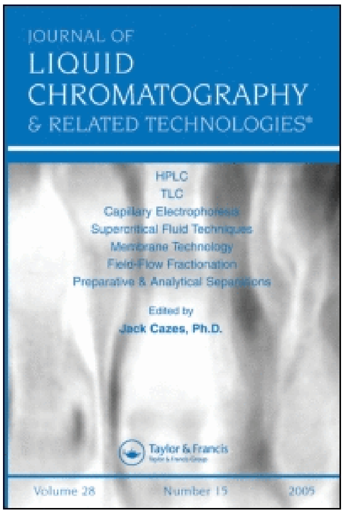

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Easy Monitoring, by Flow FFF, of the Behavior of Nanoparticles Formed from Copolymers of Sulfopropylmethacrylate and Methylmethacrylate

Maria-Anna Benincasa[a]; Valeria Mazzoni[a]
[a] Department of Chemistry, University of Rome "La Sapienza", Rome, Italy

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Easy Monitoring, by Flow FFF, of the Behavior of Nanoparticles Formed from Copolymers of Sulfopropylmethacrylate and Methylmethacrylate

Maria-Anna Benincasa and Valeria Mazzoni
Department of Chemistry, University of Rome "La Sapienza",
Rome Italy

Abstract: The size and size distribution of nanoparticles are generally important for the characterization of these materials, but are particularly needed to determine their suitability for specific applications. When nanoparticles are obtained by micelle formation from synthetic copolymers their size and structure depend on the copolymer composition and charge. Minor changes in the macromolecule primary structure may lead to dramatic changes in the aggregation behavior, hence in the nanoparticle size and size distribution. While particle diameter may be accurately measured by various sizing methods, determination of the size distribution requires a methodology which combines an adequate resolving power with size measurement capabilities. Flow FFF has shown to be able to perform both tasks in a single run. Using a very small amount of sample it yields accurate size distribution curves of most various colloidal systems. For this reason it was used here for the analysis of two copolymers of sulfopropyl methacrylate and methylmethacrylate differing for the copolymerization process. Size analysis of these samples showed a dramatic difference in their capability of forming micellar aggregates as well as in the characteristic response to the solution ionic strength.

Keywords: Nanoparticles, Field-flow fractionation, FFF, Copolymers, Sulfopropylmethacrylate, Methylmethacrylate

Address correspondence to Maria-Anna Benincasa, Department of Chemistry, University of Rome "La Sapienza", Ple A. Moro, 5, 00185 Rome, Italy. E-mail: mariaanna.benincasa@uniromal.it

INTRODUCTION

The fascinating world of applications for nanoparticles, spanning from molecular electronics to nano-scale medical devices, able to enter cell membranes to perform various functions,[1–4] has stimulated an increasing involvement of analytical techniques to fully characterize these materials. The growing importance of nanoscience is grounded on the possibility of producing materials with predefined size and physicochemical properties for targeted performance. For instance, magnetic nanoparticle-based contrast agents have become indispensable tools in cancer diagnostics for early detection of tumors, or organ sclerosis, as well as in functional magnetic resonance imaging.[5] In ophthalmology the very low effectiveness shown by topical drugs in aqueous solution has prompted investigations to improve drug adsorption and residence time. Among the various approaches undertaken to improve drug effectiveness, binding or encapsulation into copolymer nanoparticles[6] proved to increase drug uptake, thus reducing discomfort for the patient.

The use of block copolymers for the controlled synthesis of nanoscale structures[7] has proven particularly useful, since incorporation of blocks with different properties provides the driving force for the formation of the supramolecular architecture and the subsequent phase separation. Unfortunately, most widely used polymers for the production of nanoparticles, namely polyalkylcyanoacrylates and polyalkymethacrylates, give particles with low surface hydrophilicity and limited loading capabilities. The need for a good hydrophilicity associated with physicochemical stability has led to the production of nanoparticles obtained from the copolymerization of methylmethacrylate (MMA) and sulfopropylmethacrylate (SPMA)[8] The latter with its sulfonic groups strongly charged in a wide pH range provides the negative surface charge necessary for stable aqueous suspensions, while the methylmethacrylate block incorporates the hydrophobic functionality for supramolecular structure formation.

