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MERCAPTO-OXIMES AS CAPTURE MOLECULES FOR DETECTION OF NERVE AGENTS USING SURFACE-ENHANCED RAMAN SPECTROSCOPY

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14. ABSTRACT
Two compounds, 4-methylthiobenzaldehyde oxime and 4-thiobenzaldehyde oxime, were synthesized for use as capture molecules for applications using surface-enhanced Raman as a means of detection. The thioether moiety was successfully attached to the silver nanoparticle substrate as evidenced by the change in the resulting spectrum. The oxime was shown to be reactive toward the nerve agent GD (Soman) in basic solution, as monitored by ^{31}P NMR.

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PREFACE

The work described in this report was authorized under Contract No. DAAD13-03-D-0017. The work was started in July 2008 and completed in September 2008.

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SCHEME

MERCAPTO-OXIMES AS CAPTURE MOLECULES FOR DETECTION OF NERVE AGENTS USING SURFACE-ENHANCED RAMAN SPECTROSCOPY

1. INTRODUCTION

Surface-enhanced Raman spectroscopy (SERS) is emerging as a useful technique for detection and monitoring of potentially hazardous materials. To produce the SERS effect, the analyte of interest must be brought into close proximity of a nanostructured metallic substrate (typically gold or silver). Capture molecules are often used in biological detection to immobilize antibody proteins to the surfaces of SERS active substrates.[1] Capture molecules are dual-functionalized with a thiol moiety for binding to the metal surface and a reactive group for subsequent attachment to the compound of interest or analyte. The reaction between the capture molecule and the analyte produces a unique change in the SER spectrum, which can be used for identification and detection of analytes. The capture molecules prepared for this study were para-substituted benzenes with a thiol or thioether and an oxime. Oximes have been widely used for the treatment of poisoning due to exposure to organophosphorus compounds, such as chemical warfare (CW) agents.[2] The capture molecules were characterized using ^{1}H NMR, ^{13}C NMR, and gas chromatography-mass spectrometry (GC-MS). Preliminary data were collected on the reaction between the capture molecule, 4-methylthiobenzaldehyde oxime (**2**), and the nerve agent GD (Soman). Furthermore, attachment of **2** to the surface of Ag nanoparticles was confirmed with SERS.

2. EXPERIMENTAL PROCEDURES

All chemicals were purchased from Aldrich and used without further purification. The ^{1}H NMR and ^{13}C NMR were recorded on a JEOL ECX 400 MHz spectrometer. The ^{31}P NMR spectra were recorded on a Bruker DPX-300 MHz Ultrashield spectrometer with a ^{31}P switchable QNP probe. Chemical shifts are reported in parts per million (δ) and all spectra were recorded at ambient temperature. The GC-MS was run on an Agilent 6890N network GC system with an Agilent 5975 inert mass selective detector. Liquid chromatography-mass spectrometry (LC-MS) was done with an Agilent 1100 LC/MSD equipped with an electrospray source using a reversed phase gradient from 90% aqueous ammonium acetate buffer to methanol, using a Phenomenex Aqua C18 RP column. SER spectra were recorded using a high resolution (1-2 cm^{-1}) fiber optically coupled (InPhotonics RamanProbe) EIC Echelle spectrograph operating at 785 nm with a laser output of ca. 130mW.

2.1 4-Methylthiobenzaldehyde Oxime (**2**).

Compound **2** was synthesized according to a literature procedure.[3] Specifically, 0.61 g (4.0 mmol) 4-methylthiobenzaldehyde (**1**) was added to a round bottom flask, dissolved in 15 mL ethanol, and heated to 70 °C. A solution of 7.42 g $NH_2OH \cdot HCl$ dissolved in 20 mL deionized H_2O was added to the reaction flask. The reaction mixture was stirred at 70 °C for 1 hr before cooling to room temperature. The solution was diluted with 50 mL H_2O, extracted with ethyl acetate (3 × 50 mL), and dried over anhydrous $MgSO_4$. The solvent was evaporated

to give the product as a light yellow powder (600 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.08 (s, -C*H*=N-OH), 7.22-7.48 (m, aromatic), 2.49 (s, -S-CH_3), 1.98 (b, =N-O*H*). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 150.0, 141.4, 128.6, 127.4, 126.2, 15.4. Calculated: C_8H_9NOS *m/z* = $[M]^+$ 167.04; Found: EI-MS: *m/z* = $[M]^{+\bullet}$ 167.

