

**FABRICATION AND ENVIRONMENTAL APPLICATIONS OF
SILVER COATED GOLD NANORODS/ ELECTROSPUN POLYMER FIBER
MATRIX FOR USE AS
SURFACE ENHANCED RAMAN SCATTERING SUBSTRATES**

by

Shuyu Xu

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Materials Science and Engineering

Fall 2018

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TABLE OF CONTENTS

LIST OF TABLES	ix
LIST OF FIGURES	x
ABSTRACT	xv

Chapter

1	INTRODUCTION	1
1.1	Surface Enhanced Raman Scattering.....	1
1.2	SERS-active Metallic Nanoparticles	4
1.3	Plasmonic Properties of Rod-shape MNPs.....	6
1.4	Synthesis of AuNRs and Silver Coated Gold Nanorods	8
1.5	SERS Substrates	12
1.6	Dissertation Overview	16
	REFERENCES	18
2	FABRICATION OF 3D SERS SUBSTRATES COMPRISED OF AG/AUNRS AND ELECTROSPUN PCL FIBERS	28
2.1	Introduction	28
2.2	Materials and Methods	29
2.2.1	Materials	29
2.2.2	Fabrication of Ag/AuNRs-PCL SERS substrate	31
2.2.2.1	Synthesis of PSS coated Ag/AuNRs (PSS/Ag/AuNRs)	31
2.2.2.2	Electrospinning of PCL and PHBHx fibrous mats	34
2.2.2.3	Surface modification of PCL fibrous mats	36
2.2.2.4	PSS/Ag/AuNRs immobilization on a PCL fibrous mat	37
2.3	Results and Discussion	37
2.3.1	Characterization of SERS-active MNPs.....	37
2.3.2	Characterization of electrospun polymeric fibers.....	44
2.3.3	Characterization of LbL deposition on PCL fibrous mat	46

2.3.4	Characterization of the SERS substrate.....	51
2.3.5	Discussion of the immobilization process.....	52
2.4	Conclusion.....	54
	REFERENCES	55
3	EVALUATION OF AG/AUNRS-PCL SERS SUBSTRATES	58
3.1	Overview	58
3.2	Materials and Materials	58
3.2.1	Materials	58
3.2.2	SERS measurements.....	59
3.3	Results and Discussion	59
3.3.1	SERS sensitivity, reproducibility, and stability.....	59
3.3.2	SERS substrate fabricated with PHBHx.....	64
3.3.3	SERS performance evaluated by enhancement fact	65
3.3.4	Comparison of AuNR- and Ag/AuNR-based SERS substrates ..	69
3.4	Conclusion.....	75
	REFERENCES	77
4	AG/AUNRS-PCL SERS SUBSTRATE FOR ARSENIC DETECTION AND SPECIATION	81
4.1	Introduction	81
4.2	Materials and Methods	84
4.2.1	Materials	84
4.2.2	Fabrication of Ag/AuNRs-PCL SERS substrate	84
4.2.3	Quantitative measurements on arsenic species.....	85
4.2.4	SERS of <i>pAsA</i> at different pH	86
4.2.5	DFT computation of <i>pAsA</i> molecular structure	86
4.2.6	<i>In-situ</i> SERS of arsenic adsorption, desorption and reduction....	87
4.3	Results and Discussion	88
4.3.1	SERS analysis of arsenic speciation.....	88
4.3.2	Quantification of arsenic detection.....	93
4.3.3	SERS analysis of <i>pAsA</i>	98
4.3.4	<i>In-situ</i> SERS analysis of arsenic.....	111

4.4	Conclusion	115
	REFERENCES	117
5	CONCLUSIONS AND FUTURE WORK	122
5.1	Conclusions	122
5.2	Future Work.....	125
5.2.1	SERS substrate for biosensing.....	125
5.2.2	Two-dimensional correlation spectroscopy and low-frequency spectroscopy of SERS	131
	REFERENCES	136
Appendix		
	COPYRIGHT PERMISSION	139

LIST OF TABLES

Table 3.1 Peak assignments, averaged intensities, intensity variations of 4-Mpy major peaks.....	63
Table 3.2 Relative EF of Ag/AuNR to AuNR at different peaks of 4-Mpy.	73
Table 4.1 Peak assignments of <i>pAsA</i> and Rox in normal Raman and SERS spectra.	91
Table 4.2 K_{sp} of silver- and arsenate- related inorganic salts.	98
Table 4.3 Population of $pAsA^-$ and $pAsA^{2-}$ in <i>pAsA</i> solutions at different pH.....	103
Table 4.4 Pople basis sets and settings used in optimization of <i>pAsA</i> molecule.	105
Table 4.5 Total energy differences for $pAsA^-$ and $pAsA^{2-}$ molecules in different chemical adsorption sites and molecule orientations. (Courtesy to Dr. Sabino and Dr. Janotti).....	111

LIST OF FIGURES

Figure 1.1 Schematic illustration of SPR phenomenon that generates SERS activities.....	4
Figure 1.2 (A) Single SPR of spherical MNP. (B) Transverse and longitudinal SPR modes of rod-shape MNP.....	7
Figure 2.1 Synthesis of AuNRs using seed-mediated wet chemistry protocol.	31
Figure 2.2 Synthesis of Ag/AuNRs.	32
Figure 2.3 Schematic illustration of the electrospinning process.	34
Figure 2.4 PDADMAC/PSS polyelectrolytes LbL deposition onto a PCL fibrous mat.	36
Figure 2.5 UV-vis of AuNRs synthesized using different CTAB chemicals (left), and the table listing CTAB chemicals purchased from various suppliers with production numbers (right).	38
Figure 2.6 (A) UV-vis spectra of AuNRs, Ag/AuNRs and PSS/Ag/AuNRs. (B, C) TEM images of AuNRs and Ag/AuNRs. The scale bar is 20 nm.	40
Figure 2.7 TEM images of (A) AuNRs and (B) Ag/AuNRs (Au:Ag = 1.42). The scale bar is 100 nm in (A) and 20 nm in (B).	41
Figure 2.8 UV-vis spectra, digital photo (the inset) of Ag/AuNRs, with a molar ratio of Au to Ag of 4.25, 2.13, and 1.42, respectively.	42
Figure 2.9 TEM images of Ag/AuNRs (from left to right), with a molar ratio of Au to Ag of 4.25, 2.13, and 1.42, respectively.....	43
Figure 2.10 Electrospun fibers of 15 wt% PCL in different solvents: (A) DCM, (B) CHCl ₃ , (C) CHCl ₃ :DMF (v:v) = 5:1, (D) CHCl ₃ :DMF (v:v) = 2:1. The scale bar is 10 μm.	44
Figure 2.11 (A) 11 wt% PCL electrospinning in CHCl ₃ :DMF (v:v)=2:1, (B) 6 wt% PCL electrospinning in AA:FA (v:v) = 3:1. The scale bars are 10 μm and 1 μm, respectively.....	46

Figure 2.12 (A-F) SEM images of PDADMAC/PSS LbL deposition onto PCL fibers at pristine PCL, 1 st , 3 rd , 5 th , 7 th and 9 th layer respectively. The scale bar is 1 μm	48
Figure 2.13 (A) SEM image of a (PDADMAC/PSS) _{7.5} -PCL mat. (B) SEM-XEDS spectrum of the square area labeled in (A).....	49
Figure 2.14 Two layers of PSS/PDADMAC LbL deposition on a PCL mat with a smaller fiber diameter (~ 300 nm).....	50
Figure 2.15 (A, B) Digital photos of a (PDADMAC/PSS) _{3.5} -PCL mat before and after a 24-hour immobilization, respectively. (C) UV-vis spectra of PSS/Ag/AuNRs before and after a 24-hour immobilization.	51
Figure 2.16 SEM images of PSS/Ag/AuNR decorated PCL fibers. The scale bars are 1 μm and 200 nm in (A) and (B), respectively.....	52
Figure 3.1 (A) SERS spectra of 4-Mpy at concentrations from 10^{-4} M to 10^{-8} M. Inset: structure of 4-Mpy bound to Ag/AuNR. (B) Magnified SERS spectra of 4-Mpy at concentration of 10^{-7} M, 10^{-8} M and bare SERS substrate along with Raman spectra of (PDADMAC/PSS) _{3.5} -PCL mat, PSS, CTAB and CTAC powders, respectively.	61
Figure 3.2 (A) SERS spectra of 10^{-5} M 4-Mpy at 9 randomly selected spots on the Ag/AuNRs-PCL SERS substrate. (B) Peak intensities and variations of 10^{-5} M 4-Mpy measured by randomly selecting spots on the Ag/AuNRs-PCL SERS substrate.....	62
Figure 3.3 SERS spectra of 4-Mpy at a concentration of 10^{-5} M on the same batch of Ag/AuNRs-PCL substrate when freshly prepared and aged for 3 months.	63
Figure 3.4 Molecular structure of PHBHx.	64
Figure 3.5 (A) SEM images of Ag/AuNR decorated PHBHx electrospun fibrous mat. (B) SERS spectrum of 4-Mpy at a concentration of 10^{-5} M on Ag/AuNRs-PHBHx substrate.....	65
Figure 3.6 (A) Normal Raman spectrum of 0.25 M 4-Mpy aqueous solution. (B) SERS spectrum of 10^{-5} M 4-Mpy measured on Ag/AuNRs-PCL SERS substrate.....	67

Figure 3.7 (A-D) SEM images of SERS substrates: AuNR_778 nm, Ag/AuNR_703 nm, Ag/AuNR_673 nm, and Ag/AuNR_664 nm, respectively; the scale bar is 1 μm . Inset: TEM images of the corresponding SERS-active MNPs used to fabricate the SERS substrates; the scale bar is 5 nm.	70
Figure 3.8 (A) The SERS spectra of 10^{-5} M 4-Mpy measured on different SERS substrates. (B) Comparison of peak intensities between different SERS substrates.....	71
Figure 4.1 Raman Spectra of <i>pAsA</i> , Rox, AsV and AsIII powders (from top to bottom). The molecular structures of each arsenic chemical are shown in the insets.	89
Figure 4.2 SERS spectra of arsenic species adsorbed onto Ag/AuNRs-PCL substrate.....	90
Figure 4.3 SERS spectra of (A) AsV, AsIII at 1×10^{-4} M and AsV/AsIII mixture at 5×10^{-5} M, (B) <i>pAsA</i> , Rox at 5×10^{-5} M and <i>pAsA</i> /Rox mixture at 1×10^{-5} M.	93
Figure 4.4 SERS spectra of Rox with concentrations ranging from 5×10^{-8} M to 5×10^{-4} M.	94
Figure 4.5 SERS quantification of Rox against concentration at 405, 627, 813 and 1322 cm^{-1}	95
Figure 4.6 SERS spectra of AsV at concentrations ranging from 1×10^{-5} M to 5×10^{-8} M in DI water. Insert: SERS intensity of 785 cm^{-1} peak as a function of AsV concentration.....	96
Figure 4.7 SERS spectra of AsV at concentrations ranging from 10^{-5} M to 10^{-7} M prepared in common salt ions solutions. Insert: SERS intensity of 785 cm^{-1} peak as a function of AsV concentration.	97
Figure 4.8 (A) SERS spectra of <i>pAsA</i> with concentrations ranging from 5×10^{-8} M to 5×10^{-5} M. (B) SERS quantification of <i>pAsA</i> against concentration for peaks at 611 and 1593 cm^{-1} . (C) SERS spectra of <i>pAsA</i> with concentrations ranging from 5×10^{-5} M to 1×10^{-3} M. (D) SERS intensities of <i>pAsA</i> for peaks at 611 and 1593 cm^{-1} , with the whole concentration range from 5×10^{-8} M to 1×10^{-3} M.	99
Figure 4.9 Different tautomers of <i>pAsA</i> that can exist in water.....	100

Figure 4.10 <i>pAsA</i> polyprotic distribution as a function of pH.	101
Figure 4.11 (A) SERS spectra of 5×10^{-4} M <i>pAsA</i> under different pH conditions and 5×10^{-5} M <i>pAsA</i> (no NaOH added). (B) SERS intensity comparison of the 611cm^{-1} and 1593cm^{-1} peaks of <i>pAsA</i> at 5×10^{-4} M at different pH values and <i>pAsA</i> at 5×10^{-5} M (no NaOH added).	102
Figure 4.12 SERS intensity of <i>pAsA</i> peaks at 611 and 1593cm^{-1} plotted against concentration of $pAsA^{2-}$	104
Figure 4.13 (A) Optimized structure of $pAsA^-$ using DFT calculation with No. 8 basis set in Gaussian 09. (B) Calculated Raman and (C) IR spectrum of <i>pAsA</i> molecule.	106
Figure 4.14 Proposed binding structures of $pAsA^-$ and $pAsA^{2-}$ bound onto silver surface.	108
Figure 4.15 Possible adsorption sites of $pAsA^-$ or $pAsA^{2-}$ on the Ag(111) surface. The top site, the bridge site and the hollow site. (Courtesy to Dr. Sanobi and Dr. Janotti).....	109
Figure 4.16 Optimized $pAsA^-$ and $pAsA^{2-}$ on the Ag(111) surface. H, O, N, C, As and Ag are represented by the pink, red, blue, brown, green and grey spheres, respectively. (Courtesy to Dr. Sabino and Dr. Janotii)	111
Figure 4.17 <i>In-situ</i> SERS spectra of (A) AsIII and (B) AsV adsorption at a concentration of 1×10^{-4} M in DI water as a function of time. Insert: Raman intensity of (A) 725cm^{-1} and (B) 785cm^{-1} peaks with time. ...	113
Figure 4.18 <i>In-situ</i> SERS of 10^{-4} M AsV desorption in 20 mg/L (A) NaCl and (B) KH_2PO_4 solution. Inset of (B): Raman intensity of AsV peak at 785cm^{-1} as a function of time illustrating the desorption of AsV in 20 mg/L NaCl and KH_2PO_4 solution, respectively.	114
Figure 4.19 <i>In-situ</i> SERS spectra of 5×10^{-5} M Rox reduction by 2 mM $NaBH_4$	115
Figure 5.1 (A) Molecular structures of nucleobases: thymine, adenine, cytosine, and guanine. (B) Normal Raman spectra of (from up to bottom): thymine, guanine, cytosine.	127
Figure 5.2 SERS spectra of 5×10^{-5} M (A) adenine, thymine, and adenine/thymine mixture with A:T = 1:1 in molar ratio; (B) guanine, cytosine, and guanine/cytosine mixture with G:C = 1:1 in molar ratio.....	128

- Figure 5.3** (A) Digital photos of AuNRs transferred from aqueous solution (left) to CHCl_3 (right). (B) UV-vis spectra of bare AuNRs (green), PEGylated AuNRs in DI H_2O (blue) and PEGylated AuNRs in CHCl_3 (red). 130
- Figure 5.4** The ATR-FTIR spectra of (from top to bottom): PCL, PEG, electrospun membrane of PCL/PEG blends, and electrospun membrane of PCL/PEG blends after immersion in DI water for a week. 131
- Figure 5.5** (A) Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures with increasing molar concentration of CHCl_3 from 0 (pure EtOH) to 1.0 (pure CHCl_3). (B) Raman intensity of $-\text{CCl}_3$ stretching at 672 cm^{-1} with increasing CHCl_3 content. (C) Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures at low-frequency region. (D) Reduced Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures at low-frequency region..... 134
- Figure 5.6** (A) Reduced Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures after removal of Lorentzian contribution. (B) Synchronous and (C) asynchronous spectrum of serial spectra in (A). 135

ABSTRACT

Surface-enhanced Raman scattering (SERS), has been recognized as an ultra-sensitive analytical technique since its discovery in the 1970s. After five decades of development, SERS has been successfully applied in various fields. Despite the great potential of SERS for trace-level detection, non-destructive testing, and surface analysis, the lack of reproducibility of SERS substrates has prevented SERS from being a routine analytical method. To solve this problem, we have proposed a fabrication strategy for three-dimensional (3D) SERS substrates comprised of SERS-active metallic nanoparticles (MNPs) and polymeric electrospun fibrous mat. MNPs are immobilized onto the fibrous mats via electrostatic attraction which acts as a driving force. Specifically, in this dissertation, silver coated gold nanorods (Ag/AuNRs) were synthesized and used as SERS-active MNPs. To utilize non-specific electrostatic attraction as the driving force to direct the immobilization of Ag/AuNRs, opposite electrostatic charges were introduced on Ag/AuNRs and electrospun polycaprolactone (PCL) fibers. Negative charges were introduced on Ag/AuNRs surface by coating a layer of polyanion poly(sodium 4-styrenesulfonate) (PSS), while positive charges on the PCL fiber surfaces were developed through polyelectrolyte layer-by-layer (LbL) deposition leaving polycation poly(diallyldimethylammonium chloride) (PDADMAC) as the outermost layer. In the immersion of LbL modified PCL mat in the PSS coated Ag/AuNRs (PSS/Ag/AuNRs) solution, PSS/Ag/AuNRs were adsorbed onto PCL fiber surface, where a dense and uniform decoration of Ag/AuNRs was formed. This fabrication of MNPs/polymer

composite is a generalized method that can be applied to different types of MNPs and polymer materials. Ag/AuNRs with different silver coating thicknesses and poly (3-hydroxybutyrate-co-hydroxyhexanoate) electrospun fibers were all used in production of SERS substrates.

Regarding the sensitivity, reproducibility and stability, SERS performance of the Ag/AuNRs-PCL composites was evaluated using 4-mercaptopyridine (4-Mpy) as a probe molecule. A limit of detection (LOD) as low as 10^{-8} M and excellent reproducibility with less than 7% intensity variations among nine measurements were achieved. In addition, the SERS substrate had good long-term shelf stability. The SERS intensities obtained from a 3-month aged substrate had negligible differences when compared to the intensities obtained from a freshly prepared substrate. Enhancement factor, an enhancement indicator of SERS substrates, compared to normal Raman, was calculated to be 10^4 - 10^5 for the major peaks of 4-MPy. Comparison experiments were conducted using Ag/AuNRs-PCL substrates having different silver coating thicknesses and AuNRs-PCL fabricated using the same strategy. The results showed that the Ag/AuNRs-PCL substrates with different silver coating thickness had overall better SERS sensitivities than AuNRs-PCL substrate, especially for SERS-sensitive vibration modes of 4-Mpy. This could be attributed to the larger surface area of Ag/AuNRs compared to AuNRs allowing a higher loading of 4-Mpy molecules and the charge transfer between silver and gold in the bimetallic Ag/AuNRs to enhance the local electric field of Ag/AuNRs.

To explore the potential application of Ag/AuNRs-PCL SERS substrates in environmental sensing, we have carried out successful speciation and quantification of several arsenic species. Ag/AuNRs provide appropriate surface chemistry for arsenic

analysis through the formation of As-O-Ag bonds. A LOD as low as 4 ppb for various inorganic and organic arsenic species was achieved. Moreover, Ag/AuNRs-PCL substrates have been applied to investigate arsenic activities and molecular configuration on a silver surface. *In-situ* SERS observation of arsenic adsorption, desorption and reduction have been monitored. A detailed analysis of the SERS spectra of *p*-arsanilic acid (*p*AsA) under different concentrations and pH conditions provided insights on the nature of the binding structures of *p*AsA on a silver surface. *p*AsA⁻ and *p*AsA²⁻ are the *p*AsA tautomers that can effectively be adsorbed onto the silver surface but have different molecular structure interacting with the surface. *p*AsA⁻ was speculated to bind onto the silver surface in a tilted fashion while *p*AsA²⁻ was the tautomer that binds with a 90-degree angle to the silver surface and contributes most of the observed SERS intensity due to its perpendicular orientation and chemical interaction with the Ag surface. These spectra studies were supported by theoretical calculations for the bonding configurations of *p*AsA⁻ and *p*AsA²⁻ on the Ag(111) surface executed by Dr. Fernando Sabino and Dr. Anderson Janotti. All the results are strong evidence that the 3D Ag/AuNRs-PCL substrate is of great potential for SERS measurements and serving as an environmental sensor for arsenic analysis.

Chapter 1

INTRODUCTION

1.1 Surface Enhanced Raman Scattering

Conventional Raman spectroscopy is a nondestructive vibrational spectroscopic technique with molecular specificity and is complementary to infrared (IR) spectroscopy. The drawbacks of conventional Raman spectroscopy include (1) its inherently weak signal (1 scattered photon out of 10^6) which originates from inelastic scattering^[1] and (2) the luminescent interference from many samples under laser excitation. Surface enhanced Raman scattering (SERS) is a surface-sensitive resonant, scattering technique that amplifies a conventional Raman scattering signal by many orders of magnitude. SERS dramatically increases the weak scattering cross-section of conventional Raman scattering, and can improve the fingerprint specificity of vibrational spectroscopy. It was first discovered in 1974 by Fleischmann *et al.* in the investigation of pyridine adsorbed on roughened silver electrodes.^[2] Since then, both fundamental and applied SERS research have been extensively carried out. In the 1990s, studies were reported claiming ultrasensitive detection of single molecules using SERS.^[3-4] Over decades of development, SERS has grown to be a powerful analytical technique, with applications in fields such as, chemical and biomedical sensing,^[5-9] environmental detection,^[10-13] art analysis and forensic analysis.^[14-15]

The mechanism of SERS is controversial and has been debated for decades with no unambiguous explanation. Current theory holds that several resonances possibly contribute to the enhancement of SERS activities, including charge-transfer

(CT) resonance, molecular resonance, and surface plasmon resonance (SPR).^[16] CT resonance and molecular resonance arise from a chemical interaction, which is not always present for a SERS active system, but depends on the nature of analyte molecules and their excited states. CT resonance is the charge transfer between molecule and substrate, while molecular resonance is due to the excitation of a higher electronic state of the analyte molecule. Most of the SERS enhancement is contributed by SPR, which originates from an electromagnetic mechanism. SPR stems from the interaction between the incident light and the metallic nanostructure of the surface, which is usually made of coinage metals, such as silver, gold and copper. **(Figure 1.1)** When the free electrons of the metallic surface are excited by the electromagnetic field of the excitation wavelength, the electrons confined on the surface will experience a collective oscillation. The oscillation of electrons will further boost the electromagnetic field localized around the metallic surface, which acts as a dipole antenna. When the analyte molecules are in the vicinity (within few nanometers) of the metallic surface, the Raman scattering from the vibrational modes of these molecules will be largely magnified. Raman intensity, which is equal to polarizability times localized electric field ($|E|$). Since there are electric fields associated with both incident light ($|E_{inc}|$) and scattered light ($|E_{scat}|$), the Raman intensity is proportional to $|E|^4$, which is the so-called $|E|^4$ -approximation.^[17-18] When the electric field is in resonance with the SPR of the metallic nanostructures, strong SERS activities occur. The enhancement from the electromagnetic mechanism (up to $\sim 10^{11}$) is much stronger than that from the chemical mechanism ($\sim 10^3$) and always exists independent of the chemical interactions.^[19]

Theoretical studies, such as discrete dipole approximation (DDA) and finite-difference time-domain (FDTD) calculations have carried out to quantify and map the intensities of the electric field distributed among the nanoparticles.^[20-21] These calculations suggest that the highest SERS signals, known as “hot spots”,^[22-25] reside within the very small gaps (on nm scales) or around the sharp edges among the densely packed nanoparticles. A simple model of a gold colloidal dimer (radii = 30 nm) with a 2-nm gap excited by 559 nm as described by Etchegoin and Le Ru,^[26] shows that the enhancement exhibits the maximum value, approximately 10^8 , on the surface of a colloid right in the center of the hot spot. However, it dramatically decreases to approximately 10^3 at a distance of 20 nm from the hot spot along the surface from the vertical axis. It is the very small numbers of molecules sitting in hot-spot areas, where the highest SERS enhancement exists that contribute the most to the SERS intensity. The observed SERS intensity is actually a statistic result of various enhancements distributed throughout different spots of the sampled area. These facts suggest that the generation of hot spots is a necessity in order to obtain intense SERS signals.

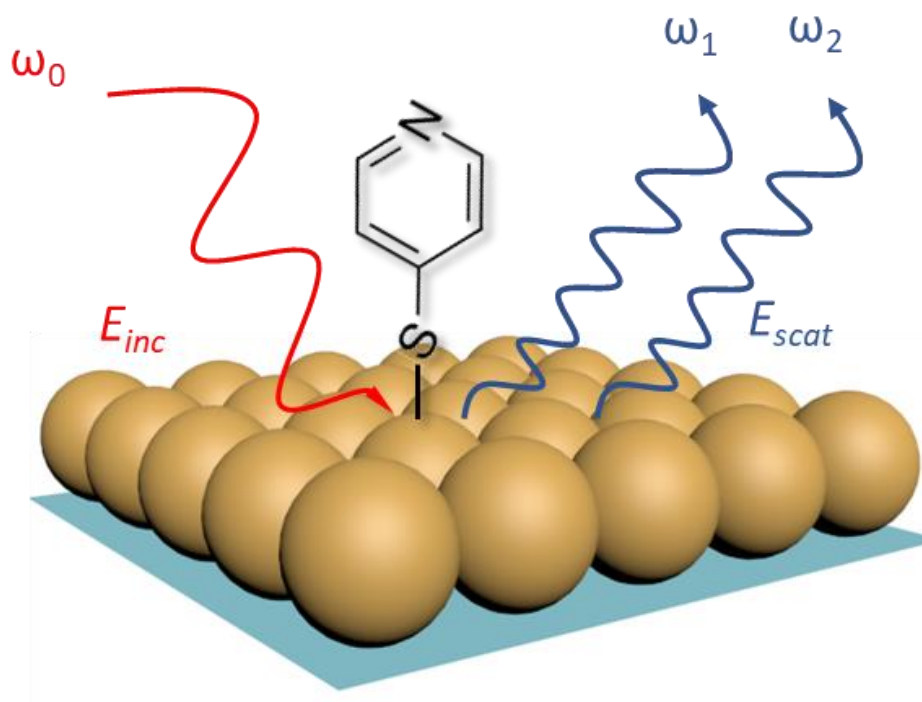


Figure 1.1 Schematic illustration of SPR phenomenon that generates SERS activities.

1.2 SERS-active Metallic Nanoparticles

Metallic nanoparticles (MNPs) show exceptional optical properties compared to their bulk forms and have a long history of observation in optical phenomena. The Late Roman Lycurgus Cup, for example, is an art work made of dichroic glass with small levels of dopants of silver and gold nanoparticles. When lit externally, the cup shows green, while when lit internally, the cup shows red.^[27] MNPs have been extensively explored for applications including electronic devices,^[28-30] optical sensors,^[31-35] and catalytic platforms.^[36-39]

The optical properties of a material are characterized by its dielectric function $\epsilon(\lambda)$, which depends on the frequency of light. $\epsilon(\lambda)$ of metals is complex, with a real part and an imaginary part. From a given dielectric function $\epsilon(\lambda)$ of a material, the

electromagnetic properties of the material can be calculated for different geometries. As an example, we take a single metal nanosphere excited in a laser field. The polarizability α , is related to the dielectric constant as follows:

$$\alpha \propto \frac{\epsilon_i - \epsilon_0}{\epsilon_i + 2\epsilon_0} \quad 1-1$$

ϵ_i is the dielectric constant of the metal, and ϵ_0 is the dielectric constant of the medium surrounding the sphere.^[40] As described in the previous section, plasmon resonance is critical for SERS activity. To obtain large induced electric fields as well as large SPR localized around the metal surface, α should be large. When the real part of ϵ_i approaches $-2\epsilon_0$, and at the same time, the imaginary part of ϵ_i is small, this condition can be met, yielding a large induced field as well as large SPR. Thus, the SPR properties of a MNP are determined by the surrounding media and by the size, shape and identity of the MNPs. For noble coinage metals, such as gold (Au) and silver (Ag), the conditions of SPR can be met in the spectral region from the near-UV (~ 300 nm) to the near-IR (~ 900 nm). In this region, the real part of their dielectric functions are always negative and large in magnitude while the imaginary part of their dielectric functions are always positive but extend over a small range of magnitude.^[41-42] Since the significant optical properties of coinage metals are present in the visible and near-IR range, silver and gold nanoparticles become the most popular materials for extensive studies in SERS research. For some other metals, such as aluminum, platinum and palladium, the SPR properties can be achieved primarily in the UV spectral range.^[43-45]

1.3 Plasmonic Properties of Rod-shape MNPs

The properties of MNPs are strongly size- and shape- dependent. Many significant efforts have been undertaken to synthesize gold and silver nanoparticles with various geometries. These range from the simplest nanospheres, to nanocubes, to anisotropic nanorods, to nanowires and on to the irregular flower-like nanoparticles.^[46-51] Spherical MNPs are the simplest and most used MNPs for both practical and theoretical studies. Regarding the plasmon resonance property, the isotropic shape of spherical MNPs results in only one single SPR peak, as shown in **Figure 1.2A**. For example, spherical gold colloids typically have the SPR peak around 500 nm, appearing ruby red in color. This SPR peak depends upon the size of the gold nanosphere.^[52] Studies have shown that when the size of gold nanosphere increases from 9 nm to 99 nm, its SPR peak has a red shift from 517 nm to 575 nm.^[53] The rod-shape MNPs, on the other hand, have superior SPR properties over spherical MNPs. Unlike spherical MNPs, the anisotropic shape of rod-shaped MNPs splits the SPR into two modes as illustrated in **Figure 1.2B**. The SPR mode originating from the plasmon oscillation along the short axis is called the transverse SPR, while the mode originating from the plasmon oscillation along the long axis is referred to as the longitudinal SPR.^[54]

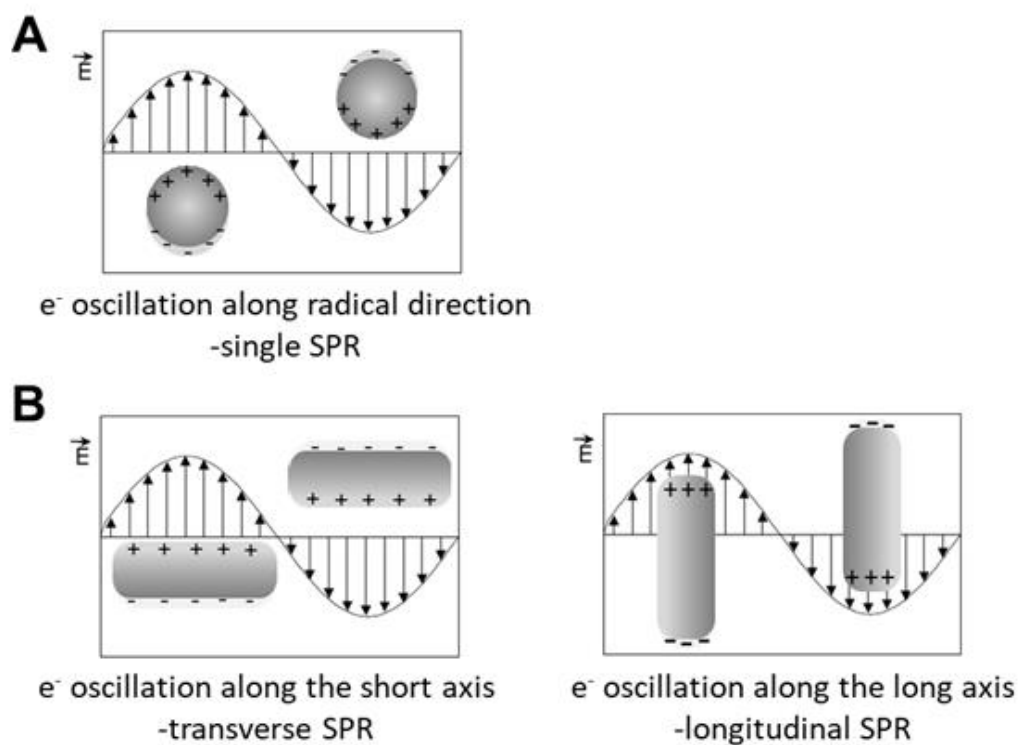


Figure 1.2 (A) Single SPR of spherical MNP. (B) Transverse and longitudinal SPR modes of rod-shape MNP.