Regardless of the methodology followed for synthesizing the copolymers, molecular weight determination, characterization of physicochemical properties, and evaluation of suitability of nanoparticles for the proposed application are always needed. The analysis of complex systems with "batch" analytical techniques gives results, which, although accurate, are always averaged over relative concentrations of different populations and weighed by the specific response of each species to the technique employed. Only the combination of these methodologies with an appropriate separation technique may provide a detailed characterization of multivariant systems. Unfortunately, some of the techniques most commonly used for the separation of polymer solutions and colloidal suspensions have often limited applicability to large molecular size samples and considerable practical difficulty.

The absence of the constraints of a stationary phase and the possibility of using different types of fields to drive the separation process have merited close consideration of field-flow fractionation (FFF) for the analysis of complex mixtures of polymers and colloidal particles.[9–12] Among the peculiarities of this relatively new technique, are the lack of strong chemical interactions, which, on the contrary, act in the separation mechanism of liquid chromatography, and the reduced extensional shear force typical of packed columns. Also, the possibility of controlling retention by varying the field strength as needed, and the rigorous theory that allows to precisely relate retention to sample properties (e.g., to molecular size) have contributed to the success of field-flow fractionation.

The biased results given by GPC for compact polymers and nanoparticles,[8,13–17] or the intrinsic difficulty of ultracentrifugation[18] have suggested the use of flow FFF (FlFFF) for the characterization of copolymers of potassium sulfopropylmethacrylate and methylmethacrylate. The choice of this particular sub-technique was dictated by its universal character that does not require samples to have any specific property to interact with the field.

Two samples of the amphiphilic diblock copolymers p(SPMA-*b*-MMA) were analyzed by aqueous flow FFF. The synthesis[19] carried out by the group of Prof. Crescenzi at the University of Rome followed, in one case, a two-step procedure with preliminary precipitation and isolation of a fifty unit block of $p(SPMA)_{50}$, and subsequent resuspension of the latter to allow reaction with a 25 unit methylmethacrylate block. Hereafter, this sample will be named p(SPMA-*b*-MMA) I. The second sample, labeled p(SPMA-*b*-MMA) II was obtained in a one-step procedure without isolation of the $p(SPMA)_{50}$ block before copolymerization with MMA.

EXPERIMENTAL

The flow FFF apparatus used here differed from that reported previously,[20] only for the detection system and the type of membrane. For a detailed description of the experimental set-up and operation, the reader is thus referred to the literature.

A SPD-10A UV-Vis spectrophotometric detector from Shimadzu (Kyoto, Japan) operating at 210 nm monitored the sample elution profile. The channel accumulation wall was lined with a thin film NADIR membrane of isotactic polypropylene from Hoechst (Wiesbaden, D). The narrow distributed polymer standards of polystyrene sulfonate of nominal molecular weight 12,900, 29,000, and 81,800 Da were provided by Polysciences (Warrington, PA). The salt solutions at ionic strength ranging from 5 to 75 mM that served as carrier liquids were obtained by dissolving Na_2SO_4 (Carlo Erba, IT) in distilled water further purified through an ion exchange/ultrafiltration device from USF (Ransbach-Baumbach, D). The p(SPMA-MMA) solutions were obtained by direct solubilization of the weighed amount of the lyophilized copolymer in the volume of distilled water necessary to have concentrations of ca 1 mg/mL. All analyses were carried out by injecting 4–6 μL of sample solution. Detailed description of the copolymers synthesis may be found in reference [19].

RESULTS AND DISCUSSION

Flow FFF of the p(SPMA-MMA) I sample, copolymerized in the two-step reaction, showed a highly reproducible bimodal profile as illustrated in Figure 1. In this figure, the profiles of three standards of polystyrene sulfonate (PSS), a polymer with the same charged group as the p(SPMA) segment, are also reported. The fractogram of the p(SPMA-*b*-MMA) I in Figure 1 reveals the presence of two well resolved components, one of which with molecular mass very close to that of the PSS 12,900. On the basis of the number of repeat units in the p(SPMA$_{50}$-*b*-MMA$_{25}$) copolymer, a molecular weight in this range is indeed easily anticipated for this macromolecule. By contrast, the more retained species in Figure 1 appears to have a molecular mass higher than ~80,000 Da.