2.2 4-Thiobenzaldehyde (**3**).

Compound **3** was synthesized according to a literature procedure.[4] Specifically, **1** (1.26 g, 8.28 mmol) was dissolved in 100 mL of $CHCl_3$ and cooled to 0 °C before 2.13 g (12.3 mmol) of m-chloroperoxybenzoic acid was added. The mixture was stirred for 1 hr at 0 °C and warmed to room temperature before 0.92 g $Ca(OH)_2$ (12.4 mmol) was added and stirred for an additional 30 min. The mixture was filtered, and the solvent was evaporated before adding 15 mL of trifluoroacetic anhydride (TFAA,108 mmol) and refluxing for 45 min. The TFAA was removed by evaporation before adding 80 mL of a 50:50 mixture of triethylamine and methanol. The solution was evaporated to dryness before diluting with $CHCl_3$ and extracting with saturated NH_4Cl solution (2 × 50 mL). The organic fractions were combined and dried over anhydrous MgSO4, and the solvent was evaporated to give a yellow solid as the crude product. The compound was purified using column chromatography with a gradient of hexanes:ethyl acetate starting at 4:1 to give 552 mg of light yellow viscous liquid (48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.88 (s, 1H, C*H*=N-OH), 7.40-7.67 (m, 4H, aromatic), 4.57 (s, 1H -S*H*). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 191.2, 144.3, 134.4, 130.1, 129.3, 57.4. Calculated: C_7H_6OS *m/z* = $[M]^+$ 138.01; Found: El-MS: *m/z* = $[M-H]^+$ 137, $[M]^{+\bullet}$ 138.

2.3 4-Thiobenzaldehyde Oxime (**4**).

Compound **4** (250 mg, 0.96 mmol) was dissolved in 10 mL of ethanol and heated to 70 °C. The 3.2 g (46 mmol) of NH_2OH•HCl was dissolved in 12 mL of water before adding it to the reaction mixture. The solution was stirred for 1 hr at 70 °C before cooling to room temperature. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The organic fractions were combined, dried over anhydrous $MgSO_4$, and evaporated to dryness to give 280 mg (96% yield) of light yellow powder. ^{1}H NMR (400 MHz, d_3-methanol) δ (ppm): 7.99 (s, 1H, C*H*=N-OH), 7.22-7.52 (m, 4H, aromatic), 4.85 (s, 1H, S*H*). ^{13}C NMR (400 MHz, d_3-methanol) δ (ppm): 148.3, 134.0, 130.0, 128.4, 127.1. Calculated: C_7H_7NOS *m/z* = $[M]^+$ 153.02; Found: EI-MS: *m/z* = $[M]^{+\bullet}$ 153.

2.4 ^{31}P NMR.

Compound **2** (20 mg, 0.12 mmol) was dissolved in a 50:50 mixture of acetonitrile and water and 1 μL of GD (5.6×10^{-3} mmol) was added. Since no change was observed after 20 min, 20 μL of diisopropylethylamine (DIPEA) was added to make the solution basic. The spectrum of the basic solution was recorded after 2 min. A control experiment was performed using the same conditions as above without the addition of **2**, and the spectrum was recorded after 30 min.

2.5 Saturation of Ag Nanoparticles.

The Ag colloid was prepared according to literature procedure.[5,6] A solution of 0.25 mg/mL **2** was prepared with 50:50 methanol/water. Added to a 1 cm quartz cuvette was 500 μL of Ag colloid, 10 μL of 1 M NaCl solution, and a blank Raman spectrum was recorded. The solution of **2** was added in 10 μL aliquots to the cuvette, shaken gently, and allowed to equilibrate for 1 min before each spectrum was recorded.