The transverse and longitudinal SPR modes have different plasmonic resonances. In the UV-vis absorption spectrum, the transverse SPR peak shows up at higher frequency due to shorter distance of electron movement, while the longitudinal SPR peak has a red shift showing up at lower frequency due to the longer distance of electron movement.^[54] For gold nanorods (AuNRs), the transverse SPR peak usually is seen in the visible region near 520 nm, which is similar to the SPR peak of gold nanospheres. The longitudinal SPR peak, on the other hand, has a much stronger optical absorption than the transverse SPR peak. Moreover, experimental results and theoretical calculations based on Mie theory indicate that the position of the

longitudinal SPR peak of AuNR is very sensitive to size of the AuNR. The longitudinal SPR peak of AuNRs can be tuned from the visible region (~ 600 nm) to the near-IR region (over 1100 nm) based on the rod anisotropy. It is determined by the aspect ratio (length/width) rather than the absolute dimension of the AuNR.^[55-57] It was found that a one unit increase in the aspect ratio of the AuNR resulted in a 100-nm red shift in the longitudinal SPR peak. For example, when the aspect ratio of AuNR increases from 2.2 to 3.5, the longitudinal SPR peak of a AuNR red shifts from 650 nm to 760 nm, while there is negligible shift in its transverse SPR peak.^[57] The high sensitivity of the longitudinal SPR peak to the aspect ratio of the AuNRs results from the large polarizability along the long axis of the nanorods.^[54] This intriguing flexibility of tuning the longitudinal SPR peak of AuNRs makes it a very popular nanomaterial for all-purpose applications, from colorimetric sensing to photothermal therapy.

1.4 Synthesis of AuNRs and Silver Coated Gold Nanorods

Many chemical approaches have been developed to synthesize AuNRs with good shape control. These methods can be cataloged into top-down methods and bottom-up methods. Top-down methods are the methods that can pattern the bulk materials (e.g. gold films). Once the bulk materials are removed, the left-over regions of the metal are the pre-designed nanostructures. Lithographic techniques are the most popular top-down methods to fabricate nanostructures, having good control on the size, geometry of the nanostructures, and the gaps among the nanostructures. The most commonly used lithographic methods include, electron-beam lithography (EBL),^[58-59] and focused ion beam (FIB) lithography^[60-61]. There are several steps in a classical fabrication protocol of EBL. A layer of an electron sensitive polymer resist (~ 100 nm

thick) is spin coated on a flat substrate. A pre-designed pattern can be drawn on the polymer resist under the exposure of electron beams. The high-energy electrons degrade the polymer resist within the pattern areas, which is then removed by a chemical developing agent, leaving the remaining resist with the desired pattern as a mask. A gold film is deposited on the top by vacuum evaporation. Then, the gold film on top of the remaining polymer resist will be removed using the so-called “lift off” technique, resulting the gold nanostructures in a pre-designed pattern. Lithographic methods are the most appropriate candidates when precise control of the nanostructures is required. They are especially useful when fundamental SERS studies are carried out as a function of gap distances and shapes of nanostructures.^[62] Although lithographic methods are the most used techniques in the fabrication of well-patterned nanostructures with high resolution (sub-10 nm), the high cost of devices, large waste of the bulk materials, and scale-up issues are the drawbacks that limit the prevalence of these techniques.

Bottom-up methods refer to the techniques using gold atoms or precursor gold ions as a source to build up the gold nanostructures. The modern synthesis of gold nanospheres dates back to the mid-1800s,^[63] but the fabrication of colloidal AuNRs, however, has not emerged until recently. The seed-mediated wet chemical method is the most popular bottom-up synthetic approach to grow AuNRs due to its simplicity of synthesis and high quality of products. The current protocol method was first reported by Murphy *et al* in 2001^[64] and further revised by El-Sayed *et al*^[65]. After development over a decade, the protocol for seed-mediated growth of gold nanorods with high monodispersity and excellent yield has been established. The seed-mediated growth of AuNRs can be summarized in two steps: seed nucleation and growth.

Briefly, small spherical gold seeds with diameters of approximately 2-4 nm are first synthesized by the reduction of gold ions with a strong reducing agent. The gold seeds are then added to the growth solution containing more gold ions, and a slow reduction of gold ions is continued using a weak reducing agent (L-ascorbic acid, L-AA) on the surface of the gold seeds. In the presence of a surfactant (hexadecyltrimethylammonium bromide, CTAB) that acts as a stabilizer and soft template in the growth solution, growth occurs and can be induced and confined along one direction. The key factors to initiate and drive the growth of AuNRs in the second step are the surfactant CTAB and some other additives, such as silver nitrate (AgNO_3)^[65] and hydrogen chloride (HCl),^[66] which synergistically induce the anisotropic growth and also control the shape (aspect ratio) of AuNRs. The mechanism of the AgNO_3 assisted seed-mediated AuNRs growth has been extensively studied.^[56, 67-69] Analysis by FTIR and TGA, El-Sayed *et al* found that the cationic CTAB molecules, at high concentration ($> 0.2 \text{ M}$), form a bilayer structure around the AuNRs.^[70] The CTAB molecules assemble in a bilayer on the AuNRs, where the long carbon tails are packed internally via hydrophobic interaction. However, the cationic headgroups, $-(\text{CH}_3)_3\text{N}^+\text{Br}^-$, on one side bind on the surface of AuNRs, and on the other side are exposed to aqueous solution. The defined bilayer structure of CTAB on the surface of AuNRs stabilizes the AuNRs by preferentially binding to the $\{110\}$ facet of the AuNRs due to the higher surface energy.^[70-71] In addition, Ag^+ ions undergo a reduction by L-AA to Ag^0 catalyzed by the gold surface with an under potential deposition (UPD) and reduction preferentially occurring on the $\{110\}$ facet over other facets. The coverage of Ag^0 on the $\{110\}$ facet inhibits the further deposition of gold which eventually confines the growth of gold anisotropically along the $[100]$ direction,

resulting in the formation of AuNRs. Experimentally, the effects of parameters on AuNRs synthesis have been systematically investigated, including the concentration of CTAB, L-AA and AgNO₃, ratio of gold seeds/gold precursor, aging time and temperature of the gold seeds, and the addition of other additives.^[72]

Short AuNRs with longitudinal SPR peaks at ~ 600-800 nm can be easily synthesized by using CTAB and AgNO₃ as shape-assisting agents as described above. With increasing amounts of Ag⁺ in the growth solution, there is an increase in the aspect ratio of as-synthesized AuNRs. As for long AuNRs with high aspect ratios (longitudinal SPR peak > 800 nm), a co-surfactant system, CTAB/benzyltrimethylhexadecylammonium chloride (BDAC) or additive, nitric acid is needed in the growth solution for synthesis of high-aspect-ratio AuNRs.^[65-66, 73] Christopher B. Murray *et al* reported an improved protocol of AuNRs synthesis using aromatic acids (e.g. 5-bromosalicylic acid, 5-BrSA) to reduce the use of CTAB to ~0.05 M.^[47] This method could yield monodispersed AuNRs with fine-tuned longitudinal SPR peaks ranging from 653 nm to 1246 nm. The mechanism has been demonstrated to involve small molecular aromatic additives that could insert into the CTAB bilayer where negatively charged -COO⁻ groups of the aromatic additives are located and could further stabilize the CTAB molecules on the gold surface promoting a tight control on the growth of AuNRs. There are many other methods with respect to AuNRs synthesis, such as photochemical reduction,^[74-75] and electrochemical methods,^[76-77] that will not be further discussed in this dissertation.

Rather than the simple, well-established, wet chemical methods for synthesis of AuNRs, the synthesis of silver nanorods takes extraordinary efforts, which often results in unsatisfying monodispersity and yield.^[78-80] Thus, synthesis of silver coated

gold nanorods (Ag/AuNRs) starting with the as-prepared AuNRs is of great interest for practical use. Silver is a more SERS-active material than gold in the visible region due to dielectric differences of the metals.^[81] Coating a silver layer onto the AuNRs offers an easy way to obtain bimetallic nanoparticles carrying the desired SERS activity of silver. Control of the thickness of the silver coating on the AuNRs provides another parameter to tune the plasmonic properties of Ag/AuNRs. The Ag/AuNRs experience blue shifts when thicker silver shells are deposited onto the contour of the AuNRs.^[82-83] Nikolai G. Khlebtsov *et al* reported that the self-assembled Ag/AuNRs SERS substrate with a 2-nm thin silver coating layer increased the analytical enhancement factor (AEF) from 10^3 for a self-assembled AuNRs substrate to 2.5×10^4 , using rhodamine 6G (Rh6G) as a probe molecule. The AEF, in this case, was a function of silver coating thickness when using laser excitation at 633 nm.^[84] Other studies also suggest Ag-Au bimetallic nanoparticles with Ag as the coating layer show superior SERS sensitivity over the monometallic nanoparticles or Au-Ag bimetallic nanoparticles with Au as the outer shell. This is thought to originate from the charge transfer between the two metallic components.^[85-86] The coating of silver onto AuNRs has been reported in the literatures.^[87-93] Typically, Ag^+ ions can be mildly reduced by L-AA and deposited onto AuNRs under alkaline conditions.

1.5 SERS Substrates

The design and fabrication of substrates is one of the major topics in the SERS community. Poor signal reproducibility is the main obstacle that prevents SERS techniques from becoming a routine analytical method. As we discussed, the objective of fabricating SERS substrates is all about the generation of aggregated MNPs carrying hot spots. A quality SERS substrate should meet three requirements: (1)

highly sensitive, (2) highly reproducible, and (3) long-term stability. Good SERS substrates, ideally, would have high selectivity but also be able to conduct multiplex detection for different analytes. Furthermore, portability and cost-efficiency are also factors when judging a good SERS substrate.

In traditional SERS analysis, metallic nanoparticle colloids are the most common SERS substrates due to ease of preparation. Typically, strong SERS signals occur once the aggregation of MNPs is induced by the analyte molecules or by adding some inorganic salts (e.g. NaCl).^[94-95] The aggregation of MNPs can be easily achieved by evaporating the colloidal solution containing MNPs mixed with analyte molecules, or spreading onto a surface containing analytes.^[96] However, one of the main shortcomings for these solution-based colloidal SERS substrates is that, the aggregation of the MNPs is difficult to control and is not stable with time leading to poor reproducibility of the SERS signals. Thus, a better fabrication strategy is solid SERS substrates. Many techniques have been developed to fabricate a solid SERS substrate, such as vapor deposition,^[97] electrochemical deposition,^[98] Langmuir-Blodgett deposition,^[99] and nanosphere lithography.^[100] By using these methods, substrates having well controlled or patterned SERS-active nanostructures can be fabricated. This results in solid SERS substrates with better reproducibility compared to colloidal SERS substrates. The solid substrates are easier to use with respect to storage and transportation than those solution-based SERS substrates. However, most of the solid SERS substrates mentioned above are only two-dimensional (2D) structures with limited sites for analyte binding. Expensive devices and sophisticated fabrication are still required for some of the more advanced techniques for preparing nanostructured substrates.

Recently, novel types of SERS substrates consisting of MNPs and polymers have been developed. The cost efficiency makes these MNPs/polymer composite SERS substrates a viable option for commercialization and practical use. Different types of MNPs and polymer combination have recently been reviewed by Rahim and Kim for plasmonic sensing applications.^[101] A few types of paper-based SERS substrates consisting of MNPs and polymer fibers have emerged. Ian M. White *et al* have developed SERS substrates by using inkjet printing to dope silver nanoparticles into a cellulose-based paper with a detection limit as low as 95 fg of Rh6G, 9 ng of heroin and 15 ng of cocaine.^[102] This paper-based substrate has already been commercialized as P-SERSTM Substrate and applied to detections of drugs, bacteria, and pesticide.^[103] Ultrasensitive detection, however, is mostly limited to qualification rather than quantification, due to the lack of good control of the nanoparticle aggregation within the substrate. Electrospinning of polymer and MNPs solution blends can fabricate fibrous substrates with MNPs embedded in the fibers.^[104] By increasing the concentration of MNPs in the solution blends, MNPs inside the fibers increase and can be well aligned along the fiber direction if the MNPs are anisotropic (e.g. AuNRs). This method also has its own limitations. To blend MNPs dispersed in aqueous solution, the polymers (e.g. polyvinyl alcohol) used are usually water soluble. If no post cross-linking is involved, the SERS substrate cannot be used to detect analytes dissolved in water. In addition, the fabrication of nanoparticles contained within polymer fibers, to a large degree, limits the size, rate and amount of analyte molecules that can penetrate inside the fiber and reach the surface of the MNPs. Another type of MNPs/polymer SERS substrate involves an *in-situ* formation of silver nanoparticles from the reduction of precursor Ag⁺ ions adsorbed on a negative charged

modified fiber surface.^[105] However, the fabrication strategy strongly relies on the design and the use of specific types of MNPs (silver nanoparticles) and polymer modification (agar/polyacrylonitrile). Thus, it would be advantageous to develop a universal strategy of SERS substrate fabrication that can decorate the MNPs on the surface of polymer fibers and do so with the controlled aggregation of MNPs.

In previous work in the Rabolt/Chase group, a strategy of SERS substrate fabrication using AuNRs and electrospun polycaprolactone (PCL) fibrous mat was developed.^[106] Polyelectrolyte layer-by-layer (LbL) deposition onto the fiber surface resulted in a uniform, positively charged fiber surface, which facilitated electrostatic binding of the poly (sodium 4-styrenesulfonate) (PSS) coated AuNRs. Thus, AuNRs were immobilized onto the fiber surface electrostatically, forming a dense and uniform monolayer, and this uniformity of immobilization guaranteed a high reproducibility for SERS signals. Using 4-mercaptopyridine (4-Mpy) as a Raman probe molecule to investigate the reproducibility, the intensity variation of SERS signals obtained from random spots on a substrate was shown to be less than 5%. This SERS substrate was not limited to chemisorbed analytes, but can also detect physisorbed analytes, such as Rh6G through electrostatic interactions, indicating a wide range of various analytes amenable to this detection method. Benefiting from the large surface area provided by the 3D fibrous architecture for AuNRs immobilization, this SERS substrate had a 6-fold increase in sensitivity compared to 2D AuNRs-PCL films and an enhancement factor of at least 10^4 in Raman intensity. Furthermore, this novel SERS substrate has already been applied to a selective and quantitative detection of trace amounts of heavy metal ions such as mercury ions (Hg^{2+}) and copper ions (Cu^{2+}).^[107]

1.6 Dissertation Overview

The successful development of a fabrication strategy for 3D AuNRs-PCL SERS substrates has led to an expanded research interest. To further explore the universal nature of this fabrication strategy, different types of SERS-active MNPs and other electrospun polymeric mats are of interest. Comparison of SERS substrates fabricated with different SERS-active MNPs would also provide perspectives for better design of future substrates. The application of SERS substrates as environmental sensors for arsenic detection is an important goal of this research.

In this chapter, SERS techniques and plasmonic properties of SERS-active MNPs have been briefly introduced. The synthesis of AuNRs, particularly using the seed-mediated growth method, has been discussed in detail. Different fabrication methods of SERS substrates have been reviewed. Due to the preparation simplicity and exceptional plasmonic properties of Ag/AuNRs as elucidated in this chapter, we will use Ag/AuNRs as the SERS-active MNPs for the SERS substrate fabrication using the same fabrication strategy in **Chapter 2** to obtain Ag/AuNRs-PCL SERS substrate. Another polymer, Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHx) will be used to produce electrospun fibrous mats to further reinforce the universal nature of our fabrication strategy. In **Chapter 3**, the SERS performance of a Ag/AuNRs-PCL SERS substrate will be investigated in detail in terms of sensitivity, reproducibility and stability by using 4-Mpy as probe molecules. The SERS performance of the Ag/AuNRs-PCL SERS substrate fabricated with Ag/AuNRs having different silver coating thicknesses will be compared with each other as well as to AuNRs-PCL SERS substrate. A specific application of Ag/AuNRs-PCL SERS substrate will be explored in **Chapter 4**, where various arsenic species will be quantitatively determined. The unusual SERS behaviors of *p*-arsanilic acid will be

fully discussed. The capability of Ag/AuNRs-PCL SERS to serve as *in-situ* monitoring platform will be demonstrated on arsenate desorption and roxarsone reduction. Conclusions and proposed future research studies will be summarized in **Chapter 5**.

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Chapter 2

FABRICATION OF 3D SERS SUBSTRATES COMPRISED OF AG/AUNRS AND ELECTROSPUN PCL FIBERS

2.1 Introduction

As introduced in **Chapter 1**, fabrication of a SERS substrate that has high sensitivity and good reproducibility, is the main challenge facing the SERS community. Poor reproducibility has hindered this powerful technique and kept it from being a routine analytical method for decades. Previously, our group has developed a 3D SERS substrate fabricated using AuNRs and PCL electrospun fibers that show high Raman signal reproducibility.^[1] AuNRs carrying negative charges were immobilized onto the PCL fiber surface modified with positive charges using an electrostatic interaction. As a result, a dense and uniform monolayer of AuNRs decorated the PCL fiber surface. Additionally, the large surface area provided by the 3D architecture of the PCL fibrous mat enabled a higher loading of SERS-active nanoparticles. This gave the 3D fibrous SERS substrate a 6-fold increase in SERS signal compared to a 2D planar substrate fabricated using AuNRs bound to the surface of a PCL film following a similar fabrication method. To prove this fabrication strategy offers a generalized and universal methodology for SERS substrate assembly, we further extended this fabrication strategy by using other materials, Ag/AuNRs, PCL electrospun fibrous mats, and PHBHx electrospun fibrous mat, as SERS-active MNPs and as a 3D scaffold respectively.

AuNRs were first synthesized following the classical, seed-mediated, wet chemistry protocol developed by Murphy^[2] and refined by El-Sayed^[3]. Silver salt ions were then reduced to form a silver coated layer around the AuNRs resulting in Ag/AuNRs.^[4] By changing the amount of Ag⁺ ions, different coating thicknesses of the silver shell were obtained. To introduce negative charges on the Ag/AuNRs, a layer with a polyanion, PSS, was deposited onto the Ag/AuNRs. In order to introduce positive charges onto the surface of electrospun PCL and PHBHx fibrous mats, PSS and a polycation, poly(diallyldimethylammonium chloride) (PDADMAC), were deposited by LbL deposition to modify the polymer fiber surfaces. Using PDADMAC as the outermost layer, this provided positive charges dispersed onto the fiber surface of the mat.

To obtain the Ag/AuNRs-electrospun fiber composite SERS substrate, the modified fibrous mat was immersed into PSS/Ag/AuNRs solution for a specified period of time resulting in immobilization of the Ag/AuNRs from the solution phase onto the solid polymer mat. Other competing processes, in addition to electrostatic attraction, exist during the immobilization process and will be discussed in detail.

2.2 Materials and Methods

2.2.1 Materials

Hexadecyltrimethylammonium bromide (CTAB, $\geq 98.0\%$, $M_w = 364.45$ g/mol) and hexadecyltrimethylammonium chloride (CTAC, $\geq 98.0\%$, $M_w = 320.00$ g/mol) were purchased from TCI, Inc. Gold chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, $\geq 99.9\%$ trace metal basis, $M_w = 393.83$ g/mol), sodium borohydride (NaBH_4 , $\geq 98.0\%$, $M_w = 37.83$ g/mol), L-ascorbic acid (L-AA, $\geq 99.0\%$, crystalline, $M_w = 176.12$ g/mol), silver

nitrate (AgNO_3 , $\geq 99.0\%$, $M_w = 169.87$ g/mol), sodium chloride (NaCl , $\geq 99.5\%$, $M_w = 58.44$ g/mol), (3-aminopropyl)triethoxysilane, (3-APTS, $\geq 98.0\%$, $M_w = 221.37$ g/mol), 4-mercaptopyridine (4-Mpy, 95%, $M_w = 111.16$ g/mol), polycaprolactone (PCL, average $M_n \sim 80,000$), poly(diallyldimethylammonium chloride) solution (PDADMAC, average $M_w \sim 200,000$ -350,000, 20 wt% in H_2O), poly(sodium 4-styrenesulfonate) (PSS, average $M_w \sim 700,000$), chloroform (CHCl_3 , $\geq 99.5\%$, $M_w = 119.38$ g/mol, $d_{20\text{ }^\circ\text{C}} = 1.49$ g/cm³), *N,N*-Dimethylformamide (DMF, anhydrous, 99.8%, $M_w = 73.09$ g/mol, $d_{20\text{ }^\circ\text{C}} = 0.95$ g/cm³), dichloromethane (DCM, anhydrous, $\geq 99.8\%$, $M_w = 84.93$ g/mol, $d_{20\text{ }^\circ\text{C}} = 1.33$ g/cm³), acetic acid (AA, $\geq 99.7\%$, $M_w = 60.05$ g/mol, $d_{20\text{ }^\circ\text{C}} = 1.05$ g/cm³) were all purchased from Sigma-Aldrich. Formic acid (FA, $M_w = 46.03$ g/mol, $d_{20\text{ }^\circ\text{C}} = 1.22$ g/cm³) was purchased from Fisher Scientific. Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHx, with 3.9 mol% HHx content, $M_w \sim 843,000$, PDI = 2.2) was donated by the Procter & Gamble Co. All the chemicals were used as received with no pretreatment. Ultrapure deionized water (DI water, 18.2 $M\Omega\cdot\text{cm}$, Milli-Q Plus System, MilliPore Co.) was used as the solvent to prepare aqueous solutions throughout these experiments.

2.2.2 Fabrication of Ag/AuNRs-PCL SERS substrate

2.2.2.1 Synthesis of PSS coated Ag/AuNRs (PSS/Ag/AuNRs)

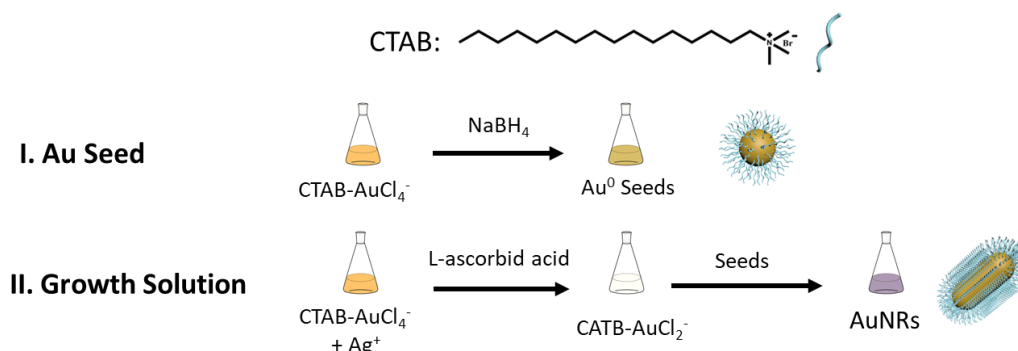


Figure 2.1 Synthesis of AuNRs using seed-mediated wet chemistry protocol.

To grow AuNRs, small spherical gold seeds with diameters of approximately 2-4 nm were first prepared. Then 0.25 mL of 10 mM HAuCl_4 solution was added to 9.75 mL of 0.1 M CTAB solution under vigorous stirring. When 0.60 mL of 10 mM freshly prepared and ice-cold NaBH_4 solution was added, the color of the solution immediately turned to light brown indicating the formation of gold nanoparticles. After continued vigorous stirring for 2 minutes, the seed solution was stored in a water bath at 28 °C for 20 minutes to allow the release of unreacted reducing agent, NaBH_4 , before further use. A growth solution was prepared by adding 4.0 mL of 10 mM HAuCl_4 , 2.0 mL of 5 mM AgNO_3 , 0.64 mL of 0.1 M L-AA respectively into 95 mL of 0.1 M CTAB solution with thorough mixing. The solution color changed from bright yellow to darker yellow and then to colorless, where Au^{3+} ions were first complexed and stabilized with surfactant CTAB and then mildly reduced to Au^+ ions by the weak reducing agent, L-AA. When 0.2 mL of seed solution was added to the growth

solution and vigorously stirred for 30 seconds, the solution changed color from colorless to purplish black within 10 minutes. This solution was stored and incubated in the water bath at 28 °C overnight with no perturbation for the complete growth of AuNRs. This synthesis of AuNRs is summarized in **Figure 2.1** shown above.

III. Silver Coating:

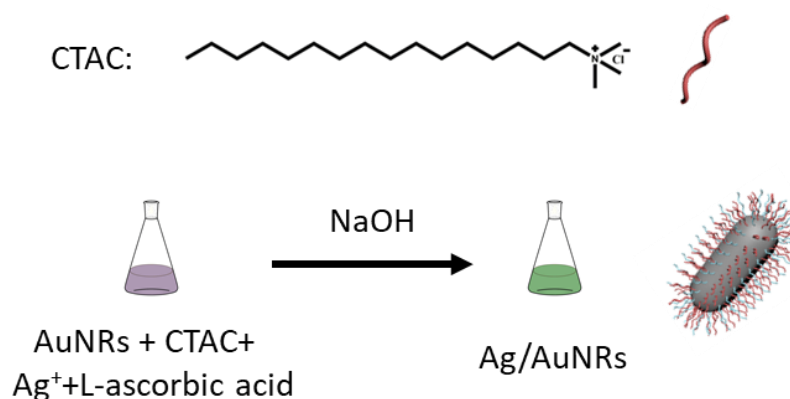


Figure 2.2 Synthesis of Ag/AuNRs.

To synthesize Ag/AuNRs, 12 mL of as-synthesized AuNRs was centrifuged at 12,000 rpm for 15 minutes. After carefully removing the supernatant containing excess CTAB, the precipitate of AuNRs was dispersed into 60 mL of 80 mM CTAC and stirred for 15 minutes at 28 °C. To the AuNRs/CTAC solution, 1.2 mL of 1 mM AgNO₃ solution, 1.2 mL of 0.1 M L-AA, and 2.4 mL of NaOH were added in sequence. The solution turned from purplish black to light brown yellow indicating the reduction of Ag⁺ ions to Ag⁰, after the NaOH was added to trigger the reduction. The reaction was completed within 10 minutes. By increasing the amount of 1 mM AgNO₃

added in the solution, Ag/AuNRs with a different silver coating thickness were prepared. This process was characterized by the solution color change from purplish black (pure AuNRs solution) to bright green and then dark green when 2.4 mL and 3.6 mL of 1mM AgNO₃ were added respectively. The scheme of silver coating on the AuNRs is summarized in **Figure 2.2**.

The 60 mL of as-prepared Ag/AuNRs solution was centrifuged at 12,000 rpm for 15 minutes. After the supernatant containing excess CTAB and CTAC was removed, the remaining precipitate of Ag/AuNRs was dispersed into 6 mL of 2 mg/mL PSS stock solution prepared in 1 mM NaCl solution. The solution was stirred to facilitate the PSS adsorption for 1.5 hours. Afterwards, the solution was centrifuged at 12,000 rpm for 10 minutes twice to remove the excess PSS and NaCl, and re-dispersed into DI water before future immobilization. The final PSS/Ag/AuNRs solution was at a concentration ($\sim 3 \times 10^{14}$ particles/L) that was twice that of the original AuNRs concentration.

The SERS-active MNPs (AuNRs, Ag/AuNRs, PSS/Ag/AuNRs) were characterized using UV-vis spectroscopy (Agilent Cary 60 UV-vis spectrometer) and transmission electron microscopy (TEM, JEOL JEM-3010). To prepare samples for UV-vis extinction measurements, typically, 1 mL of SERS-active solution was centrifuged at 12,000 rpm for 15 minutes and re-dispersed into 1 mL of DI water. This process was repeated and transferred to an Eppendorf UVette (transparent from 230 nm to 1600 nm). To characterize the morphology of the SERS-active MNPs, TEM samples were prepared. After 1 mL of SERS-active solution was centrifuged at 12,000 rpm for 15 mins, the process was repeated and then re-dispersed into 50 μ L DI water. A drop of 5 μ L concentrated solution was then transferred onto a carbon coated copper

grid (CF-200-Cu, 200 mesh, Electron Microscopy Sciences) and dried under ambient conditions. The TEM measurements were conducted under bright field mode at a 300-kV accelerating voltage. To confirm the presence of a PSS coating on the Ag/AuNRs, 60 μ L of MNPs solution was diluted into 10 mL of DI water and transferred into a disposable, folded capillary cell (DTS1070, Malvern Panalytical). Then, zeta potentials of AuNRs, Ag/AuNRs and PSS/Ag/AuNRs were measured using a Zetasizer Nano ZS (Malvern Panalytical). The refractive index of CTAB, CTAC, and PSS were set as 1.34, 1.38 and 1.39 respectively.

2.2.2.2 Electrospinning of PCL and PHBHx fibrous mats

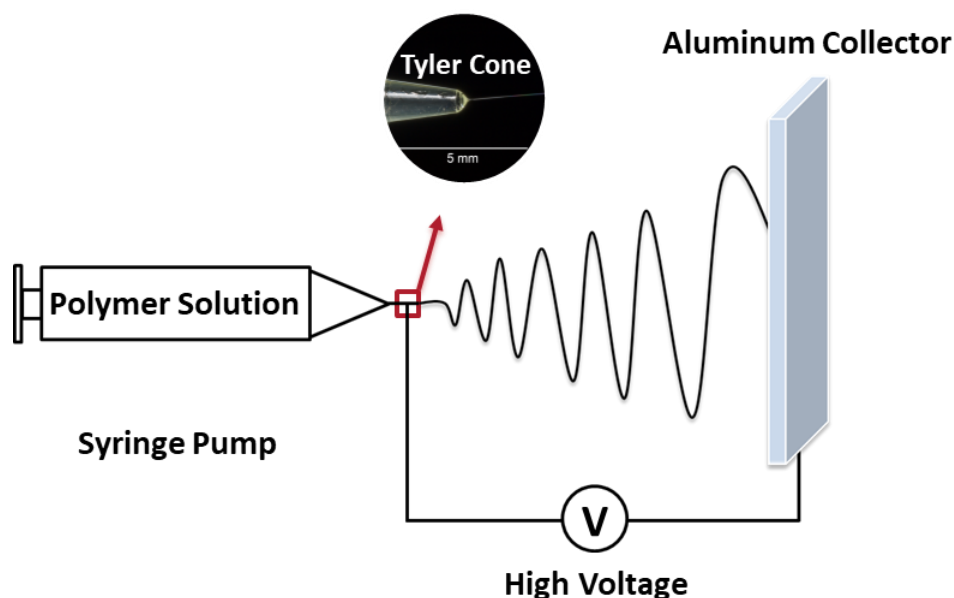


Figure 2.3 Schematic illustration of the electrospinning process.

A PCL fibrous mat was produced using electrospinning. Electrospinning is a simple but versatile technique, which produces micron to nanometer diameter fibers. The basic principle of electrospinning is shown in **Figure 2.3** and described in literatures.^[5-8] Briefly, a polymer solution (or melt) is supplied at a given flow rate to a syringe needle tip. When a high voltage is applied between the needle tip and a conductive collector, the solution (or melt) droplet surface at the needle tip is charged and distorts to a conical shape, known as Taylor cone. Once the electrostatic repulsive forces overcome the surface tension of the drop, a single filament thread is ejected, and undergoes stretching and whipping as it moves toward the collector. In these trajectories, solvent evaporates, and fibers are formed before being collected at the target. In this study, a 15 wt% PCL in CHCl_3 solution was loaded into a 3-mL BD disposable syringe (Dia. 8.66 mm) equipped with a 21-gauge (0.71 mm, BSTEANTM) blunt needle tip and supplied to the tip by a syringe pump (NE-300 model, New Era Pump System, Inc.) at 0.5 mL/h. A voltage difference of 15 kV (CZE1000R, SPELLMAN Power Supply) was applied between the needle tip and a flat aluminum foil collector with the distance set to be 25 cm. A thin, uniform and bead-free fibrous mat of PCL was produced after 20-minutes of electrospinning. Using CHCl_3 /DMF mixtures with different ratios to prepare the PCL solutions, fibrous mats with various fiber diameters were obtained. PHBHx fibrous mats were obtained using the same electrospinning setup and similar experimental conditions.

The morphology of PCL and PHBHx fibrous mats were characterized using scanning electron microscopy (SEM, Auriga 60 CrossBeam). All SEM images were taken with the InLens mode with an accelerating voltage of 3 kV. The surface area of

a pristine PCL fibrous mat was estimated using a Brunauer-Emmett-Teller (BET) physisorption apparatus (Micromeritics ASAP 2000 Plus).

2.2.2.3 Surface modification of PCL fibrous mats

To modify the PCL fibrous mats and introduce positive charges, LbL deposition was used to coat polyelectrolytes onto a PCL mat, and this is schematically described in **Figure 2.4**.