In the amphiphilic block copolymer of sulfopropylmethacrylate and methylmethacrylate, hydrophilicity is ensured by the charged sulfonic groups, which also account for the polyelectrolitic nature of this macromolecule. It is well established in polymer chemistry that the solution ionic strength has a remarkable effect on the molecular conformation of flexible chain polyelectrolytes, inducing contraction of the polymer coil as the salt concentration increases.[21] Changes in retention level registered in the flow FFF of polyelectrolytes at different ionic strength are shown to depend on modifications of the macromolecule's conformation induced by the added

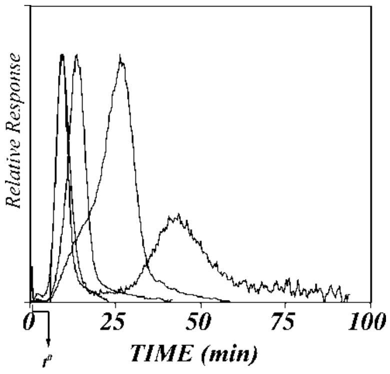

Figure 1. Flow FFF elution curves of the p(SPMA-*b*-MMA) I sample overlaid on the profiles for three polystyrene sulfonate standards. Nominal molar mass of the standards was in order of elution: 12.9 kDa, 29.0 kDa, and 81.8 kDa. Flow conditions for all samples were $\dot{V} =$ 0.2 mL/min and $\dot{V}_c = 1.2$ mL/min, using an aqueous solution of sodium sulfate at 5 mM ionic strength as eluent.

salt and modulated by its concentration.[22] In particular, the lower values of the molecule hydrodynamic diameter obtained from flow FFF measurements for flexible chain polyelectrolytes when the solution ionic strength is increased are attributed to the effective higher compactness of the macromolecule under these conditions. By contrast, neutral polymers, which are not expected to substantially change their molecular parameters under different ionic strength conditions, do not show remarkable variations in retention by flow FFF.[23] Therefore, for a full characterization of the p(SPMA-*b*-MMA) I, this sample was analyzed in solutions of increasing ionic strength. In spite of the fact that, due to the predicted polyelectrolitic nature of the SPMA-MMA copolymer, changes in the FIFFF retention time with ionic strength were expected for this sample, the elution profile of the p(SPMA-*b*-MMA) I appears unresponsive to the salt concentration in solution as can be seen in Figure 2. This behavior reveals a hydrodynamic size of the macromolecular coil independent of salt concentration. However, it is noted in this figure that, while neither the shape nor the retention time of both peaks are affected by the electrolyte concentration, as ionic strength increases the relative area of the later eluting component consistently increases at the expense of that of the less retained peak, totally compensating the decrease in this peak area. It thus appears that ionic strength induces the formation of larger size species rather than affecting the macromolecule's compactness, as generally observed for flexible chain polyelectrolytes in flow FFF. Although molecular aggregation, which is favored by ionic strength, could be claimed as the most obviously responsible of this phenomenon, it can not be invoked here to account for the formation of species of well defined size, as those under the peak in Figure 2 appear. Indeed, molecular

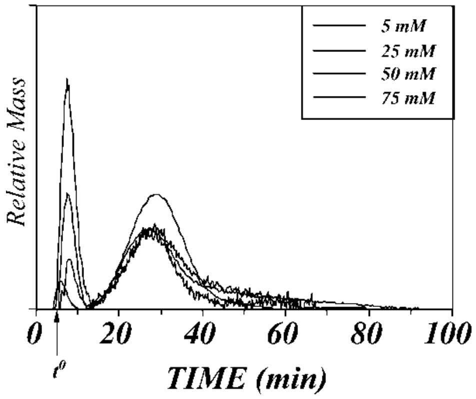

Figure 2. Area normalized flow FFF fractograms of the p(SPMA-*b*-MMA) I sample obtained using Na_2SO_4 aqueous solutions at the ionic strength shown in legend. Flow conditions for all runs were: $\dot{V} = 0.2$ and $\dot{V}_c = 0.6$ mL/min. The sample injected mass was between 3.2 and 4.8 μg.

aggregation, acting with a random mechanism, generally produces mixtures of species of different size, which generate broad and asymmetric FFF profiles[24] very dissimilar from those of Figure 2.

