Analysis and Characterization of Multifunctional Oligothiophene-Silica Hybrid Nanomaterials by AF4-MALS-FD

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ABSTRACT

Synthesis and applications of new functional nanoparticles are topics of increasing interest in many fields of nano(bio)technology. Chemical modifications of inorganic nanoparticles are often necessary to improve their features as spectroscopic tracers or chemical sensors, and to increase water solubility and biocompatibility for applications in nano(bio)technology. Analysis and characterization of structured nanoparticles are then key steps for their synthesis optimization and final quality control. In this work we present results indicating that Asymmetrical Flow Field-Flow Fractionation (AF4) online coupled with Multi-Angle Light Scattering (MALS) and Fluorescence Detection (FD) can become the unparallel methodology for size and optical characterization of new, structured, fluorescent nanomaterials.

Keywords: asymmetrical flow field-flow fractionation (AF4), multi-angle light scattering (MALS), fluorescence detection (FD), nanoparticle size characterization

1 INTRODUCTION

The application of nanotechnology to disease treatment, diagnosis, monitoring, and to the control of biological systems has recently been referred to as "nanomedicine". In order to overcome the limitations of molecular imaging and gene/drug delivery, nanoparticles (NPs) with different compositions, and unique physical and biological properties have been designed. However, recent indications from institutions such as the US Food and Drug Administration and the European Commission have focused that major issues in current nanomedicine lie in the lack of homogeneous, pure and well-characterized nanomaterials, also because of the lack of well-assessed, robust, routine methods for their quality control analysis and characterization.

Size and optical characterization is a key step in the quality control process of NP synthesis and application. At present, the most commonly used techniques for direct determination of NP size are scanning or transmission electron microscopy (SEM, TEM). However, these techniques are labor intensive and NPs are observed in dried conditions under a high-energy electron source that can significantly modify their native size/morphology. Light scattering (LS) techniques are also broadly used to size-analyze dispersed particles. Dynamic LS (DLS) measures the Stokes diffusion coefficient (D) of NPs in the dispersing medium, which is correlated to the NP hydrodynamic radius (rh) [1]. DLS analysis is fast, and NPs can be directly analyzed in any dispersing medium. However, in case of samples with complex, multimodal particle size-distributions (PSD), accuracy of DLS-based analysis may be also limited. Static, multi-angle LS (MALS) also gives independent information on the NP root-mean-square (rms) radius (or radius of gyration, rg) [2]. However, as in the case of DLS, MALS accuracy is reduced for samples with complex and multimodal PSD.

The accuracy of PSD analysis of complex NP samples may be enhanced by hyphenating DLS or MALS with size-separation methods such as size-exclusion chromatography (SEC), or asymmetrical flow field-flow fractionation (AF4). SEC has already been proposed to size-separate complex NP samples, including QDs [3]; however, its selectivity is quite limited for very-large Mr analytes such as NPs. Moreover interactions with the stationary phase can alter NP retention, morphology, and recovery, with consequently severe alteration of PSD analysis results. AF4 is a flow-assisted technique ideally suited to size-separate dispersed analytes over a broad size range [4,5]. Unlike SEC, the typical NP size range is perfectly centered within the application range of AF4: polystyrene NPs have been used as standard analytes to develop flow field-flow fractionation instrumentation and methods. Over more than 10 years, online asymmetrical flow field-flow fractionation (AF4) coupled with MALS has been widely applied to size separate and characterize NPs [6-9]. AF4 has also demonstrated to be an useful tool in the challenging task of the optical characterization of fluorescent NPs. In fact, AF4 allows the separation of the NPs from the unbounded fraction of the fluorophore in order to accurately assess the spectroscopic behavior of the fluorophore molecules that are actually included in the NPs. In the literature, AF4 with offline fluorescence spectroscopy had already been used to

characterize SiO_2NPs covalently modified with fluorescent units [10].

In this work AF4 with online MALS and fluorescence detection (FD) was evaluated for the analysis and optical characterization of new, structured, fluorescent nanomaterials. For this purpose, silica nanoparticles (SiO_2NPs) doped with oligothiophene (Tf) fluorophores were studied. AF4-MALS-FD was used to i) separate the unbound reagents from the synthesized NPs, ii) determine the PSD of the NPs in dispersion, and iii) simultaneously determine the fluorescent properties of the purified NPs.

2 EXPERIMENTAL

Blue, green, and orange silane-ended TFs were prepared starting from succynimidyl ester precursors (TFsNHS) and then incorporated in the silica NPs. Oligothiophene-doped silica NPs (TF-SiO2NPs) were prepared by sol-gel synthesis according to the Van Blaaderen-modified Stöber procedure [11]. Mono- (B, G, O), bi- (BG, BO, GO, with equimolar TFs ratios) and tri-component (BGO1:1:1 and BGO3:1:1) SiO2NPs were analyzed.