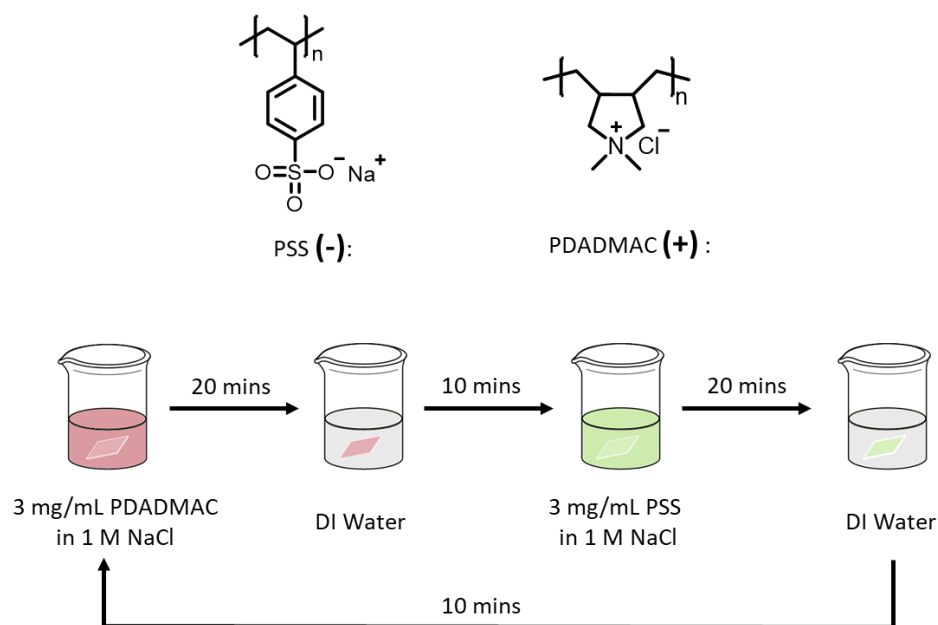


Figure 2.4 PDADMAC/PSS polyelectrolytes LbL deposition onto a PCL fibrous mat.

Specifically, a pristine PCL mat was immersed into 3 mg/mL PDADMAC in 1 M NaCl solution for 1 hour and then immersed into DI water for a 20-minute washing. Afterwards, the mat was alternately immersed into 3 mg/mL PSS in 1 M NaCl solution and 3 mg/mL PDADMAC in 1 M NaCl solution for 20 minutes. 10-minute

washing steps were used in between immersions until the desired number of layers of polyelectrolytes were deposited leaving PDADMAC as the outermost layer. The modified PCL mat was immersed into DI water overnight with two changes of fresh water to wash out the excess polyelectrolytes and NaCl before further immobilization. The LbL modified PCL fibrous mat is denoted as (PDADMAC/PSS)_N-PCL, where N is the number of deposition cycles of the LbL deposition of the PDADMAC/PSS pair, thus 2N is the number of deposited polyelectrolytes layers counted in total. The change in morphology of the fiber surface in the PCL mat during the LbL deposition was characterized by SEM following similar procedures described in the previous section.

2.2.2.4 PSS/Ag/AuNRs immobilization on a PCL fibrous mat

The surface modified PCL fibrous mat was cut into a rectangular shape (typically 1.0 cm×4.0 cm) and immersed into 6 mL of the PSS/Ag/AuNRs solution for the immobilization of Ag/AuNRs onto the PCL fiber surface. After the desired period of time, the mat was washed twice with DI water and left to dry under ambient conditions. The fabricated SERS substrates were characterized by UV-vis and SEM.

2.3 Results and Discussion

2.3.1 Characterization of SERS-active MNPs

Though the recipes for AuNRs synthesis have been developed and refined for a decade, many parameters (concentrations, surfactants, time, etc.) during synthesis require careful control. The importance of the surfactant CTAB to the quality of synthesized AuNRs is well known. Even CTAB chemicals from the same brand and same production number but different batches could result in a large variation in

AuNRs quality.^[9] Trace amounts of the impurity (usually iodide) left in the CTAB during industrial production can prevent the formation of AuNRs.^[10] Therefore, before the synthesis of AuNRs, CTAB from different vendors, production numbers, and lot numbers (Lot#) were tested by synthesizing the AuNRs using the same gold seed solution (prepared using CTAB_1) under identical experimental conditions. UV-vis spectra were recorded to evaluate the qualities of AuNRs and these are shown in **Figure 2.5**.

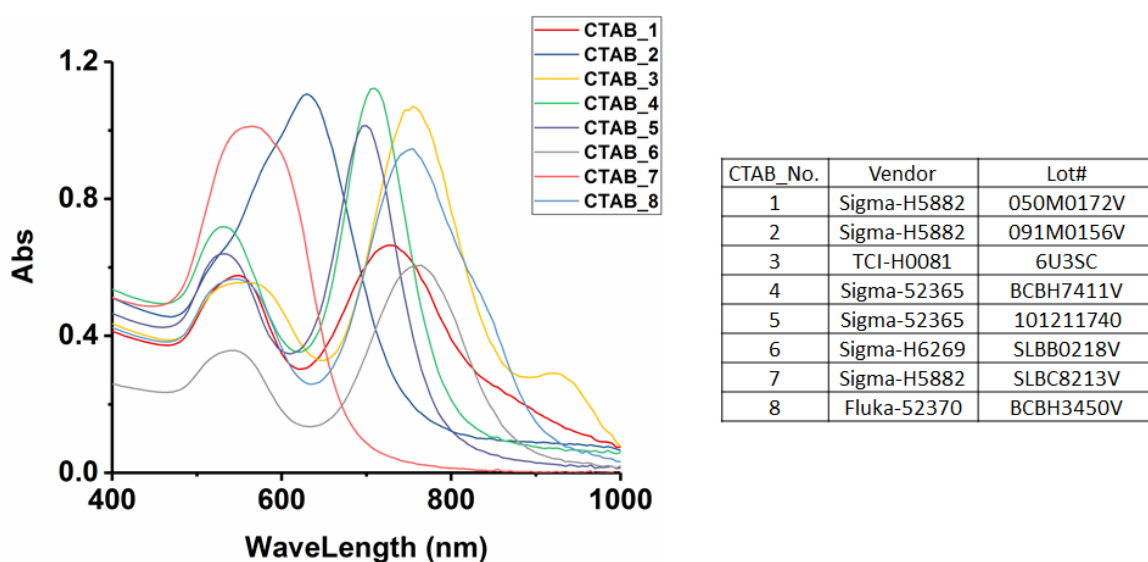


Figure 2.5 UV-vis of AuNRs synthesized using different CTAB chemicals (left), and the table listing CTAB chemicals purchased from various suppliers with production numbers (right).

The UV-vis spectra showed that CTAB from different sources did have significant effects on the synthesis of AuNRs. For CTAB surfactants from Sigma-H5882, the colloidal solutions contained few AuNRs as shown by a single SPR peak (CTAB_1 and CTAB_2) or produced a low yield of AuNRs as suggested by the

comparable intensities of transverse and longitudinal SPR peaks (CTAB_7). CTAB from Sigma-H6269 (CTAB_6) also resulted in a low AuNR yield. For CTAB surfactants that generated AuNRs with acceptable qualities (CTAB_3, CTAB_4, CTAB_5 and CTAB_8), the longitudinal SPR peaks and yields varied among the group. A small peak around 930 nm in the UV-vis spectrum of CTAB_3 (TCI-H0081) could originate from small aggregated clusters of AuNRs generated during synthesis. In this study, surfactants (CTAB and CTAC) produced from TCI Inc were used throughout all the MNP synthesis.

The UV-vis absorption spectra of AuNRs, Ag/AuNRs and PSS/Ag/AuNRs aqueous solutions are shown in **Figure 2.6A**. The longitudinal SPR peak of AuNRs is typically centered at approximately 770 nm and the transverse SPR peak is found near 515 nm. After silver coating, the longitudinal peak of Ag/AuNRs has a blue shift to 705 nm, while the transverse SPR peak of Ag/AuNRs only exhibits a very slight blue shift. A further PSS coating had no change on the position of the longitudinal peak, which indicated that no aggregation occurred during the coating process. Zeta potentials for AuNRs, Ag/AuNRs and PSS/Ag/AuNRs were measured as +31.1 mV, +49.6 mV and +39.8 mV, respectively. The inversion of the zeta potential before and after PSS coating demonstrated successful PSS adsorption onto the Ag/AuNRs.

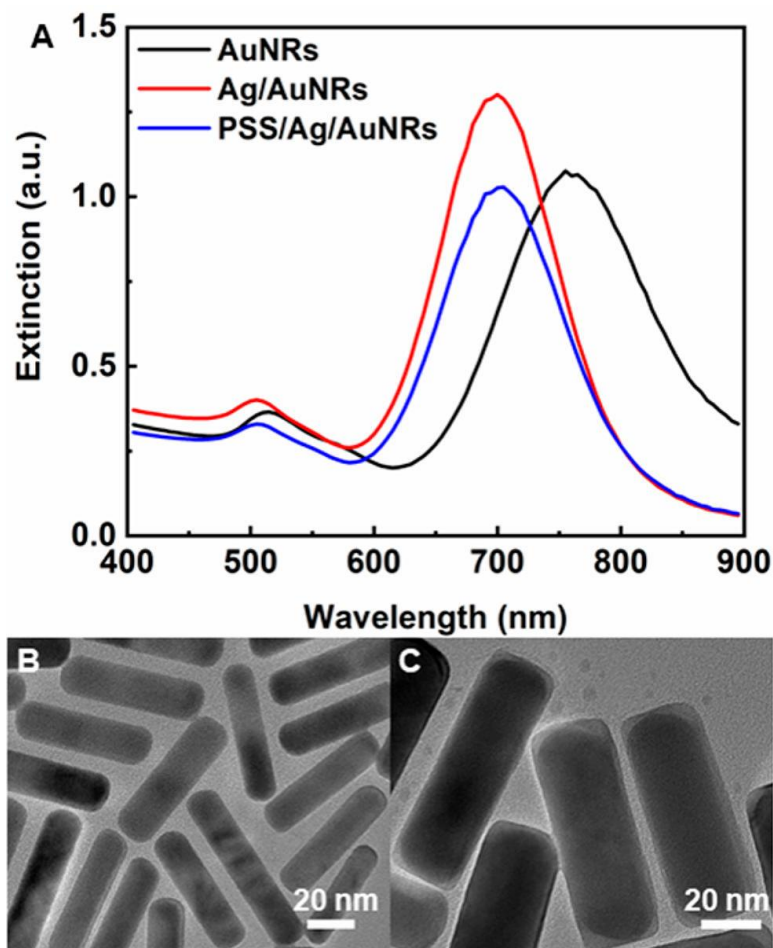


Figure 2.6 (A) UV-vis spectra of AuNRs, Ag/AuNRs and PSS/Ag/AuNRs. (B, C) TEM images of AuNRs and Ag/AuNRs. The scale bar is 20 nm.

The TEM images of AuNRs and Ag/AuNRs are shown in **Figure 2.6B-C**. The dimensions of the as-synthesized AuNRs were estimated to be 58.5 nm in length and 16.7 nm in width with an average aspect ratio of 3.5. After a silver coating, a thin silver layer (approximately 3 nm) contoured the AuNRs. In the AuNRs synthesis, the center of the longitudinal SPR peak of as-synthesized AuNRs typically ranged from 800 nm to 760 nm with the corresponding aspect ratio varying from 3.8 to 3.5, due to

slight variations (i.e. temperature) during synthesis. TEM images of AuNRs and Ag/AuNRs with larger scale are shown in **Figure 2.7**.

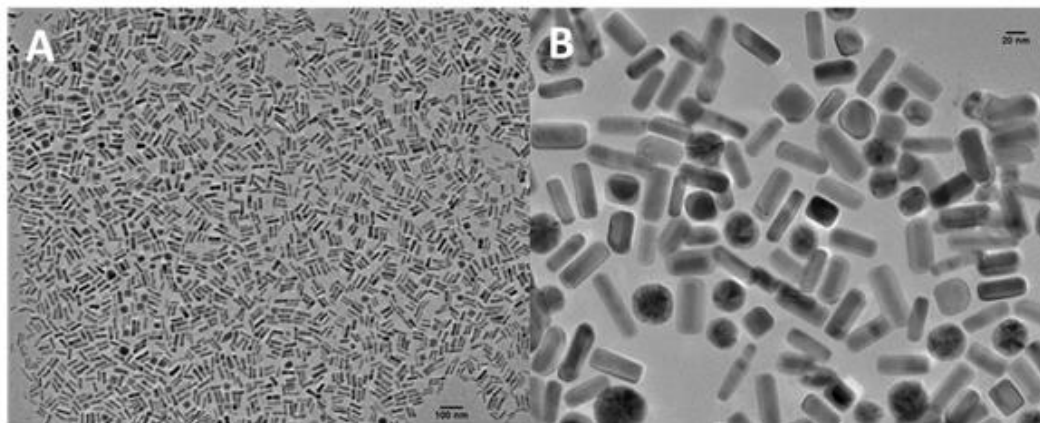


Figure 2.7 TEM images of (A) AuNRs and (B) Ag/AuNRs (Au:Ag = 1.42). The scale bar is 100 nm in (A) and 20 nm in (B).

AgNO_3 was the source of Ag^+ ions for coating Ag onto the AuNRs in this study. With an increasing amount of AgNO_3 added, the color of the Ag/AuNRs solution turns from light brown to bright green (digital photo inserted in **Figure 2.8**). The longitudinal SPR peak of Ag/AuNRs gradually blue shifts to approximately 650 nm as the UV-vis spectrum shows in **Figure 2.8**. A thicker silver coating layer is deposited onto the surface of the AuNRs as the TEM images show in **Figure 2.9**.

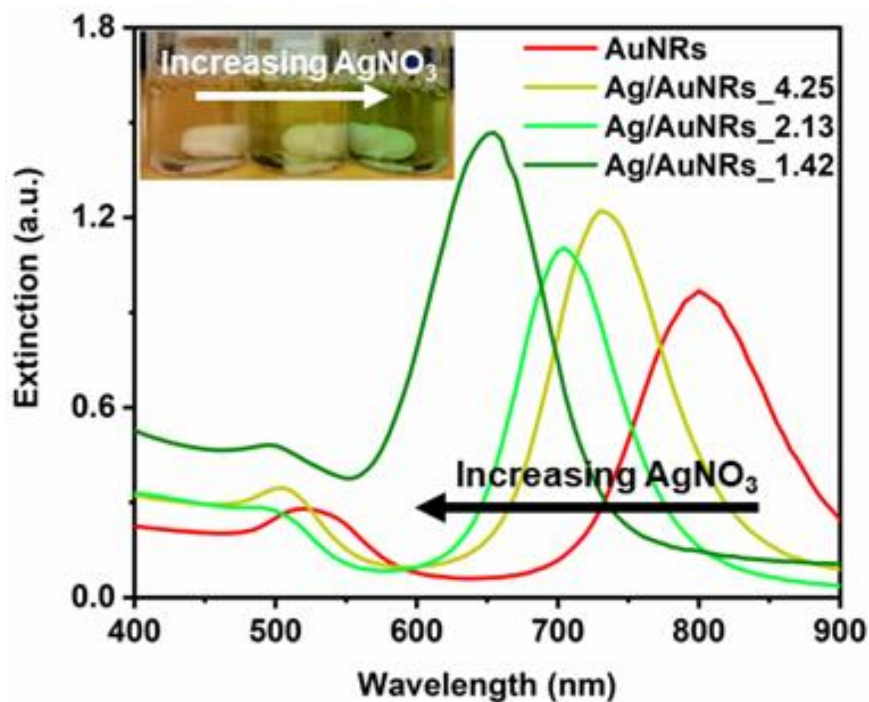


Figure 2.8 UV-vis spectra, digital photo (the inset) of Ag/AuNRs, with a molar ratio of Au to Ag of 4.25, 2.13, and 1.42, respectively.

The thickness of the silver coating layer increases from about 3 nm to over 7 nm as the amount of AgNO_3 is increased. In **Figure 2.9** (right), the AuNR core is observed to distort its cylindrical shape in 7 nm silver coated AuNR, becoming thicker at both ends, and thinner in the middle, like a dog bone. In addition, the silver coating seems to thicken more than lengthen. However, since the same recipe is used in the synthesis of silver coatings, the crystalline properties of the silver layers should be identical. Thus, variations in shapes and sizes of the MNPs could be ascribed to the experimental variations (i.e. concentration) from batch to batch during MNPs synthesis. Overall, the molar ratio of silver to gold in the Ag/AuNRs calculated from

the estimated dimensions in the TEM results corresponds to the amounts of reagents, particularly AgNO_3 and HAuCl_4 used in the synthesis.

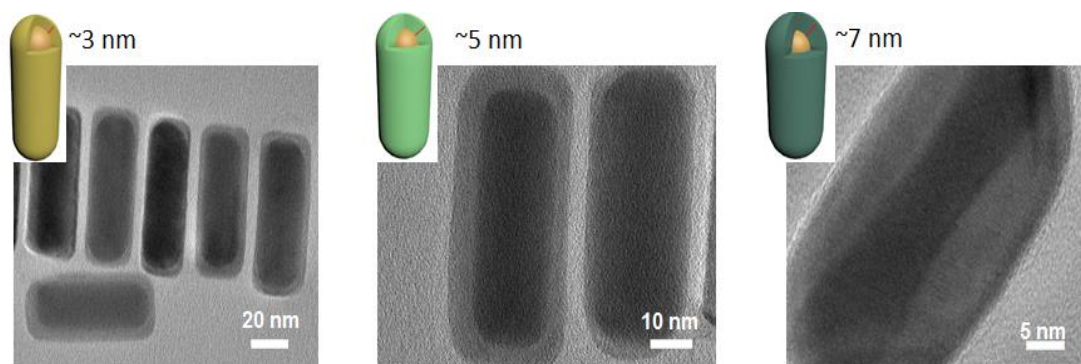


Figure 2.9 TEM images of Ag/AuNRs (from left to right), with a molar ratio of Au to Ag of 4.25, 2.13, and 1.42, respectively.

A few other silver coating methods have been tried before the protocol described above was developed. For example, Ag^+ ions can be reduced to Ag^0 by L-AA at elevated temperature (60 °C) instead of adding NaOH as the reaction trigger.^[11] Other surfactants, such as polyvinylpyrrolidone (PVP),^[12-14] and CTAB^[15-16] were used as a stabilizer/template during silver coating. None of these recipes showed appropriate control over the coating growth of silver onto AuNRs. By using CTAC as a surfactant, not only can CTA^+ adsorb onto the MNPs as a stabilizer, but also Cl^- can complex with Ag^+ ions to form AgCl nanoparticles. These were suspended in the solution as a silver source, which in large degree slows down the reduction of Ag^+ ions to Ag^0 , yielding a uniform and well controlled silver coating contoured around the AuNRs.^[4, 17]

2.3.2 Characterization of electrospun polymeric fibers

Using DMC as solvent, a PCL fiber with an approximate diameter of 4 μm was obtained. In addition, using a solvent mixture of CHCl_3 /DMF for electrospinning, PCL fibrous mats with various fiber diameters ranging from several micrometers to hundreds of nanometers were obtained. (**Figure 2.10**)

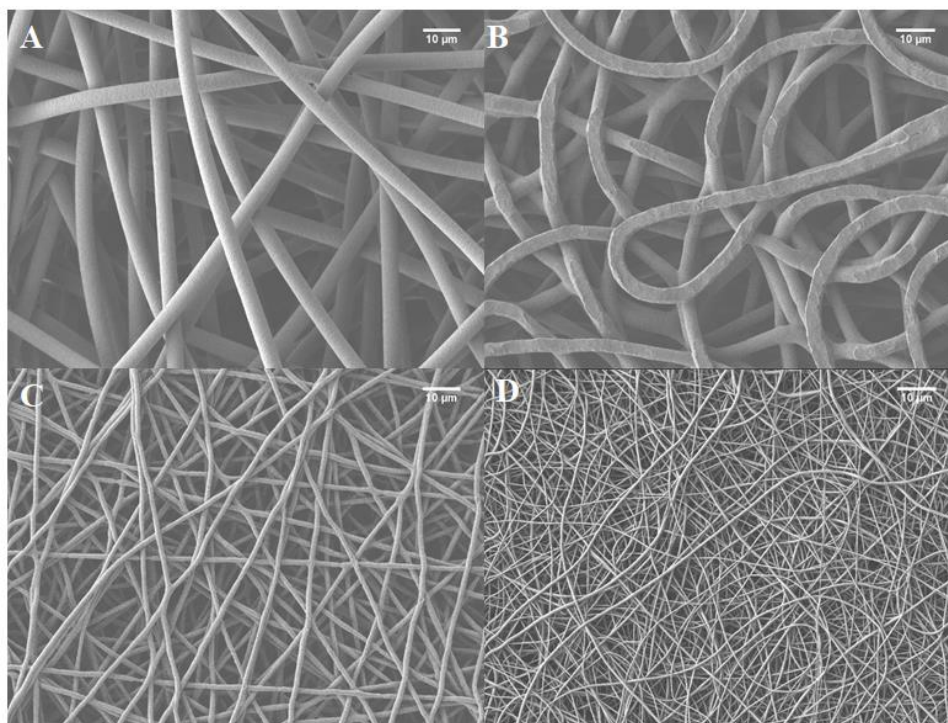


Figure 2.10 Electrospun fibers of 15 wt% PCL in different solvents: (A) DCM, (B) CHCl_3 , (C) CHCl_3 :DMF (v:v) = 5:1, (D) CHCl_3 :DMF (v:v) = 2:1. The scale bar is 10 μm .

The higher the DMF content in the solvent mixture, the thinner the PCL fibers. DMC and CHCl_3 are solvents that evaporate quickly, which makes the liquid jet dry faster, increasing the viscosity of the liquid jet. The viscoelastic force counteracts the

electrostatic repulsive force, leading to a thicker fiber.^[18] Since DMF has a higher dielectric constant compared to DMC and CHCl_3 , it has higher resistance to an electric field. Hence, in the electric field, a liquid jet of DMF accumulates more charges resulting in a high electrostatic repulsive force yielding much thinner fibers.^[19]

When using lower concentrations of PCL in CHCl_3 /DMF with a larger DMF content for electrospinning, PCL fibers with diameters of approximately 200 nm were formed. Occasionally, bundled fibers are found to form ridges in the electrospun mat. This could be attributed to the high boiling point of DMF in a high humidity atmosphere when electrospinning. In this situation, the solvent would not completely evaporate before the fiber reaches the collector. The continued evaporation of DMF on the mat generates a hydrodynamic drag force which drives the formation of ridges in the PCL fiber bundles. When using acetic acid (AA) and formic acid (FA) as co-solvents for electrospinning, PCL fibers with diameters less than 100 nm containing beads, can be formed. (**Figure 2.11**) It could be the difference in dielectric constants between the polymer and solvent leading to a difference in distribution of charges in the polymer and solvent, which could result in the formation of beads.^[18]

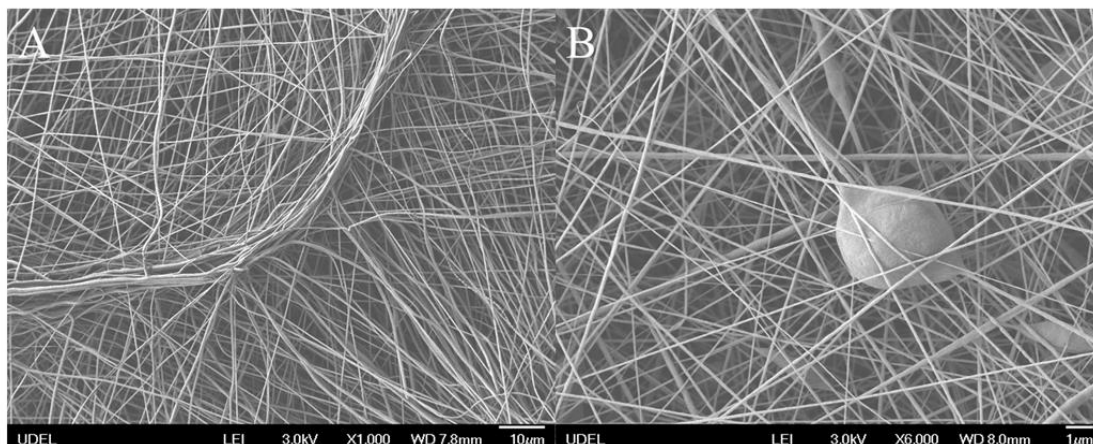


Figure 2.11 (A) 11 wt% PCL electrospinning in $\text{CHCl}_3\text{:DMF}$ (v:v)=2:1, (B) 6 wt% PCL electrospinning in AA:FA (v:v) = 3:1. The scale bars are 10 μm and 1 μm , respectively.

2.3.3 Characterization of LbL deposition on PCL fibrous mat

To immobilize PSS/Ag/AuNRs onto a PCL fiber surface via electrostatic interactions, positive charges were introduced onto the fiber surfaces of a PCL mat by LbL deposition of polyelectrolytes. Among various methods of surface treatment like plasma treatment^[20] and chemical modification^[21], polyelectrolytes LbL assembly has the best feasibility and the fewest limitations on the shape and material of the substrates. The concept of LbL self-assembly was first developed by Decher *et al* in 1997.^[22] A typical polyelectrolytes LbL self-assembly process involves alternatively immersing substrates into polycation and polyanion solutions with washing steps in between. By repeating the dipping cycles, a thin and fuzzy assembled film of polyelectrolyte multilayers will be coated onto the substrate.

Since the assembly of polyelectrolytes is driven by the force of electrostatic attraction, the properties and structures of self-assembled polyelectrolyte multilayers can be significantly affected by the charge density of the molecules and the ionic

strength of the solutions.^[23] In this study, a strong polyelectrolyte pair, PSS and PDADMAC, were selected to conduct the LbL deposition. Both components of the pair are completely dissociated in aqueous solution. Thus, the deposition of a PSS/PDADMAC pair is less dependent on pH than a weak polyelectrolyte pair, such as poly (acrylic acid) (PAA) and poly (allylamine hydrochloride) (PAH), where the ionization of PAA and PAH is pH-dependent.^[24-25] The ionic strength of the solution is critical to the thickness and morphology of LbL assemblies. Studies have shown that PSS and PDADMAC can be readily assembled and kept stable in NaCl solution at concentrations below 2 M. A higher ionic strength leads to the dissociation and mass loss of PSS/PDADMAC self-assembled films.^[26]

The PSS/PDADMAC LbL deposition onto PCL fiber surfaces has been well studied in our previous investigation where changes of surface chemistry, morphology, and water contact angle have been elucidated in detail.^[1] The PDADMAC/PSS deposition can be readily carried out on the PCL mats with various diameters, ranging from several microns down to hundreds of nanometers. To confirm the success of PDADMAC/PSS deposition, SEM images were taken at different stages of as polyelectrolytes deposition. (**Figure 2.12**). As the number of LbL deposition cycles increases, the surface morphology of PCL fibers (approximately 1 μm in diameter) changes from rough (pristine PCL mat, **Figure 2.12A**) to smooth (7 layers and 9 layers of polyelectrolyte deposition in **Figure 2.12E** and **Figure 2.12F**, respectively). This is indicative of an increased amount of polyelectrolyte deposited onto the PCL fiber surface. Energy dispersive X-ray spectroscopy (XEDS) of a 15-layer deposition on PCL is shown in **Figure 2.13B**. The existence of sulfur from PSS is confirmed at 2.3 keV, the K_{α} peak of nitrogen featured in PDADMAC is around

0.392 keV, which was probably covered by the K_{α} peak of elemental carbon. In our case, a seven-layer deposition of polyelectrolyte is sufficient for the following experiments. So, the modified PCL fibrous mat is designated as (PDADMAC/PSS)_{3.5}-PCL denoting three and a half cycles of PDADMAC/PSS deposited onto PCL fibers with 7th PDADMAC layer as the outermost coating layer.

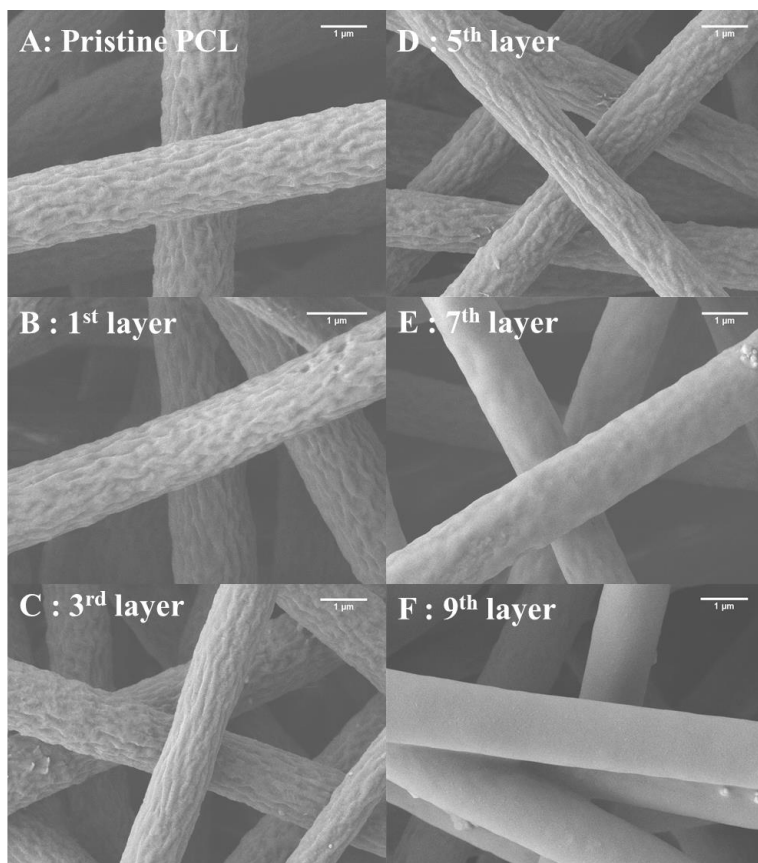


Figure 2.12 (A-F) SEM images of PDADMAC/PSS LbL deposition onto PCL fibers at pristine PCL, 1st, 3rd, 5th, 7th and 9th layer respectively. The scale bar is 1 μm .

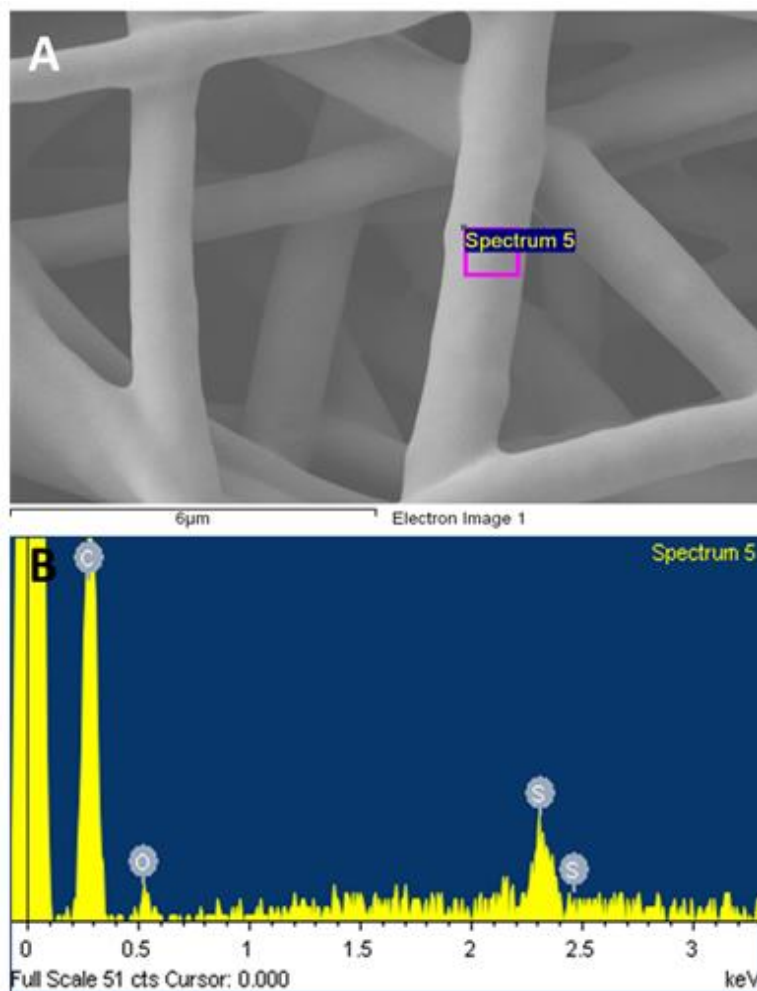


Figure 2.13 (A) SEM image of a (PDADMAC/PSS)_{7.5}-PCL mat. (B) SEM-XEDS spectrum of the square area labeled in (A).

It is worth mentioning that we have observed increased difficulty in carrying out polyelectrolytes LbL deposition onto PCL mats with thin fibers where the surface tension of the polyelectrolytes solution droplets at those denser and smaller pores restricts the polyelectrolytes solution from penetrating inside the PCL mats. The reduction in penetration of polyelectrolytes resulted in a less uniform surface modification of the fibrous mat. The thinner the fibers are, the more the mat behaves

like a planar substrate, where polyelectrolytes are likely to form a film on the outside layer of the mat as shown in **Figure 2.14**. In addition, it takes a longer time to wash the NaCl off the mat containing smaller fiber diameters. Cleaning the salt off the mesh is critical for the following immobilization step since the inorganic salt can induce severe aggregation and precipitation of the MNPs, leading to a nonuniform decoration of MNPs on the fiber surface. Considering the time efficiency and uniformity of the substrate fabrication, a PCL fibrous mat with fiber diameters of approximately 3 μm was typically used in this study unless explicitly stated.

