## USE OF GOLD NANOPARTICLE CONTAINING POLY (ALLYLAMINE HYDROCHLORIDE)/ POLY (ACRYLIC ACID) MULTILAYER FILMS AS SERS SUBSTRATES

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**BİRSU KÖLEMEN** 

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#### Approval of the thesis:

## USE OF GOLD NANOPARTICLE CONTAINING POLY (ALLYAMINE HYDROCHLORIDE)/ POLY (ACRYLIC ACID) MULTILAYER FILMS AS SERS SUBSTRATES

Submitted by **BİRSU KÖLEMEN** in partial fulfillment of the requirements for the degree of **Master of Science in Chemistry Department, Middle East Technical University** by,

Prof. Dr. Mevlüde Gülbin Dural Ünver Dean, Graduate School of Natural and Applied Sciences Prof. Dr. İlker Özkan Head of Department, Chemistry Assoc. Prof. Dr. Gülay Ertaş Supervisor, Chemistry Dept., METU Assist. Prof. Dr. İrem Erel Göktepe Co-Supervisor, Chemistry Dept., METU **Examining Committee Members:** Prof. Dr. Şefik Süzer Chemistry Dept., Bilkent University Assoc. Prof. Dr. Gülay Ertaş Chemistry Dept., METU Assist. Prof. Dr. İrem Erel Göktepe Chemistry Dept., METU Assoc. Prof. Dr. Emren Nalbant Esentürk Chemistry Dept., METU Assoc. Prof. Dr. Ali Çırpan Chemistry Dept., METU

Date: 25.03.2015

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Birsu Kölemen

Signature:

## ABSTRACT

## USE OF GOLD NANOPARTICLE CONTAINING POLY (ALLYLAMINE HYDROCHLORIDE)/ POLY (ACRYLIC ACID) MULTILAYER FILMS AS SERS SUBSTRATES

Kölemen, Birsu

M.S., Department of Chemistry Supervisor: Assoc. Prof. Dr. Gülay Ertaş Co-supervisor: Assist. Prof. Dr. İrem Erel Göktepe

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Surface-enhanced Raman spectroscopy (SERS) is a surface sensitive vibrational spectroscopy technique that results in the enhancement of Raman scattering by molecules adsorbed on rough metal surfaces. It is a powerful, sensitive and nondestructive technique which is used in chemical, material and life sciences including biosensing, catalysis, spectroelectrochemistry, criminology, single molecule SERS applications and many others. Nanoparticle (NP) embedded multilayer films using Layer by Layer (LbL) technique is one of the types of SERS substrates that have unique properties. Herein, pH-controllable exponentially grown (rougher) and linear (Poly(allylamine hydrochloride)/Poly(acrylic grown (smoother) acid)) polyelectrolyte-NP multilayer film systems were studied for sensing benzenethiol (BT) molecule (up to  $10^{-7}$ M). The effects of number of polyelectrolyte layers and deposition times of gold (Au) NPs on SERS signals were studied and the enhancements in Raman signals are similar for all the studied systems. Characterizations of these surfaces are made by UV-vis Spectrometer, Zeta sizer, Atomic Force Microscope (AFM) and Raman Spectrometer.

**Keywords:** Surface-enhanced Raman spectroscopy, Layer-by-Layer technique, SERS substrate, pH-controllable polyelectrolyte.

# ALTIN NANOPARÇACIK İÇEREN POLİ (ALLİLAMİN HİDROKLORÜR)/ POLİ (AKRİLİK ASİT) ÇOK KATMANLI FİLMLERİN YGRS ALTTAŞI OLARAK KULLANILMASI

Kölemen, Birsu

Yüksek Lisans, Kimya Bölümü Tez Yöneticisi: Doç.Dr. Gülay Ertaş Ortak Tez Yöneticisi: Yrd. Doç.Dr. İrem Erel Göktepe

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Yüzeyde-güçlendirilmiş Raman spektroskopisi (YGRS) yüzeye hassas titreşimsel spektroskopi tekniği olup pürüzlü metal yüzeye tutunan moleküllerin Raman saçılmalarını arttırmayla sonuçlanır. Kimya, malzeme ve canlı bilimlerinde (biyosensör, kataliz, spektroelektrokimya, kriminoloji, tek molekül YGRS vb.) kullanılan güçlü, hassas ve zararsız bir tekniktir. Katman katman kaplama tekniği (KKK) kullanılarak hazırlanan nanoparçacık (NP) gömülü çok katmanlı filmler özgün özelliklere sahip bir çeşit YGRS alttaşıdırlar. pH kontrollü üssel büyüyen (daha pürüzlü) ve lineer büyüyen (daha düz) (Poli(allilamin hidroklorür)/Poli(akrilik asit)) polielektrolit-nanoparçacık çok katmanlı film sistemleri ile benzentiyol molekülünün 10<sup>-7</sup>M'a kadar kantitatif olarak Raman sinyallerinin ölçülebileceği gösterildi. Bu sistemler için, YGRS sinyallerine, polielektrolit katman sayısı etkisi ve altın (Au) NP kaplama süresi etkisi incelenmiş ve çalışılan filmlerde benzer sinyal artışları hesaplanmıştır. Bu sistemler, UV-görünür Bölge Spektrometresi, Zetasizer, Atomik Kuvvet Mikroskobu (AKM) ve Raman Spektrometresi ile karakterize edilmiştir.