The formation of nanoparticles of well defined stoichiometry, and with narrow size distribution from free molecules of poly($SPMA_{50}$-*b*-MMA_{25}) I in solution, better explains the behavior registered for this sample in Figure 2. The amphiphilic character predictable for a copolymer made of two blocks (the p(SPMA) and p(MMA)) of completely different hydrophilicity, allows the formation of core shell nanoparticles having a hydrophobic center generated by interactions between the p(MMA) blocks and a hydrophilic corona of charged p(SPMA) terms.[6,8] The size as well as the charge of these nanoparticles is dictated by the copolymer composition.[8]

The presence of nanoparticles in the second peak of Figure 2 is further supported by the size distribution analysis accomplished according to the FFF theory.[25,26] Conversion of the time based fractograms into the corresponding size distribution functions reported in Figure 3, substantiates that each particle population of the p(SPMA-MMA) I sample, separated by flow FFF, has a well defined size range that does not change in solutions of different ionic strength, in spite of the fact that for the polyelectrolitic character of part of the copolymer some variation in retention time could be expected. On the other hand, the results of Figure 3, confirming that the solvent properties influence the relative amount of each sample component, indicate for nanoparticles formed from p(SPMA-MMA) I, a typical micelle behavior, with the critical concentration decreasing as more electrolyte is added.

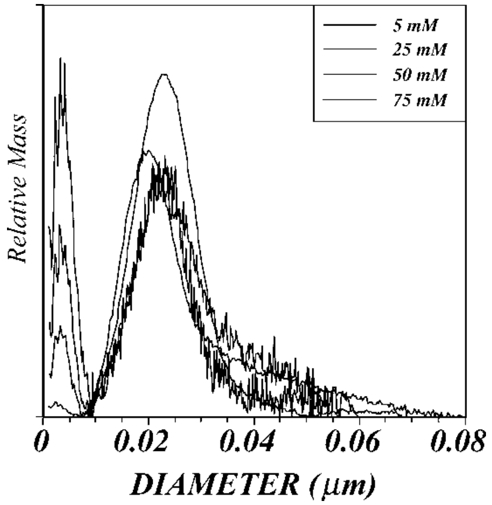

Figure 3. Size distributions for the p(SPMA-*b*-MMA) I obtained after the flow FFF analyses shown in Figure 2.

The mean diameter obtained from the first moment of the peaks in Figure 3, and their standard deviations, are reported in Table 1. It appears that the average size of the p(SPMA-*b*-MMA) I nanoparticles slightly, but consistently, decreases as ionic strength increases from 25 to 75 mM. This trend, typical of electrically charged macromolecules,[22–24] reveals the polyelectrolitic nature of the particle layer in contact with the solution, which decreases in thickness following decreased repulsion between the SPMA units. This behavior is perfectly consistent with the expected charged properties of the nanoparticles' corona. The diameter value at 5 mM ionic strength only apparently contrasts with data at the other salt concentrations. As a matter of fact, it has been shown that FlFFF retention of electrically charged samples at very low ionic strength is affected by interparticles repulsion, which by increasing the thickness of the eluting zone, reduces elution time. This decrease translates into lower measured particle diameters.[22] Altogether the mean diameter of the p(SPMA-*b*-MMA) I nanoparticles is reproducibly centered around 21–25 nm, a result in very good agreement with measurements obtained by dynamic light scattering.[19]

It is worth mentioning that standard deviations in Table 1 are a function of the width of the eluting band which is measured by the plate height. Several components affect band broadening in FFF systems,[27] among which, however, longitudinal diffusion may be considered negligible for macromolecular species because of their low diffusivity. By contrast, the nonequilibrium contribution due to diffusion of particles of a given size into streamlines of different velocity depends, among others, on the thickness of the eluting zone which could be reduced by a stronger field. In the case of flow FFF, a higher cross-flow velocity would decrease the band broadening due to nonequilibrium effects. If non-ideal contributions are minimized in an ideal working system the major component affecting band width for highly retained peaks remains the sample polydispersity. Standard deviation for the p(SPMA-*b*-MMA) I sample in Figure 1, obtained with a cross-flow rate of 1.2 mL/min, is only 3.8 nm, a much lower value than those reported in Table 1, which were measured at the same longitudinal flow velocity, but with a 0.5-fold lower cross-flow rate.

