

DETECTION OF POLYCYCLIC AROMATIC HYDROCARBONS USING SURFACE ENHANCED RAMAN SPECTROSCOPY ON MODIFIED SILVER SURFACES



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I hereby recognize and pledge to fulfill my responsibilities as defined by the Honor Code and maintain the integrity of both myself and the college community as a whole.

Pledge:_____

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Abstract:

Polycyclic Aromatic Hydrocarbons are carcinogenic environmental pollutants. In this work, SERS and SEIRA were utilized to determine the effectiveness of Ag-BNNS as a supporting substrate for PAH detection. Calixarene was tested as a potential surface modification on Ag colloids to better detect PAHs. Fluorescence Spectrophotometry was used to monitor the guest-host complexing of pyrene and calixarene. Enhancement of adsorbed substrates was seen in SERS using Ag-BNNS. Fluorescence results were inconclusive in determining the K_{eq} value of the pyrene-calixarene complex.

Introduction:

Polycyclic Aromatic Hydrocarbons

Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds which contain multiple rings that have aromatic characteristics. Chart 1 shows examples of multiple PAHs.

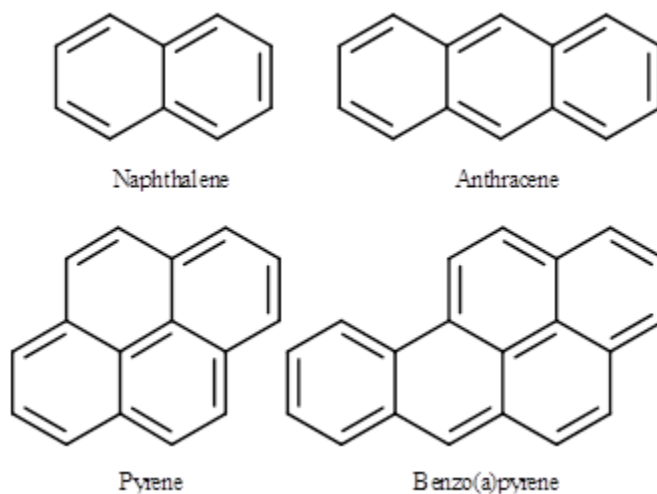


Chart 1. Molecular Structures of Multiple PAHs

PAHs form from the incomplete combustion of fossil fuels such as coal, oil, and gasoline.^[1] These compounds have been found to be carcinogenic, mutagenic, and teratogenic.^[1] PAHs are found in water systems, such as oceans and lakes, as well as in the atmosphere and soil.^[2] In these water systems, due to hydrophobic properties, PAHs aggregate on suspended matter in bodies of water.^[1] In spite of their hydrophobic nature, they exist in aqueous media at very low concentrations and have been detected in the Baltic Sea at ppt concentrations.^[3] Several methods currently exist to detect PAHs within these environments, such as Gas Chromatography and Mass Spectroscopy (GC/MS).^[3] However, these methods require pre-concentration steps, causing them to be time-consuming as well as costly.^[4] GC/MS is also a difficult method to attempt *in situ* detection of PAHs, creating a need for a new screening method. Surface

Enhanced Raman Spectroscopy (SERS) allows for *in situ* detection of analytes in aqueous media due to the lack of the broad OH peak seen in other forms of spectroscopy, as well as enhancement of analyte spectra, eliminating the need for pre-concentration steps.^[3] Infrared spectroscopy can be used as a complementary tool to prove chemical concepts in the development of reusable surfaces on which PAHs can be detected at low concentrations using *in situ* SERS.^[1] PAHs are environmental pollutants which need to be monitored in our water systems, atmosphere and soil due to their carcinogenic properties. The formation of a sturdy, reproducible and reusable surface on which these analytes can be detected is important due to the lack of an effective, low cost *in situ* screening tool.

Raman and IR Spectroscopy

Raman and Infrared (IR) spectroscopy are both analytical techniques which detect the presence of molecules based on molecular vibrations. The two forms of spectroscopy are complementary because different vibrations are active in each. In IR spectroscopy light is incident on a molecule. If the oscillation frequency of the electromagnetic field matches that of a molecular vibration which causes a change in dipole then energy is absorbed. The intensity of light transmitted through the sample is observed at a range of incident light frequencies. The decrease in intensity of the frequencies absorbed by the sample result in a “peak” in the recorded intensity vs. frequency spectrum.^[5] Peaks on the spectra represent specifically vibrations due to polar bonds and non-symmetric vibrations. A greater change in the dipole moment of the bond results in a larger absorption cross section and this along with higher analyte concentrations will produce a larger peak on the spectra.

Raman spectroscopy is based on the scattering of the incident light due to the molecular vibrations.^[5] In the explanation of Raman, light will be treated as a particle. A photon is emitted

with high energy and thus a short wavelength. This photon then “collides” with the molecule in an inelastic collision that transfers energy to a vibrational mode thus exciting the symmetric and asymmetric stretches of that bond. The photon then scatters with a lower energy and thus a longer wavelength and is detected. A Raman shift is calculated so that the spectra shows the difference in energy between the photon which was incident and the photon detected, thus the amount of energy absorbed by the bond. A spectrum is generated by graphing the intensity of scattered photons as a function of the Raman shift.

Surface Enhanced Raman Spectroscopy and Surface Enhanced IR Absorption Spectroscopy

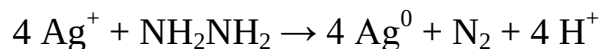
In chemistry, the detection of single molecules as well as low concentrations of analytes, such as PAHs, is sometimes necessary. In these cases, more specialized analytical techniques such as Surface Enhanced Raman Spectroscopy (SERS) and Surface Enhanced IR Absorption Spectroscopy (SEIRA) are needed. Both SERS and SEIRA use electromagnetic enhancement provided by metallic surfaces. Most commonly silver, gold and copper are used. Two mechanisms for this enhancement exist which are widely accepted. The first is the Electromagnetic Enhancement (EM).^{[6] [7] [8]} This enhancement is due to strong local enhancement of the electric field around the metal due to excitation of the surface plasmons. This causes enhancement of the spectra for molecules near the metallic surface. SERS can give an enhancement factor from 10^6 - 10^8 .^[9] The EM enhancement mechanism has been found to provide an enhancement of 10^6 , accounting for a large percentage of the overall enhancement.^[10] The second mechanism for enhancement is Charge Transfer (CT) which accounts for the rest.^{[6] [7] [8]} Surface Enhanced IR Absorption Spectroscopy is much less sensitive than SERS. SEIRA has enhancement factors from 10-100, compared to the 10^8 enhancement of SERS.^[11] The SEIRA enhancement is due to an effect similar to that of plasmon resonance in SERS, known as phonon