Anahtar Kelimeler: Yüzeyde-güçlendirilmiş Raman spektroskopisi, Katman-Katman Kaplama Tekniği, YGRS alttaşı, pH kontrollü polielektrolit.

To my princess Sesim,

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## **TABLE OF CONTENTS**

ABSTRACTv				
ÖZvii				
ACKNOWLEDGEMENTS x				
TABLE OF CONTENTS xii				
LIST OF FIGURES xiv				
CHAPTERS				
LIST OF ABBREVATIONS				
1 INTRODUCTION				
1.1 Raman Spectroscopy1				
1.1.1 Raman Selection Rules				
1.2 Surface Enhanced Raman Spectroscopy (SERS)				
1.2.1 SERS Enhancement Mechanisms				
1.2.2 SERS Substrates7				
1.3 Polyelectrolytes				
1.4 Nanoparticles 11				
1.4.1 Synthesis of Gold Nanoparticles				
1.5 Nanoparticle Embedded Polyelectrolyte Films				
1.6 Applications of SERS 15				
1.6.1 PE-NP Films for SERS Applications 15				
1.7 Aim of the Study 17				
2 EXPERIMENTAL PART				

	2.1	Materials19			
	2.2	Polyelectrolyte Multilayer Formation: LbL Technique19			
	2.3	Synthesis of Au Nanoparticles			
	2.4	Coating of Polyelectrolyte Films with Au NPs21			
	2.5	Characterization Techniques			
3	RE	SULTS AND DISCUSSION			
	3.1	Characterization of Spherical Au NPs			
	3.2	The Multilayer Films of (PAH/PAA) <sub>n</sub> 25			
	3.3	Multilayer Growth			
	3.4	UV-vis Spectroscopy of Citrate Capped Au NPs on (PAH/PAA) <sub>n</sub> Films			
	with I	Different Deposition Periods for Exponentially and Linear Grown Systems27			
	3.5	AFM Measurements			
	3.5.	1 AFM of Exponential System			
	3.5.	2 AFM of Linear System			
3.6 SERS of Citrate Capped Au NPs Containing (PAH/PAA) <sub>n</sub> Fi					
	rent Deposition Periods for Exponentially and Linear Grown Systems36				
	3.6.	1 Reproducibility of SERS Signals41			
	3.6.	2 Enhancements of Raman Signals for Different Concentrations of BT			
	vers	sus Neat Raman Signal43			
	3.7	SERS Maps on Linear and Exponentially Grown Films43			
4	CO	NCLUSIONS47			
R	REFERENCES				

## **LIST OF FIGURES**

## FIGURES

Figure 1. Elastic (Rayleigh) and inelastic (Raman) scattering. Energy levels of				
scattering processes (a), Raman and Rayleigh spectra (b)2				
Figure 2. Electromagnetic enhancement factors (in log scale) of dimers (left and				
middle) and isolated single (right) silver nanoparticles. Copyright $\ensuremath{\mathbb{C}}$ 2000, American				
Physical Society. Reprinted with permission from ref (25). <sup>25</sup>				
Figure 3. Tunable LSPRs of Cu, Au and Ag NPs. Copyright © 2012, Elsevier.				
Reprinted with permission from ref (16). <sup>16</sup>				
Figure 4. Langmuir Blodgett Technique				
Figure 5. Layer by Layer Technique				
Figure 6. (PAH/PAA) LbL films at various pHs 10				
Figure 7. Possible surface modifications of Au NPs				
Figure 8. Golden Timeline Copyright © 2013, American Physical Society.				
Reprinted with permission from ref (117). <sup>117</sup>				
Figure 9. Number of SERS papers as a function of time. Copyright $\mathbb O$				
2012, Elsevier. Reprinted with permission from ref (16). <sup>16</sup>				
Figure 10. LbL grown $(PE/NP)_n$ film preparation with desired number of layers (a).				
SER spectra of LbL grown PE/NP film with 1, 3, 5, 7 and 11 layers (b). Copyright				
$\ensuremath{\mathbb{C}}$ 2005, American Chemical Society. Reprinted with permission from ref (158) <sup>158</sup> .				
Figure 11. Chemical structures of PAH (a) and PAA (b)				
Figure 12. Schematic representation of adsorbed Au NPs on $(PAH/PAA)_n$				
multilayer films				
Figure 13. UV-Vis spectrum of Au NPs				
Figure 14. Hydrodynamic size distributions of Au NPs by Dynamic Light Scattering				
method for 6 measurements				
Figure 15. Zeta potentials of Au NPs for 6 measurements				

