Stabilization of DAPI in base-catalyzed sol-gels using SERS

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Introduction

Background

Sol-gels are made of a highly porous, low-density gel matrix of metal-oxygen bonds. Sol-gels are capable of trapping solution contents within the pores of the framework and could possibly be used as substrates in surface-enhanced Raman spectroscopy (SERS) detection.[1] Other types of sol-gels have been used as coating agents for interface materials.[2]

Overview

The target molecule in this work is 4',6-diamidino-2-phenylindole (DAPI, Figure 1), a fluorescent dye that binds to DNA. SERS is capable of detecting a target molecule that has been adsorbed to the surface of metals to increase the intensity of that target molecule.[3,4] The metal used to increase the SERS intensity of DAPI was silver nanoparticles.

UV-vis and SERS of a solution containing silver nanoparticles and DAPI were shown to diminish over 24 h. The stabilization of the solution was observed after applying a silica sol-gel matrix. The sol-gel framework in this study consisted of tetramethyl orthosilicate (TMOS) that was hydrolyzed using ammonium hydroxide.

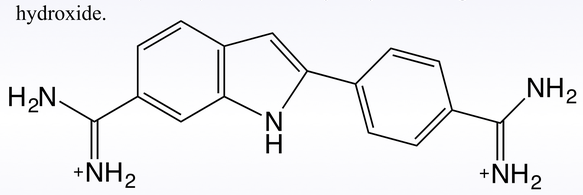


Figure 1: 4',6-diamidino-2-phenylindole (DAPI).

Methods

Chemicals

Silver nitrate (99.8%), tetramethyl orthosilicate (TMOS, 98%), methanol (≥99.9), ammonium hydroxide (30%), sodium citrate (90%), and 4',6-diamidino-2-phenylindole (DAPI, ≥98%) were purchased from Sigma Aldrich (Milwaukee, WI).

Base-Catalyzed Hydrolysis of Silica Sol-gels

Methanol (2.21 mL), deionized water (0.712 mL), and ammonium hydroxide (0.0052 mL, 30%) were combined and added to a beaker containing methanol (2.21 mL) and TMOS (1.92 mL). The solution was sonicated for ten minutes and silver colloid (3.75 mL) was added. After an additional ten minutes of sonication, DAPI (3.75 mL) of various concentrations was added. After three more minutes of sonication, gels were poured into plastic cuvettes. A blank sol-gel was made by replacing DAPI with deionized water.

Silver Colloid Synthesis

All glassware and storage containers were cleaned with Aqua Regia (3:1, 12 M HCl:16 M HNO_3) and rinsed with deionized water. Silver nitrate (50 mL, 1 mM) and deionized water (25 mL) were heated while stirring. Once boiling, sodium citrate (7.0 mL, 1%) was added and refluxed (30 min). Colloid solution was stored in the dark at room temperature.

Instrumentation

Raman spectra were produced using a custom-built Raman spectrometer with a 532 nm laser. Spectra were acquired using KestralSpec with 10 second acquisitions, a slit width of 50 nm and automatic background subtraction. Absorbance measurements of sol-gels were analyzed between 300 and 700 nm using a Hitachi U-3000 spectrophotometer. All assays were run against a deionized water blank to measure the absorption of silver nanoparticles.

Results

Table 1. Intensity of SERS peak at 1621 cm^{-1} for sol-gels containing DAPI and silver nanoparticles as a function of time.

Text

DAPI Concentration (μM)	Intensity (x10^3 counts)		Intensity Percent Increase (%)
	Initially	After 24 h	
0	-	-	-
10	16.40	75.73	361.8
20	62.09	92.51	49.0
50	323.2	323.4	0.1
100	362.2	432.0	19.3

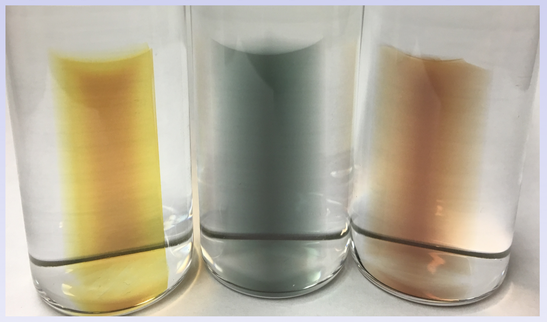


Figure 2. Sol-gels containing silver nanoparticles and DAPI, 0 μM (left), 20 μM (middle), and 10 μM (right).

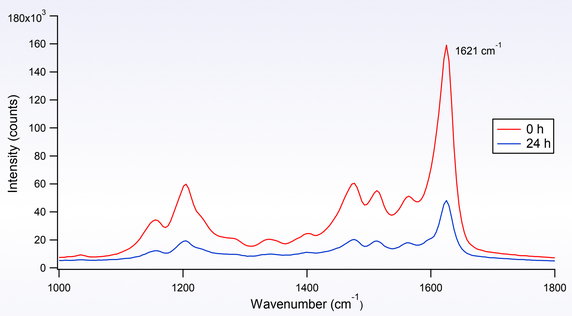


Figure 3. SERS spectra of DAPI (8 μM, 11 mL) and silver nanoparticle (4 mL) solution after initial mixing and after 24 h.