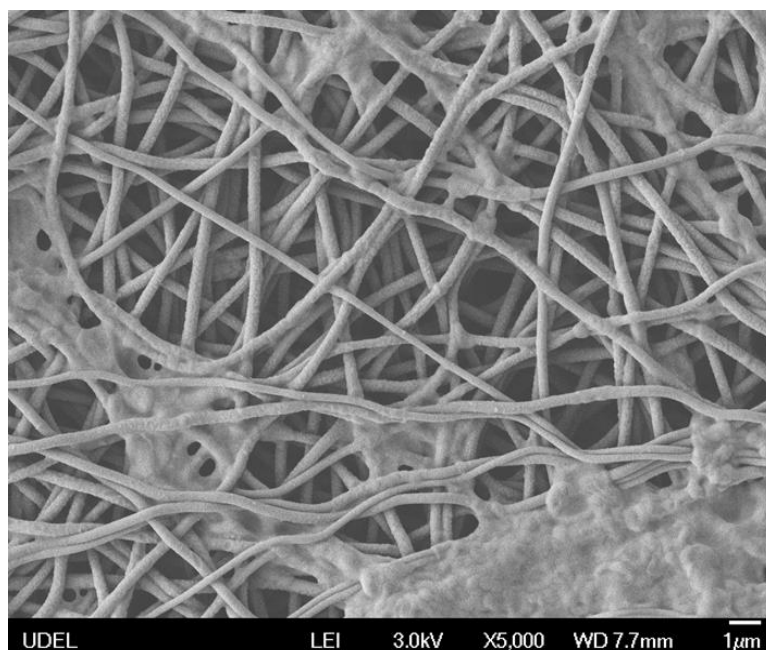


Figure 2.14 Two layers of PSS/PDADMAC LbL deposition on a PCL mat with a smaller fiber diameter (~ 300 nm).

2.3.4 Characterization of the SERS substrate

After 24 hours of immersion in the PSS/Ag/AuNR solution, the (PDADMAC/PSS)_{3.5}-PCL mat turned from white (**Figure 2.15A**) to dark green (**Figure 2.15B**) in color, and the UV-vis absorbance of the PSS/Ag/AuNRs solution decreased to zero, as shown in **Figure 2.15C**.

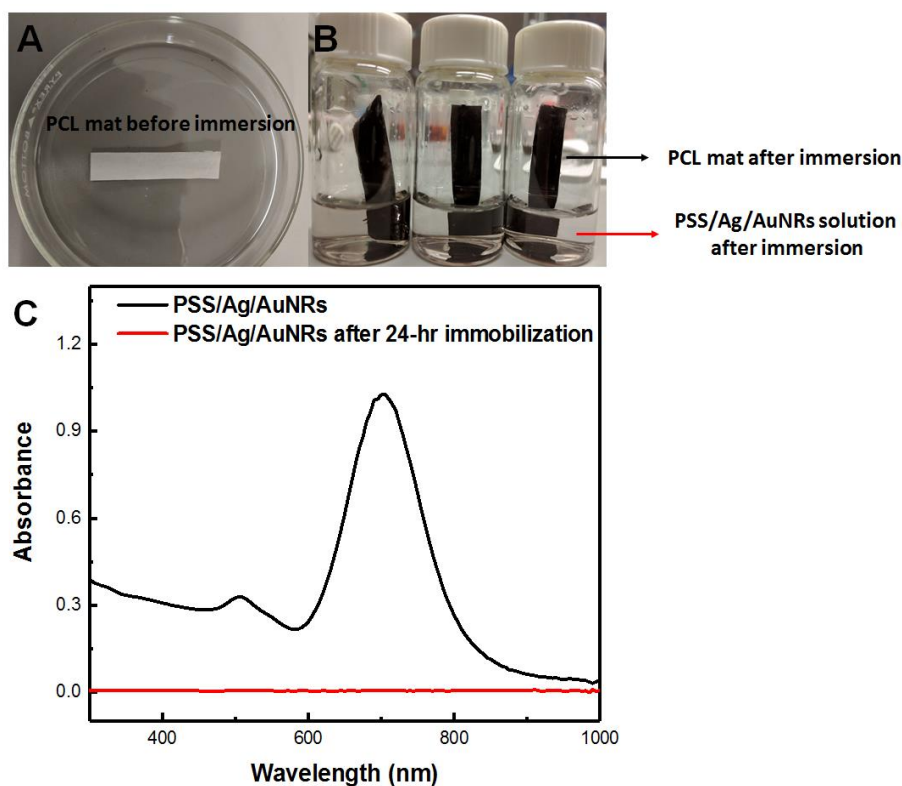


Figure 2.15 (A, B) Digital photos of a (PDADMAC/PSS)_{3.5}-PCL mat before and after a 24-hour immobilization, respectively. (C) UV-vis spectra of PSS/Ag/AuNRs before and after a 24-hour immobilization.

The lack of UV-vis absorbance of the PSS/Ag/AuNRs solution after the immobilization indicated that an effective immobilization process had occurred with a

complete transfer of PSS/Ag/AuNRs from the colloidal solution to the solid PCL mat. Under moderate shaking (reciprocating shaker, CMS EQUATHERM) that facilitates the penetration and accessibility of the PSS/Ag/AuNRs to the inner fiber surfaces, the immobilization process can be completed within hours, dramatically increasing the fabrication efficiency. A densely packed monolayer assembly of PSS/Ag/AuNRs immobilized on the PCL fiber surface is shown in the SEM images in **Figure 2.16**.

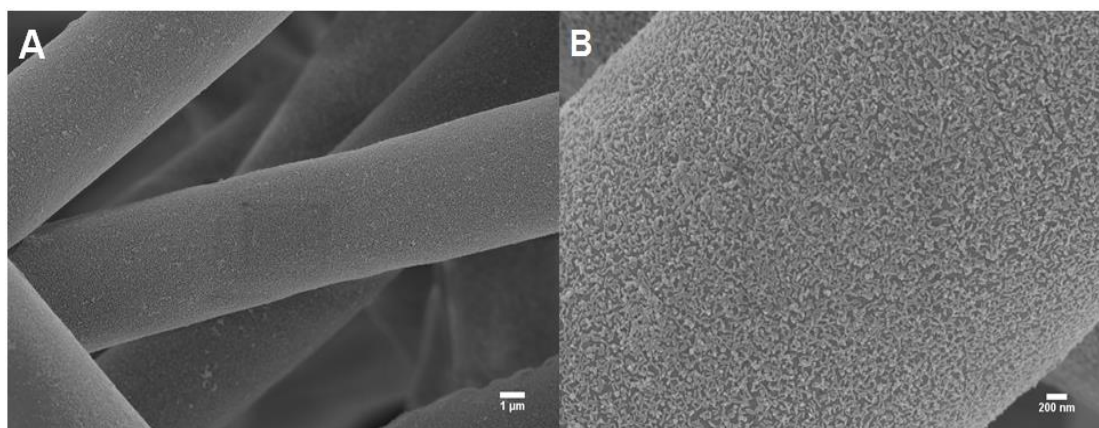


Figure 2.16 SEM images of PSS/Ag/AuNR decorated PCL fibers. The scale bars are 1 μm and 200 nm in (A) and (B), respectively.

2.3.5 Discussion of the immobilization process

Direct immobilization of Ag/AuNRs without a PSS coating was first attempted, since the as-synthesized Ag/AuNRs are stabilized with surfactants (CTAB and CTAC) and the zeta potential was above +40 mV. This theoretically suggested that a stable colloidal solution existed and was ready for immobilization onto a negatively charged surface.^[27] Uniform decoration of Ag/AuNRs onto PCL fibrous mat, was not obtained consistently, and Ag/AuNRs were found to be partially aggregated and precipitated during the immobilization process. Since the surfactant molecules adsorbed on the

MNPs form bilayers, similar to liposomes,^[28] and undergo a dynamic adsorption/desorption equilibrium, we concluded that direct immobilization of MNPs was not robust. When MNPs without PSS coating directly interact with the negatively charged fiber surface, the surfactants on MNPs could slip off from the surface of MNPs and adsorb onto the negatively charged fiber surface, leading to resultant desorption of the surfactants and aggregation of the MNPs in the solution. Therefore, a PSS coating on the MNPs is preferred to further protect and stabilize the MNPs during immobilization.

The immobilization of PSS/Ag/AuNRs on the fiber surface in solution is a subtle process. Parameters, including centrifugation cycles, speed, and time need to be engineered and optimized to obtain densely packed coverage of MNPs on the fiber surface. Multiple competing processes exist during immobilization. One is the competition between the free surfactant molecules left in solution after centrifugation and surfactants deposited on the MNPs. Once the free surfactants, which have better mobility than those on the MNPs, adsorb onto the fiber surface, the surface area of fibers occupied by free surfactants is no longer available for immobilization due to the electrostatic repulsion. Consequently, this results in more sparse coverage of MNPs. Another competition is between the affinity of MNPs among themselves and their affinity with the PCL mat. It should be noted that there exists a range of zeta potential for colloids that make the MNPs associate with each other, but neither necessarily leads to a considerable precipitation.^[29] The affinity of colloids with themselves rather than with a substrate can result in a very low density of MNPs on the fiber surface.

Ionic strength of the PSS solution is also critical for effective PSS coating. A high ionic strength can directly induce aggregation of MNPs, a moderate ionic

strength, however, is needed for an effective PSS coating. The negatively charged side groups on PSS molecule are repulsive and force the molecular chain to extend itself, while rod-shaped MNPs are highly curved. Thus, for a specific molecular weight of PSS, a certain ionic strength is needed to partially screen the electrostatic charges on the chain and help to relax the chain conformation needed for a complete wrap around the contour of the particle. The higher molecular weight of a polyelectrolyte, the higher ionic strength is needed for deposition onto the curved MNPs.^[30]

2.4 Conclusion

Immobilization of MNPs on a solid substrate is a very interesting topic which should be a focus of further in-depth fundamental studies. However, due to the centrifugation involved during the MNPs fabrication and separation, it is difficult to measure the free surfactants left over in the MNPs solution after supernatant removal, which increases the difficulty to quantify the surfactants and inter-MNPs interactions in solution. We found that adjusting the centrifugation speed and time for the four cycles during PSS/Ag/AuNR preparation is an easier way to tune the properties of MNPs in the solution, and the zeta-potential can be monitored after each cycle of centrifugation.

In this chapter, the SERS substrate comprised of Ag/AuNRs and PCL electrospun fibers have been successfully fabricated. Ag/AuNRs and PCL fibrous mats were modified by polyelectrolytes to introduce the negative charges and positive charges needed for electrostatic attraction. With this electrostatic interaction as the driving force, Ag/AuNRs were immobilized on the PCL fiber surfaces. A uniform and dense monolayer of Ag/AuNRs was deposited onto the PCL fibrous mat. In the next chapter, the SERS performance of this Ag/AuNRs-PCL substrate will be evaluated.

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Chapter 3

EVALUATION OF AG/AUNRS-PCL SERS SUBSTRATES

3.1 Overview

This chapter presents the evaluation of Ag/AuNRs-PCL SERS substrates fabricated in **Chapter 2**. To evaluate the SERS performance of this substrate, the sensitivity, reproducibility and long-term stability have been investigated in detail using 4-Mpy as a probe molecule. The non-specific nature of the immobilization strategy used in this study, has been demonstrated by the fabrication using another polymeric material, PHBHx. The quantification of the enhancement of the SERS signals is calculated by introducing the concept of enhancement factor (EF). Finally, the SERS performance of substrates fabricated with different MNPs, from pure AuNRs to Ag/AuNRs with different silver coating thicknesses, have been compared. Several factors that could affect the SERS performance have been discussed to explain the variation in the SERS performance among different substrates.

3.2 Materials and Materials

3.2.1 Materials

4-mercaptopyridine (4-Mpy, 95%, $M_w = 111.16$ g/mol) was purchased from Sigma-Aldrich. Ethanol (EtOH, 200 proof) was purchased from Fisher Scientific. All chemicals were used as received.

3.2.2 SERS measurements

To evaluate the SERS sensitivity, a SERS substrate was cut into identical small pieces and immersed into 9 mL of 4-Mpy/EtOH solutions with various concentrations of 4-Mpy, ranging from 10^{-4} M to 10^{-8} M for 2 hours. Afterwards, the SERS substrates were washed with EtOH and dried under ambient conditions prior to Raman measurements. To evaluate the reproducibility of the substrates, a SERS substrate immersed into 10^{-5} M 4-Mpy/EtOH solution was tested. SERS measurements were taken on the randomly chosen spots on the substrate.

To estimate the EF of the Ag/AuNRs-PCL SERS substrate, a normal Raman spectrum of a 0.25 M 4-Mpy/H₂O solution sealed in 5 mm O.D. NMR tube (Fisher Scientific) was also measured. Laser power was 81.3 mW with a 30-second exposure.

The SERS measurements were performed using a Kaiser Holospec 1.8 Raman Spectrograph equipped with a TE cooled Andor CCD and an ONDAX 785-nm laser and probe head. The Raman instrument uses an 800 lines/mm transmission holographic grating, and the numerical aperture is 0.9 with a magnification of 65 $\times$. The size of the laser spot was estimated to be approximately 10 μ m. Spectra were recorded using 785 nm excitation with approximately 1 mW laser power and a 30-second exposure. Each spectrum is an average of three measurements on randomly selected sampling spots of a substrate unless otherwise stated.

3.3 Results and Discussion

3.3.1 SERS sensitivity, reproducibility, and stability

The SERS performance of a Ag/AuNRs-PCL SERS substrate was tested by using 4-Mpy, which chemically bonds to the silver coating surface via the formation of a Ag-S bond. The SERS spectra of 4-Mpy at various concentrations are shown in

Figure 3.1A. The demonstration of the sensitivity of this substrate is shown by the detection of 4-Mpy at a concentration as low as 10^{-8} M. The major peaks of 4-Mpy, such as a ring breathing mode at 1008 cm^{-1} and $\nu(\text{ring breathing})/\nu(\text{CS})$ at 1098 cm^{-1} are still detectable. An expanded view of the spectra at low concentration (10^{-7} M and 10^{-8} M) is shown in **Figure 3.1B** along with the Raman spectra of a modified PCL mat, a bare SERS substrate, PSS powder, surfactants CTAB and CTAC, respectively. When the concentration of 4-Mpy is lowered, the background of the bare SERS substrate appeared. Compared to the Raman spectra of the chemicals involved during the fabrication of the SERS substrate, the background signals arise primarily from the PSS coating and surfactants surrounding the Ag/AuNRs, the background present on the PCL mat is negligible. We will show in the following sections and chapters that specific peaks from these background signals can be selected as a reference for background subtraction to consistently yield neater spectra and better quantification for trace level detection due to the high signal reproducibility.

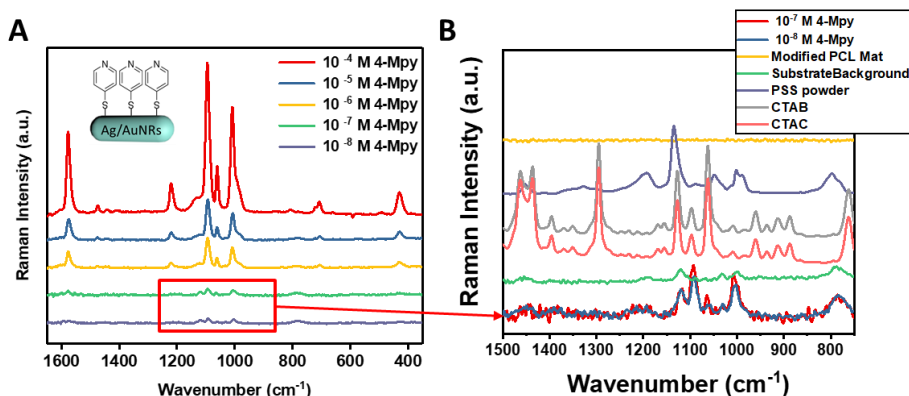


Figure 3.1 (A) SERS spectra of 4-Mpy at concentrations from 10^{-4} M to 10^{-8} M. Inset: structure of 4-Mpy bound to Ag/AuNR. (B) Magnified SERS spectra of 4-Mpy at concentration of 10^{-7} M, 10^{-8} M and bare SERS substrate along with Raman spectra of (PDADMAC/PSS)_{3.5}-PCL mat, PSS, CTAB and CTAC powders, respectively.

The reproducibility of the substrate was tested by randomly selecting spots on the substrate and measuring Raman spectra. The SERS spectra measured are shown in **Figure 3.2A**, with the average and variations of peak intensities of 4-Mpy shown in **Figure 3.2B** and summarized in **Table 3.1** along with the peak assignments.^[1-2] The relative standard deviations (RSD) for all the major peaks of 4-Mpy are less than 7%, which demonstrates excellent reproducibility within the same SERS substrate. This results from uniform monolayer coverage through Ag/AuNR immobilization and, in turn, reinforces the fact that uniformity can be achieved by this immobilization strategy.

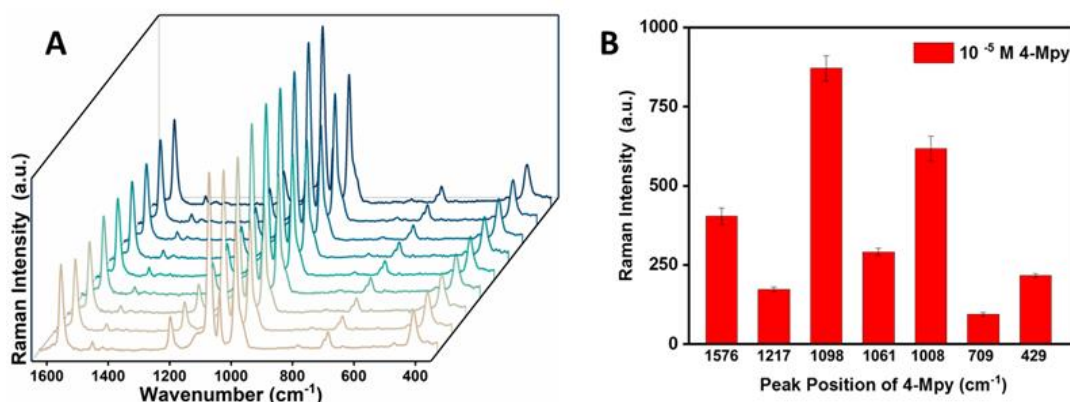


Figure 3.2 (A) SERS spectra of 10^{-5} M 4-Mpy at 9 randomly selected spots on the Ag/AuNRs-PCL SERS substrate. (B) Peak intensities and variations of 10^{-5} M 4-Mpy measured by randomly selecting spots on the Ag/AuNRs-PCL SERS substrate.

In addition to sensitivity and reproducibility, the shelf life of Ag/AuNRs-PCL substrates is of concern, since it is well-known that silver is easily oxidized. Oxidation of the silver surface can severely impact the SERS performance of the substrate. We tested the SERS performance of a Ag/AuNRs-PCL substrate from the same batch after aging under ambient conditions for 3 months. As shown in **Figure 3.3**, the SERS signals obtained on a 3-month-aged substrate are comparable in sensitivity to measurements on a freshly prepared substrate. The long shelf life of the Ag/AuNRs-PCL SERS substrate could be attributed to the protection provided by the surfactants and PSS coating, which hinder the transport of oxygen to the surface of Ag/AuNRs thus inhibiting oxidation.

Table 3.1 Peak assignments, averaged intensities, intensity variations of 4-Mpy major peaks.

Peak (cm ⁻¹)	Symmetry	Assignment ^[1-2]	Peak Intensity	RSD (%)
1576	8B ₂	ν (CC) with deprotonated nitrogen	404 $\pm$ 27	6.6
1217	12A ₁	β (CH)/ δ (NH)	173 $\pm$ 8	4.6
1098	12A ₁	ν (ring breathing)/ ν (CS)	871 $\pm$ 40	4.6
1061	18A ₁	β (CH)	292 $\pm$ 11	3.8
1008	1A ₁	ring breathing	618 $\pm$ 39	6.4
709	6A ₁	β (CC)/ ν (CS)	95 $\pm$ 5	5.6
429	7A ₁	δ (CS)/ γ (CCC)	217 $\pm$ 6	2.7

ν , stretching; β , bending; δ , in-plane deformation; γ , out-of-plane deformation; RSD, relative standard deviation.

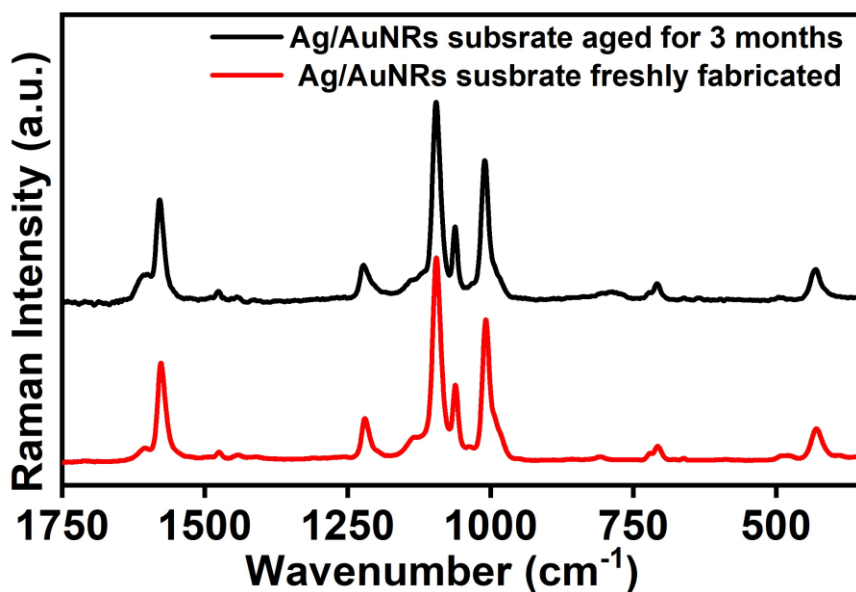


Figure 3.3 SERS spectra of 4-Mpy at a concentration of 10^{-5} M on the same batch of Ag/AuNRs-PCL substrate when freshly prepared and aged for 3 months.

3.3.2 SERS substrate fabricated with PHBHx

To reinforce the universal nature of the nonspecific, electrostatic immobilization strategy, the fabrication protocol has been readily implemented on a polymeric scaffold made of PHBHx. PHBHx is one of the microbial synthesized polyesters in the polyhydroxyalkanoate (PHA) family. These bio-based plastics are one of the most promising bio-based materials due to their good biodegradability and biocompatibility. Various properties of PHAs have been reported, as well as an extensive set of applications ranging from rigid plastics to tissue scaffolds.^[3-5] Polyhydroxybutyrate (PHB) homopolymer has a very high crystallinity ranging up to 75-85% which makes PHB a stiff and brittle material. When 3-hydroxyhexanoate (3HHx) is randomly polymerized with PHB, PHBHx copolymer is obtained. The molecular structure of PHBHx is shown in **Figure 3.4**. Because of the presence of propyl side chains due to 3HHx, the crystalline structure of PHB can be interrupted in the copolymer to different degrees.^[6] By tuning the side chain length on 3HHx and its composition in the copolymer, PHBHx polymers with various thermal and mechanical properties can be produced.

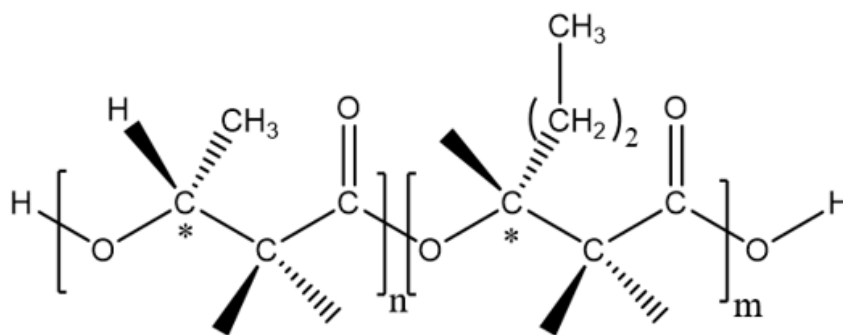


Figure 3.4 Molecular structure of PHBHx.

Using PHBHx with 3.9 mol% HHx content, electrospun mats of PHBHx fibers with fiber diameters of approximately 2 μm were fabricated following the same electrospinning procedure as used in the previously discussed PCL mats. The immobilization strategy of Ag/AuNRs developed for the PCL fiber surface was readily implemented onto a PHBHx fiber surface with minor tuning of experimental parameters. As seen in **Figure 3.5A**, a very dense monolayer of Ag/AuNRs decorates the PHBHx fiber surface, and a robust SERS signal response of the substrate is demonstrated on 10^{-5} M 4-Mpy, shown in **Figure 3.5B**.

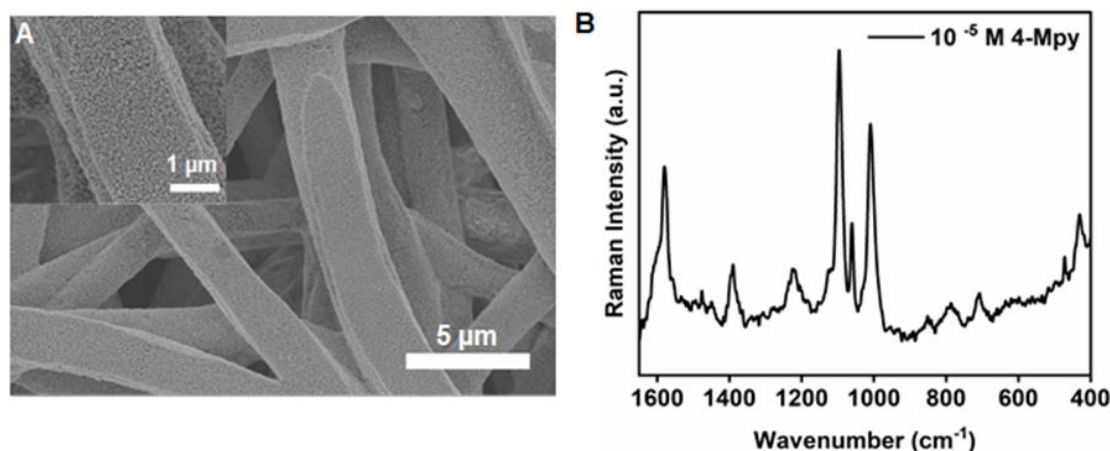


Figure 3.5 (A) SEM images of Ag/AuNR decorated PHBHx electrospun fibrous mat. (B) SERS spectrum of 4-Mpy at a concentration of 10^{-5} M on Ag/AuNRs-PHBHx substrate.

3.3.3 SERS performance evaluated by enhancement factor

In principle, any measured enhancement of SERS signals can be quantified. When it was discovered that the nature of SERS enhancement is essentially an increase in the scattering cross-section of the molecules relative to conventional

Raman scattering, the concept of EF was introduced. In fact, the description of EF is a complicated topic, which is dependent on parameters including the analyte molecules, substrate, and excitation wavelength and laser power.^[7] More importantly, different definitions are required for different scenarios. For example, the electromagnetic enhancement is not evenly distributed within the SERS substrate. The enhanced electromagnetic field is localized among the very small regions called hot spot, which contribute most of the SERS enhancement. To describe a SERS enhancement for a given molecule at a specific point, a single molecule enhancement factor (SMEF) is needed, which is defined in equation as:^[7]

$$SMEF = \frac{I_{SERS}}{\langle I_{RS} \rangle} \quad 3-1$$

where I_{SERS} is the SERS intensity of the single molecule of concern, and $\langle I_{RS} \rangle$ is the averaged normal Raman intensity per molecule for the same probe. In our case and many other cases, SERS measurements were carried out on the laser illuminated spots of a SERS substrate where clearly more than one probe molecule are located in the illuminated area. A classical EF from the SERS substrate point of view is used and described in the following equation:^[8-11]

$$EF = \frac{I_{SERS}/N_{SERS}}{I_{RS}/N_{RS}} \quad 3-2$$

where I_{SERS} and I_{RS} are intensities obtained from SERS substrate and normal Raman scattering respectively under one unit of excitation, N_{SERS} and N_{NR} are the average number of molecules in the scattering volume of SERS and normal Raman measurements respectively.

The SERS spectrum and the normal Raman spectrum of 4-Mpy are shown in **Figure 3.6**. In the normal Raman spectrum of 4-Mpy, the peaks at 1001 cm^{-1} and 1116 cm^{-1} are assigned to a ring breathing mode, $\nu(\text{ring breathing})$, and a trigonal ring breathing mode with C-S stretching, $\nu(\text{ring breathing})/\nu(\text{CS})$, corresponding to peaks at 1008 cm^{-1} and 1098 cm^{-1} in the SERS spectrum, respectively. The variations of the peak positions between normal Raman spectrum and SERS spectrum are due to the bonding of 4-Mpy onto the silver surface leading to shifts of the vibration modes.

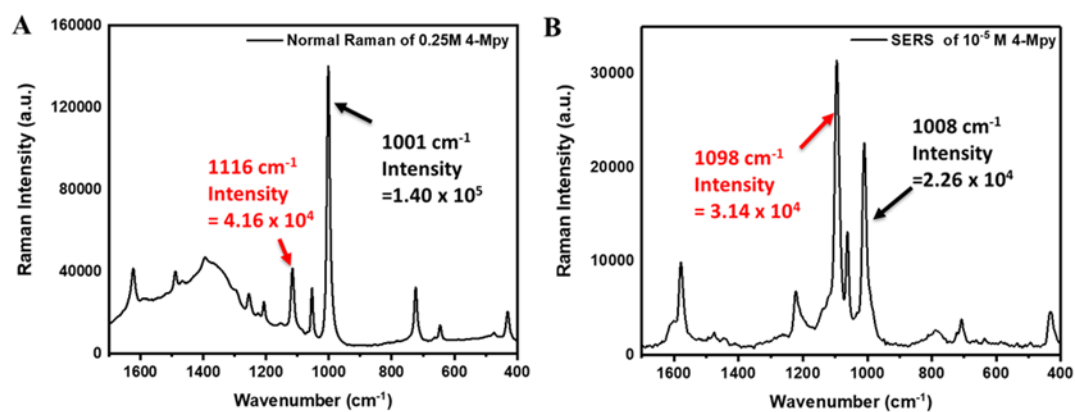


Figure 3.6 (A) Normal Raman spectrum of 0.25 M 4-Mpy aqueous solution. (B) SERS spectrum of 10^{-5} M 4-Mpy measured on Ag/AuNRs-PCL SERS substrate.