3. RESULTS AND DISCUSSION

Our approach was to create a simple molecule that contains a nucleophilic moiety, as well as a thiol for attachment to SERS active metal surfaces, such as Ag nanoparticles. An oxime was chosen as the nucleophile because it has been shown to successfully react with the phosphorus center of CWAs.[7] The affinity of mercaptans toward SERS active metals like Au or Ag has been well documented.[8]

The synthesis of **2** was achieved by reacting commercially available 4-methyl-thiobenzaldehyde (**1**) with hydroxylamine hydrochloride in an ethanol/water solution at 70 °C. Oxime formation was verified in the ^{1}H NMR, where the aldehyde proton had shifted from 9.91 ppm to the benzylic region at 8.08 ppm. Compound **2** was used without further purification, as it was shown to be ca. 98% pure by GC-MS. Compound **3**, a precursor to **4**, was synthesized according to a procedure by Coombs and coworkers,[4] and after removing the methyl group, the oxime **4** was synthesized using the same procedure as compound **2**. The reactions are shown in the Scheme. Although compound **4** was not used in these preliminary tests, future work will include reacting **4** with agent and saturation studies with silver colloid.

3.1 ^{31}P NMR Studies with GD.

To examine the reaction between the oxime and GD, compound **2** was dissolved in 1 mL of a 50:50 mixture of acetonitrile and water, and 1 μL GD was added to the solution. A ^{31}P NMR spectrum was taken 20 min after the addition of GD, and no reaction had occurred. However, it is well known that oximes require activation, often through the addition of a base, to become nucleophilic enough to react with G-agents.[9] Immediately after the addition of 10 μL of the base DIPEA, a signal appeared in the spectrum corresponding to the product of a reaction between GD and **2**, and the original GD signal had disappeared (Figure 1). The peak at 24 ppm corresponds to a small amount of the hydrolysis product, pinacolyl methyl-phosphonic acid (PMPA). A control experiment was performed to confirm that the signal in the ^{31}P NMR was indeed due to a reaction between GD and **2** and not a simple product of hydrolysis. For the control, 1 μL of GD was added to the 50:50 acetonitrile/water mixture. The spectrum, which was recorded after a period of 30 min, revealed the presence of residual GD, as well as a new peak from the hydrolysis product PMPA at 24 ppm (Figure 1). By comparing the control spectrum to that of the reaction product of **2** with GD, it is evident that hydrolysis of GD in basic solution is much slower, and suggests that GD reacts preferentially with **2**. Further confirmation of the reaction between **2** and GD was provided by running the NMR solution on LC-MS, where both the $[M + H]^+$ and $[M + Na]^+$ peaks were identified at $m/z = 330$ and $m/z = 352$, respectively.