resonance. In SEIRA there are enhanced optical fields which occur at the surfaces of the particles being detected. These fields occur only when illuminated at the resonance frequency of the surface phonons.^[12] SERS has distinct advantages over SEIRA, such as the ability to detect molecules at lower concentrations due to the greater enhancement factors associated with SERS. Along with this, SERS provides a less broad OH peak in the presence of water. This allows for detection of analytes and enhancement of spectra in aqueous media. SEIRA requires phonons to be excited in a small wavelength range so that enhancement can be seen. This often requires several optimization steps. Along with this, because of the low enhancement factors seen in SEIRA, the molecular density of the sample is important. A thin liquid film on an IR salt plate hit with a 1 mm light beam interacts with an order of 10^{18} molecules (Appendix 1). SEIRA experiments to detect small concentrations of PAHs on modified metal surfaces at most will interact with 10^{13} molecules.^[13] This weak enhancement due to lack of an optimized wavelength as well as low molecular density in solid IR spectroscopy can be problematic when using SEIRA as a tool for low concentration analyte detection. Much of the chemistry surrounding formation of a surface on which PAHs can be detected using SERS should be detectable using SEIRA although using SEIRA would require a significant increase in concentration of analyte due to the lowered enhancement factor available.

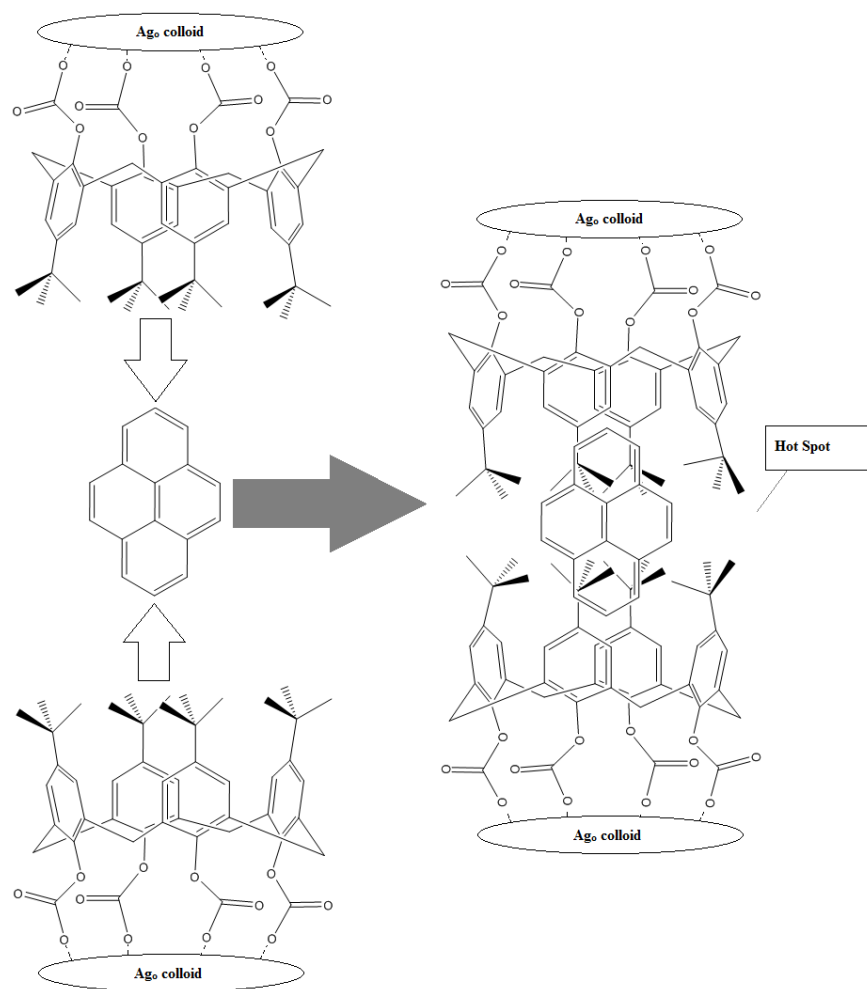
Silver Colloids

Metallic surfaces, normally silver, gold or copper, are necessary for the spectral enhancement of a molecule in SERS and SEIRA. These metals are used because of their stability in air.^[14] They also have localized surface plasmon resonances which cover most of the visible and near infrared range.^[14] This is where the majority of Raman measurements occur, making these three metals the most convenient to use in Raman spectroscopy.^[14] SERS has been used in

conjunction with thin films of these metals as well as silver and gold colloids. The formation of these colloids is achieved by the reduction of silver acetate using hydrazine, as shown in the following reaction:



The aggregation of these colloids is an important factor related to the level of enhancement of analytes spectra as well as reproducibility of results. The degree of aggregation must be monitored. If there is too little colloid aggregation, a true colloid layer does not exist and therefore enhancement is severely damaged.^[15] If perfect aggregation of colloids is achieved, the metal nanoparticles can aggregate in such a way to produce “hot spots.”^{[4] [16]} Scheme 1 shows an example of “hot spot” formation. The spot between two functionalized metal nanoparticles is referred to as a “hot spot” because the spectral enhancement is doubled by the close proximity to two metal nanoparticles, which is felt by the analyte.^[16]



Scheme 1. Formation of "hot spot" around PAH pyrene by host-guest interaction with 4-tert-butylcalix[4]arene's

Uniform aggregation of metallic colloids across the surface is also necessary. A colloid surface with uniform aggregation has uniform enhancement of analyte spectra across that surface allowing small concentrations to be detected consistently. This allows for reproducibility of results.

Supporting Substrates

Colloids can be aggregated in a more uniform manner by the use of supporting substrates such as hexagonal boron nitride (h-BN) and polyaniline layers.^{[6] [17] [18]} Chart 2 shows the molecular structure of h-BN.

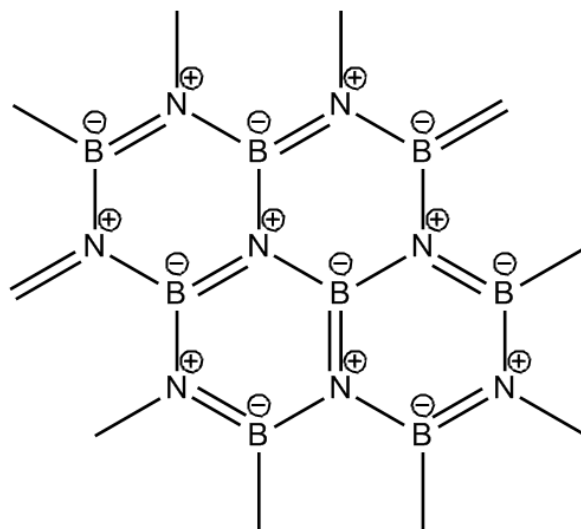


Chart 2. Hexagonal boron nitride

For aggregation of silver colloids on h-BN, silver acetate is first reduced by hydrazine in solution, as shown previously. The aggregation of the already reduced silver colloids on h-BN is due to the physical and electrostatic adsorption between the h-BN and silver colloids.^[18] Along with this, h-BN has high stability in hazardous environments.^[7] It is inert to oxidation and is thermally stable up to 700 °C.^{[6] [18] [19]} The uniformity of the silver colloid layer on h-BN is directly related to the concentration of reduced silver in solution. Polyaniline layers have been shown to create more uniform layer than h-BN when the membrane is doped with camphorsulfonic acid. This is due to the reduction of the silver acetate occurring on the surface of the membrane allowing for quick adsorption to the surface across the membrane.^[17] Both analytes have the ability to create uniform colloid layers. Polyaniline membranes have thus far shown greater uniformity in colloid aggregation. Depending on the concentration of reduced

silver in solution h-BN may have the same ability. If similar uniform colloid layers can be seen from both, h-BN will allow for both thermal and chemical stability.