One can calculate the EF of the $\nu(\text{ring breathing})$ mode of 4-Mpy. The laser power was 81.3 mW recorded in the normal Raman measurement and 1.2 mW recorded in SERS measurement. Therefore, the intensity obtained for each measurement was calculated as:

$$I_{SERS} = 22639 \quad 3-3$$

$$I_{RS} = 140192 \times \frac{1.2 \text{ mW}}{81.3 \text{ mW}} = 2069 \quad 3-4$$

The number of Ag/AuNRs present in the PCL mat (S_{PCL} , 40 mm×10 mm) during the immobilization was around 4×10^{12} . This was calculated using the dimension of as-synthesized AuNRs as estimated from TEM images, where the average length of AuNRs was 58.5 nm and the average diameter of AuNRs was 16.7 nm. The sampling spot (S_{laser}) of the laser was 10 μm in diameter. We assume that the laser can pass through the SERS substrate, which means the pass-through length of the laser light in the SERS measurement is exactly the thickness of the substrate (typically less than 200 μm measured by a caliper). Thus, the number of sampled Ag/AuNRs ($N_{Ag/AuNR}$) was estimated as:

$$N_{Ag/AuNR} = 7.826 \times 10^5 \quad 3-5$$

Using a silver coating thickness of 3 nm, the surface area of a Ag/AuNR ($S_{Ag/AuNR}$) is calculated as $5.41 \times 10^{-11} \text{ cm}^2$. When 4-Mpy adsorption onto the gold surface saturates, the 4-Mpy is chemisorbed perpendicularly on the gold surface to form a monolayer with a molecular density equal to $5 \times 10^{-10} \text{ mol/cm}^2$.^[12] Using this density, the number of molecules of 4-Mpy (N_{SERS}) sampled within the laser scattering volume of the SERS measurement then is determined to be:

$$N_{SERS} = N_{Ag/AuNR} \cdot S_{Ag/AuNR} \cdot \rho = 1.27 \times 10^{10} \quad 3-6$$

For the normal Raman scattering measurement, the scattering volume sampled is determined by the pass-through length of laser light, which was fixed by a NMR tube with an inner diameter of 5 mm. Knowing the concentration of the aqueous 4-Mpy solution, the number of molecules of 4-Mpy (N_{RS}) sampled within the laser scattering volume of the normal Raman measurement is calculated as:

$$N_{RS} = 5.91 \times 10^{13} \quad 3-7$$

Hence, the EF of $\nu(\text{ring breathing})$ can be calculated as:

$$EF_{\nu(\text{ring breathing})} = \frac{I_{SERS}/N_{SERS}}{I_{RS}/N_{RS}} = 5.1 \times 10^4 \quad 3-8$$

Similarly, the EF of $\nu(\text{ring breathing})/\nu(\text{CS})$ was calculated following the same method:

$$EF_{\nu(\text{ring breathing})/\nu(\text{CS})} = \frac{I_{SERS}/N_{SERS}}{I_{RS}/N_{RS}} = 2.4 \times 10^5 \quad 3-9$$

3.3.4 Comparison of AuNR- and Ag/AuNR-based SERS substrates

In our previous studies, we have compared the SERS performance of a 3D AuNRs-PCL fibrous SERS substrate with a 2D AuNRs-PCL planar SERS substrate fabricated with the same immobilization strategy but on a 2D PCL film.^[13] The result indicated that the SERS signals obtained from the 3D fibrous SERS substrate had a 6-fold relative increase to the signals obtained from the 2D planar SERS substrate. The increase of signal from the fibrous substrate suggested that it was the larger surface area, provided by the 3D architecture of the PCL fibrous mat that allowed a larger loading of the SERS-active MNPs. This makes the fibrous SERS substrate more sensitive than the 2D planar substrate.

To investigate how different SERS-active MNPs affect the sensitivity of the 3D structured SERS substrate, the SERS performance of various substrates fabricated with AuNRs and Ag/AuNRs having different silver coating thicknesses have been compared. The SEM images of the substrates are shown in **Figure 3.7** and labeled with the type of the SERS-active MNPs used to fabricate the substrates followed by the wavelength of the longitudinal SPR peak measured in the solution phase.

“Ag/AuNR_673” nm, for instance, denotes a SERS substrate fabricated with Ag/AuNRs having a longitudinal SPR peak at 673 nm in solution.

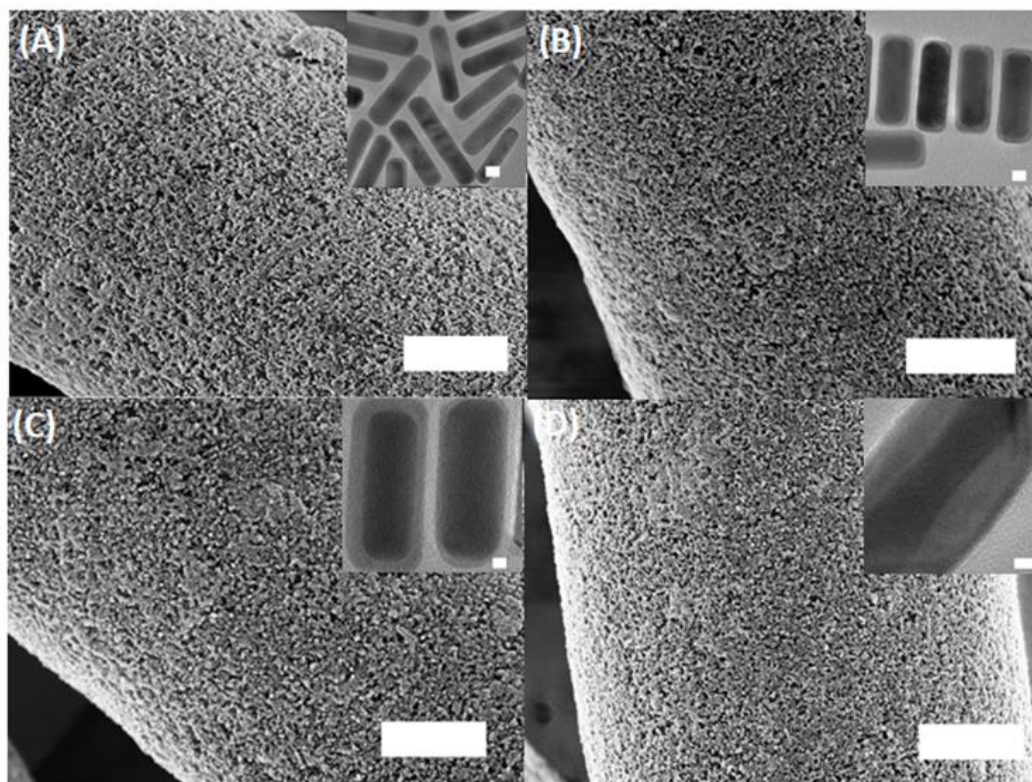


Figure 3.7 (A-D) SEM images of SERS substrates: AuNR_778 nm, Ag/AuNR_703 nm, Ag/AuNR_673 nm, and Ag/AuNR_664 nm, respectively; the scale bar is 1 μ m. Inset: TEM images of the corresponding SERS-active MNPs used to fabricate the SERS substrates; the scale bar is 5 nm.

The SERS spectra of 10^{-5} M 4-Mpy on these substrates were measured randomly on three different spots, averaged and are shown in **Figure 3.8A**. The peak intensities for 4-Mpy on these substrates are shown in **Figure 3.8B**.

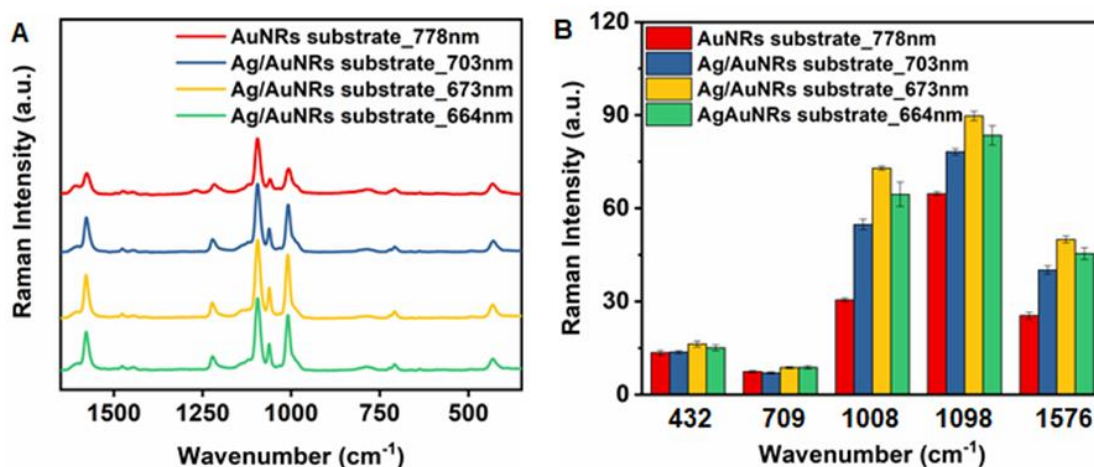


Figure 3.8 (A) The SERS spectra of 10^{-5} M 4-Mpy measured on different SERS substrates. (B) Comparison of peak intensities between different SERS substrates.

The spectra from **Figure 3.8** showed that the SERS intensities obtained from Ag/AuNR-based SERS substrate are higher than the SERS intensities from the pure AuNR-based SERS substrate. Interestingly, the increased intensities for different peaks of 4-Mpy varied. The intensities for the peaks of 4-Mpy particularly at 1008 cm⁻¹, 1098 cm⁻¹ and 1576 cm⁻¹, which are assigned to $\nu(\text{ring breathing})$, $\nu(\text{ring breathing})/\nu(\text{CS})$ and $\nu(\text{CS})$ with deprotonated nitrogen respectively, are higher and show greater variation among the substrates. The intensities for the peaks of 4-Mpy at 432 cm⁻¹ and 709 cm⁻¹, show less variation in the SERS intensities among the different substrates. In the studies of Guo *et al*, the trend we described above was observed for SERS spectra of 4-Mpy on laser-ablated gold and silver metal substrates.^[1] However, the authors never discussed this interesting phenomenon. The SERS comparison, in our case, for 3D SERS substrates is even more complicated since several factors can

affect the observed SERS intensities. In the following section, we will discuss these factors one by one.

First, there is the issue of the density of MNPs dispersed on the substrates. It is known that generation of narrow gaps between MNPs is critical to obtain intense SERS signals. In other words, the distance dependence of the SERS intensities suggests that the SERS intensities decay dramatically once the gaps broaden among the MNPs. Take a spherical metallic dimer for example, where the theoretical calculation predicts that, the SERS intensity decays with d^{-10} , where d is the distance between the two spheres.^[14-16] To assure that the performance difference is not governed by a difference in the density of NPs immobilized, we note that the SEM images have confirmed that there is little, if any, difference in the densities of MNPs for different substrates (**Figure 3.7**). Although the spatial resolution of the SEM images is insufficient to provide quantitative information on spacings between the rod-shape MNPs on the 3D substrates, the size of the PCL mat and the number of nanoparticles for fabrication have all been well controlled. The surface area of PCL fibrous mesh was examined using BET surface area measurements ($0.90 \pm 0.10 \text{ m}^2/\text{g}$). From the UV-vis spectra before and after immobilization, the number of nanoparticles adsorbed onto substrates can also be well estimated.

Second, there is the issue of surface area of the SERS-active MNPs. Once the silver coats the exterior surface of the AuNR to form the Ag/AuNR, the optical properties change. The most straightforward change is that the surface area gets enlarged. Thus, more 4-Mpy molecules can be loaded onto the surface of the MNPs which increases the SERS signals. One way to eliminate the factor of surface area is to use the EF we have defined in the previous section. This parameter gives the intensity

generated per molecule in SERS compared to the intensity generated per molecule in normal Raman scattering. To compare the EF of Ag/AuNR-based substrate to the EF of AuNR-based substrate, the equation is:

$$\frac{EF_{Ag/AuNR}}{EF_{AuNR}} = \frac{I_{Ag/AuNR}/N_{Ag/AuNR}}{I_{AuNR}/N_{AuNR}} \quad 3-10$$

where $I_{Ag/AuNR}$ and I_{AuNR} are the SERS signals obtained from Ag/AuNR-based and AuNR-based SERS substrate respectively, and $N_{Ag/AuNR}$ and N_{AuNR} are the molecules sampled in the Ag/AuNR-based and AuNR-based SERS substrate respectively. Since the number of MNPs sampled under the laser illumination and the density of saturated 4-Mpy are identical for both Ag/AuNR- and AuNR-based substrate, the equation above is transformed to:

$$\frac{EF_{Ag/AuNR}}{EF_{AuNR}} = \frac{I_{Ag/AuNR}/S_{Ag/AuNR}}{I_{AuNR}/S_{AuNR}} = \frac{I_{Ag/AuNR}}{I_{AuNR}} \times \frac{d_0^2 + 2d_0 \cdot l_0}{(d_0 + 2\Delta)^2 + 2(d_0 + 2\Delta)(l_0 + 2\Delta)} \quad 3-11$$

where $S_{Ag/AuNR}$ and S_{AuNR} represent the surface area of a Ag/AuNR and AuNR, respectively. d_0 , l_0 and Δ are the diameter of AuNR, length of AuNR, and thickness of the silver coating layer, respectively. Briefly, the equation indicates that the increase in SERS intensity is not the same as EF. The EF of various Ag/AuNR-based substrates divided by EF of AuNR-based substrate are calculated and summarized in **Table 3.2**.

Table 3.2 Relative EF of Ag/AuNR to AuNR at different peaks of 4-Mpy.

	432 cm ⁻¹	709 cm ⁻¹	1008 cm ⁻¹	1098 cm ⁻¹	1576 cm ⁻¹
EF ₁ /EF ₀	0.67	0.62	1.18	0.79	1.04
EF ₂ /EF ₀	0.63	0.61	1.24	0.72	1.02
EF ₃ /EF ₀	0.47	0.51	0.90	0.55	0.76

Here EF_0 is the EF of SERS substrate AuNR_778 nm, and EF_1 , EF_2 and EF_3 are the EFs of SERS substrates Ag/AuNR_703 nm, Ag/AuNR_673 nm and Ag/AuNR_664 nm respectively. Most of $EF_{Ag/AuNR}/EF_{AuNR}$ values shown in **Table 3.2** are less than 1, which means the EFs obtained from Ag/AuNR-based substrate are less than EFs obtained from AuNR-based substrate. This result could simply be attributed to the 785-nm excitation wavelength that is in the spectral range that is usually more favorable for gold than silver due to their dielectric difference.^[17] The larger surface area provided by the Ag/AuNRs makes the eventual SERS signals observed from Ag/AuNR-based substrate larger than those observed from the AuNR-based substrate. It should be noted that the EF_1/EF_0 and EF_2/EF_0 for peaks at 1008 cm^{-1} and 1576 cm^{-1} are larger than or closer to 1, even at 785 nm laser excitation. This increase of EF, could be due to the properties of the bimetallic junction where CT occurs between the bimetallic nanostructures. When silver is coated onto the AuNRs, the surface is covered gradually by metallic silver, so the surface of the nanorods becomes gradually silver-like, allowing CT from the gold core to silver shell. Given the Fermi levels of Au (-5.0 eV) and Ag (-4.6 eV),^[18] there is likely an increase in the localized electromagnetic fields and the SERS enhancement for analytes around the MNPs.

This raises the question why the increase showed up at some specific peaks for a given Ag/AuNR-based substrate? The 4-Mpy molecule is known to exhibit C_{2v} symmetry with two in-plane vibrational modes A_1 , B_2 , and two out-of-plane vibrational modes A_2 and B_1 .¹²⁻¹³ As SERS selection rules suggest,^[19] in-plane modes of 4-Mpy are much more sensitive to the localized electromagnetic field than those out-of-plane modes. Thus, the intense peaks found in the SERS spectrum of 4-Mpy, A_1 mode of $\nu(\text{ring breathing})$ at 1008 cm^{-1} and B_2 mode $\delta(\text{CS})/\gamma(\text{CCC})$ of at peak

1576 cm^{-1} , experienced more enhancement than other peaks when the electromagnetic field is augmented by the CT between the two components of the bimetallic MNPs.

Third is the issue of SPR properties of aggregated SERS-active MNPs. It should be noted that the longitudinal SPR of SERS-active MNPs for SERS substrate fabrication were measured from solution phase and not directly from the solid SERS substrate. Usually, the aggregated clusters of SERS-active MNPs exhibit a red shift compared to a well-dispersed colloidal solution.^[20] The measurement of the red shift of the MNPs assembled monolayer was not able to be carried out. In our study, the EF of the SERS substrate Ag/AuNR_664 nm is the smallest compared to that of the other two Ag/AuNR-based SERS substrates. This could be attributed to the gradual offset of longitudinal SPR peak for the Ag/AuNRs relative to the laser wavelength, namely 785 nm, which could decrease the coupling effects between the Ag/AuNRs and laser light, decreasing the SERS intensities. In addition, the thickest silver coating (over 7 nm) of Ag/AuNRs could make the Ag/AuNRs behave like pure silver MNPs, whose dielectric properties are no longer favored in the near infrared spectral region at 785 nm.

3.4 Conclusion

In this chapter, the performance of Ag/AuNRs-PCL SERS substrates have been well evaluated. Using 4-Mpy as a chemisorbed probe molecule, the Ag/AuNRs-PCL SERS substrates have been demonstrated to be highly sensitive, reproducible and stable. The fabrication of SERS substrates with PHBHx electrospun fibrous mat has further proved the universal nature of electrostatic immobilization. Lastly, the performance of SERS substrates fabricated with different MNPs, AuNRs and Ag/AuNRs with different silver coating thicknesses, has been compared and discussed. The results showed that the overall SERS performance of Ag/AuNR-based

substrates is better than that of AuNR-based substrate. The reasons that the observed SERS intensities of Ag/AuNR-based substrate increased are complicated. The enlarged surface area of Ag/AuNRs, which provided more sites for 4-Mpy loading, could be the major reason responsible for the increased SERS intensities. Additionally, CT among the bimetallic MNPs could result in a larger EF of Ag/AuNRs for specific vibrational modes of 4-Mpy compared to AuNRs even at 785 nm excitation. In fact, the performance of a SERS substrate is affected by many factors, including the properties of MNPs (e.g. SPR), the type of analyte molecules, the adsorption efficiency of analyte molecules onto substrate surface, and the laser excitation used. To further elucidate the comparison between different SERS substrates, more fundamental studies should be conducted on a simplified substrate (e.g. 2D glass slide).

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Chapter 4

AG/AUNRS-PCL SERS SUBSTRATE FOR ARSENIC DETECTION AND SPECIATION

4.1 Introduction

Arsenic is a metalloid element that is naturally found in water, mines and soils in a variety of forms. Arsenic is now one of the most widely found toxic contaminants in the environment throughout the world. Concerns with the hazards of arsenic in the environment and to human beings have led the World Health Organization (WHO) to set a stringent standard with a maximum of 10 ppb ($\mu\text{g/L}$) in drinking water. However, it has been reported that drinking water containing arsenic pollution at concentrations above the WHO standard has affected over a million people in 50 countries. This is especially true in developing countries in South Asia (e.g. Bangladesh) and South America (e.g. Chile).^[1-2] The exposure to arsenic in food and drinking water increases significantly the risks of carcinogenesis, reproductive problems and cardiovascular disease.^[3-4] Different forms of arsenic have different toxicities and a variety of uses. Arsenate (AsV) and arsenite (AsIII) are the most dominant and highly toxic forms of arsenic found in water. AsV is the main species found in the aerobic environment, while AsIII is prevalent in a more reductive environment, and can be 50-100 times more toxic than AsV .^[5] On the other hand, organoarsenic, such as *p*-arsanilic acid (*pAsA*), roxarsone (Rox) and acetarsone, are widely used as feed additives in poultry farming to improve feed efficiency and control diseases. The majority of these antimicrobial drugs, however, are not metabolized in poultry, and are excreted

chemically unchanged in farming litter. Along with the farming litter used as fertilizer in agricultural planting, these organic arsenic compounds will finally degrade to other organic/inorganic arsenic species, such as AsV, AsIII, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) with higher mobility and toxicity that accumulate in the soil, underground water and rice.^[6-9]

The toxicity, mobility and degradation of various arsenic species are known to be closely related to their chemical forms, oxidation states, and valences. Therefore, speciation of arsenic pollution is sometimes much more important than a simple detection of the total amount of arsenic. Many analytical techniques have been well developed and are routinely used in analytical labs for arsenic detection. These include atomic fluorescence spectroscopy (AFS), atomic absorption spectroscopy (AAS), inductively coupled plasma-atomic emission spectrometry or mass spectrometry (ICP-AES or ICP-MS), as well as other wet chemical methods.^[10] Although a high sensitivity has been reached, most of these techniques only give results for the total arsenic content with no differentiation of arsenic species. Coupled with effective separation methods such as ion chromatography (IC) or high-performance liquid chromatography (HPLC) with a carefully designed column, the above noted techniques can determine the quantification and speciation of arsenic analysis.^[11-14] However, these instrumental techniques are still expensive, bulky and the sample preparation is sophisticated and time-consuming. This limitation has led to the need for a fast, sensitive and reproducible analytical method for arsenic analysis. This situation makes SERS a highly promising and attractive technique for ultrasensitive arsenic detection and speciation in environmental sensing applications.

In recent years, SERS has been extensively used in environmental analysis and monitoring,^[15-16] including detection of arsenic in water.^[4] Extended X-ray absorption fine structure (EXAFS) analysis has suggested AsV is chemically adsorbed onto a silver surface by the formation of As-O-Ag bonds^[17] and the difference in Raman shift ($\sim 60 \text{ cm}^{-1}$) of $\nu_s(\text{As-O})$ between AsV and AsIII helped identify arsenic in different valences.^[18] This discovery makes silver based nanostructures excellent SERS platforms for arsenic detection. Silver colloids of various shapes and modifications have been studied for arsenic detection.^[18-20] In addition, silver nanofilms fabricated by mirror reactions^[21-23] and two-phase interfacial self-assembly^[24] have also been utilized for arsenic detection using SERS. A limit of detection (LOD) of approximately 1 ppb has been reached for both AsV^[18] and AsIII^[19]. However, previous SERS sensors for arsenic detection used either colloidal suspensions or 2D solid substrates. A 3D substrate fabricated using MNPs/polymer composites has not been reported as a SERS sensor for arsenic analysis. Previously, the 3D AuNRs-PCL SERS substrates fabricated in our lab have been applied to heavy metal ion detection, particularly, Cu^{2+} and Hg^{2+} .^[25] To detect free Cu^{2+} and Hg^{2+} in solution, ion-binding ligands, trimercaptotriazine (TMT) and 2,5-dimercapto-1,3,4-thiadiazole dimer (di-DMT) were conjugated to the AuNRs via thiol groups and used as bridging molecules to capture and detect the Cu^{2+} and Hg^{2+} , respectively. By monitoring certain SERS spectral changes of TMT and di-DMT, such as peak position shifts and peak intensity changes, a rigorous quantitative analysis of Cu^{2+} and Hg^{2+} was carried out. In this study, we continued the application of SERS to the environmental sensing for arsenic analysis using the Ag/AuNR-based SERS substrate. Since arsenic species can anchor

to the silver via the formation of As-O-Ag bonds, the arsenic species can be directly detected, in our case, by using Ag/AuNRs as the SERS-active MNPs.

In this chapter, quantitative SERS analysis of different arsenic species is reported. *In-situ* observations of arsenic adsorption, desorption, and reduction have been carried out as well. More importantly, by analyzing SERS spectra of *pAsA* at different concentrations and pH, different binding structures of *pAsA* adsorbed on a silver surface were hypothesized and theoretical calculations based on density functional theory (DFT) were carried out in order to explain the spectral observations for *pAsA*. These studies demonstrate the power and versatility of the SERS technique with our unique 3D Ag/AuNR-based substrates for arsenic analysis in environmental sensing applications.

4.2 Materials and Methods

4.2.1 Materials

Cetyltrimethylammonium bromide (CTAB) and cetyltrimethylammonium chloride (CTAC) were purchased from TCI, Inc. 3-nitro-4-hydroxyphenylarsonic acid (roxarsone, Rox) was purchased from Fisher Scientific, and 4-aminophenylarsonic acid (*p*-arsanilic acid, *pAsA*) was purchased from Alfa Aesar. All the other chemicals were purchased from Sigma-Aldrich. All chemicals were used as received. Deionized (DI) water (18.2 M Ω ·cm; Millipore Co.) was used for solution preparations unless otherwise described.

4.2.2 Fabrication of Ag/AuNRs-PCL SERS substrate

The fabrication of Ag/AuNRs-PCL SERS substrate has been described in detail in **Chapter 2** and our recent publication.^[26] Specifically, 5-nm thick silver layer

coated AuNRs with a width of approximately 25 nm and a length of about 70 nm were used as SERS-active MNPs for substrate fabrication and arsenic analysis. This substrate resulted in the optimum SERS performance as discussed in **Chapter 3**.

4.2.3 Quantitative measurements on arsenic species

Arsenic (Rox, *p*AsA and AsV) solution samples were prepared at various concentrations with identical volumes of 10 mL. A piece of SERS substrate was cut into identical pieces (typically 2 mm×10 mm). Each piece was immersed into a sample solution containing a different concentration of arsenic for 2 hours, and then taken out and washed with DI water to remove loosely bound analyte molecules. The substrates were dried under ambient temperature and humidity before SERS measurements. The SERS measurements were performed using the same Raman spectrometer as described in detail in **Chapter 3**. For each sample, measurements were recorded on three randomly selected spots on the substrate using ~ 0.8 mW laser power with a 10-s exposure for 10 accumulations. To test arsenic detection in a more complex environment, AsV solutions containing common salt ions (30 μM Na^+ , 10 μM K^+ , 10 μM Mg^{2+} , 10 μM Ca^{2+} , 10 μM Cl^- , 10 μM HPO_4^{2-} , 10 μM SO_4^{2-} , 20 μM NO_3^- , 10 μM H_2PO_4^-) were prepared at different concentrations. The sample preparation and SERS measurements were similar to the procedures described above.

To achieve rigorous data analysis, spectra were processed before plotting in the following sequence: each spectrum was the average of three measurements. All the spectra were baseline corrected. A background spectrum was then subtracted, and the result was offset to read zero in regions where there were no Raman peaks. The background spectrum was obtained using a bare substrate immersed in a blank

solution (DI water or common salt ions solution without arsenic analyte) under the same experimental conditions.

4.2.4 SERS of *pAsA* at different pH

To explore the effect of pH on the SERS spectra of *pAsA*, 5×10^{-4} M *pAsA* solutions at different pHs were prepared. The pH of a solution was adjusted by adding appropriate amounts of 0.1 M NaOH and the pH of the resultant solution was measured with a pH Meter (Fisher Scientific, accumet AB15 Basic). The preparation of SERS samples, acquisition of SERS spectra and data processing followed the previous description.

4.2.5 DFT computation of *pAsA* molecular structure

To obtain the optimized molecular structure and simulated Raman spectrum of *pAsA*, DFT calculation was carried out using Gaussian 09. B3LYP was used as DFT hybrid functionals in the calculation. Different basis sets were applied from small to large, and a solvation model and two water molecules were added to mimic the proper hydrated environment.

To produce the silver surface that interacts with the negatively charged *pAsA* tautomers, computer modelling calculations were carried out by Dr. Fernando Sabino and Dr. Anderson Janotti in our department. The first-principles calculations based on DFT^[27-28] that were performed to explore the possible configurations of *pAsA* on the silver surface are briefly described as follows: the generalized gradient approximation functional proposed by Perdew, Burke and Ernzerhof (PBE)^[29] was used, with van der Waals correction as proposed by S. Grimme^[30-31], which includes two and three body terms (D3).^[31-32] The projected augmented wave (PAW)^[33] method was used to

describe the interaction between valence electrons and the ionic cores as implemented in VASP code.^[34-35] The PAW potentials were used with the following valence configurations: Ag(4d¹⁰5s¹), As(4s²4p³), C(2s²2p²), H(1s¹), N(2s²2p³), O(2s²2p⁴). The total energy calculations were performed using a plane wave basis set with a cutoff energy of 470 eV and a convergence tolerance of 1×10⁻⁶ eV. For ionic relaxations, the structures were optimized until the forces on each atom were smaller than 0.01 eVÅ⁻¹. A Ag(111) slab based on the calculated equilibrium lattice parameter of bulk Ag was constructed, considering that the Ag(111) is the most stable surface.^[36] The slab consists of a 5×5 repetition of the Ag(111) surface unit cell, and is composed of 5 layers, where the two bottom layers were frozen, and the three top most layers were allowed to relax. A vacuum region of 20 Å was used to separate two neighboring slabs in the z direction, considering that the length of *pAsA* is shorter than 7.3 Å. Integrations over the Brillouin zone were performed using a 2×2×1 Γ -centered mesh of special k points.

4.2.6 *In-situ* SERS of arsenic adsorption, desorption and reduction

To conduct *in-situ* SERS measurements, a piece of SERS substrate was immersed in 10 mL of AsV (or AsIII) solution in a petri dish and placed in the Raman instrument. 180-degree backscattering was employed for real-time adsorption monitoring. Raman spectra were recorded *in-situ* on the same sample spot of the substrate in the solution, with 1-s exposure for 60 accumulations. As for arsenic *in-situ* desorption, a piece of SERS substrate was immersed in 10⁻⁴ M AsV solution for 2 hours, washed with DI water and dried. The substrate was then immersed into 10 mL of a 20 mg/L NaCl and KH₂PO₄ solution, respectively, and placed in the Raman spectrometer for desorption monitoring. For arsenic *in-situ* reduction of Rox, a piece

of SERS substrate was immersed in 5×10^{-5} M Rox solution in DI water for 2 hours, washed with DI water and dried. The substrate was then immersed into a 2 mM NaBH_4 solution in the Raman spectrometer for reduction monitoring. Raman acquisition of the *in-situ* AsV desorption and the Rox reduction was the same as for the arsenic adsorption experiments.

4.3 Results and Discussion

4.3.1 SERS analysis of arsenic speciation

Normal Raman spectra of the four arsenic compounds were collected for reference purposes. **Figure 4.1** shows normal Raman spectra of the arsenic species studied in this chapter, followed by the SERS spectra of the arsenic species adsorbed onto the Ag/AuNRs-PCL substrate (**Figure 4.2**). The tentatively assigned Raman modes and observed peak frequencies for *pAsA* and Rox for both the normal Raman spectra and the SERS spectra are summarized in **Table 4.1**.^[37]

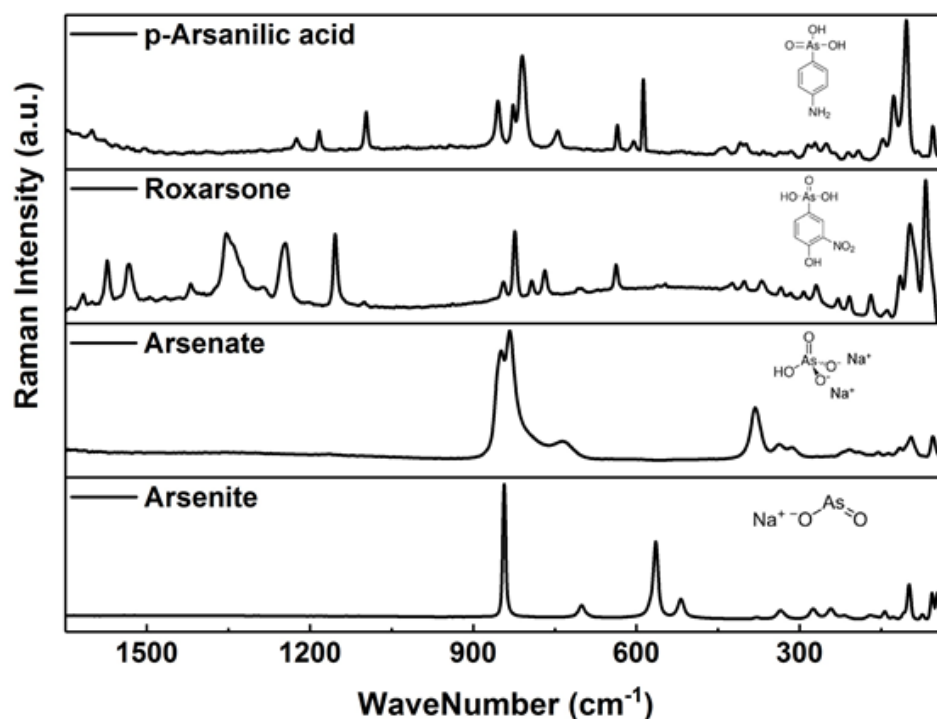


Figure 4.1 Raman Spectra of *p*AsA, Rox, AsV and AsIII powders (from top to bottom). The molecular structures of each arsenic chemical are shown in the insets.

As seen from **Figure 4.2**, all the arsenic species could be adsorbed onto Ag/AuNRs-PCL substrates and gave excellent SERS spectra. Moreover, all the arsenic species have their own spectroscopic features. In the normal Raman spectrum of Rox, the peak frequencies of the symmetric and the asymmetric stretching modes for AsO_x are found at 824 cm^{-1} and 844 cm^{-1} respectively. A weak band attributable to an As-OH vibration is found at 744 cm^{-1} and assigned to an As-O stretching mode for As-OH. An As-C stretching vibration is found at 637 cm^{-1} . In the SERS spectrum of Rox, the symmetric and the asymmetric stretching modes of AsO_x shift to lower

wavenumbers, 797 cm^{-1} and 813 cm^{-1} respectively, which suggests a covalent chemical interaction of As-O with the silver surface forming the As-O-Ag bonds. Supporting evidence for complexation of As-O with the silver surface comes from the observation of band near 200 cm^{-1} , which is attributed to Ag-O stretching. In addition, the stretching mode of As-C is strongly enhanced in the SERS spectrum and shifts by 10 cm^{-1} to lower frequency at 627 cm^{-1} .