The NP samples were all analyzed using an Agilent 1100 liquid chromatography system (Agilent Technologies, Palo Alto, CA) equipped with an Eclipse 3 Separation System (Wyatt Technology Europe, Dernbach, Germany). Online detection of the eluted species was performed with a DAWN HELEOS 18-angle light scattering (Wyatt Technology Corporation, Santa Barbara, CA), and a 1200 fluorescence detector (Agilent Technologies). The software package Wyatt Eclipse @ ChemStation Version B.03.01 (Wyatt Technology Europe) was used to set and control the flow rate values and the fluorescence detector. Astra V software (Wyatt Technology Corporation) controlled the MALS detectors. All the samples were analyzed in 50:50% v/v EtOH/H2O with 0.025% SDS as carrier solution. The solutions were filtered on 0.1 μm cellulose membrane filters (Millipore) prior to use. Samples were focused for 2 min at 0.5 mL/min and, then, eluted at 0.65 mL/min detector flow rate and 0.15 mL/min cross flow rate. The channel was 250 μm thick, 240-mm long, with a breath decreasing from 16.0 mm (inlet end) to 4 mm (outlet end). Regenerated cellulose (Wyatt Technology Europe) with 10 kDa molar mass cut-off was used as accumulation membrane. Injected sample volumes were: 10 μL for B NPs; 150 μL for all the other NP samples.

3 RESULTS AND DISCUSSION

A challenging task in the synthesis and characterization of fluorescent NPs is their separation from the unreacted fraction of the fluorophore. Figure 1 shows the fractographic profiles of B NPs by AF4-MALS-FD. The fluorescence trace (thin-line) was recorded setting the excitation wavelength to 350 nm, and the emission wavelength to 465 nm. These are respectively the wavelengths at which the absorption maximum and the emission maximum of the blue oligothiophene dye are observed. The fractographic profile shows: (i) a sharp peak at the void time, and (ii) a broad band at retention time values ranging from 3 to 13 min. According to the AF4 theory, the florescence signal at the void time can be ascribed to unbounded fluorophore molecules that are too small to be retained in the AF4 channel, whereas the fluorescent signal between 3 and 13 min can be ascribed to those fluorophore molecules that are associated to NPs. No evidence of aggregates was found upon fractionation. The goal of NP separation from the unbounded fluorophore molecules present in the sample was then achieved. MALS detection allowed the size-characterization of the NP population in terms of r_g. The MALS detection gave an r_g distribution (thick line) ranging from 30 to 60 nm, with an r_g of about 30 nm at the NP band maximum.

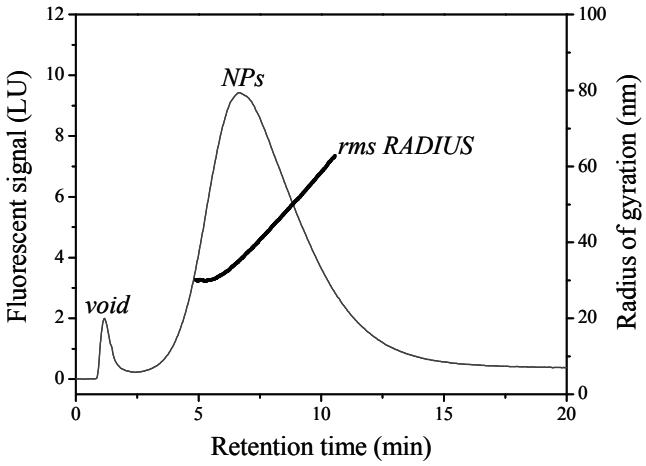

Figure 1. AF4-MALS-FD fractogram of B NPs. Thin line: fluorescence signal (λex: 350 nm, λem: 465 nm); thick line: rg values determined by MALS detection. AF4 conditions are given in the Experimental section.

The MALS signal processed in correspondence with the elution time of the NP bands gave a number-averaged radius (<rms>n) value of 38.0 ± 0.8 nm. The polidispersity index was also determined and expressed as Mz/Mn, where Mz and Mn are, respectively, the z-average and the number-average molar mass values. The Mz/Mn value found was 1.33 ± 2%.

Online fluorescence detection allowed for on real-time recording emission spectra of the eluted species. Figure 2 (full line) reports the emission spectrum at tr = 6.7 min, i.e. in correspondence with the fractionated NP band maximum. It is compared with the emission spectrum of the blue Tf dye in solution (dashed line). The emission maximum wavelength and the absorption band shape coincide. This is a further confirm of the presence of bounded fluorophores in the fractionated NPs, and it allows for a straightforward comparison of the spectroscopic properties of the free and bound fluorophore.

