

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

Recent reports by the FDA and the European Union acknowledge the lack of rugged analysis and characterization methods for nanomaterials as a major limiting factor to the final establishment of nanotechnologies. We present here results indicating that AF4-MALS-FD can become the unparalleled technique for the analysis and optical characterization of new, structured, fluorescent nanomaterials.

This study was performed on silica nanoparticles (SiO<sub>2</sub>NPs) doped with oligothiophene (TFs).

TFs are molecular dyes characterized by high chemical and optical stability and bright emission over the entire visible range. Two main aspects must be considered to optimize the synthesis of TF-SiO<sub>2</sub>NPs: a) NP aggregation, which may affect NP diffusivity and, then, the ability of NPs to permeate cell membranes; b) actual inclusion and self-organization of the dyes inside the NPs, which determine the spectroscopic properties.

We determined the NP aggregation state exploiting the unique capabilities of an Eclipse Separation System with on-line MALS detection (DAWN HELEOS) to size-separate and size-characterize NPs in liquid dispersion.

Figure 1 reports the results obtained by injecting TF-SiO<sub>2</sub>NP samples (A, B) at different concentrations. Sample A was 10 times less concentrated than sample B. Hydrodynamic radius ( $r_h$ ), root mean square radius ( $r_{rms}$ ), and  $r_{rms}/r_h$  values suggest that the sample A mostly contained single, spherical TF-SiO<sub>2</sub>NPs. Non-spherical aggregates were found for the more concentrated sample B.

Spectroscopic properties of TF-SiO<sub>2</sub>NPs were assessed by AF4 with on-line fluorescence detection (Agilent Technologies). AF4 was exploited to separate TF-SiO<sub>2</sub>NPs from non-included TFs.

In Figure 2 the fluorescence intensity as a function of the emission wavelength and retention time is reported for SiO<sub>2</sub>NPs including two different TFs. The shift in the NP emission spectrum, due to the occurrence of FRET between the different TFs trapped in the same NPs, unambiguously proves the purity of TF-SiO<sub>2</sub>NPs upon AF4 fractionation.

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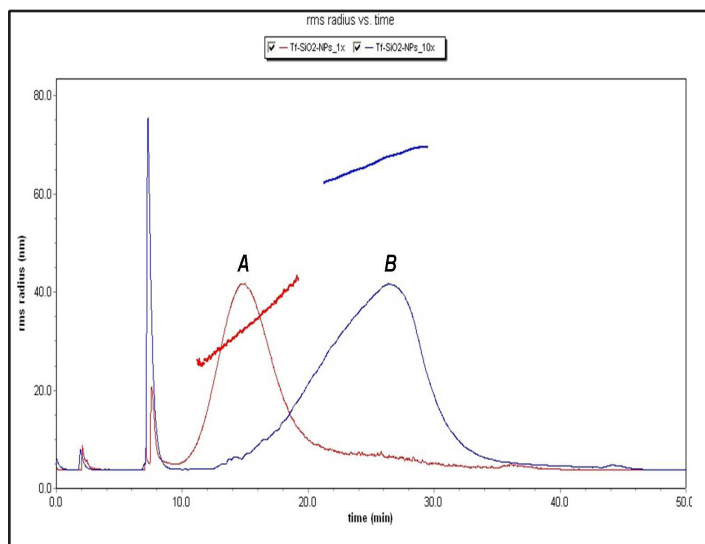


Figure 1. AF4-MALS fractograms of oligothiophene-doped silica NPs injected at different concentrations. (A) Diluted sample; injection volume: 200  $\mu$ L. (B) Tenfold concentrated sample; injection volume: 20  $\mu$ L. Experimental conditions: channel dimensions = 24 cm x 21.5 mm x 350  $\mu$ m (length x width x thickness); membrane = reg. cellulose 10 kDa; mobile phase = 5mM Tris buffer (pH 8.7); detector flow = 1.0 mL/min, cross-flow (start) = 0.25 mL/min, cross-flow (end) = 0.00 mL/min, cross-flow gradient time = 40 min.

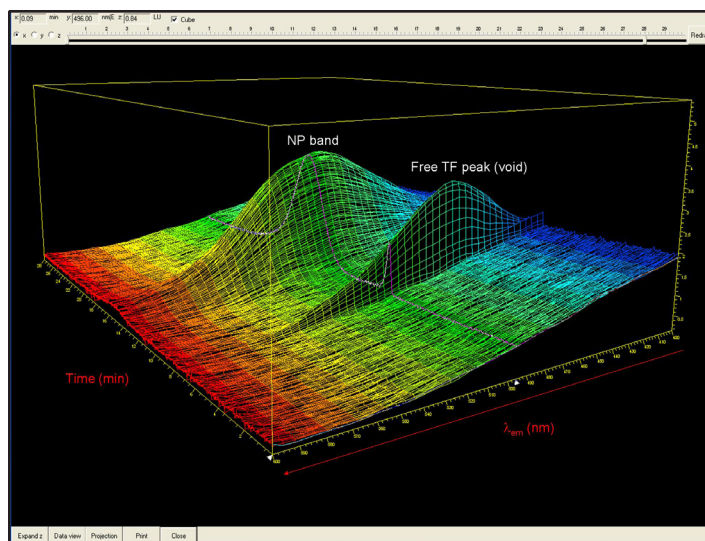
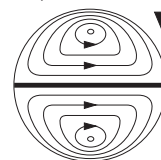


Figure 2. Fluorescence emission intensity as a function of emission wavelength and retention time of SiO<sub>2</sub>NPs including blue and green TFs ( $\lambda_{exc} = 325$  nm). Experimental conditions: channel dimensions = 24 cm x 21.5 mm x 250  $\mu$ m (length x width x thickness); membrane = reg. cellulose 10 kDa; mobile phase = 50:50% v/v EtOH/H<sub>2</sub>O; detector flow = 0.65 mL/min, cross-flow = 0.15 mL/min.



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