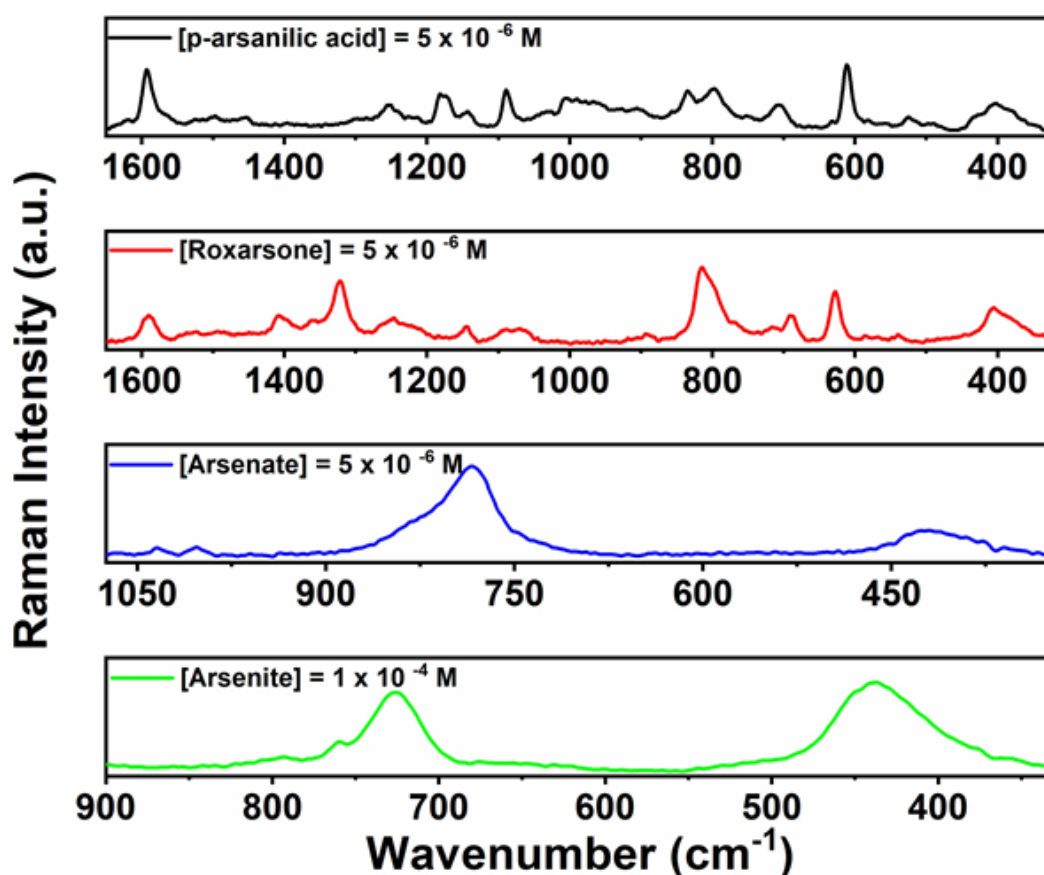


Figure 4.2 SERS spectra of arsenic species adsorbed onto Ag/AuNRs-PCL substrate.

Table 4.1 Peak assignments of *pAsA* and *Rox* in normal Raman and SERS spectra.

Assignments ^[37]	<i>pAsA</i> (cm ⁻¹)		Assignments ^[37]	<i>Rox</i> (cm ⁻¹)	
	Normal Raman	SERS		Normal Raman	SERS
$\nu(\text{Ag-O})$	210		$\nu(\text{Ag-O})$	229	
16a(a')		400	$\gamma(\text{CC})$		407
	587		$\nu(\text{As-C})$	637	628
$\nu(\text{As-C})$	605	611	4b(a'')		690
	635		$\nu_s, \nu_{as}(\text{As-OH})$	744	
4b(a')		708	$\nu_s(\text{AsO}_x)$	824	799
$\nu_s, \nu_{as}(\text{As-OH})$	745	742	$\nu_{as}(\text{AsO}_x)$	844	817
$\nu_s(\text{AsO}_x)$	810	795	$\nu(\text{CN})$		1146
$\nu_{as}(\text{AsO}_x)$	855	834	9(a')	1154	
NH ₂		983	$\nu_s(\text{NO}_2)$	1342	1322
18(a')		1002	$\nu(\text{CC})$	1419	
7(a')	1097	1089	$\nu_{as}(\text{NO}_x)$	1533	1490
9a(a')	1183	1182	$\nu(\text{CC})$	1573	
19b(b''), $\nu(\text{CC})+\delta(\text{C-H})$		1448	$\nu_s(\text{As=O}), \text{As}(\text{OH})\text{O}_2^-$		1591
$\nu_s(\text{As=O}), \text{As}(\text{OH})\text{O}_2^-$		1593	8a(a'), 8b(a'), $\nu_{as}(\text{NO}_2)$	1617	

The above changes of the arsenic involved Raman modes can be also observed by comparing the normal Raman spectrum of *pAsA* with its SERS spectrum. Similarly, the symmetric and the asymmetric stretching modes of AsO_x in *pAsA* shift from 855 cm⁻¹ and 810 cm⁻¹ to 834 cm⁻¹ and 795 cm⁻¹, respectively after adsorption onto the silver surface. In addition, the As-C stretching mode shifts from 635 cm⁻¹ to 611 cm⁻¹. Thus, the SERS features of the arsonic acid group are very good identifiers and confirm the presence of organic arsenic species. As for the inorganic arsenic species, the SERS spectrum of AsV shows a major peak at 785 cm⁻¹ which is assigned to $\nu_1(\text{A}_1)$ symmetric As-O stretching. The minor peak around 425 cm⁻¹ is the superposition of $\nu_2(\text{A}_1)$ and $\nu_5(\text{E})$ stretching modes.^[18] The shoulder at 825 cm⁻¹ is

tentatively assigned to an asymmetric stretching mode of As-O. In the SERS spectrum of AsIII, a peak at 725 cm^{-1} is assigned to the symmetric As-O stretching vibration. The minor peak appearing as a shoulder at 765 cm^{-1} is attentively assigned to the asymmetric stretching mode of As-O.

The arsenic species are readily distinguishable in the mixture, as shown in **Figure 4.3**. From the spectrum of the mixture of AsIII and AsV in **Figure 4.3A**, it is observed that peaks of AsV and AsIII both exist in the mixture with a 60 cm^{-1} difference in the $\nu(\text{As-O})$ found between the AsV and AsIII. Due to the high sensitivity of the SERS substrate, less than 1 mW of laser power was used for all the Raman measurements for arsenic analysis, which effectively prevented the oxidation of AsIII to AsV under laser illumination. For the organoarsenic species shown in **Figure 4.3B**, a broad shoulder assignable to NH_2 at 983 cm^{-1} originating from the *p*AsA and an intense stretching mode of NO_2 at 1322 cm^{-1} found in Rox are specific markers to further distinguish them in the spectrum of their mixture. Interestingly, the peak position of the As-C stretching vibration in the mixture of *p*AsA and Rox is at 623 cm^{-1} , which is in between the As-C stretching peaks of pure *p*AsA at 611 cm^{-1} and pure Rox at 628 cm^{-1} , reflecting a spectral combination of *p*AsA and Rox.

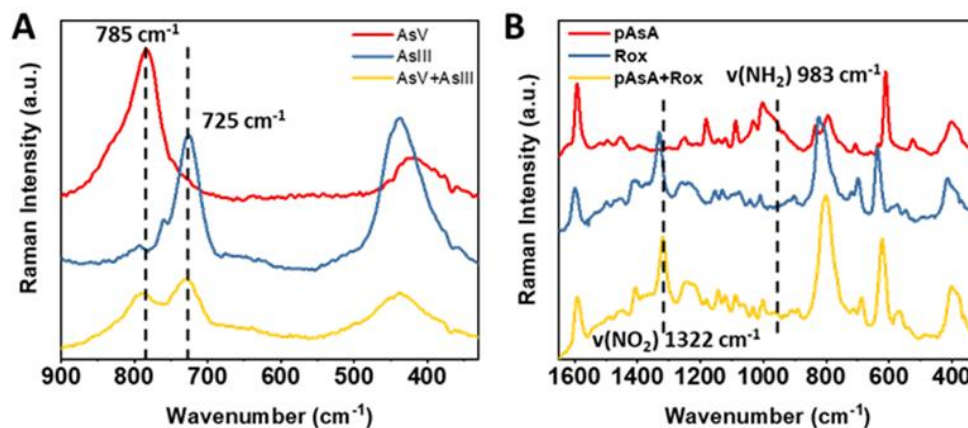


Figure 4.3 SERS spectra of (A) AsV, AsIII at 1×10^{-4} M and AsV/AsIII mixture at 5×10^{-5} M, (B) pAsA, Rox at 5×10^{-5} M and pAsA/Rox mixture at 1×10^{-5} M.

4.3.2 Quantification of arsenic detection

To investigate the SERS of Rox with respect to the concentration, the SERS spectra of Rox for decreasing concentration are shown in **Figure 4.4**. Peaks assigned to the As-C stretching at 627 cm^{-1} , the asymmetric stretching of AsO_x at 813 cm^{-1} , the stretching of NO_2 at 1322 cm^{-1} , and the asymmetric stretching modes of the $\text{As}=\text{O}$ and $\text{As}(\text{OH})\text{O}_2^-$ at 1591 cm^{-1} are readily detectable at the lowest concentration, 5×10^{-8} M (~ 3.8 ppb). The result illustrates the high sensitivity of this Ag/AuNRs-PCL SERS substrate for the detection of arsenic with LOD much lower than the WHO standard of 10 ppb.

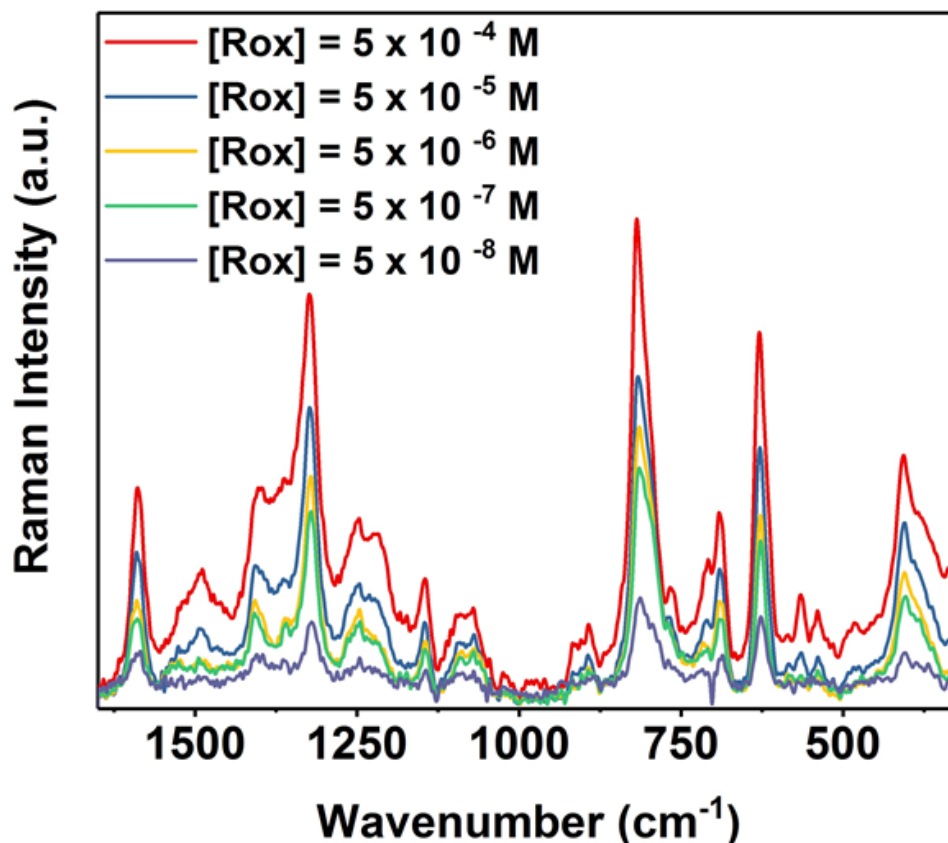


Figure 4.4 SERS spectra of Rox with concentrations ranging from 5×10^{-8} M to 5×10^{-4} M.

As all the intensities in the Rox SERS spectra (**Figure 4.4**) are decreasing with decreasing concentration, no specific peak can be used as an independent reference for normalization and quantification. Due to the excellent reproducibility of the SERS substrate, the background of a bare SERS substrate can be consistently measured and subtracted from SERS spectra of analytes, which results in better quality spectra for quantification. As **Figure 4.5** shows, after spectral processing (as described in **Section 4.2.3**) all the major peaks of Rox are quantitatively changing as a logarithmic function of concentration, which is consistent with a Langmuir adsorption model.

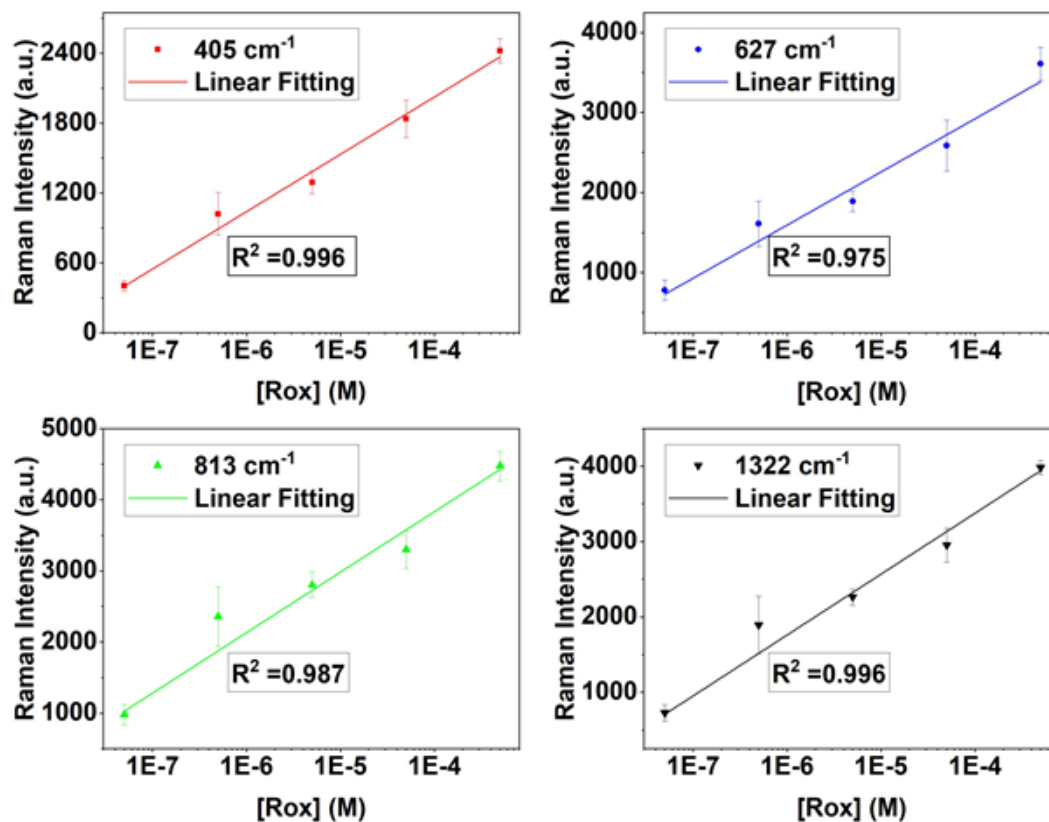


Figure 4.5 SERS quantification of Rox against concentration at 405, 627, 813 and 1322 cm^{-1} .

Similarly, for the inorganic arsenic species, the major peak of As-O stretching at 785 cm^{-1} was used as the identifier of AsV for quantification. A LOD of approximately 4 ppb was also obtained in this case as well. (**Figure 4.6**)

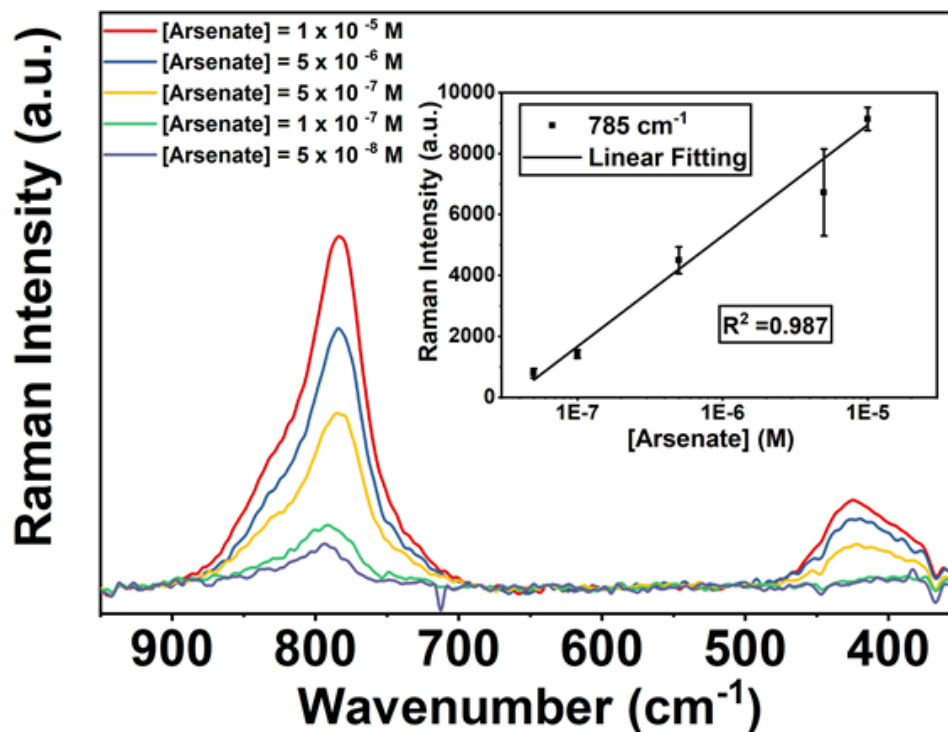


Figure 4.6 SERS spectra of AsV at concentrations ranging from 1×10^{-5} M to 5×10^{-8} M in DI water. Insert: SERS intensity of 785 cm^{-1} peak as a function of AsV concentration.

To test the SERS quantification of AsV in a more complex environment, SERS measurements of AsV prepared in a common salt ionic solution were carried out. The results in **Figure 4.7** show that the inorganic salt ions perturbed the SERS detection of AsV to some degree, but a LOD of 8 ppb is still possible. The reduction in SERS signal stems either from salt ion complexation with AsO_4^{3-} during adsorption, or by contamination of the silver surface sites.

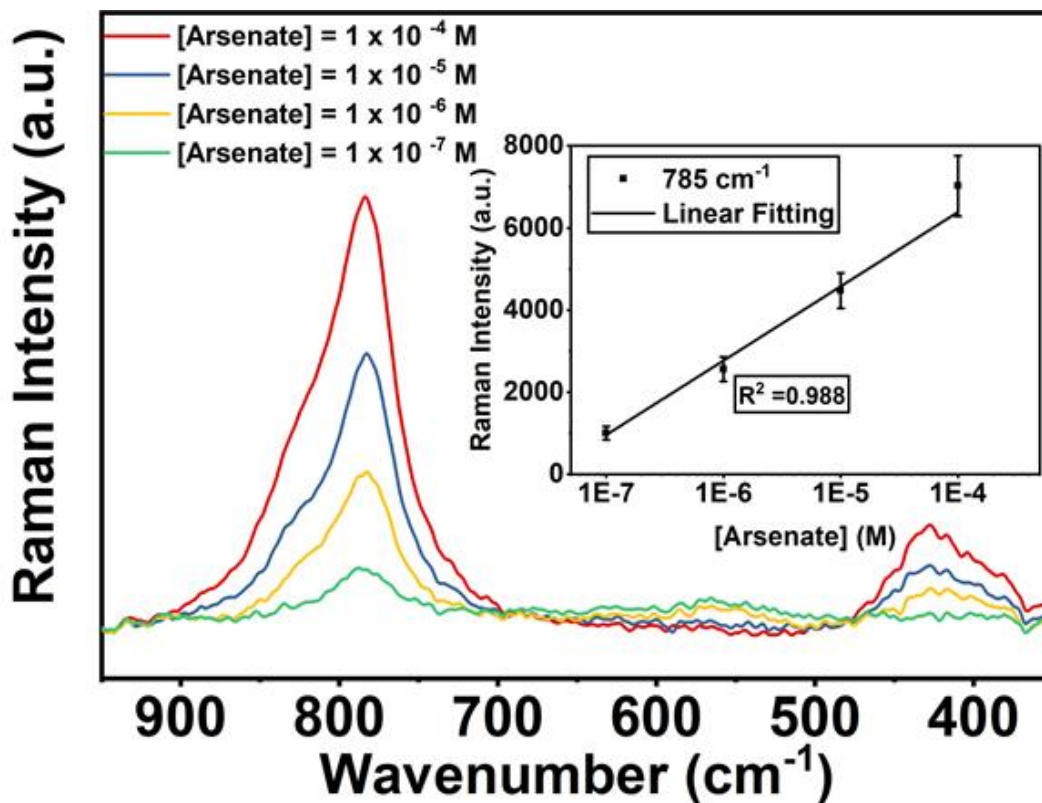


Figure 4.7 SERS spectra of AsV at concentrations ranging from 10^{-5} M to 10^{-7} M prepared in common salt ions solutions. Insert: SERS intensity of 785 cm^{-1} peak as a function of AsV concentration.

In the common salt ionic solution, cations like Mg^{2+} and Ca^{2+} can bind with AsO_4^{3-} , competing for attachment to the silver surface, to form $\text{Mg}_3(\text{AsO}_4)_2$ and $\text{Ca}_3(\text{AsO}_4)_2$ reducing the free AsV that can adsorb onto the silver surface.^[21] On the other hand, anions like Cl^- and PO_4^{3-} can compete with AsO_4^{3-} to complex with silver and form AgCl and Ag_3PO_4 , reducing the number of effective sites on the silver surface for AsV adsorption. This interference can lead to poor adsorption efficiency of AsV, which eventually results in a lower SERS sensitivity for AsV detection. To avoid complexation by inorganic salt ions in hard water, some preprocessing of

samples might be required, such as adding F^- to form precipitates with Mg^{2+} and Ca^{2+} which could then facilitate the SERS performance for arsenate detection.^[17] The solubility product constant (K_{sp}) of silver- and arsenate- related salts from available literatures are summarized in **Table 4.2**.^[17, 21, 38-40]

Table 4.2 K_{sp} of silver- and arsenate- related inorganic salts.

Compound	K_{sp}
Ag_3PO_4	8.9×10^{-17}
$AgNO_3$	52
Ag_2SO_4	1.4×10^{-5}
Ag_2CO_3	8.1×10^{-12}
$AgCl$	1.8×10^{-10}
Ag_3AsO_4	1.0×10^{-22}
$Ca(AsO_4)_2$	6.8×10^{-19}
$Mg_3(AsO_4)_2$	2.1×10^{-20}
CaF_2	3.5×10^{-20}
MgF_2	5.2×10^{-11}

4.3.3 SERS analysis of *pAsA*

When the relationship of the SERS intensity of *pAsA* to its concentration was first studied over the range of 5×10^{-8} M to 5×10^{-5} M, a low LOD and good quantification were achieved (**Figure 4.8A-B**). At higher concentrations up to 1×10^{-3} M, however, shown in **Figure 4.8C** the SERS intensities of *pAsA* continued to decrease. The SERS intensity at 1×10^{-3} M is comparable to the SERS intensity obtained at 1×10^{-7} M in **Figure 4.8D**.

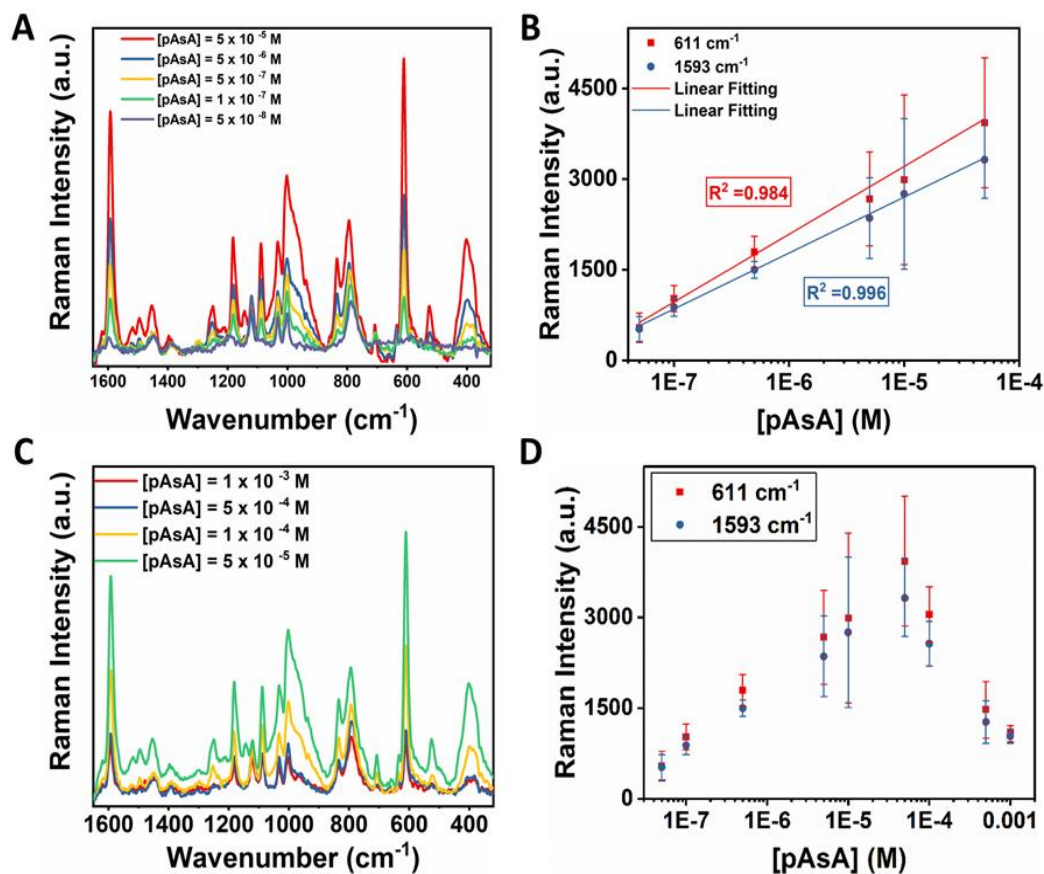


Figure 4.8 (A) SERS spectra of *pAsA* with concentrations ranging from 5×10^{-8} M to 5×10^{-5} M. (B) SERS quantification of *pAsA* against concentration for peaks at 611 and 1593 cm^{-1} . (C) SERS spectra of *pAsA* with concentrations ranging from 5×10^{-5} M to 1×10^{-3} M. (D) SERS intensities of *pAsA* for peaks at 611 and 1593 cm^{-1} , with the whole concentration range from 5×10^{-8} M to 1×10^{-3} M.

Although good quantification was obtained at concentrations below 5×10^{-5} M, the decreased Raman intensity at higher concentrations of *pAsA* was unexpected. As more and more sites on the surface are occupied, the Raman intensity should approach a limiting value as the surface becomes saturated, which was observed for 4-Mpy in our previous study.^[41] To elucidate the unexpected behavior of intensity with

increasing concentration for *pAsA*, we considered the possible molecular structures of *pAsA* in detail. As a polyprotic acid, *pAsA* has three different pKa values (pKa₁ = 1.9, pKa₂ = 4.1 and pKa₃ = 9.2), and several tautomers can co-exist in aqueous solution.^[42] With increasing pH, the dominant form of *pAsA* in water changes from protonated *pAsA*, noted as *pAsA*⁺, to neutral *pAsA*, and upon a further increase in pH, to deprotonated *pAsA*, noted as *pAsA*⁻ and *pAsA*²⁻, as shown in **Figure 4.9**.

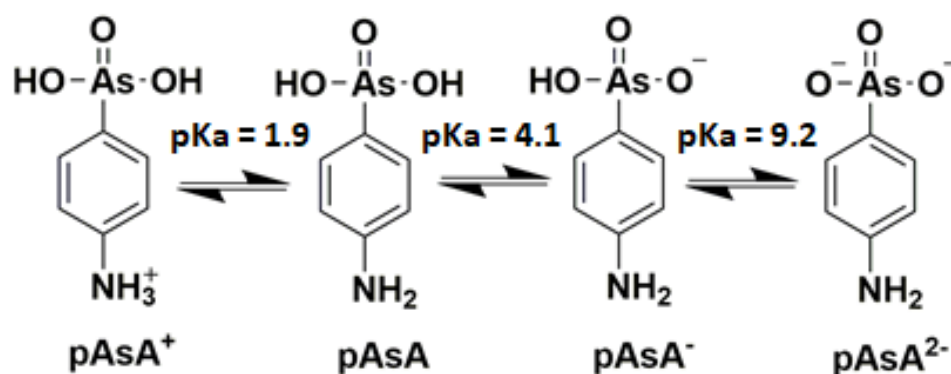


Figure 4.9 Different tautomers of *pAsA* that can exist in water.

Using the known values of pKa, one can calculate the equilibrium fraction of each *pAsA* species as a function of pH by the following equations, since the molar percent of the different *pAsA* species existing in water is only related to pH.^[43]

$$\delta(pAsA^+) = \frac{[H^+]^3}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad 4-1$$

$$\delta(pAsA) = \frac{K_{a1}[H^+]^2}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad 4-2$$

$$\delta(pAsA^-) = \frac{K_{a1}K_{a2}[H^+]}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad 4-3$$

$$\delta(pAsA^{2-}) = \frac{K_{a_1}K_{a_2}K_{a_3}}{[H^+]^3 + K_{a_1}[H^+]^2 + K_{a_1}K_{a_2}[H^+] + K_{a_1}K_{a_2}K_{a_3}} \quad 4-4$$

Therefore, the population of different tautomers of *pAsA* can be determined and plotted at every specific pH as shown in **Figure 4.10**. At concentrations above 5×10^{-4} M, the pH of the *pAsA* solution is below 3.9. **Figure 4.10** shows that the dominant species in the solution are *pAsA*⁺ and *pAsA*. As previously discussed, *pAsA* molecules anchor to the silver surface via As-O-Ag bonds, and neither *pAsA*⁺ nor *pAsA* can significantly adsorb onto the silver surface. As the concentration of *pAsA* decreases, the pH rises to above 6. More *pAsA* molecules are now in the deprotonated forms, *pAsA*⁻ and *pAsA*²⁻, which are more inclined to interact with the silver surface via the negatively charged As-O⁻.

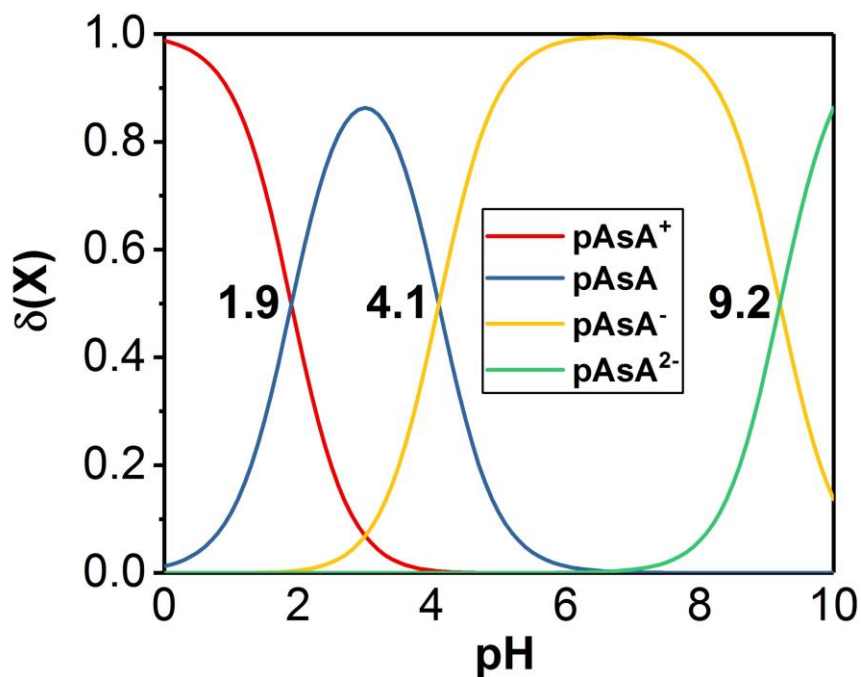


Figure 4.10 *pAsA* polyprotic distribution as a function of pH.

