Examination of 4’,6-diamidino-2-phenylindole (DAPI) in Silica Xerogels through Fluorescence Spectroscopy

Nalea Trujillo*, Elizabeth J. O. Atkinson, and Brian D. Gilbert

Department of Chemistry, Linfield College, McMinnville, Oregon 97128

Introduction

Fluorescence spectroscopy is a spectroscopic technique that depends on luminescence of a molecule’s electrons. The excitation and emission peaks are unique to molecules and thus used for characterization. 4’,6-diamidino-2-phenylindole (DAPI) was used as the target molecule for fluorescence spectroscopy. DAPI is a fluorescent molecule that has traditionally been used in biosenesors as a stain known to bind strongly to the A-T rich regions of DNA. DAPI was synthesized in silica xerogels, a 3-dimensional matrix of colloidal particles dispersed in a liquid that agglomerated together to form a network and supercritically dried. Identifying the most optimal concentration of DAPI in silica xerogels can be further used to advance biosensor technologies and other applications.

Materials and Methods

Silver colloid preparation:

Glassware was cleaned with Aqua Regia (3:1 concentrated HCl to concentrated HNO_3) and rinsed with excess amounts of DI water before use. $AgNO_3$ (1mM, 50 mL) and DI water (25 mL) were added to a 250 mL round-bottom flask and boiled under a condenser while stirred. $Na_3C_6H_5O_7$ (1%, 7.0 mL) was added and continued boiling (30 minutes). The mixture was then removed from heat and let cool to room temperature.

Acid-base catalyzed sol-gels protocol (makes 4 gels):

DAPI (6.41 mL), HCl (0.04 M, 0.035 mL), tetramethyl orthosilicate (4.62 mL) were combined and sonicated until solution was clear and homogeneous. Silver colloid (4.18 mL) was added and mixture was sonicated one minute before pouring into plastic cuvettes.

Fluorescence Spectroscopy settings:

Excitation wavelength set at 342 nm, emission start wavelength at 375 nm, emission end wavelength at 680 nm, excitation slit at 5.0 nm, emission slit at 5.0 nm, PMT V at 700 V, scan speed at 1200 nm/min, delay at 0 s, and response at 0.5 s.

Results

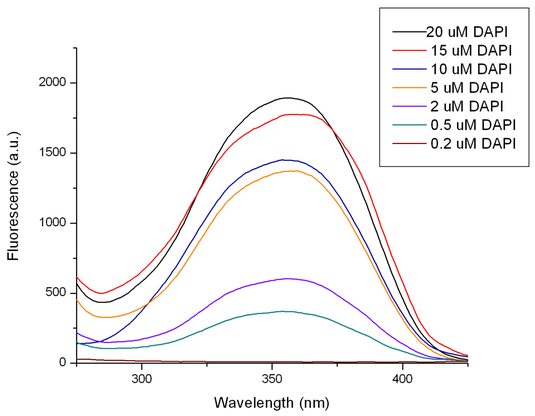

Figure 1. Flourimeter excitation spectra of acid-catalyzed xerogels with a constant Ag nanoparticle concentration (4.18 mL of Ag colloid solution in 15.245 mL sol-gel solution) against varying concentrations of DAPI.

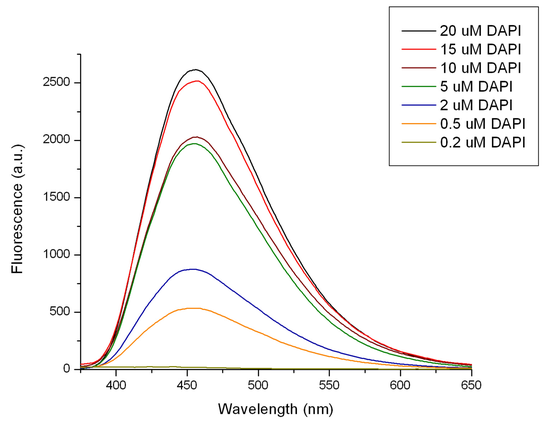

Figure 2. Flourimeter emission spectra of acid-catalyzed xerogels with a constant Ag nanoparticle concentration (4.18 mL of Ag colloid solution in 15.245 mL sol-gel solution) against varying concentrations of DAPI.

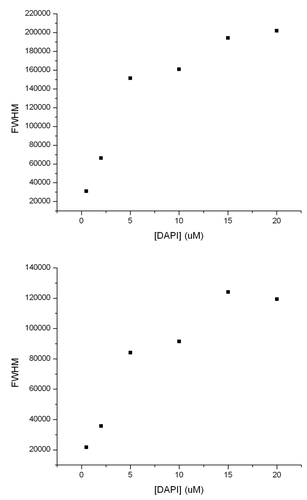

Figure 3. Full-width half maximum of a.) excitation spectra and b.) emission spectra against varying DAPI concentrations of acid-catalyzed xerogels with a constant Ag nanoparticle concentration (4.18 mL of Ag colloid solution in 15.245 mL sol-gel solution).

Literature Cited

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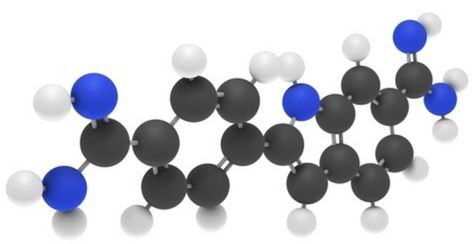

Figure 4. 4’,6-diamidino-2-phenylindole

Discussion

Using fluorescence spectroscopy, excitation at 375 nm was used to observe the fluorescence of DAPI in acid catalyzed xerogels containing Ag nanoparticles to characterization the target molecule in these silica gel substrates (Figures 1 and 2).

As observed in Figure 1, the excitation peaks directly increased with concentration of DAPI. The most concentrated gel (20 uM DAPI) had the highest excitation intensity relative to the less-concentrated gels that decreased, respectively. As seen in Figure 2, the emission peaks also directly increased with concentration of DAPI. Again, the most concentrated gel (20 uM DAPI) had the highest emission peak intensity. This suggests that the silica gel substrate becomes more prominent with increasing concentrations of DAPI; possibly an outlet to advancing biosensors.

The excitation and emission peaks allow calculations of full-width at half maximum and plotted against varying concentrations of DAPI which increases with increasing concentration(Figure 3), resulting in the expected correlation between the two variables.

Previous experiments have shown that the DAPI can also be detected using surface-enhanced Raman spectroscopy, thus, silica substrates can be fabricated containing DAPI and Ag Nanoparticles that can be detected by either spectroscopic technique.

Acknowledgments

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For further information

Please contact Elizabeth Atkins at eatkins@linfield.edu for further information.

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