Surface Modifications

Along with aggregation of the colloids, in order for spectral enhancement to be seen, analytes must also have an affinity for those metallic surfaces. PAH's lack this affinity for metal surfaces and thus surface modifications must be made in order for the PAHs to be close enough to feel the electromagnetic enhancement from the metallic surface.^[20] Modification of the surface by attachment of calixarenes has the capability to trap the PAH near the metal surface so that it can be detected using SERS or SEIRA. Calixarenes are “cup-like” structures that are synthetic cyclooligomers with a defined upper and lower rim.^{[1][4]} A basic calixarene structure can be seen in Chart 3.

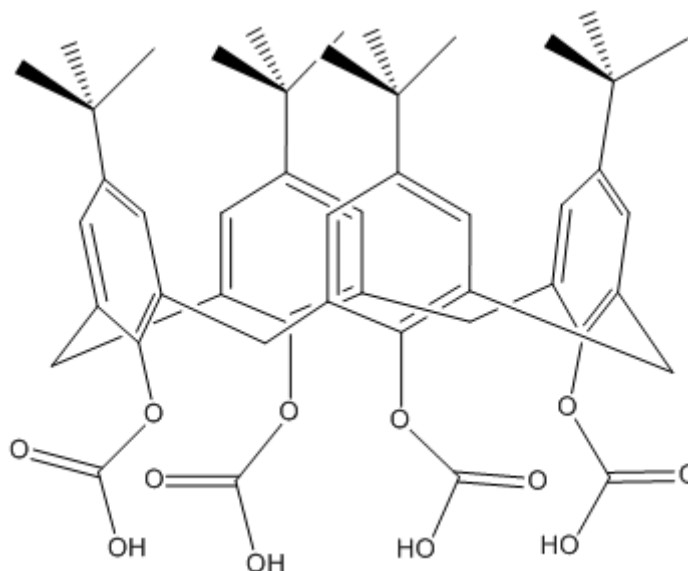


Chart 3. Molecular structure of 4-tert-butylcalix[4]arenes

The most common upper rim formation is made of 4-tert-butyl groups, as can be seen in Chart 3. Multiple lower rim formations exist based upon the type of adsorption onto the metal surface that is desired. Thiol groups have shown strong adsorption onto silver or gold surfaces along with carboethoxy groups.^{[1][20]} Carboethoxy groups have been shown to adsorb by a

bidentate interaction with the metallic surface.^[22] However, upon PAH insertion into the hydrophobic upper cavity, this bidentate interaction becomes monodentate due to steric hindrance. A possible solution to the loss of the bidentate interaction is to change the lower rim formation to carboxylate groups. Due to the lack of steric hindrance these groups should have the capabilities of forming a bidentate interaction with the metallic surface even upon insertion of the PAH thus creating stronger adsorption.

Along with creating a strong attachment to the surface, calixarenes must also interact with PAHs to trap them near the surface. The upper rim of the calixarene, as well as the hydrophobic internal cavity allows for the insertion of the PAHs to form a guest-host inclusion complex.^[23] The stability of the bound PAH calixarene formation is attributed to multiple forces, including, hydrophobic interactions with the PAH, release of water molecules from the hydrophobic cavity, van der Waals forces, and the release of calixarene bond strain.^[23] The calixarene is a size specific substrate and therefore allows for size-selective specificity on detection. This size-specificity can change based on the structure of the calixarene, but also due to the concentration of calixarene in a sample.^[4] The closer the calixarenes are together the smaller the upper rim opening. This is one easy way to manipulate the host to detect certain analytes over others. This size-specificity can be particularly important for *in situ* detection. Size-specificity not only can allow one PAH to be detected over another, but also for specific PAHs to be detected in the presence of other SERS active pollutants in the *in situ* environment.^[21] Calixarenes have been used to detect PAHs at concentrations varying from 10^{-2} M to 10^{-8} M.^{[1] [4] [11] [16] [20]}

Fluorescence

The insertion of PAHs into the calixarene cavity is necessary for detection by SERS and SEIRA. Monitoring the rate at which this happens is an important factor for accurate detection of PAHs. Fluorescence Spectrophotometry has been used successfully to monitor guest-host interactions, like those seen between PAHs and calixarenes.^{[24] [25]} In fluorescence spectrophotometry fluorophores absorb light exciting themselves to a higher electronic state. Light is then emitted as fluorescence as the fluorophore returns to the ground state.^[26] Figure 1 shows a Jablonski diagram depicting absorbance and emission.

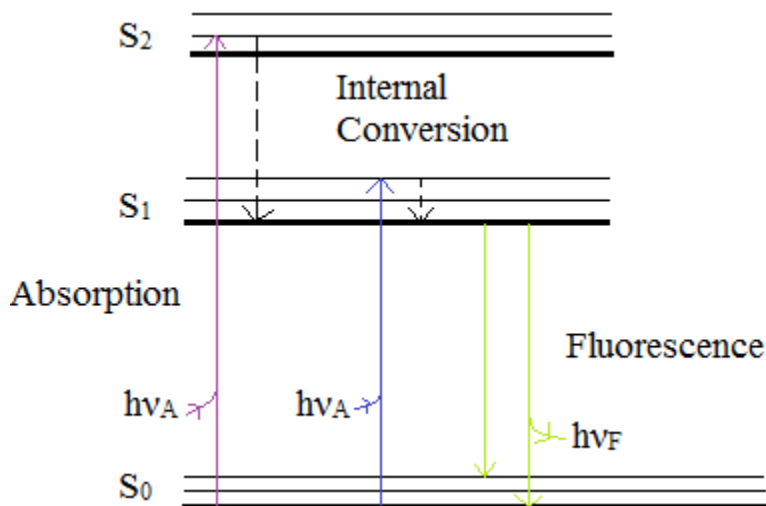


Figure 1. Jablonski diagram depicting absorption and fluorescence emissions from ground state to excited state

PAHs fluoresce due to their aromatic nature. The rate at which interaction occurs between the calixarene and PAH can be monitored by the intensity of the fluorescence. As more PAHs complex with calixarene cavities, less fluorescence from those PAHs should be seen.