To confirm the predominance of the deprotonated forms of *pAsA* rather than the protonated or the neutral *pAsA* in terms of adsorption and SERS activities, a higher concentration of *pAsA* at 5×10^{-4} M was chosen for further study under different pH conditions. Various small amounts of 0.1 M NaOH were added to 5×10^{-4} M *pAsA* to increase the pH of the solution from 3.90 (no NaOH added) to 4.81 (50 μ L NaOH added). The resultant SERS signal shows a gradual increase in intensity with pH as shown in **Figure 4.11**. This proves that the SERS intensities of *pAsA* are truly related to its tautomer population. It should be noted that no higher pH of the *pAsA* was obtained in this study, since the amount of NaOH was calculated to assure that the pH value is totally contributed by the tautomers of *pAsA* and not the extra OH⁻ from NaOH left in the solution. In other words, for the highest pH value of 4.81 obtained in this case, the amount of NaOH added into the solution turned all *pAsA* in the solution into *pAsA*⁻Na⁺ salt as the initial solute.

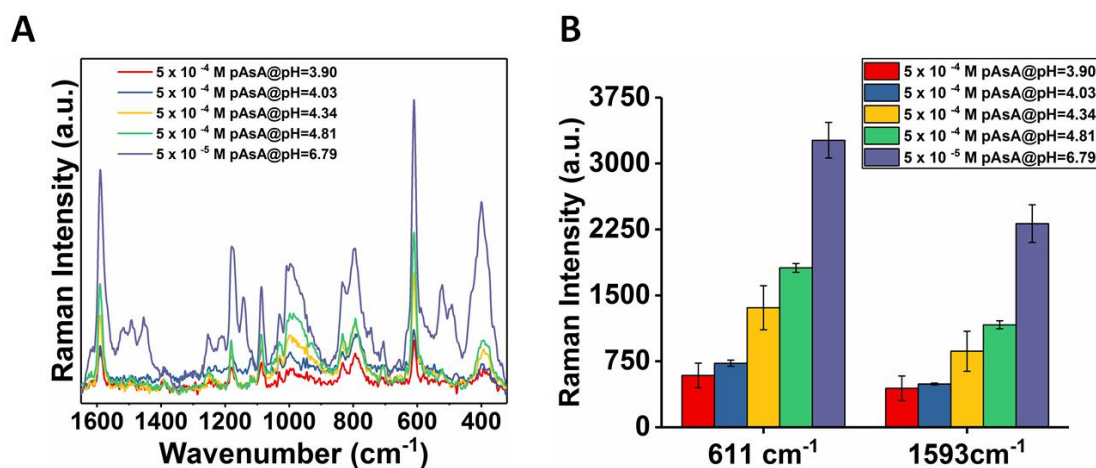


Figure 4.11 (A) SERS spectra of 5×10^{-4} M *pAsA* under different pH conditions and 5×10^{-5} M *pAsA* (no NaOH added). (B) SERS intensity comparison of the 611 cm^{-1} and 1593 cm^{-1} peaks of *pAsA* at 5×10^{-4} M at different pH values and *pAsA* at 5×10^{-5} M (no NaOH added).

Interestingly, the SERS intensities of 5×10^{-4} M *pAsA* obtained at different pH values compared to the SERS intensity of 5×10^{-5} M, the SERS intensities obtained at the former were still much weaker than the SERS intensity obtained at the latter, which has a pH of approximately 6.79. In addition, there are two deprotonated tautomers of *pAsA*: *pAsA*⁻ and *pAsA*²⁻. The roles these two tautomers play in the *pAsA* adsorption and SERS response require further elucidation. The concentrations of different *pAsA* tautomers calculated from the solution concentration and pH values are shown in **Table 4.3**. [*pAsA*⁻] existing in 5×10^{-4} M solutions under different pH conditions were all about an order of magnitude higher than [*pAsA*⁻] that existed in 5×10^{-5} M solution. This trend is opposite to the observed trend of SERS intensities, shown in **Figure 4.11B**. This result suggests that the *pAsA*⁻ was not the predominant tautomer giving rise to SERS intensities in the 5×10^{-4} M solutions. Hence, [*pAsA*²⁻], the species responsible for the SERS intensities, which changed from 1 nM to 17 nM with pH increasing from 3.90 to 4.81, reaching the highest at approximately 200 nM in 5×10^{-5} M solution that is consistent with the change of SERS intensities.

Table 4.3 Population of *pAsA*⁻ and *pAsA*²⁻ in *pAsA* solutions at different pH.

[<i>pAsA</i>] / M	pH	$\delta(pAsA^-)$	[<i>pAsA</i> ⁻] / M	$\delta(pAsA^{2-})$	[<i>pAsA</i> ²⁻] / M
5×10^{-4}	3.90	3.8E-01	1.9E-04	1.9E-06	9.6E-10
	4.03	4.6E-01	2.3E-04	3.1E-06	1.5E-09
	4.34	6.3E-01	3.2E-04	8.8E-06	4.4E-09
	4.81	8.4E-01	4.2E-04	3.4E-05	1.7E-08
5×10^{-5}	6.79	9.9E-01	5.0E-05	3.9E-03	1.9E-07

Moreover, the SERS intensities exhibited a linear relationship between the Raman intensity and the logarithmic function of $[pAsA^{2-}]$, as shown in **Figure 4.12**. Thus, the $pAsA^{2-}$, we believe, is the tautomer that contributes most of the SERS intensity even though only a trace amount of $pAsA^{2-}$ (nM-level) exists in the $pAsA$ solutions.

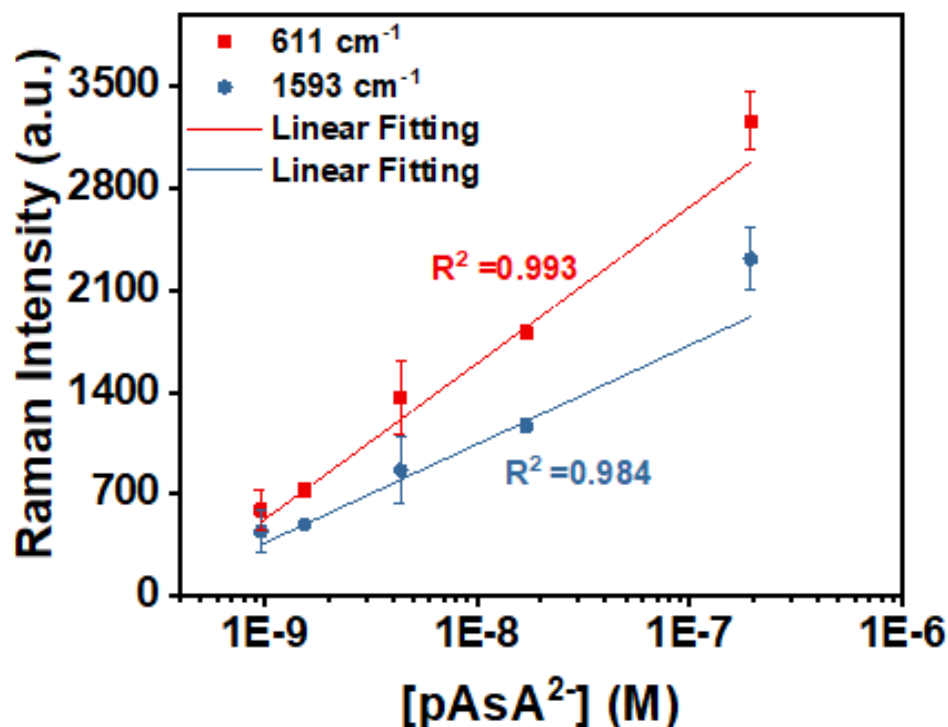


Figure 4.12 SERS intensity of $pAsA$ peaks at 611 and 1593 cm^{-1} plotted against concentration of $pAsA^{2-}$.

The coexistence of both monodentate and bidentate adsorption structures of the monophenyl substituted organoarsenic species adsorbed on ferrihydrite has been suggested by an EXAFS study.^[44] The structure of the bound $pAsA$ has been

calculated for both monodentate and bidentate adsorption structures that can complex with hydrated iron oxide clusters to form outer- and inner- sphere complexes based on DFT.^[9] Calculations of optimized geometry and Raman frequencies of *pAsA* have been carried out in Gaussian 09 based on DFT. Different Pople basis sets, from small to large have been used in the calculation and are listed in **Table 4.4**. In basis sets from No.5 to No.8, a dispersion model GD3BJ, IEFPCM solvation model, and two explicit water molecules were applied step by step to approach a more realistic micro-environment around the *pAsA* molecule.^[45]

Table 4.4 Pople basis sets and settings used in optimization of *pAsA* molecule.

No.	Pople Basis Sets
1	Restricted B3LYP 6-31 G(d,p)
2	Restricted B3W91 6-31+G (d,p)
3	Restricted B3LYP 6-31+G(d,p)
4	Restricted B3LYP 6-311+G (d,p)
5	Restricted B3LYP 6-311+G (d,p)_GD3BJ
6	Unrestricted B3LYP 6-311+G (d,p)_GD3BJ
7	Unrestricted B3LYP 6-311+G(d,p) _GD3BJ_scrf = (IEFPCM, solvent=water)
8	Unrestricted B3LYP 6-311+G(d,p) _GD3BJ_scrf = (IEFPCM, solvent=water) + two H ₂ O

The optimized configuration of *pAsA* molecule and calculated vibrational frequencies using basis set No.8 are shown below in **Figure 4.13**. From the molecular structure of *pAsA*⁻ in **Figure 4.13A**, the length of the As-O bond (As11O14) in As-OH is 1.85 Å, while the other two As-O bonds have lengths of 1.66 Å (As11O13) and

1.67 Å (As11O12), respectively. This indicates the negative charge of As-O⁻ is delocalized with As=O to form the resonance structure [O⁻As=O]. The angle of C14As11C3 is 103°, and the phenyl group is sitting right on top of the tetrahedral structure of -AsO₃.

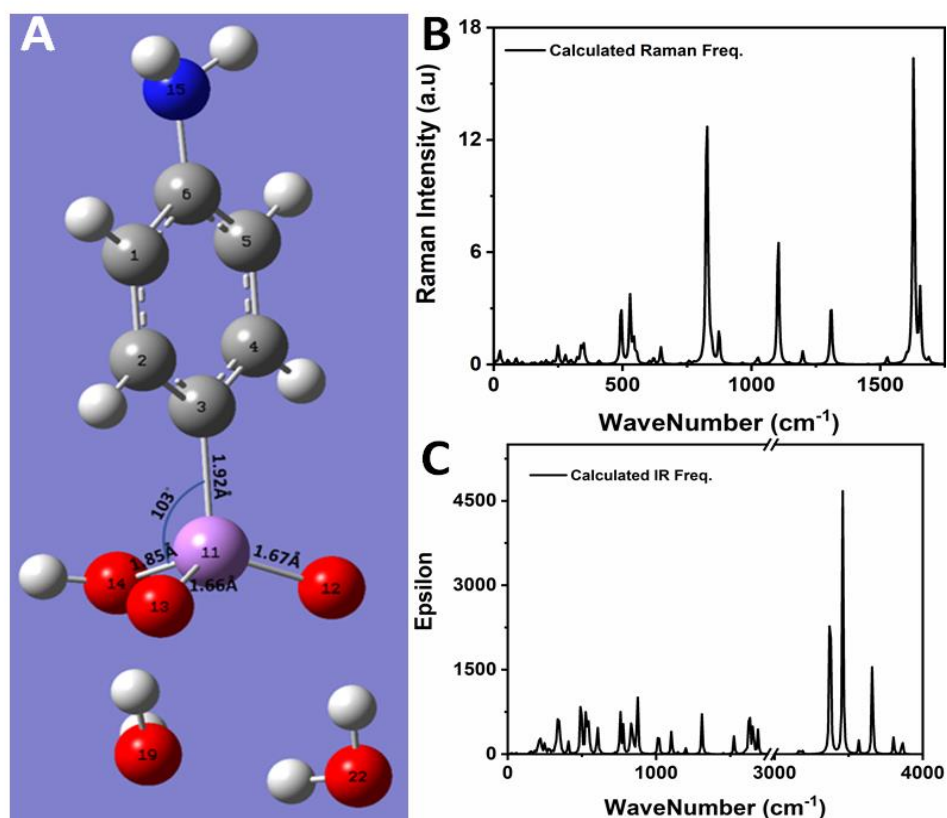


Figure 4.13 (A) Optimized structure of *pAsA*⁻ using DFT calculation with No. 8 basis set in Gaussian 09. (B) Calculated Raman and (C) IR spectrum of *pAsA* molecule.

With the optimized *pAsA*⁻ structure, we speculate that it is the different bindings of *pAsA*⁻ and *pAsA*²⁻ adsorbed on the silver surface which result in a large

difference in their contributions to the observed SERS intensities, illustrated in **Figure 4.14**. The monodentate structure is energetically favored for $pAsA^-$ bound to the silver surface, while the structure for the fully deprotonated species $pAsA^{2-}$, is more likely to bind to the silver surface in a stand-up fashion. When $pAsA^{2-}$ is bound to the silver surface, the orientation of the phenyl group is perpendicular to the surface. The polarizability change of the phenyl breathing vibrational mode is also perpendicular to the silver surface, and results in a high SERS signal as suggested by SERS selection rules.^[46] When $pAsA^-$ is bound to the silver surface in a monodentate fashion, the phenyl group is tilted. The polarizability change is now tipped away from the surface normal and less coupled to the SERS electromagnetic field localized around silver surface, this results in a much weaker SERS signal, even though $pAsA^-$ could be the major species adsorbed on the silver surface. Unlike 4-Mpy where the SERS spectrum changes with respect to peak positions when different tautomers were adsorbed onto a silver surface under different pH,^[47] the different binding structures of $pAsA^-$ and $pAsA^{2-}$ to the silver surface lead to small changes in peak positions, but large changes in peak intensities. This suggests that the changes in the orientation angles do not always cause considerable changes in Raman frequency.^[48]

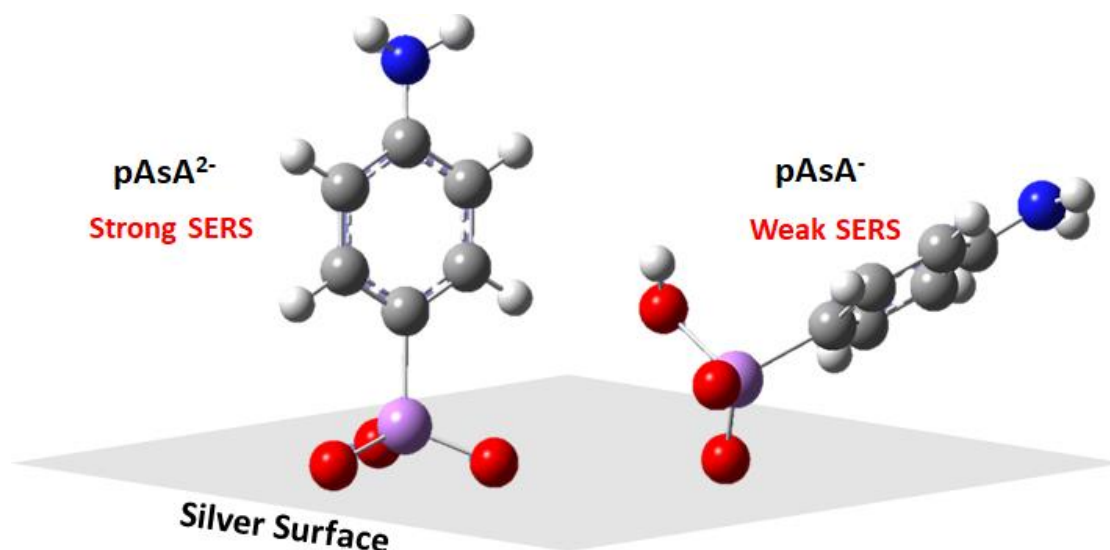


Figure 4.14 Proposed binding structures of $pAsA^-$ and $pAsA^{2-}$ bound onto silver surface.

In addition, the appearance of a para atom or group of the ring structure in the Raman spectrum has always been used as an indicator of a parallel adsorption of a molecule onto a silver surface.^[47, 49] In the aqueous solution of 4-Mpy at lower pH, for instance, the peak at 708 cm^{-1} assigned to $\beta(CC)/\nu(CS)$ and the $\nu(CC)$ at 1612 cm^{-1} associated with the protonated nitrogen atom indicated that sulfur and nitrogen atoms both interacted with the silver surface leading to a flat adsorption of the pyridine ring on the surface.^[47] In our case, however, this seems not necessarily true. As the Raman bands of $pAsA$ become visible, the band at 983 cm^{-1} attributed to $-NH_2$ was also observed but most of the SERS intensity is due to the adsorbed $pAsA^{2-}$, whose benzene ring is oriented perpendicular to the silver surface.

An atomic slab of silver crystal surface cannot be simulated in Gaussian 09. To introduce the silver slabs that can interact with $pAsA$ molecules, we collaborated with

Dr. Fernando P. Sabino and Dr. Anderson Janotti in our department, who we worked with on the further calculation of molecule-surface interaction executed in VASP. Several surface adsorption sites and molecule orientations were explored, considering the bonds of As-O, angles of O-As-O, and distance between Ag-Ag atoms. For $pAsA^-$ and $pAsA^{2-}$, two molecular orientations were considered: (1) the molecule with its long axis perpendicular to the Ag surface (straight configuration); (2) the molecule with its long axis forming an acute angle with the Ag surface (tilted configuration). In both cases, the O in the base of the molecule can bind to the Ag surface in three different configurations: (A) O on the top site, where the O atom is located on the top of an Ag atom (top site); (B) O on the bridge site, where the O atom is located in the middle of the line connecting two nearest neighbor Ag atoms in the same layer, i.e., in the middle of the Ag-Ag chemical bond(bridge site); and (C) O on a hollow site, at the center of a triangle formed by three neighboring Ag atoms (hollow site). These configurations are shown in the **Figure 4.15**.

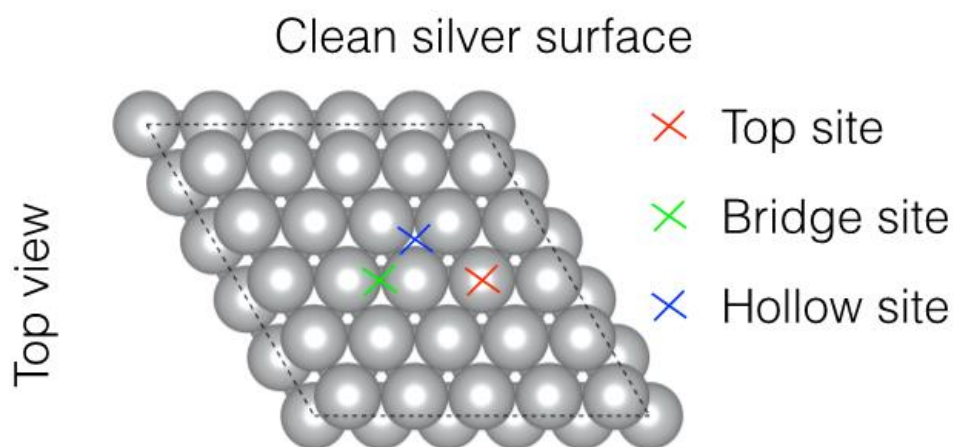


Figure 4.15 Possible adsorption sites of $pAsA^-$ or $pAsA^{2-}$ on the Ag(111) surface. The top site, the bridge site and the hollow site. (Courtesy to Dr. Sanobi and Dr. Janotti)

DFT calculations for $pAsA^-$ and $pAsA^{2-}$ on the Ag(111) surface indicate that both the optimized $pAsA^-$ and $pAsA^{2-}$ tend to adsorb onto the Ag surface through the bottom O atom anchoring to the bridge site, but with two different orientations. The monodentate structure is energetically more favorable for $pAsA^-$ in a tilted configuration, as shown in **Figure 4.16**. The phenyl group of $pAsA^-$ forms an angle of 44° with the surface plane. The tilted configuration for $pAsA^-$ is 151 meV lower in energy than the straight configuration. In contrast, the deprotonated species $pAsA^{2-}$ tends to adsorb in a straight configuration, i.e., the phenyl group is almost perpendicular to the surface, forming an angle of 85° with the surface plane. We find that starting with a tilted configuration, the $pAsA^{2-}$ spontaneously relaxes to the straight configuration. Thus, the small energy difference for $pAsA^{2-}$ on bridge site for straight (0.00 meV) and tilted (1.33 meV) configurations is associated with the similar final optimized structures. The total energy differences between the various configurations tested are listed in **Table 4.5**.

The calculation of the energetically favored orientations of the adsorbed $pAsA^-$ (tilted) and $pAsA^{2-}$ (straight) interacting with the Ag(111) surface matched our experimental observation and spectral interpretation very well. These results provide supporting evidence that, again, the trace amount of $pAsA^{2-}$ bound to the Ag silver contributes most of the SERS signals, even though the tilted $pAsA^-$ could be the majority component that adsorbed onto the substrate.

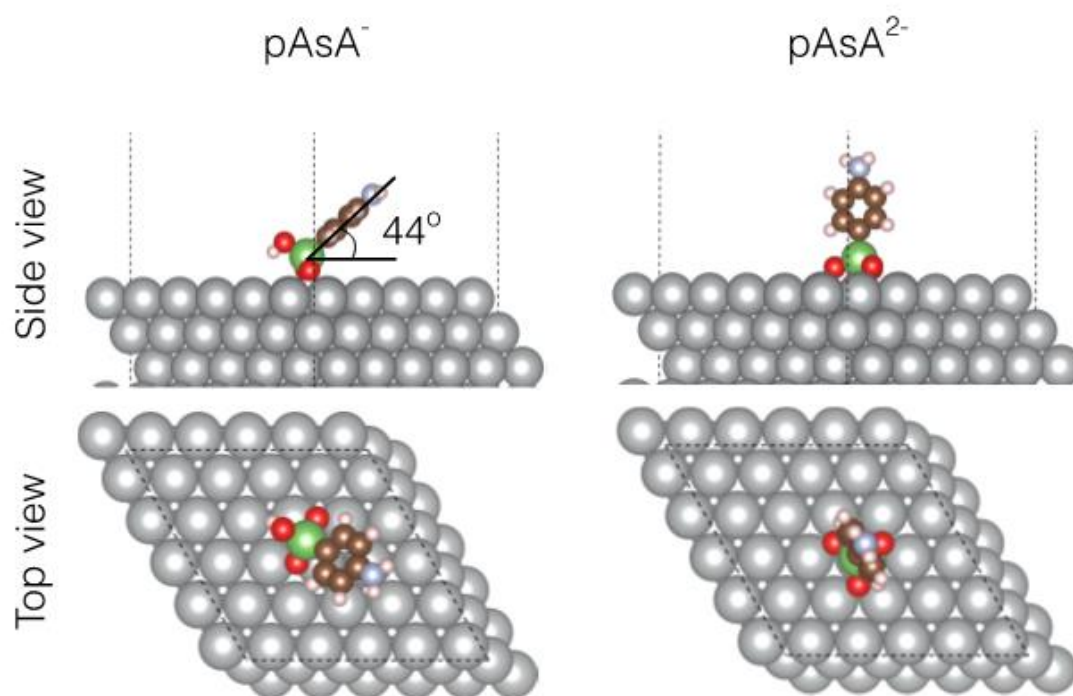


Figure 4.16 Optimized $pAsA^-$ and $pAsA^{2-}$ on the Ag(111) surface. H, O, N, C, As and Ag are represented by the pink, red, blue, brown, green and grey spheres, respectively. (Courtesy to Dr. Sabino and Dr. Janotii)

Table 4.5 Total energy differences for $pAsA^-$ and $pAsA^{2-}$ molecules in different chemical adsorption sites and molecule orientations. (Courtesy to Dr. Sabino and Dr. Janotti)

	Total Energy Differences (meV)					
	$pAsA^-$			$pAsA^{2-}$		
	Top	Bridge	Hollow	Top	Bridge	Hollow
Straight	200.44	151.25	144.15	48.61	0.00	77.12
Tilted	72.93	0.00	23.56	86.43	1.33	86.43

4.3.4 *In-situ* SERS analysis of arsenic

In addition to arsenic quantification and speciation, the Ag/AuNRs-PCL SERS substrate also serves as a platform for *in-situ* observation of arsenic adsorption,

desorption and reduction. The *in-situ* SERS spectra of AsIII and AsV adsorption are shown in **Figure 4.17**. The peak at 725 cm^{-1} was assigned to As-O stretching of AsIII, while a small peak around 785 cm^{-1} was due to the As-O stretching of AsV, coming from partially oxidized AsIII, which can coexist in solution under ambient conditions. As the adsorption time increased, the SERS intensities for AsIII bands reached a maximum within the first 30 minutes and then decreased until reaching a steady state after 80 minutes. The reason for the SERS intensity decrease is not yet clear, and it has been observed during *in-situ* SERS of AsV adsorption as well. Considering the high affinity and solubility of AsV and AsIII to water, we speculate that after fast AsIII adsorption onto the silver surface, further solvation could change the refractive index around the micro-environment of the AsV and AsIII molecules resulting in SERS intensities decreasing over a period of time.

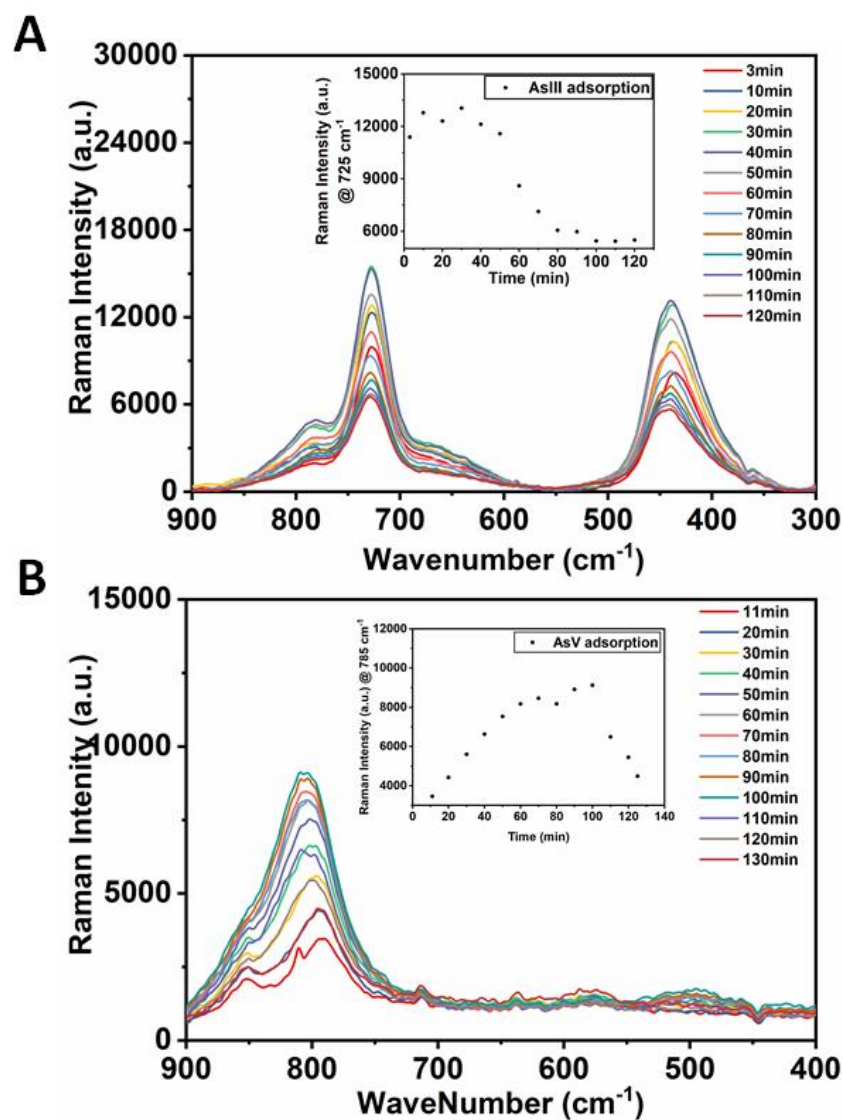


Figure 4.17 *In-situ* SERS spectra of (A) AsIII and (B) AsV adsorption at a concentration of 1×10^{-4} M in DI water as a function of time. Insert: Raman intensity of (A) 725 cm^{-1} and (B) 785 cm^{-1} peaks with time.

As for *in-situ* desorption of AsV, different desorbing agents KH_2PO_4 and NaCl were used to compare the desorption efficiency. As seen in the insert in **Figure 4.18**, H_2PO_4^- desorbed AsV from the SERS substrate more efficiently than Cl^- . More than

80% of the AsV was removed from the substrate by H_2PO_4^- within 30 minutes, while only about 50% of the AsV desorbed from the substrate in the Cl^- case after a 90-minute desorption.

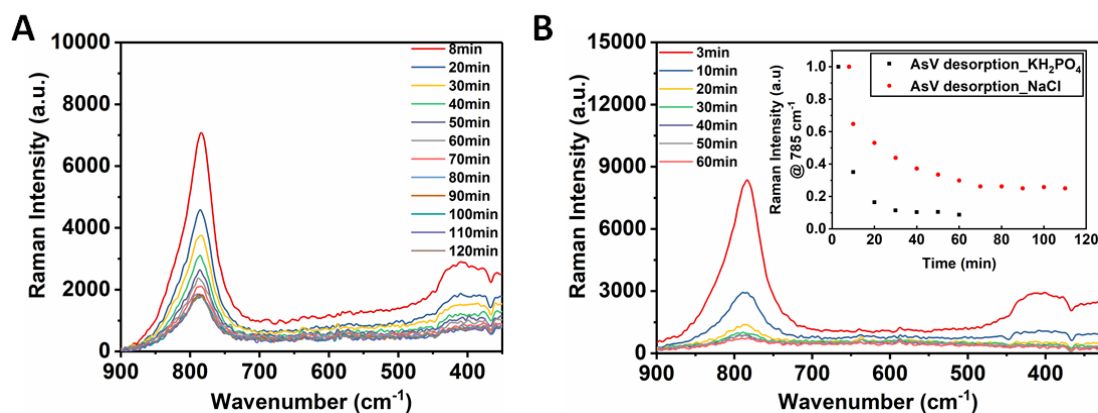


Figure 4.18 *In-situ* SERS of 10^{-4} M AsV desorption in 20 mg/L (A) NaCl and (B) KH_2PO_4 solution. Inset of (B): Raman intensity of AsV peak at 785 cm^{-1} as a function of time illustrating the desorption of AsV in 20 mg/L NaCl and KH_2PO_4 solution, respectively.

As for *in-situ* reduction of Rox by NaBH_4 shown in **Figure 4.19**, the NO_2 symmetric stretching vibration, $\nu_s(\text{NO}_2)$, at 1322 cm^{-1} quickly disappeared within 1 minute and a new peak at 983 cm^{-1} , assigned to NH_2 , showed a gradual increase in intensity with time indicating reduction of the NO_2 by NaBH_4 accompanied by the formation of NH_2 .

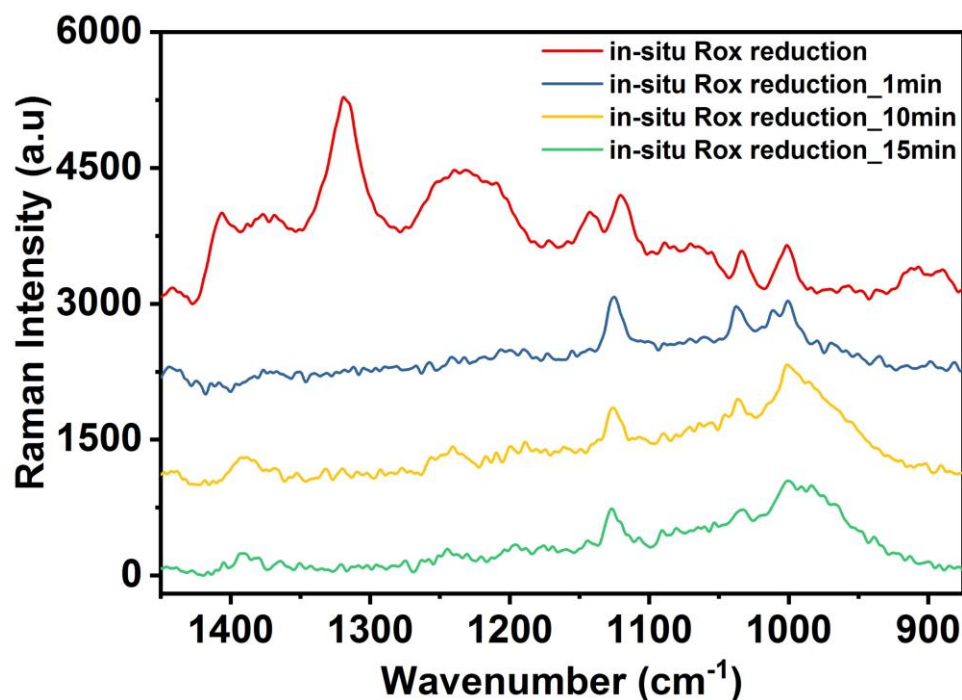


Figure 4.19 *In-situ* SERS spectra of 5×10^{-5} M Rox reduction by 2 mM NaBH_4 .