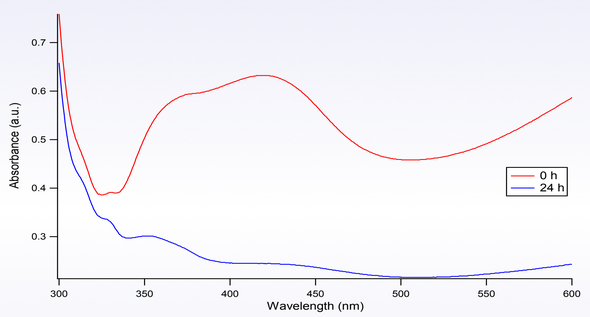


Figure 4. UV-vis spectra of DAPI (8 μM, 11 mL) and silver nanoparticles (4 mL) solution after initial mixing and after 24 h.

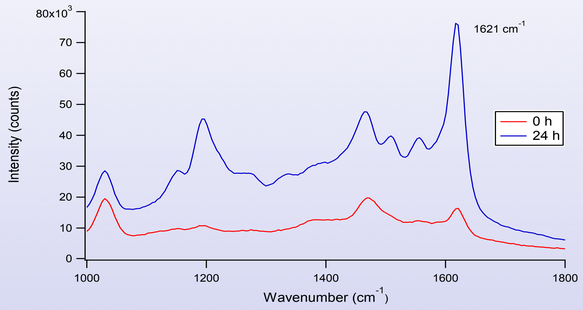


Figure 5. SERS spectra of a sol-gel containing DAPI (10 μM) and silver nanoparticles initially and after 24 h.

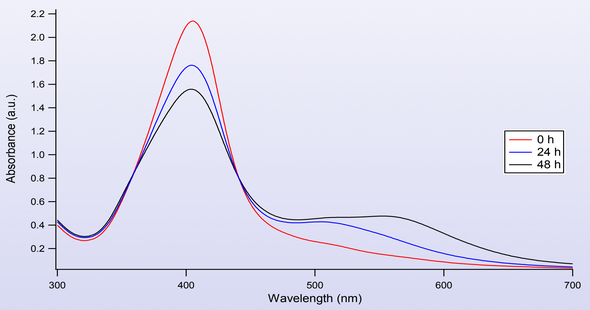


Figure 6. UV-vis spectra of a sol-gel containing DAPI (10 μM) and silver nanoparticles initially, after 24 h, and after 48 h.

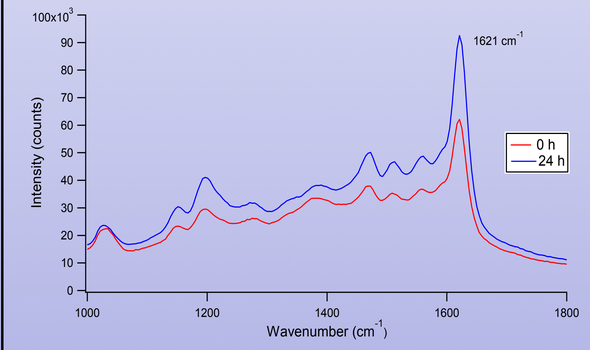


Figure 7. SERS spectra of a sol-gel containing DAPI (20 μM) and silver nanoparticles initially and after 24 h.

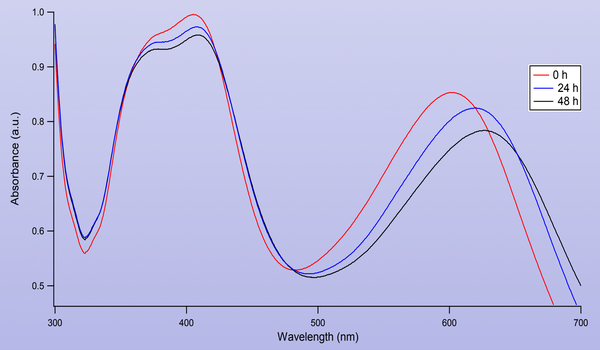


Figure 8. UV-vis spectra of sol-gel containing DAPI (20 μM) and silver nanoparticles initially, after 24 h, and after 48 h.

Discussion

Figures 3 and 4 show intensity and absorbance decreases over time for a solution of DAPI (11 mL, 8 μM) and silver nanoparticles (4 mL). These results suggest that there is less DAPI in the solution 24 h after mixing and a greater size-dispersion of silver nanoparticles. The color change in the silver nanoparticles when adding DAPI (Figure 2) also indicates silver nanoparticle diameter increases.[5]

The blank sol-gel had no DAPI peak at 1621 cm^{-1} in the Raman spectra (data not shown). There was no change in the UV-vis spectra over time, indicating no precipitation of silver nanoparticles, which suggests that DAPI is causing the aggregation of the silver in solution.

As the concentration of DAPI was increased in the sol-gels, the SERS spectra intensity increased over time (Table 1). Higher DAPI concentrations corresponded to an increase in intensity of DAPI specific peaks. Sol-gels with DAPI concentrations greater than 50 μM became saturated and showed no significant percent increase in intensity. Figure 4 shows the UV-vis spectra have the opposite trend. As DAPI concentrations increased, the sol-gels absorbance decreased and shifted to the right. The decrease in absorbance suggests that the silver is aggregating over time. Although the sol-gels and solution spectra were similar, the absorbance decrease for the solution happened at a much faster rate.

Conclusions

- Controlled silver nanoparticle aggregation occurred in base-catalyzed sol-gels that was necessary for SERS.
- DAPI and silver nanoparticle precipitation was prevented over time using base-catalyzed sol-gels vs. in solution.
- Base-catalyzed sol-gels Raman detection of DAPI was achieved and persisted over time vs. in solution.
- SERS intensity of DAPI (1621 cm^{-1}) increased initially due to silver nanoparticle aggregation and was stabilized using sol-gels vs. solution.

References

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