In order to detect low concentrations of PAHs, surface modifications must be made to colloids which are uniformly aggregated on a supporting substrate. While each surface modification has its merits, a calixarene with a carboxylate lower rim formation has been chosen due to the proposed stability from a bidentate interaction with the surface as well as the

simplicity of a one-step adsorption method. Uniform aggregation of the silver colloids using the supporting substrate h-BN is theoretically possible with varying reduced silver concentrations. SERS is the most effective detection tool, however availability of a working Raman is not always possible. As stated previously, different stretching and bending vibrations are IR vs. Raman active. SERS more strongly detects PAHs and thus gives the ability to monitor changes to the PAH caused by interaction with calixarenes.^[11] However, SEIRA is better suited for detection of the calixarene due to the polar groups it is comprised of.^[11] There are conformational changes to the calixarene upon interaction with the PAH.^[11] SEIRA therefore can show attachment of PAHs by detecting the conformational changes in the calixarene at a high enough molecular density as well as optimal excitation wavelength. Due to this, SEIRA shows the possibility of being able to prove the chemistry behind the concept and be an effective second option depending on the availability of a working Raman. Fluorescence experiments can provide important supporting information about the interaction between the calixarene and PAH.

Methods and Materials:

Preparation of Quartz Slides. Quartz slides were tested by pipetting water onto the surface to determine if they were hydrophobic or hydrophilic. Hydrophobic slides were then soaked in 2M NaOH in ethanol overnight to render them hydrophilic. The slides were then retested and set aside for later use.

Preparation of Solutions.^[18] An 80 M suspension of BNNS was made by sonicating h-BN (200 g) in deionized water (100 mL) using a 2510 Branson sonicator. The sonicated solution was then centrifuged at 3000 xg using a Sovvall RC-5B Refrigerated Superspeed Centrifuge. The supernate was pipetted off and stored as BNNS solution. A 10 mM solution of silver acetate was prepared. Silver acetate (0.1670 g) was dissolved in deionized water (100 mL). A 2 mM hydrazine solution was prepared daily by pipetting a 1.03 g/L hydrazine solution (9.7 μ L) into a 100 mL volumetric flask and diluting with deionized water.

Preparation of Ag decorated BNNS. In a vial BNNS (1 mL), and 2 mM hydrazine (1.25 mL) were combined. Two aliquots of 10 mM silver acetate solution (0.5 mL each) were added at 10 minute intervals. This solution was then vacuum filtered onto a 0.8 μ m pore size isopore membrane filter. The membrane was then placed onto a hydrophilic quartz slide and dried under N₂. The slide was washed with approximately 20 mL of methylene chloride at least 3-6 times over the course of one hour by pipetting each aliquot onto the quartz slide to dissolve the membrane and allow attachment of the Ag-BNNS layer to the quartz slide. The slide was stored in a desiccator between washes and until use.

Attachment of Cysteamine then Calixarene. A 5.8 mM solution of cysteamine hydrochloride was prepared. Cysteamine hydrochloride (0.0658 g) was dissolved in deionized water (100 mL). The cysteamine hydrochloride solution (250 μ L) was pipetted onto the Ag-

BNNS layer on a quartz slide. The surface was rinsed with a 0.1 M solution of NaOH to ensure that the surface was terminated with the free amine. SERS spectra were then taken. A 1 mM solution of 4-tert-butylcalix[4]arene tetraacetic acid was prepared. 4-tert-butylcalix[4]arene tetraacetic acid (0.0072 g) was dissolved in dichloromethane (4 mL). A 4.25 mM solution of EDC was prepared. EDC (0.0033 g) was dissolved in dichloromethane (4 mL). The 1 mM solution of 4-tert-butylcalix[4]arene and the 4.25 mM solution of EDC were combined. An O-ring was placed around the cysteamine decorated Ag-BNNS layer on the quartz slide. The combined solution was pipetted onto the quartz slide within the O-ring and air dried. Raman spectra were attempted to be taken at this point using a Thermo Scientific NXR FT-Raman Module, however technical difficulties with the Raman prevented spectra being seen.

Attachment of Calixarene. A 1 mM solution of 4-tert-butylcalix[4]arene tetraacetic acid was prepared. 4-tert-butylcalix[4]arene tetraacetic acid (0.054 g) was dissolved in dichloromethane (30 mL). A quartz slide with a Ag-BNNS layer was soaked in the calixarene solution overnight. SERS spectra were then taken.

Preparation of SEIRA active colloid powder. In five vials simultaneously, BNNS (5 mL), and 2 mM hydrazine (6.25 mL) were combined. Three aliquots of 10 mM silver acetate solution (1.0 mL each) were added at 2 minute intervals. This solution was then vacuum filtered onto a 0.8 μm pore size isopore membrane filter. A thick powder layer was produced. The powder layer was scraped off of the filter paper into a mortar and ground.

Attachment of Calixarene to colloid powder. A 1 mM solution of 4-tert-butylcalix[4]arene tetraacetic acid was prepared. 4-tert-butylcalix[4]arene tetraacetic acid (0.0074 g) was dissolved in dichloromethane (4 mL). The colloid powder was added to the calixarene solution and stirred by stir plate overnight. The colloid solution was rinsed with

dichloromethane and transferred to microfuge tubes. This solution was centrifuged for 20 minutes at 12000 $\times g$ using a Sorvall Pico Centrifuge. The solvent was pipetted off and a drop of dichloromethane was added to each microfuge tube to transfer the colloid powder back to the vial. SEIRA spectra was taken and a large dichloromethane peak was seen. The vial was placed in the oven at 100° C to evaporate the dichloromethane. SEIRA spectra was taken using a Thermo Scientific Nicolet 6700 FT-IR with the Smart iTR attachment.

Preparation of Solutions for Fluorescence. A 0.1 mM solution of pyrene was prepared. Pyrene (0.002 g) was dissolved in dichloromethane (100 mL). This solution was diluted to a 0.01 mM solution with dichloromethane. A 10 mM solution of pyrene was prepared. Pyrene (0.1019 g) was dissolved in dichloromethane (50 mL). This solution was diluted to a 1 mM solution with dichloromethane. A 1 mM solution of 4-tertbutylcalix[4]arene was prepared. 4-tert-butylcalix[4]arene (0.009 g) was dissolved in dichloromethane (2 mL). Stock solutions were combined in the glass fluorescence cuvette to give a total volume of 4 mL, a final pyrene concentration of 5 μM for Trial 1 and 500 μM for Trial 2 and final calixarene concentrations ranging from 10 μM to 500 μM . Total volume was made up using dichloromethane. Fluorescence data was taken using an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer.

Results and Discussion:

Lack of uniform Ag colloid layer:

Previous work in our group showed good SERS enhancement of 4-MBA on Ag-colloids in solution.^[27] Due to this Ag-BNNS substrates were exposed to 5.8 mM solution of 4-MBA in dichloromethane. SERS spectra of these surfaces showed good enhancement of the ring breathing modes in 4-MBA as can be seen in Figure 2.