4.4 Conclusion

In this chapter, we have described the first successful speciation and quantification of the different arsenic species using a Ag/AuNR decorated 3D polymer SERS substrate. A LOD of 4 ppb in DI water and 8 ppb in common salt ionic solution of various arsenic species has been observed. Moreover, these SERS substrates have shown the versatility to investigate arsenic activities: *in-situ* SERS monitoring of arsenic adsorption, desorption and reduction, providing an excellent sensing platform for both fundamental and applied studies. Significantly, a detailed analysis of the SERS spectra of *pAsA* has provided insightful information on the nature of the binding structures of *pAsA* onto the silver surface, from where we can draw the conclusion that $pAsA^-$ and $pAsA^{2-}$ are the *pAsA* tautomers that can effectively be

adsorbed onto the silver surface via formation of As-O-Ag bonds. We also concluded that $pAsA^{2-}$ is the tautomer that contributes the most SERS intensity due to its orientation and chemical interaction with the silver surface. These results were corroborated by DFT calculations for the bonding configurations of $pAsA^-$ and $pAsA^{2-}$ on the Ag(111) surface executed in collaboration with Dr. Sabino and Dr. Janotti. All the results have explicitly indicated that this 3D Ag/AuNRs-PCL SERS substrate is of great potential for being used as an environmental sensor for arsenic analysis.

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Chapter 5

CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

In **Chapter 1** of this dissertation, SERS was introduced as an effective analytical technique and both fundamentals and applications were described. In spite of the great potential of practical use in a wide range of applications, such as chemical, biomedical and environmental detection, the fabrication of high performance SERS substrates is still a major focus of research in the SERS community, decades of development after it was discovered. The lack of reproducibility for SERS substrates has impeded SERS from becoming a routine analytical method. To solve this problem, we have proposed a fabrication strategy involving 3D membranes comprised of SERS-active MNPs and polymeric electrospun fibrous mats, as a novel nanocomposite material for use as a SERS substrate. In the research described in this dissertation, the main goals are: (1) to utilize non-specific electrostatic attraction as the driving force to direct the immobilization of Ag/AuNRs, as SERS-active MNPs, onto electrospun PCL fibrous mats as the 3D Ag/AuNRs-PCL SERS substrate; (2) to evaluate the SERS performance of the Ag/AuNRs-PCL substrate in terms of sensitivity, reproducibility and stability, and extend the fabrication strategy to other materials; (3) to compare the SERS performance of the Ag/AuNRs-PCL substrates and AuNRs-PCL substrate fabricated with the same strategy; and (4) to apply the Ag/AuNRs-PCL SERS substrate to environmental sensing for arsenic detection and speciation.

In **Chapter 2**, the fabrication of Ag/AuNRs-PCL composites have been described in detail. The Ag/AuNRs were prepared by coating a silver layer onto AuNRs synthesized using a seed mediated wet chemical method. By increasing the Ag^+ ion concentration during the silver coating, Ag/AuNRs with different silver coating thicknesses were obtained. To utilize the non-specific electrostatic interaction as the driving force to direct the immobilization of Ag/AuNRs onto PCL electrospun fibrous mat, opposite electrostatic charges were introduced on Ag/AuNRs and electrospun PCL fibers. Ag/AuNRs were further coated with a layer of polyelectrolyte PSS, which is negatively charged, while positive charges were deposited on PCL mats via LbL deposition of a polyelectrolyte pair PDADMAC/PSS leaving the polycation PDADMAC as the outermost layer. By immersing the LbL modified PCL mat in the PSS/Ag/AuNRs solution, Ag/AuNRs were spontaneously adsorbed onto the PCL fiber surface driven by electrostatic attraction. A dense and uniform decoration of Ag/AuNRs covered the PCL fibrous mat and was characterized by UV-vis spectroscopy and SEM. This generalized fabrication strategy for preparing MNPs/polymer composites was further extended to other materials. Ag/AuNRs with different silver coating thicknesses and PHBHx electrospun fibers were also used in production of SERS substrates. By engineering and optimizing the parameters, the fabrication of Ag/AuNRs-PCL substrates turned out to be a highly efficient process. With respect to sensitivity, reproducibility and stability, SERS performance for the Ag/AuNRs-PCL substrates was tested using 4-Mpy as a probe molecule in **Chapter 3**. A detection limit as low as 10^{-8} M and excellent reproducibility with less than 7% RSD among measurements were achieved. In addition, the SERS substrate had a shelf life of over three months with no loss of SERS sensitivity compared to the freshly

prepared SERS substrate. EF were calculated to be 5.1×10^4 for $\nu(\text{ring breathing})$ and 2.4×10^5 for $\nu(\text{ring breathing})/\nu(\text{CS})$ of 4-MPy. Comparison was also made between Ag/AuNRs-PCL substrates having different silver coating thicknesses and AuNRs-PCL substrate. The results showed that the Ag/AuNRs-PCL substrates overall have better SERS sensitivities than AuNRs-PCL substrate, especially for SERS-sensitive vibrational modes of 4-Mpy. This result could be attributed to the larger surface area of Ag/AuNRs compared to that of AuNRs. It has resulted in higher loading of 4-Mpy molecules and the CT between silver and gold in the bimetallic Ag/AuNRs leads to an increase in the local electric field of Ag/AuNRs. The enhancement of a SERS substrate is a function of many factors, which include but are not limited to the SPR properties of MNPs, the type of analyte molecules, the adsorption efficiency of analyte molecules, and the laser excitation wavelength. More systematic studies in the future are required to further elucidate the difference in the SERS performance between Ag/AuNR-based and AuNR-based substrates.

To explore the environmental application of Ag/AuNRs-PCL SERS substrates, in **Chapter 4** we have carried out successful speciation and quantification of arsenic species. Ag/AuNRs provide appropriate surface chemistry for arsenic analysis through the formation of As-O-Ag bonds. A LOD as low as 4 ppb of Rox, *p*AsA and AsV was achieved. Moreover, Ag/AuNRs-PCL substrates have applied to investigate arsenic activities and molecular configuration on a silver surface. *In-situ* SERS during arsenic adsorption, desorption and reduction have been monitored. SERS observation showed that H_2PO_4^- desorbed the AsV more efficiently than Cl^- , and a reduction of Rox from NO_2 to NH_2 by NaBH_4 was complete within 1 minute. In addition, detailed analysis of the SERS spectra of *p*AsA under different concentrations and pH conditions provided

insights on the nature of the binding structures of $pAsA^-$ on the silver surface. $pAsA^-$ and $pAsA^{2-}$ were demonstrated to be effectively adsorbed onto the silver surface but have different molecular configurations interacting with the surface. Such a difference results in different contributions to signal intensities. $pAsA^-$ binds onto the silver surface in a tilted fashion while $pAsA^{2-}$ binds in a fashion normal to the silver surface and contributes most of the observed SERS intensity due to its perpendicular orientation and chemical interaction with the Ag surface. These spectral studies were supported by DFT calculations for the bonding configurations of $pAsA^-$ and $pAsA^{2-}$ on the Ag(111) surface executed in collaboration with Dr. Sabino and Dr. Janotti. It was found that the angle formed from phenyl group of $pAsA$ with silver surface is 44° for $pAsA^-$ but 85° for $pAsA^{2-}$.

In summary, we have successfully completed the major goals that were set for this dissertation work. The results and discussion in the last chapters are strong evidence to show that the 3D Ag/AuNRs-PCL substrates are of great potential for SERS measurements and can serve as an environmental sensor for arsenic analysis. The fabrication of this MNPs/polymer composite membrane is a generalized protocol which can produce SERS substrates with different MNPs for different uses and have significant potential for commercialization.

5.2 Future Work

5.2.1 SERS substrate for biosensing

SERS has been extensively applied to biosensing for rapid detection of biomolecules, such as nucleic acids, proteins, and even more complicated systems like human blood.^[1-5] Most of these applications were achieved using MNPs colloids or

metallic substrates, e.g. gold substrate. Few paper-based SERS substrates have been applied to biosensing. To explore the potential biomedical application of the 3D Ag/AuNRs-PCL SERS substrates, detection of nucleobases was tested as a simple example. The molecular structures of the nucleobases are shown in **Figure 5.1** along with their normal Raman spectra for reference. It is well known that these nucleobases can complex via complementary binding through hydrogen bonding following certain rules: thymine pairs with adenine (T-A) and cytosine pairs with guanine (C-G). The SERS spectra of the four nucleobases and their mixture obtained from the Ag/AuNRs-PCL substrate are shown in **Figure 5.2** as well as the mixed solution of T-A and C-G respectively. Among the four nucleobases, A, C and G have strong interaction with a silver surface, while T has the least affinity to bind to Ag/AuNRs. This observation is presumably due to the lack of participation of nitrogen heterogeneous ring in binding to silver surface, which is consistent with the previous report.^[6] In adenine, for example, there are nitrogen atoms in the ring and amino groups as sites to interact with the silver surface, which has a feature at 741 cm^{-1} assigned to a ring breathing mode, and a band at 1337 cm^{-1} assigned to the ring stretching mode.^[7-8] In the SERS spectra of a nucleobase pair T-A, the ring breathing mode of adenine shifted from 741 cm^{-1} to 737 cm^{-1} , which could result from the formation of hydrogen bonds. Similarly, the ring breathing mode of cytosine shifted from 801 cm^{-1} to 796 cm^{-1} after a complexation with guanine. Overall, the Ag/AuNRs-PCL substrate appears to be a good platform to identify nucleobases for detection of nucleic acids either through direct binding or hybridization with complementary sequences.

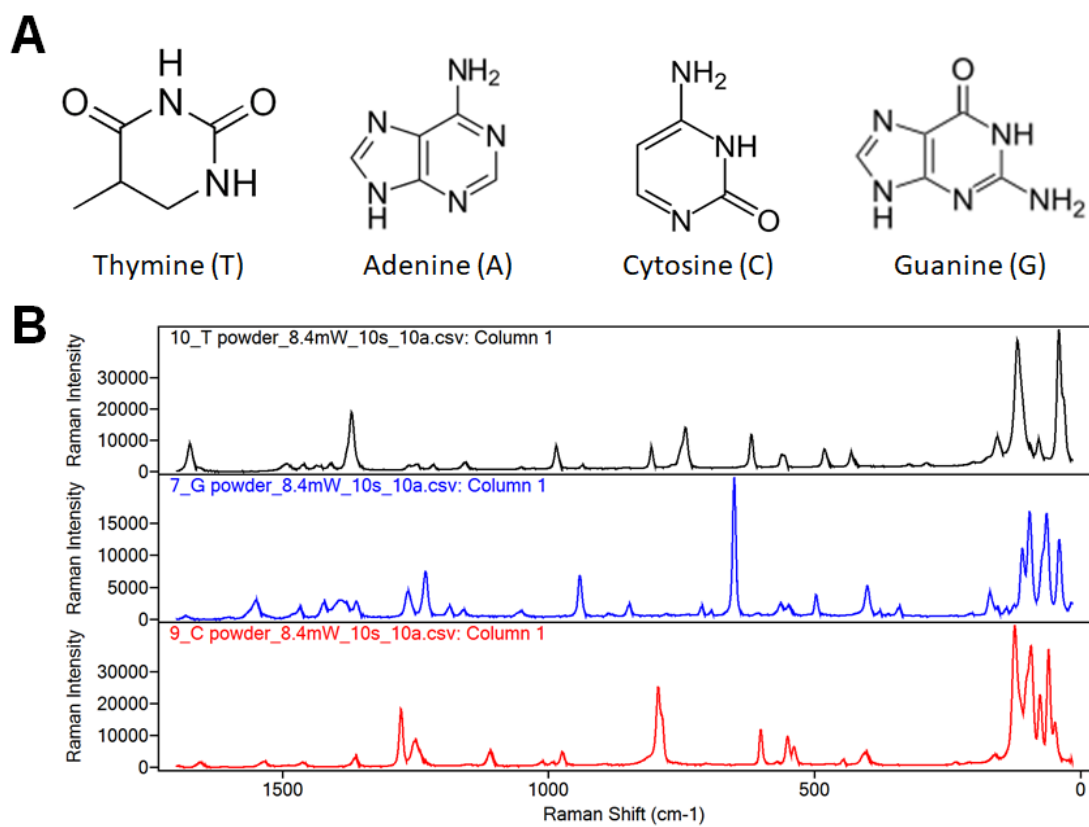


Figure 5.1 (A) Molecular structures of nucleobases: thymine, adenine, cytosine, and guanine. (B) Normal Raman spectra of (from up to bottom): thymine, guanine, cytosine.

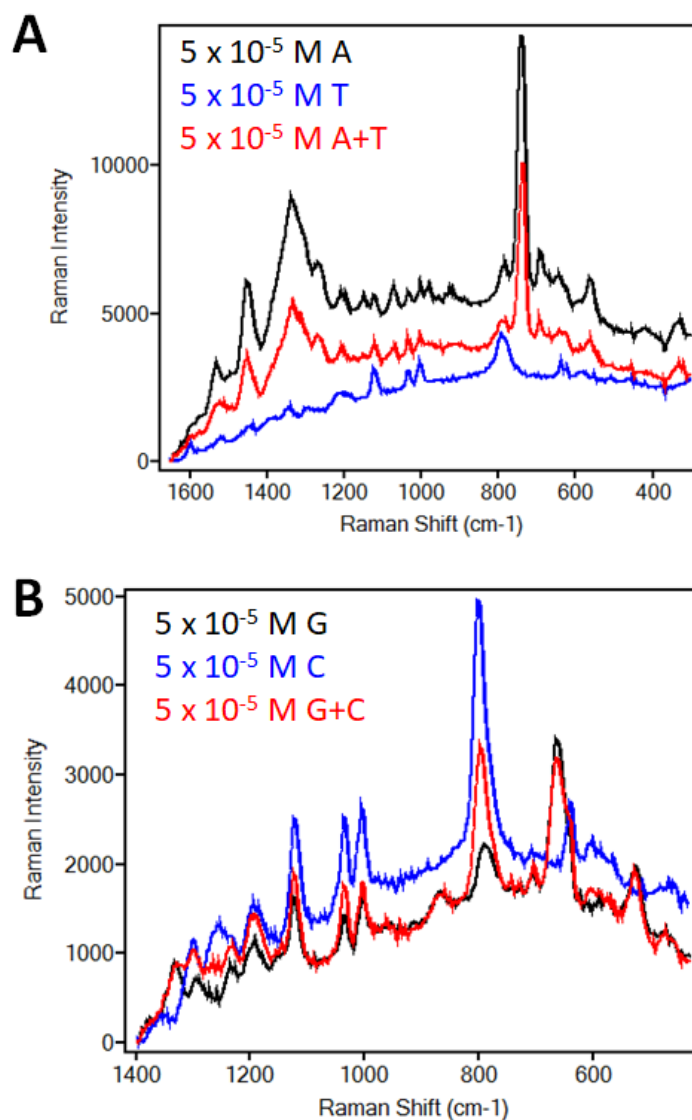


Figure 5.2 SERS spectra of $5 \times 10^{-5} \text{ M}$ (A) adenine, thymine, and adenine/thymine mixture with A:T = 1:1 in molar ratio; (B) guanine, cytosine, and guanine/cytosine mixture with G:C = 1:1 in molar ratio.

Western blot is a widely used protocol involving gel electrophoresis to separate protein mixtures. Followed by SERS, a label-free and effective bioanalytical method can be developed for protein detection.^[9-10] In western blot, a nitrocellulose (NC)

membrane is used for the electrophoresis. After proteins are blotted, silver colloids are stained inside the NC membrane. Our fabrication strategy can be incorporated with western blot, in this case, to produce membranes for the development of “Western-SERS”. Replacing PCL with NC for electrospinning in our SERS substrate fabrication could be an easy trial. Alternatively, MNPs/NC solution blends can be electrospun for membrane fabrication. A few issues need to be taken into consideration. First is the preparation of MNPs/NC solution blends, which need organic solvents. Protected by thiolated poly(ethylene glycol) (PEG) and 1-dodecanethiol (DDT), MNPs are readily transferred from aqueous solution to various organic solvents with the absence of aggregation.^[11] **Figure 5.3A** shows the transfer of AuNRs from aqueous solution to an organic solvent, CHCl_3 , where the dark purple color of AuNRs appears in the CHCl_3 once the transfer is completed. The solvent transfer of AuNRs is stabilized by the combination of PEG and DDT. In the solvent exchange, the AuNRs in the aqueous solutions are first stabilized by the addition of thiolated PEG, which results in no change in the SPR peaks of AuNRs as shown in **Figure 5.3B**. After transferring into the organic solvent, the longitudinal SPR peak of AuNRs does not broaden, indicating negligible aggregation of AuNRs in the CHCl_3 . The red-shift of the AuNRs after solvent exchange results from the refractive index difference between water and CHCl_3 around the AuNRs.

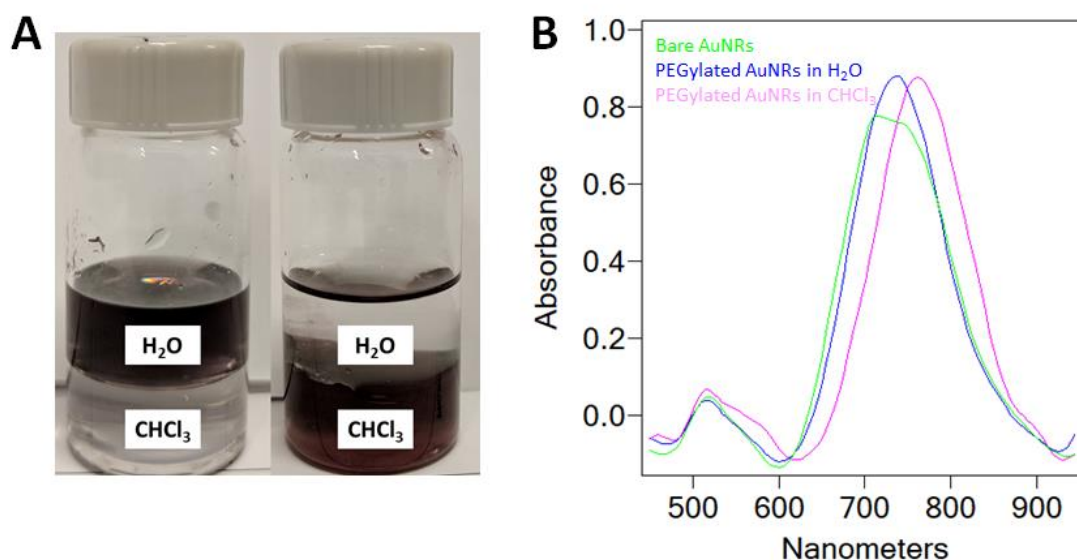


Figure 5.3 (A) Digital photos of AuNRs transferred from aqueous solution (left) to CHCl₃ (right). (B) UV-vis spectra of bare AuNRs (green), PEGylated AuNRs in DI H₂O (blue) and PEGylated AuNRs in CHCl₃ (red).

The second point to consider is the penetration of analytes. One possible solution involves blending some water-soluble polymers in the MNPs/NC solution. By dissolving these polymers back into water, fiber surfaces could be made more permeable to facilitate the penetration of analytes. For instance, dialyzing the electrospun fibrous membrane of PCL/PEG blends (80k/2k, wt:wt = 5:2) in DI water, the removal of short PEG chains can be easily completed. As shown in **Figure 5.4**, the FTIR of electrospun fibrous membrane of PCL/PEG blends contains peaks of both PCL and PEG. The immersion of the membrane in DI water washes off the PEG molecules in the membrane with a loss of PEG peak intensities, such as C-O stretching at 1250 cm⁻¹ and O-H bending at 1350 cm⁻¹.

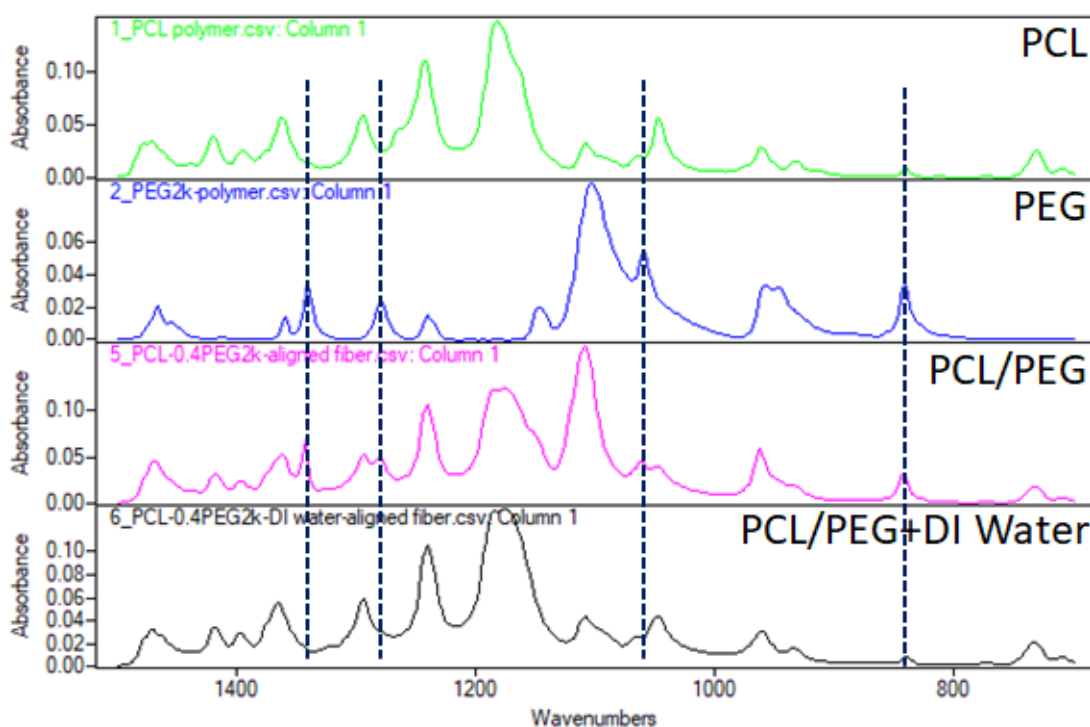


Figure 5.4 The ATR-FTIR spectra of (from top to bottom): PCL, PEG, electrospun membrane of PCL/PEG blends, and electrospun membrane of PCL/PEG blends after immersion in DI water for a week.

5.2.2 Two-dimensional correlation spectroscopy and low-frequency spectroscopy of SERS

In the SERS spectrum of *pAsA* and Rox mixture of **Figure 4.3B**, As-C stretching at 623 cm^{-1} for a *pAsA*/Rox mixture is located between As-C stretching of pure *pAsA* at 611 cm^{-1} and Rox at 628 cm^{-1} . Obviously, the As-C stretching in a *pAsA*/Rox mixture is a combination of As-C of pure *pAsA* and Rox. The competition or synergy in the adsorption of *pAsA* and Rox can be further investigated in the future by studying a series of *pAsA* and Rox mixtures with different concentration ratios. Two-dimensional correlation spectroscopy (2D-COS) is a powerful tool to study the dynamic spectra or a series of spectra under perturbation (e.g., concentration,

temperature, mechanical, *etc*). The basic concept of perturbation-based 2D spectroscopy was first proposed by Dr. Isao Noda in 1986.^[12-14] After decades of evolution, 2D-COS has been developed as an extensively used mathematical tool to analyze vibrational spectra, as well as other types of spectra or even in other analytical methods, e.g. microscopy. Fundamentals and applications of 2D-COS have been discussed in detail in the book written by Dr. Isao Noda and Dr. Yukihiro Ozaki.^[15] 2D-COS largely increases the resolution of spectra by spreading the spectroscopic signals along the 2D dimensions but has no requirement for specialized instrumental or experimental setups. Briefly, 2D-COS consist of two orthogonal components: the synchronous correlation spectrum which indicates how similarly separate peaks change under the perturbation, while the asynchronous correlation spectrum is a measure of the out-of-phase changes in peak signals under the perturbation. Therefore, analysis of synchronous and asynchronous spectra provides details about the perturbation-induced dynamic changes occurring in the system. At the same time, low frequency Raman spectroscopy has raised great interest due to the development of volume holographic gratings in recent years.^[16-17] The vibrational modes detected in the low frequency spectral region directly measure the subtle interactions and long range interactions existing in a system (e.g. weak hydrogen bonding existing in solvents). Often these could give significant information complementary to other characterization techniques, such as neutron scattering. Here, we give an example of how 2D-COS applied to binary solvent mixtures, particularly in the low frequency Raman region. Hopefully, the method will extend to SERS studies on multiplex detection of analytes.

Binary solution mixtures, such as CHCl_3 and EtOH, which are conventionally regarded as simple, almost ideal mixtures, have been examined using 2D-COS. If CHCl_3 and EtOH are entirely miscible, no cross peaks in the asynchronous 2D correlation spectrum should be expected. In reality, however, we proved this is not the case. In **Figure 5.5A**, the Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures were recorded. In the normal Raman spectral region, the peak at 672 cm^{-1} for $-\text{CCl}_3$ stretching increases linearly with increasing CHCl_3 concentration, (**Figure 5.5B**) which indicates the behavior like an ideal solution mixture between CHCl_3 and EtOH with no interactions between the molecules.

Figure 5.5C showed the low frequency region of $\text{CHCl}_3/\text{EtOH}$ mixtures. The central Rayleigh wing contributions arising from the relaxational and rotational diffusion effects still dominates the very low frequency region which hinders the interpretation of the Raman spectra. Reduced Raman spectra defined in the following equation are used to correct and remove the thermal population effects, instrument response, ν^4 scattering and stokes/anti-stokes symmetry.^[18-20]

$$R(\nu) = I(\nu)\nu \left[1 - \exp\left(-\frac{h\nu c}{kT}\right) \right] \quad 5-1$$

where $R(\nu)$ refers to the reduced spectrum, and $I(\nu)$, ν , h , c , k and T represent the Raman intensity, frequency, Planck constant, velocity of light, Boltzmann constant and absolute temperature respectively. **Figure 5.5D** is the reduced spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures obtained from **Figure 5.5C**. Removal of Lorentzian contribution by curve fitting was further applied to reduce the effects of diffusion and relaxation. The resultant spectra are shown in **Figure 5.6A** along with the synchronous and asynchronous spectra obtained after 2D-COS was applied. (**Figure 5.6B-C**) The results indicate a clear deviation from ideal solution behavior, where obvious

asynchronous correlation cross peaks were observed. The presence of asynchronous peaks suggests the presence of some subtle concentration-dependent molecular interactions, such as restricted translation ($\sim 70 \text{ cm}^{-1}$) and libration ($\sim 110 \text{ cm}^{-1}$), resolved from Raman signals processed with 2D-COS.^[21-22]

In summary, the 2D-COS and low-frequency Raman spectroscopy undoubtedly are powerful tools to study molecular interactions in high resolution. A combination of SERS with 2D-COS and low-frequency Raman spectroscopy should be a simple but versatile method to deal with multiple components in the analytes for multiplex detection.

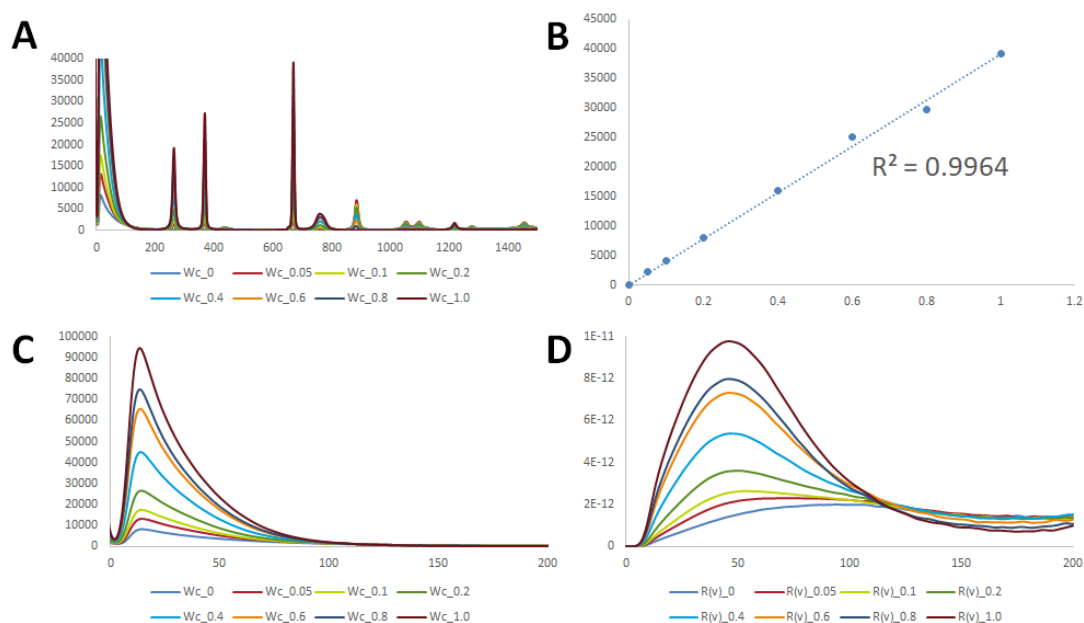


Figure 5.5 (A) Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures with increasing molar concentration of CHCl_3 from 0 (pure EtOH) to 1.0 (pure CHCl_3). (B) Raman intensity of $-\text{CCl}_3$ stretching at 672 cm^{-1} with increasing CHCl_3 content. (C) Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures at low-frequency region. (D) Reduced Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures at low-frequency region.

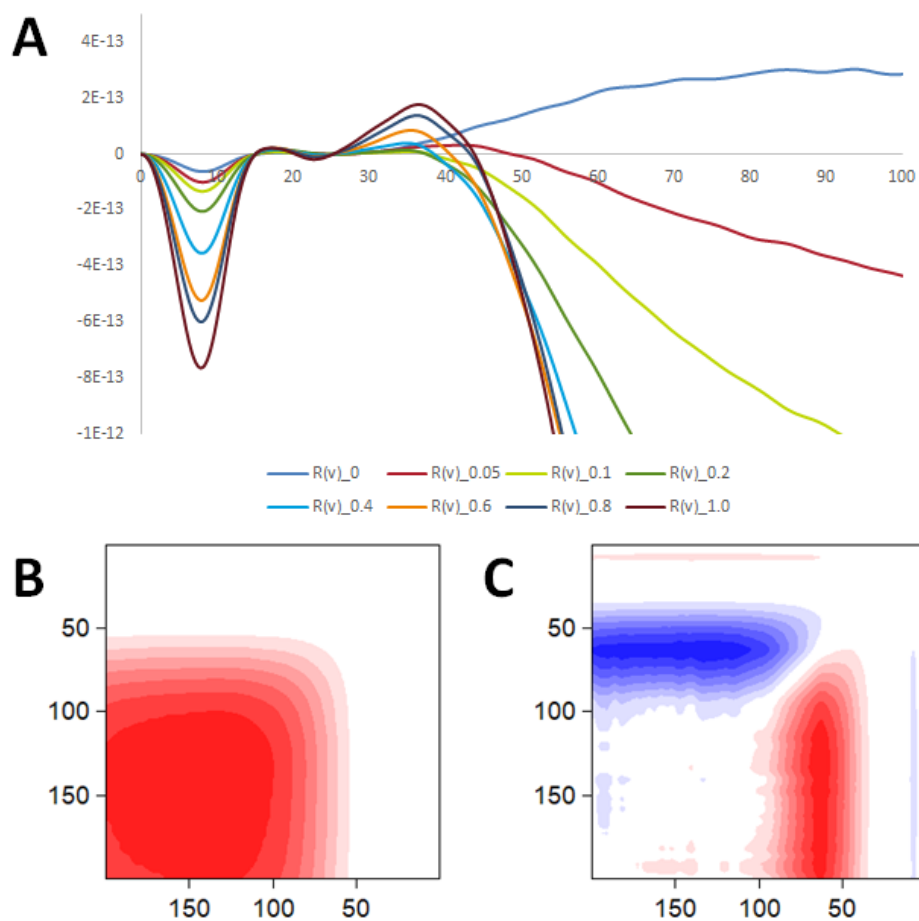


Figure 5.6 (A) Reduced Raman spectra of $\text{CHCl}_3/\text{EtOH}$ mixtures after removal of Lorentzian contribution. (B) Synchronous and (C) asynchronous spectrum of serial spectra in (A).

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Appendix

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