3.2 SERS of **2**.

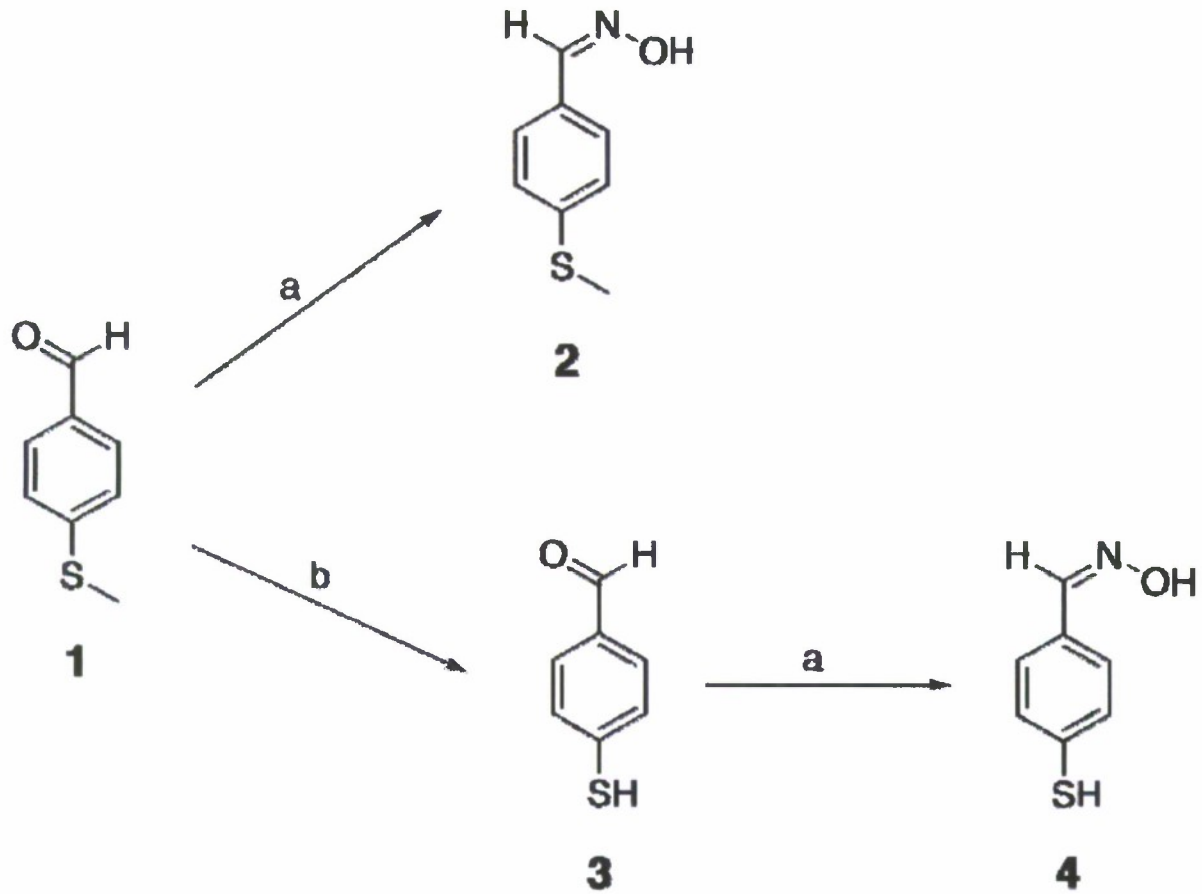
Ag colloid solutions were prepared according to the modified procedure of Lee and Meisel,[5] detailed in the work of Guicheteau and coworkers.[6] A normal Raman spectrum was obtained from the solid **2** (Figure 2). To observe the SER effect, a 1 mg/mL solution of **2** in methanol was added to 500 μL of Ag colloid, and a spectrum was recorded. For comparison, a spectrum of **2** dissolved in methanol (without Ag colloid) was also recorded and only a few broad peaks, primarily attributed to methanol, can be observed. In contrast, the SER spectrum of **2** shows many of the same peaks observed in the solid sample, although they appear to be slightly shifted and somewhat broadened. Clearly visible are the aromatic C—C vibrations at ca. 1600 cm^{-1}, the aromatic C—S vibration at ca. 1100 cm^{-1}, and the peak at ca. 1250 cm^{-1} due to the methyl group, among many others.

3.3 Saturation of **2** to Silver Colloid.

Preliminary saturation experiments were performed to calculate the amount of surface coverage for molecule **2** needed to form a monolayer around the silver nanoparticles. A Langmuir isotherm was constructed using peak area measurements from the 1185 cm^{-1} mode (Figure 3). A noticeable leveling off is observed between the 28th and 31st additions yielding a surface coverage concentration of approximately 0.13 mg/mL. Additional experiments need to be performed in the lower end of the curve (below 0.05 mg/mL) and in the area of potential saturation.

4. CONCLUSION

Two compounds bearing thiols and oximes were synthesized for use as surface-enhanced Raman spectroscopy (SERS) capture agents. Compound **2** was shown to react quickly with GD under basic conditions, as monitored by ^{31}P NMR. As evidenced by the SER spectrum, compound **2** was also shown to bind with Ag nanoparticles in aqueous solution.



Scheme. Synthesis of SERS Capture Molecules (a) $NH_2OH \bullet HCl$, EtOH, H_2O, and (b) i: m-CPBA, $CHCl_3$, $Ca(OH)_2$, ii: TFAA, iii: TEA/MeOH.