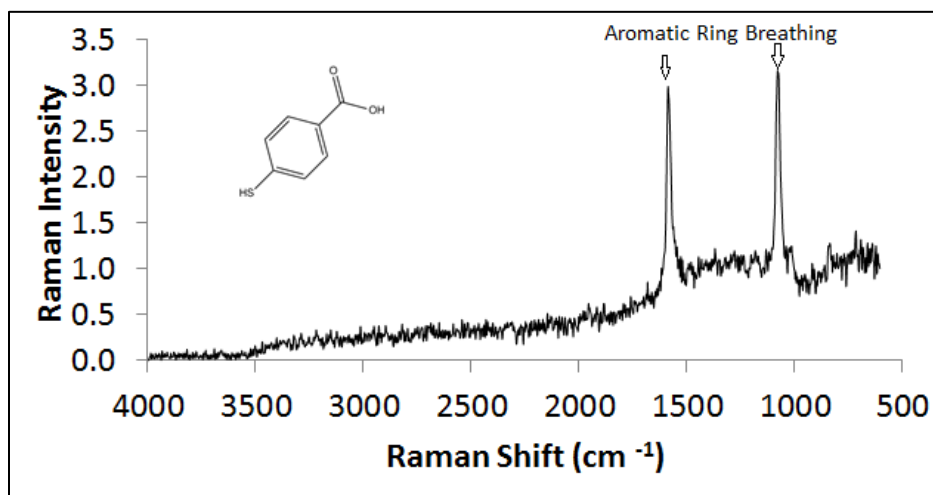


Figure 2. Raman spectra of 4-MBA attachment to Ag-BNNS layer. Aromatic ring breathing modes seen at 1600 cm⁻¹ and 1100 cm⁻¹.

The stretch around 1600 cm⁻¹ and 1000 cm⁻¹ coincide with aromatic ring breathing. Due to this, it is known that the Ag-BNNS layer has the potential as a colloid layer to give spectral enhancement to analytes for detection. However, the enhancement was very sensitive to the position of the incident laser on the surface suggesting that the surface was not uniform and uniform aggregation of silver colloids had not been achieved.

This was further given validation by Scanning Electron Microscope (SEM) images, which were taken of the Ag-BNNS that were prepared. Figure 3 shows the SEM image of the Ag-BNNS surface.

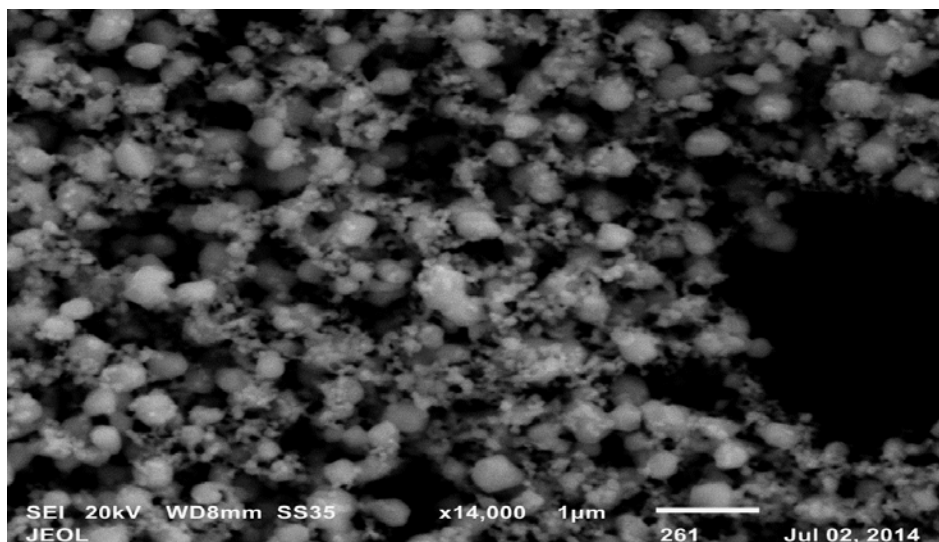
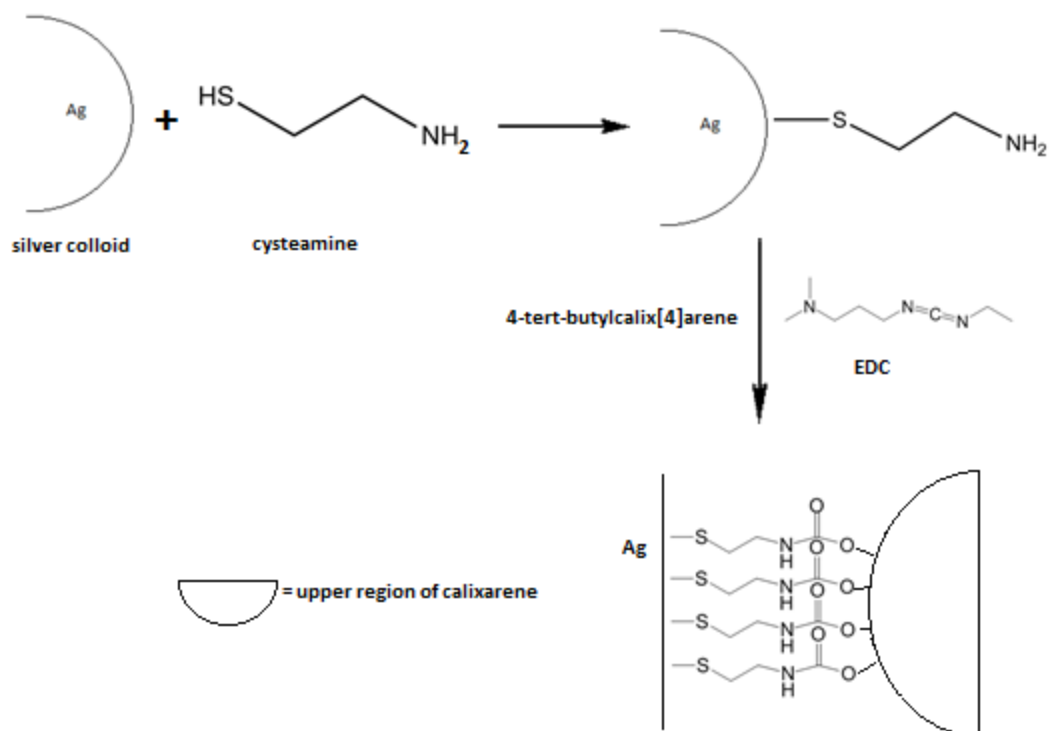


Figure 3. Scanning Electron Microscope image of Ag colloids on Boron Nitride Nanosheets (BNNS) structure. Ag colloids are the round structures seen. Non-uniform aggregation is seen due to the lack of Ag colloids in parts of the image.