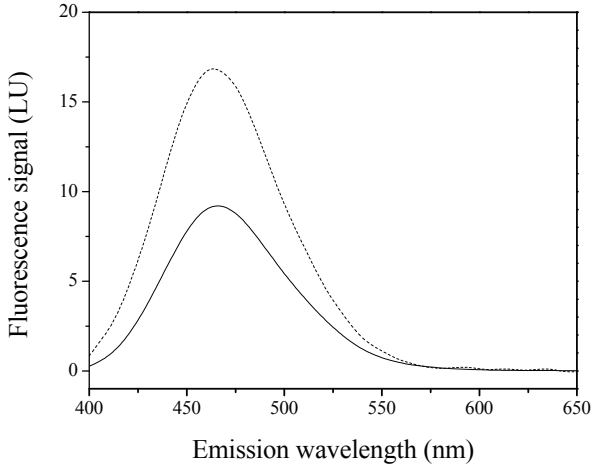

Figure 2. Emission spectra of the B NPs eluted at 6.7 min (solid line), and of a solution of the blue Tf dye (dashed line). The excitation wavelength was set at 350 nm.

The same analysis was repeated for each type of TF-SiO2NP synthesized: B, G, O, BG, BO, GO, BGO1:1:1, and BGO3:1:1 NPs. All the resulting fractographic profiles presented the same sharp peak at the void time due to the unreacted dye molecules, and the same broad band at higher retention time due to elution of the NPs.

Online FD allowed for real-time recording of the emission spectra of the eluted species. For single component NPs, the emission spectra recorded in correspondence with the void time values were identical to the spectra recorded in correspondence with the elution time values of the NPs. On the contrary, for bi-component and tri-component TFs-SiO2 NPs, in correspondence with the NP retention time the emission signatures deriving from the FRET process between co-assembled TFs were observed. This finding proved the simultaneous encapsulation in a single NP of blue, green, and orange TFs (only two or all of them) when bi-component or tri-component NPs are synthesized. Figure 3 reports the emission spectra obtained from the analysis of BG NPs, here taken as multi-component model sample. Upon excitation at 325 nm, the fluorescence spectrum recorded in correspondence with the void time (dashed line) shows an emission maximum at 465 nm, which is characteristic of the B dye. In correspondence with the NP retention time (dashed line), the fluorescence is dominated by the emission of G. This means that an energy transfer process from the B excited state towards G takes place. This finding definitely proved the existence of a population of multi-component NPs.

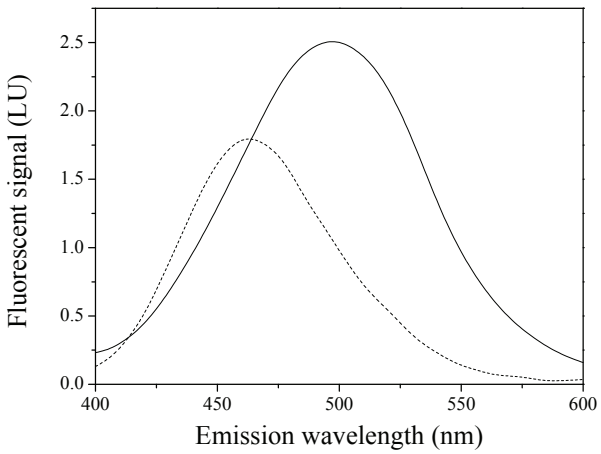

Figure 3. Emission spectra of the BG NPs eluted in correspondence with the NP band maximum (solid line), and of the unbounded blue and green Tf molecules eluted at the void time (dashed line). The excitation wavelength was set at 350 nm.

4 CONCLUSIONS

AF4-MALS-FD is here shown to be a powerful tool to analyze and characterize fluorescent NPs of increasing interest in nano-biotechnology. AF4 gave time-resolved size-fractionation of the NPs, and separated the unbound constituents which were present in the sample. The coupling of AF4 with MALS detection gave the r_g distribution of the NPs, and the associated polidispersity in terms of the Mz/Mn index. Moreover, online fluorescence detection allowed i) the identification of the fluorescent species bounded to the NPs, and ii) the determination of the fluorescent properties of the NPs.

We can then conclude that AF4-MALS-FD may soon become a methodology to be implemented into comprehensive approaches for the analysis and characterization of structured NPs. We can support this belief by at least three reasons. Firstly, the "gentle" separation mechanism of AF4 does not alter the NP morphology, and allows the characterization of complex structured nanomaterials in their native conditions. Secondly, AF4 can separate aggregates from single NPs, and MALS detection sensitivity increases with increasing particle size. These features make AF4-MALS an ideal tool to study the presence of aggregates and the stability of NPs in different dispersions. Thirdly, AF4 can separate the unbound constituents of the structured NPs, which are possibly present in the sample. This feature can be exploited to optimize the synthesis of structured NPs, and to obtain pure NP fractions to be used for further characterization procedures.

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