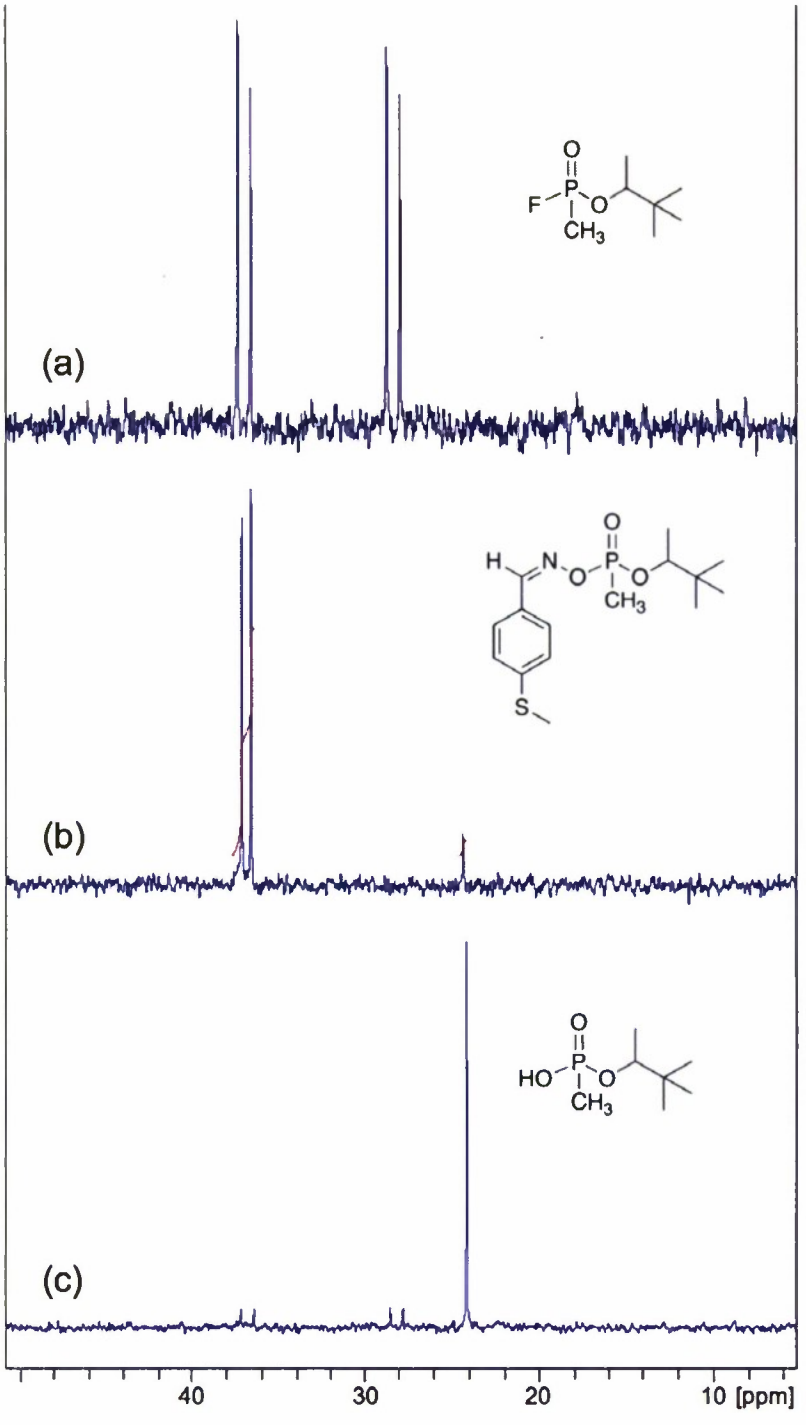


Figure 1. ^{31}P NMR of (a) GD with **2** and No Base after 20 Min (b) GD with **2** and DIPEA 2 Min after Adding Base (c) GD with DIPEA 30 Min after Addition of Base, Pinacolyl Methylphosphonic Acid (PMPA). All solutions were a 50:50 mixture of acetonitrile and water.