Due to this discovery it was determined that for multiple step reactions in which SERS spectra are taken after each step, the slide must remain within the spectrometer throughout so that the same point on the surface is imaged after each step. An attempt was made to make a more uniform silver colloid layer by dissolving multiple Ag-BNNS layers onto one quartz slide, however variation in the spectral enhancement from point to point continued to be seen.

Cysteamine attachment:

Based on the success of the thiol-Ag interaction between 4-MBA and the Ag-BNNS surface, attachment of cysteamine was attempted in the hopes that further reaction to form an amide bond between the cysteamine and the carboxylate groups on the calixarene would provide a robust attachment of the calixarene to the surface as shown in Scheme 2.



Scheme 2. Cysteamine and 4-tert-butylcalix[4]arene attachment to Ag colloid surface

The covalent attachment of the calixarene to cysteamine via the amide bond formation forms a stronger linkage and therefore more durable connection allowing greater reproducibility in SERS results. Figure 4 shows enhancement of 5.8 mM solution of cysteamine and the IR spectra taken of solid cysteamine hydrochloride as comparison.

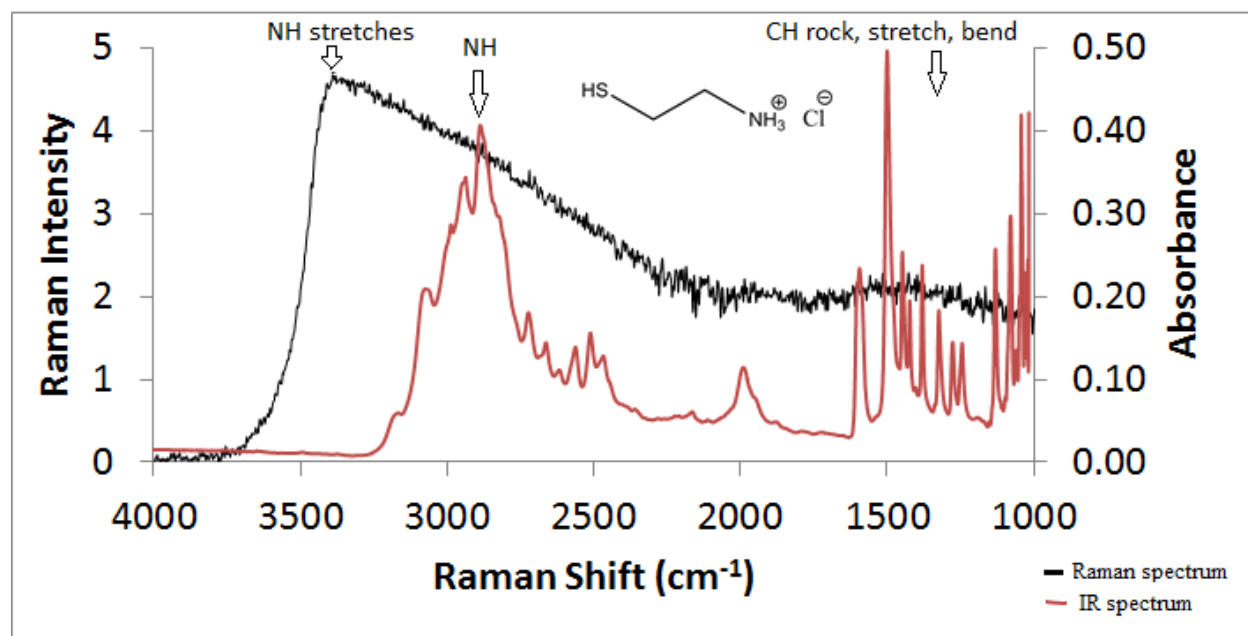


Figure 4. Raman spectra of cysteamine adsorbed onto the Ag-BNNS layer. NH stretches seen at 3400 cm^{-1} . IR spectra of solid cysteamine. NH stretch seen from 3300 cm^{-1} to 2500 cm^{-1} . CH rocks, stretches and bends seen below 1600 cm^{-1} . Raman intensity shown on left y-axis. IR Absorbance shown on right y-axis.

The peak at 3400 cm^{-1} corresponds to the NH stretch in cysteamine. No spectrum is available for the attachment of calixarene due to the Raman experiencing technical difficulties. The NH stretches correspond between the Raman and IR spectra of cysteamine, with a slight downshift of the amine in the cysteamine adsorbed onto the surface due to H-bonding which is not found in the salt, as seen in the solid cysteamine IR. The CH rocking, stretching and bending is not seen in the Raman spectra (Figure 4) due to noise.

4-tert-butylcalix[4]arene tetraacetic acid attachment:

Calixarene was attempted to be directly adsorbed to the metal colloid surface by the carboxylate. This non-covalent interaction between the calixarene and the Ag colloid surface represents a simpler one step method for adsorption of the substrate onto the metallic surface. Figure 5 shows detection of 4-tert-butyl calix[4]arene tetraacetic acid on Ag-BNNS layer and IR spectrum of solid 4-tert-butyl calix[4]arene tetraacetic acid.

