far CD-MS-based techniques have been applied with success to very high molar mass biological[28−31] and synthetic polymer[25,26,32,33] samples and might become an alternative characterization technique to size exclusion chromatography. The possibility of determining the molar mass of such giant macromolecular systems drove us to consider the particularly challenging molar mass determination of large size supramolecular structures. In this letter, we report the development of charge detection mass spectrometry (CD-MS) for fast, direct, and accurate determination of the absolute molar mass of polymer nanoparticles, constituted of self-assembled amphiphilic block copolymers. A series of molar mass distributions in the megadalton to gigadalton range was synthesized and studied by transmission electron microscopy, dynamic light scattering, and charge detection mass spectrometry. The pertinence of CD-MS for mass determination, as compared to other analytical techniques, is demonstrated.

Self-assembled nanometric micelles were prepared via a CRP technique, namely, RAFT (reversible addition−fragmentation chains transfer)-mediated[10,34] emulsion polymerization of styrene in simple batch conditions, which relies on the use of highly efficient water-soluble macroRAFT agents (possessing high transfer constants). Those macroRAFT agents are random poly(methacrylic acid-*co*-poly(ethylene oxide) methyl ether methacrylate) (P(MAA-*co*-PEOMA)) copolymers with a trithiocarbonate reactive group[35−37] as summarized in Scheme 2. Such hydrophilic polymer precursors are composed of methacrylic acid (MAA) subunits in the acidic form and poly(ethylene oxide) methyl ether methacrylate (PEOMA) subunits with the PEO side chains containing 19 ethylene oxide units. The composition is either MAA/PEOMA 50:50 mol/mol (samples 1 and 4) or MAA/PEOMA 67/33 mol/mol (samples 2, 3, and 5) (see Table ST1 in Supporting Information). The final product is an in situ created suspension of self-assembled P(MAA-*co*-PEOMA)-*b*-polystyrene amphiphilic block copolymer nanoobjects, in which the polystyrene blocks form the hydrophobic core and the poly(methacrylic acid-*co*-poly(ethylene oxide) methyl ether methacrylate) blocks compose the stabilizing hydrophilic shell. Due to the nature and high molar mass of the polymer chains forming the core, the nanoobjects are in a frozen state, and their organization (and hence their molar mass) is not affected by dilution in water or polar solvents.[38]

We synthesized five self-assembled amphiphilic block copolymer samples. These nanoobjects are spherical (as confirmed by transmission electron microscopy (TEM) analysis, see Figure 1), and their dimension and thus their molar mass were tuned by changing the molar ratio of methacrylic acid/poly(ethylene oxide) methyl ether methacrylate of the macroRAFT agent and the initial concentration ratio of the macroRAFT agent to styrene (see ref 36 and Table ST1 in Supporting Information for the overall characteristics of the particles).[36]

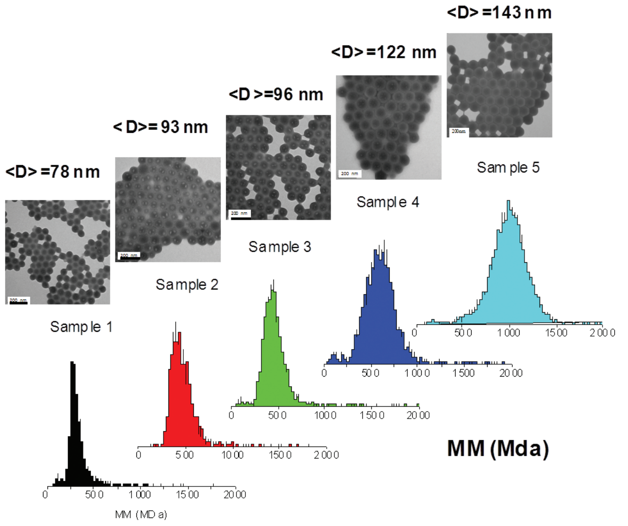


Figure 1. Histogram mass spectra obtained from CD-MS measurements and the corresponding transmission electron microscopy images of the five samples studied in this work.