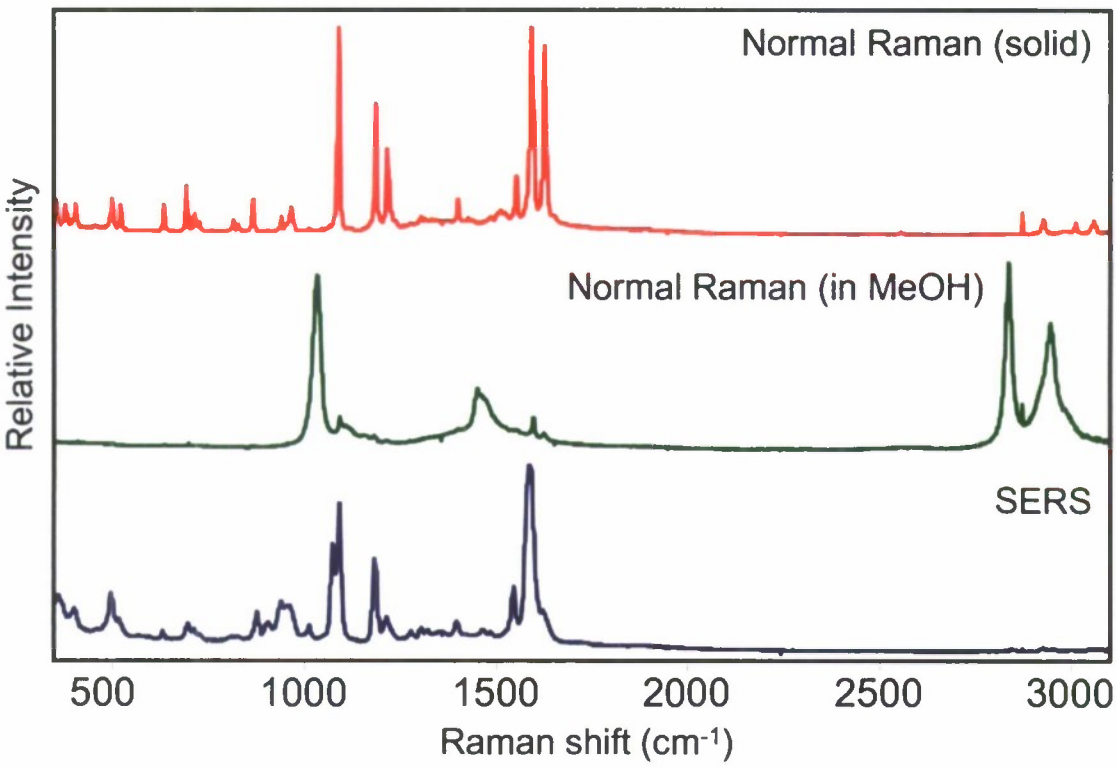


Figure 2. Normal Raman of Solid **2** (Top) and **2** Dissolved in MeOH (Center), and Surface-Enhanced Raman of **2** in Ag Colloid Solution (Bottom).

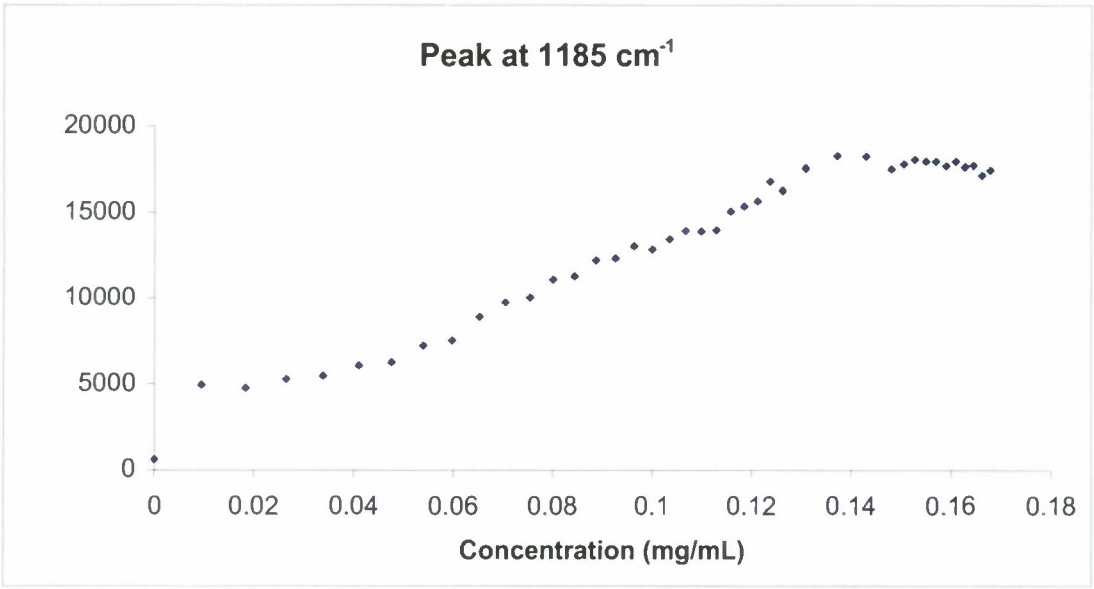


Figure 3. Preliminary Saturation Curve of **2** onto Ag Nanoparticles.

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