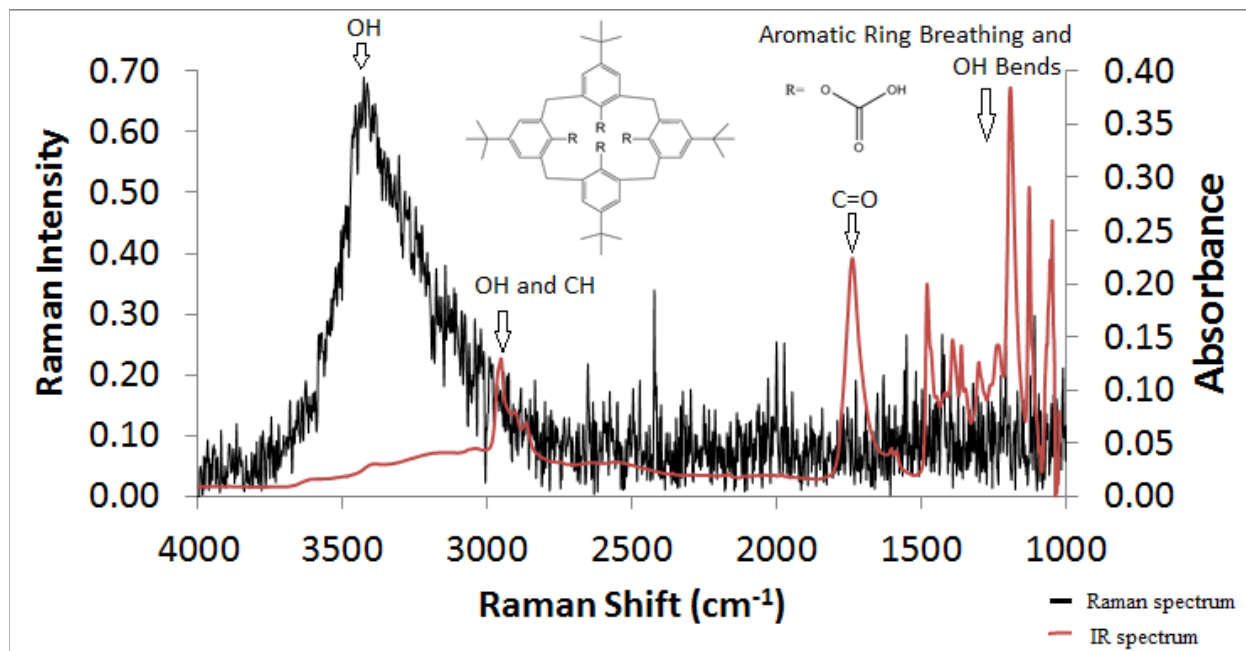


Figure 5. Raman spectrum of 4-tert-butylcalix[4]arene tetraacetic acid adsorbed onto the Ag-BNNS layer. OH stretches seen at 3400 cm^{-1} . IR Absorbance spectrum of solid 4-tert-butylcalix[4]arene. OH stretches seen from 3500 cm^{-1} to 2500 cm^{-1} . CH stretches seen at 3000 cm^{-1} . C=O stretch seen at 1750 cm^{-1} . Aromatic ring breathing modes and OH bends seen below 1500 cm^{-1} . Raman intensity shown on left y-axis. IR Absorbance shown on right y-axis.

The peak at 3400 cm^{-1} corresponds to OH stretches. The enhancement value of the Raman spectrum is low. No comparison was able to be made between other spectra of the same surface due to the Raman experiencing technical difficulties. It is possible that there was poor colloid aggregation at the location that the spectra was taken on this surface, and that with better aggregation at another location on the surface greater enhancement would have been seen. The OH and potentially CH stretches correspond between the Raman and IR spectra of calixarene. The C=O stretch, aromatic ring breathing modes and OH bends are not seen in the Raman spectra (Figure 5) due to noise.

SEIRA enhancement of calixarene:

Due to the technical difficulties which were experienced with the Raman spectrometer, enhancement of the calixarene adsorbed onto the metal surface was attempted to be seen by

Surface Enhanced Infrared Absorption Spectroscopy. While this spectroscopy has been shown to have much lower enhancement values than SERS, the polar groups in the calixarene are IR active and therefore it was hoped that attachment of the calixarene onto the metal surface could be proven by this method at high enough concentrations of calixarene. However, detection of calixarene onto the metal surface was not seen in SEIRA experiments done. It is believed that calixarene is in fact adsorbing to the metal surface based on alternative supporting evidence such as the SERS spectra shown in Figure 5. Multiple reasons exist for the lack of calixarene spectra by SEIRA. One is that phonons in a very specific wavelength range must be excited for each vibration or stretch for enhancement to be seen.^[12] This can be difficult in IR spectroscopy and therefore requires multiple optimizations. Overall the enhancement of SEIRA is weak and therefore a higher concentration of analyte is needed in order to be detected.^[11] Since PAHs adsorbed directly onto colloid surfaces which were tested in the IR in powder form, there was a much smaller molecular density than other possible IR testing states, such as liquid films on salt plates (Appendix 1). Due to this, increased calixarene concentration was not enough to overcome the decreased enhancement which is seen in SEIRA. With proper optimization of the nanoparticle shape and size SEIRA may have the potential to be used as a detection method in the future.

Fluorescence of PAHs:

In an attempt to understand the interaction between calixarene's and PAHs, fluorescence experiments were done. Pyrene was chosen as the PAH in each of the experiments. Pyrene fluoresces due to its aromatic nature. A low concentration of pyrene (10^{-5} M) was first used. As increased concentrations of calixarene were added it was hypothesized that the fluorescence spectra of pyrene would decrease in intensity. As calixarene concentration increased the

formation of the pyrene-calixarene guest-host complex would also increase due to a higher availability of calixarene. This complex would form based on the hydrophobic interactions between pyrene and the internal cavity of the calixarene. As pyrene inserted into the calixarene cavity it was proposed that quenching would occur and less pyrene would be “seen.” Upon testing no significant decrease in pyrene fluorescence was seen with increased concentrations of calixarene. Figure 6 shows a separate peak in the fluorescence spectra due to excimer formation at increased concentrations of pyrene.

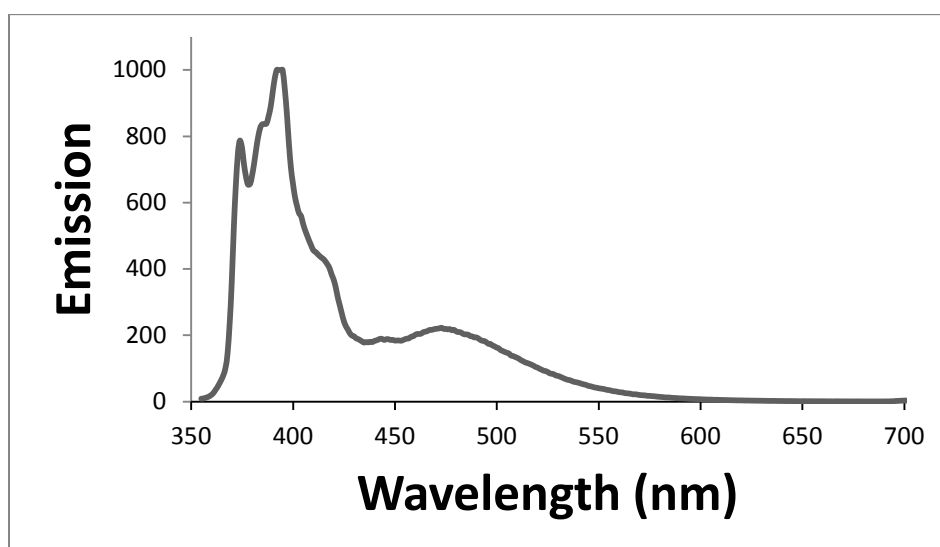


Figure 6. Fluorescence emission spectra of pyrene. Monomer fluorescence can be seen from 350 nm to 430 nm. Excimer fluorescence can be seen from 430 nm to 600 nm.

New experiments were run with higher concentrations of pyrene (10^{-3} M) in an attempt to see a reduced excimer peak as insertion of the pyrene into the calixarene cavity may be more favorable than dimer formation due to the hydrophobic interactions between the pyrene and calixarene. Two trials were run and no decrease in pyrene excimer fluorescence intensity was seen with increased calixarene concentration. Figure 7 shows the fluorescence intensity of Trial 1 and Trial 2.