Table 1. Mean diameters and standard deviations of nanoparticles in the p(SPMA-*b*-MMA) I sample measured by flow FFF

Ionic strength (mM)	Diameter (nm)	Standard deviation (nm)
5	22.9	5.8
25	24.3	6.3
50	21.5	5.7
75	20.9	6.3

A study similar to that described above was carried out on another SPMA-MMA copolymer sample obtained by sequential polymerization of the MMA block after the 50 unit block of sulfopropylmethacrylate was synthesized. The fractograms of the p(SPMA-*b*-MMA) II collected using the same carrier liquids as for the p(SPMA-*b*-MMA) I sample are shown in Figure 4. Several differences are noted in the flow FFF behavior of this sample. Beside the peak at low retention level, no other species of narrowly disperse size is evident. However, the presence of components of increasing size is manifested by the broad peak tail registered at each ionic strength. It also appears that, in the p(SPMA-*b*-MMA) II sample the formation of large size species at the expenses of the low molecular weight material is favored by increased ionic strength. However, contrary to the first sample analyzed, this process does not produce narrowly disperse particles in the p(SPMA-*b*-MMA) II, but rather a mixture of compounds of different hydrodynamic diameter. Irregular and unreproducible FFF profiles, similar to those of Figure 4, have been detected in several samples prone to forming multimolecular aggregates.[24]

The model for the formation of nanoparticulate, introduced for the p(SPMA-*b*-MMA) I, is based on the assumption that this copolymer contains only two blocks, each one made of same repeat units different from those of the other block. In case the reaction does not proceed sequentially producing one block after the other as expected, a number of different copolymers may be formed having blocks made by one monomer type interrupted by units of the other monomer.[19] These compounds may still give rise to aggregates but with a variable stoichiometry that will depend on the block composition, in particular, on the number and position of the hydrophobic

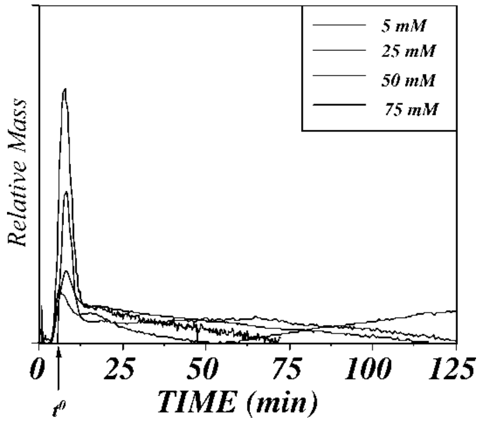

Figure 4. Flow FFF fractograms of the p(SPMA-*b*-MMA) II sample in aqueous Na_2SO_4 at the ionic strength reported in the legend. Flow conditions were the same as in Figure 2 for all runs. Sample load 3.2–4.0 μg.

MMA units within the charged chains of p(SPMA). In salt solutions where interparticle electrostatic repulsion is reduced, and thinning of the charged p(SPMA) corona increases, these randomly positioned MMA segments may generate very large multi-micellar clusters. The long and asymmetric peak tails of Figure 4 account well for the different mechanism of micelle formation occurring with the p(SPMA-MMA) II copolymer to some extent randomly polymerized.

CONCLUSIONS

The analysis and characterization of products obtained from polymerization reactions that do not yield pure compounds may be a complicated and time-consuming operation. Flow FFF allows separation and accurate characterization of mixtures of polymers and nanoparticles in a single analysis. The flow FFF investigation of these types of samples is particularly informative when the relative amount of species in a sample is dependent on solution properties such as ionic strength. FlFFF analysis of two amphiphilic copolymers of sulfopropyl methacrylate and methylmethacrylate has revealed that changes in the block copolymerization sequence lead to a very different capability of the copolymer for forming ordered supra-molecular structures. When the two blocks are separately synthesized before copolymerization, the final product appears to form stable nanoparticles of definite size whose distribution does not change with the solution ionic strength. By contrast, the copolymer obtained by sequential polymerization of the blocks in the same pot shows totally different flow FFF profiles that reveal the capability of this sample for forming only random aggregates, rather than nanoparticles of specific size. This discrepancy is indicative of a different, and in part disordered, structure of the copolymer.

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