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APPLICATION NOTE

# AN2613: Analysis and characterization of fluorescent nanomaterials by FFF-MALS-FD

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## Summary

Recent reports by the FDA and the European Union acknowledge the lack of robust characterization methods for nanomaterials as a major limiting factor to the final establishment of nanotechnologies. We present here results indicating that field-flow fractionation (FFF) with online multi-angle light scattering (MALS) and fluorescence detection (FD) can become an unparalleled technique for the analysis and optical characterization of novel structured fluorescent nanomaterials.

## Introduction

This study was performed on silica nanoparticles ($SiO_2$ 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 to be considered to optimize the synthesis of TF-$SiO_2$ NPs: a) NP aggregation, which may affect NP diffusivity and thus, the ability of NPs to permeate cell membranes, and b) actual inclusion and self-organization of the dyes inside the NPs, which determine the spectroscopic properties of NPs. FFF-MALS separates the particles by size and determines the size of each eluting fraction. Online fluorescence detection together with size measurements identifies free TFs versus TF-doped NPs and highlights the differences in their spectra.

## Materials and Methods

We determined the NP aggregation state exploiting the unique capabilities of an Eclipse™ FFF System with a DAWN® online MALS detector to size-separate and size-characterize NPs in liquid dispersion, where MALS provides the rms radius $R_g$. A WyattQELS™ dynamic light scattering (DLS) module was embedded in the DAWN for determination of hydrodynamic radius $R_h$. MALS and DLS data were analyzed with ASTRA® light scattering software.

Experimental conditions for size determination of the silica NPs (Figure 1) were: FFF: channel dimensions = 24 cm x 21.5 mm x 350 µm (length x width x thickness); membrane = regenerated cellulose, MWCO 10 kDa; mobile phase = 5 mM tris buffer (pH 8.7); detector flow = 1.0 mL/min. Experimental conditions for fluorescence measurements (Figure 2) were: FFF: channel dimensions = 24 cm x 21.5 mm x 250 µm (length x width x thickness); membrane = regenerated cellulose, MWCO 10 kDa; mobile phase = 50:50% v/v EtOH/$H_2O$; detector flow = 0.65 mL/min, crossflow = 0.15 mL/min, fluorescence excitation wavelength: $\lambda_{ex}$ = 325 nm.

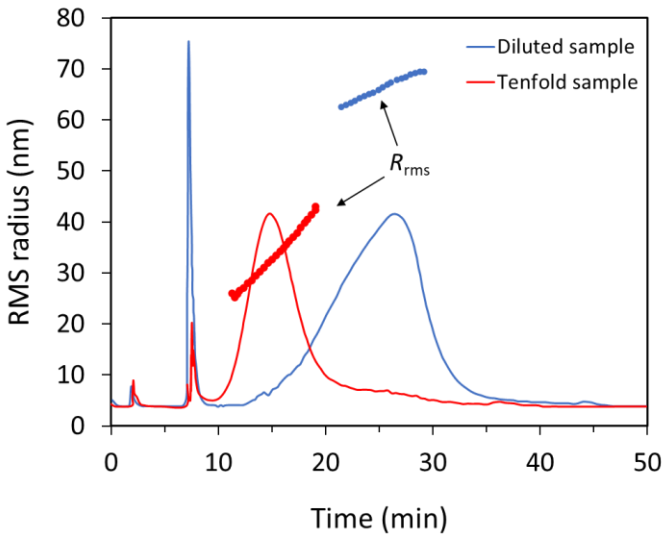


Figure 1. FFF-MALS fractograms of oligothiophene-doped silica NPs injected at different concentrations. Diluted sample injection volume: 200 µL.

## Results and Discussion

Figure 1 reports the results obtained by injecting TF-$SiO_2$ NP samples at different concentrations. The 'tenfold' sample was 10 times more concentrated than the 'diluted' sample. $R_h$ (not shown), $R_{rms}$, and $R_{rms}/R_h$ values (not shown) suggest that the diluted sample contained mostly single, spherical TF-$SiO_2$ NPs. Non-spherical aggregates were found for the more concentrated sample.

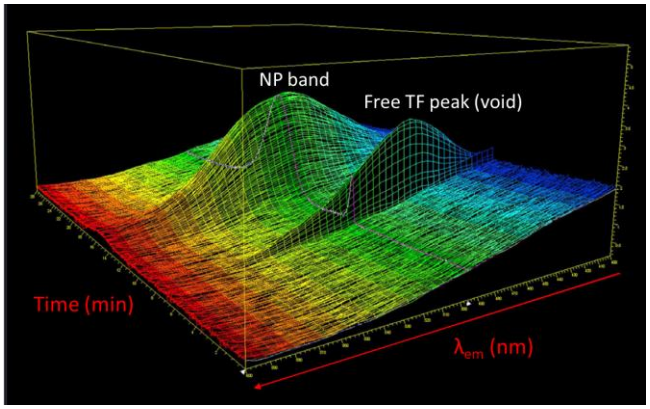


Figure 2. Fluorescence emission intensity as a function of emission wavelength and retention time of SiO2 NPs including blue and green TFs.

Spectroscopic properties of TF-$SiO_2$ NPs were assessed by FFF with on-line fluorescence detection. AF4 was employed to separate TF-$SiO_2$ NPs from non-included TFs. In Figure 2 the fluorescence intensity as a function of the emission wavelength and retention time is reported for $SiO_2$ NPs incorporating two different TFs. The shift in the NP emission spectrum, due to the occurrence of Förster resonance energy transfer (FRET) between the different TFs trapped in the same NPs, unambiguously proves the purity of TF-$SiO_2$ NPs upon fractionation with Eclipse.

## Conclusions

Structured fluorescent nanomaterials are slated to become valuable medical diagnostic tools and therefore require robust characterization methods. FFF-MALS-FD, providing size-based separation followed by different structural and chemical analytics, is shown to constitute a key component in the characterization toolbox for these NPs. FFF is an automated separation method that makes use of HPLC components like autosamplers and pumps, while offering 21 CFR Part 11 – compliant software, making it eminently suitable for the requirements of producers of medical diagnostic NPs.

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