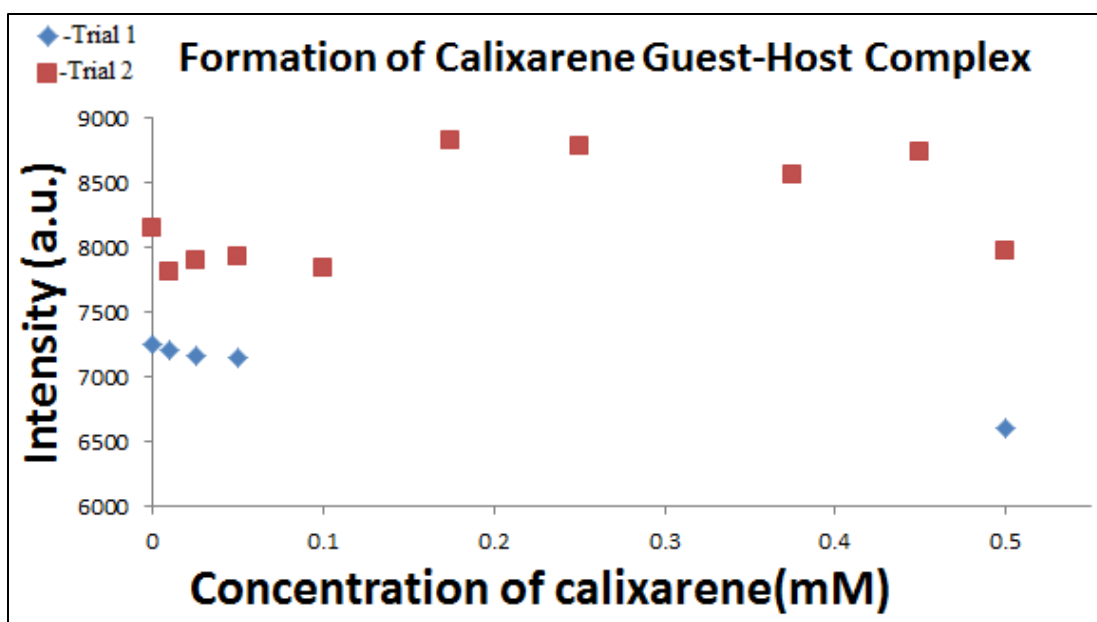


Figure 7. Graph of pyrene fluorescence intensity as concentration of calixarene increases, no consistent trend was seen between Trial 1 ([pyr] = 5 μ M) or Trial 2 ([pyr] = 500 μ M).

This lack of a decrease in the intensity of pyrene excimer fluorescence could be due to the following possibilities. No decrease in fluorescence could be due to a low K_{eq} value for the formation of the guest-host complex between the calixarene and the pyrene. This would mean that too few interactions are occurring for calixarene quenching of the pyrene to be seen. Much higher concentrations of calixarene would need to be used for this decrease to be observed. Calixarene could potentially not quench the fluorescence of the pyrene. Due to this, there could be formation of the pyrene-calixarene complex, but no change in fluorescence would be detected. A new system, such as a working Raman spectrometer, would need to be utilized to monitor this interaction. A slight trend in Trial 2 may point to a third explanation in which formation of the guest-host complex between calixarene and pyrene corresponds to an increased intensity of fluorescence. By this, calixarene would not quench fluorescence of the pyrene, but enhance it.^{[28] [29]} As higher concentrations of calixarene were added in Trial 2, a general increase

in the intensity of the fluorescence can be seen. Continued experimentation is necessary to confirm these potential findings from Trial 2.

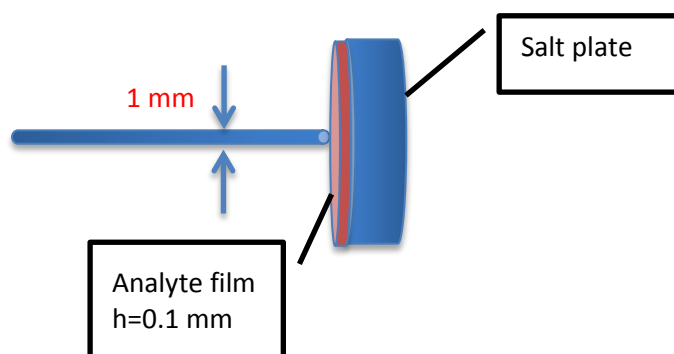
Conclusion:

PAHs are environmental pollutants which lack an effective, inexpensive, *in situ* method of detection. Surface Enhanced Raman Spectroscopy has shown promise for detecting small concentrations of analytes. A uniform colloid layer must exist in order for reproducible results to be seen. This uniform layer was not prevalently seen using BNNS as a supporting substrate. Exploring other scaffolding options on which to aggregate silver colloids may be an important factor in continuing this research. Surface Enhanced Infrared Absorption Spectroscopy showed promise in providing supporting information for the detection of calixarene adsorption on to the silver colloid surface as well as monitoring guest-host complexing between the pyrene and calixarene. SEIRA, however, lacked the enhancement and optimization necessary to detect low concentrations of analytes such as PAHs in aqueous environments. With continued optimization of SEIRA as well as coupling this spectroscopy method with a new enhancement surface, detection of PAHs may be possible. Fluorescence experiments on guest-host complexing formation between pyrene and calixarene showed promise. Continued experimentation of this system could lead to conclusive results regarding the K_{eq} value of this complex formation. Due to calixarene's ability for size-dependent guest-host formation and therefore detection as well as its multiple available lower rim formations which adsorb readily to metal surfaces, continued experimentation on its guest-host complexing with PAHs is important. The continued use of calixarene as a surface modification for detection of PAHs depends greatly on the results found from continued Raman and Fluorescence experiments.

Appendix:

I. Calculations made for molecular density of liquid film on a salt plate versus colloid layer which interacts with light beam:

Liquid film:



Illumination volume of liquid film:

$$V_{liq\ film} = \pi r^2 h = 3.14 * 10^{-5} cm^3$$

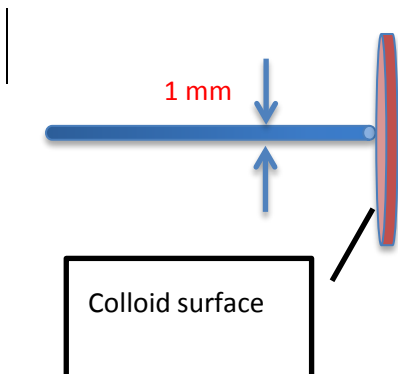
Density and molecular mass of water used for simplicity.

Number of molecules in liquid film:

$$N_{illuminated} = \frac{V_{liq\ film} * density}{MW}$$

$$= 1.05 * 10^{18} \text{ molecules}$$

Colloid layer:



$$\theta = 10^{15} \text{ molecules/cm}^2 \text{ [13]}$$

$$N_{illuminated} = \theta A$$

$$= 3.14 * 10^{13} \text{ molecules}$$

Thus an enhancement factor of at least 10^5 is required to give signals on a surface that are comparable to those in “normal” IR spectroscopy.

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