We have obtained molar mass information for the five self-assembled amphiphilic block copolymer samples diluted in a water−methanol mixture (50:50 v/v). As mentioned in introduction, CD-MS measures *m*/*z* and *z* (the charge) for thousands individual ions in less than one minute. With our current setup, mass measurements can be performed in the 1−2000 megadalton range. Figure S1 in the Supporting Information displays the raw data obtained by CD-MS, which are plotted in a two-dimensional graph (z vs m/z) for sample 5. This (m/z-z) image illustrates the unique type of data that CD-MS can provide. The mass of each ion is obtained from a combination of both the charge and m/z values. Histograms of mass for the five samples are depicted in Figure 1. Each displayed histogram represents the measurement of about 10000 individual ions sampled during a period of 120 s. The mean charge on the micellar ions range from 1.3×10^{-16} C (∼800 e) to 2.5×10^{-16} C (∼1560 e) and can be compared to the Rayleigh's limiting charge.[39] This is an upper limit when spherical ions are formed as charged residues from spherical droplets, but smaller values are also possible. The Rayleigh's limiting charge q_R for a droplet having the surface tension γ of the solvent can be expressed as a function of the molar mass (MM in Da) by $q_R = 0.06375\ (\mathrm{MM})^{1/2}$ assuming density 1 g/cm^3 and $\gamma = 0.06375$ N/m (for a mixture of water/methanol). The observed mean charge on the micellar ions is about 70−80% of that held by a water/methanol (50:50, v/v)) droplet of the same size charged to the Rayleigh limit. The molar mass distributions of the self-assembled amphiphilic block copolymer samples exhibit essentially monomodal distributions with maxima ranging from 300 to 1000 MDa with a full width at half-maximum of a distribution ranging from ∼200 to 500 MDa. The mass width is due to the dispersion in the number of copolymer chains per particle. The mass distributions shown in Figure 1 can be characterized statistically by the weight average molar mass (M_w) and the number average molar mass (M_n) leading to the determination of polydispersity index (PDI = M_w/M_n). The analysis of molar mass distributions leads to PDIs ranging from 1.04 to 1.11 for the samples (see Table 1). Such low polydispersity indexes confirm the efficiency and quality of the RAFT-mediated aqueous emulsion polymerization to synthesize self-assembled amphiphilic block copolymer nano-objects. To our knowledge, it is the first time that MMDs and PDIs of intact synthetic polymer particles are directly determined by mass spectrometry. It is moreover of great interest to obtain such information for structures that are more complex than simple latex particles obtained via traditional emulsion polymerization.

Table 1. Number Average Molar Mass (M_n), Weight Average Molar Mass (M_w), and Corresponding Polydispersity Index (PDI = M_w/M_n) Calculated from CD-MS Molar Mass Distributions of the Studied Samples[a]

sample	M_n (MDa)	M_w (MDa)	PDI = M_w/M_n
1	299	331	1.11
2	448	469	1.05
3	452	472	1.04
4	591	636	1.08
5	984	1020	1.04

[a] M_n and M_w are calculated according to IUPAC definitions.[40] Mass distribution windows were restricted up to M_i values with n_i reaching 5% of n_{max} (where n_i is the number of macroions with molar mass M_i, and n_{max} is the highest n_i of the histogram).

As already mentioned in the introduction, most of the characterization techniques give a measurement of the particle dimensions from which a "volume" can be estimated and the average molar mass has to be derived with the knowledge of density of the components. A comparison of mass measurements derived from either TEM or dynamic light scattering (DLS) with our direct molar mass determination from CD-MS is given in Table ST2 in the Supporting Information. Average volumes were converted in molar masses assuming perfectly spherical nanoobjects and a homogeneous density of 1. For the five samples, the MMs obtained by CD-MS are bracketed by those obtained by TEM and DLS. The MMs obtained by CD-MS are systematically higher than those derived by TEM and lower than those extracted by DLS, as expected. Convergence between MMs extracted from TEM and CD-MS is observed for heavier samples. A hydrodynamic volume is accessible by dynamic light scattering, which overestimates the real size and thus the molar mass of the nanoobjects, while on the other hand, TEM presents a better contrast for the core hydrophobic moieties which leads to an underestimation of the real mass of the nanoobjects.

To conclude, the CD-MS-based analyses presented here provide for the first time a direct measurement of molar mass distributions of synthetic polymer particles constituted of self-assembled amphiphilic block copolymers in the 100−1000 MDa range. Beyond MM measurements and the evaluation of the accuracy of CD-MS as compared to complementary techniques, CD-MS provides molar mass distribution and PDI values. These analyses represent a breakthrough and demonstrate the great potential of charge detection mass spectrometry in the characterization of supramolecular organized polymers. They may drive significant advances in the understanding of both the formation and final structure of those nanoobjects. We indeed intend to use the method to further investigate the early times of the reaction and gain information on the nucleation step, based on simultaneous chain growth and self-assembly.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, composition and molar mass characteristics of the amphiphilic block copolymers constitutive of the analyzed nanoparticles and the average particle diameter of the latter from DLS and TEM, molar mass comparative data (CD-MS, DLS, TEM), and mass-to-charge ratio vs charge ($m/z - z$) image of individual ions from sample 5. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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