Figure 16. Functional groups of PAH (pH= 6.5), PAA (pH= 4.0) polyelectrolytes.26					
Figure 17. Thickness values of the exponential grown $(PAH/PAA)_n$ multilayer					
films					
Figure 18. UV-vis absorption spectra of Au NPs absorbed on exponentially grow					
$(PAH/PAA)_n$ multilayer films with increasing number of layers (L). Deposition time					
of Au NPs (a) 12h (b) 6 h (c) 2 h28					
Figure 19. UV-vis absorption spectra of Au NPs absorbed on linear grown					
(PAH/PAA) <sub>n</sub> multilayer films with increasing number of layers. Deposition time of					
Au NPs (a) 12h (b) 6 h (c) 2 h					
Figure 20. 2D (a) and 3D (b) images of (PAH/ PAA) <sub>9</sub> exponential system without					
<i>Au NPs</i>					
Figure 21. 2D (a) and 3D (b) images of (PAH/ PAA) <sub>29</sub> exponential system without					
Au NPs. The loop and tail structure of (PAH/PAA)29 can be seen for both small					
$(5x5\mu m^2)$ and large $(25x25\mu m^2)$ surface area					
Figure 22. 2D (a) and 3D (b) images of (PAH/ PAA) <sub>9</sub> exponential system with Au					
<i>NPs</i>					
Figure 23. 2D (a) and 3D (b) images of (PAH/ PAA) <sub>29</sub> exponential system with Au					
<i>NPs</i>					
Figure 24. 2D (a) and 3D (b) images of (PAH/PAA) <sub>9</sub> linear system <i>without Au NPs</i> .					
Figure 25. 2D (a) and 3D (b) images of (PAH/PAA) <sub>29</sub> linear system without Au					
<i>NPs</i>					
Figure 26. 2D (a) and 3D (b) images of (PAH/PAA) <sub>9</sub> linear system with Au NPs34					
Figure 27. 2D (a) and 3D (b) images of (PAH/PAA) <sub>29</sub> linear system with Au NPs. 34					
Figure 28. 2D (a) and 3D (b) images of (PAH/PAA) <sub>39</sub> linear system without Au					
<i>NPs</i> 35					
Figure 29. 2D (a) and 3D (b) images of (PAH/PAA) <sub>49</sub> linear system without Au					
<i>NPs</i>					
Figure 30. 2D (a) and 3D (b) images of (PAH/PAA) <sub>39</sub> linear system with Au NPs. 36					
Figure 31. 2D (a) and 3D (b) images of (PAH/PAA) <sub>49</sub> linear system with Au NPs.36					
Figure 32. SER spectra of 1.0 x 10 <sup>-5</sup> M BT molecule on Au NP containing					
exponentially grown $(PAH/PAA)_n$ multilayer films with different Au-NP deposition					
times ((a) 12h (b) 6 h (c) 2 h). Spectra are shifted vertically for clarity					

# LIST OF ABBREVATIONS

Au NP	Gold nanoparticle
BT	Benzenethiol
СМ	Chemical Mechanism
DI	Deionized
EM	Electromagnetic Mechanism
L	Layer
LbL	Layer by Layer
PAA	Poly (acrylic acid)
РАН	Poly (allylamine hydrochloride)
PE	Polyelectrolyte
Ra	Roughness
SERS	Surface Enhanced Raman Spectroscopy

## **CHAPTER 1**

## INTRODUCTION

#### 1.1 Raman Spectroscopy

When a radiation strikes a surface, it can interact with the atoms and/or molecules in various ways. These particles (atoms/molecules) can absorb (UV and IR light) or scatter the incoming light.<sup>1</sup> When light is scattered from a molecule, the scattered photons have the same energy as the incident photon mostly (elastically scattered radiation or Rayleigh scattering). However, small fraction of the scattered radiation have different energy (or wavelength) compared to incident radiation, which causes wavelength shift depending on the chemical structure of the molecule. This inelastic scattering is called Raman scattering as it was discovered by C. V. Raman in 1928.

Raman shift is basically the energy difference between the incident and scattered light as shown by the equation below;

$$\Delta E = (1/\lambda_{incident}) - (1/\lambda_{scattered})$$

where  $\Delta E$  is the Raman shift in cm<sup>-1</sup>,  $\lambda_{incident}$  is the wavelength of incoming light in centimeters and  $\lambda_{scattered}$  is the wavelength of scattered light in centimeters.<sup>2</sup>



Figure 1. Elastic (Rayleigh) and inelastic (Raman) scattering. Energy levels of scattering processes (a), Raman and Rayleigh spectra (b).

Raman scattering is mainly divided into two sub-groups, which are Stokes and anti-Stokes scattering. When the scattered radiation is at lower frequency than the excitation radiation, it is called Stokes scattering. In other words, in the case of Stokes scattering molecule is excited from its lower vibrational energy level (V = 0) and relaxes back to higher energy levels at its ground state (V=1) as shown in Figure 1. In anti-Stokes Raman, the scattered radiation is at a higher frequency than the source radiation. This time, excitation occurs from the higher energy levels followed by relaxation to lowest energy level (V=0) as shown in Figure 1. Since more molecules are found in the lowest energy level of ground state (V=0) than the higher energy levels, the intensity of anti-Stokes line is lower than the intensity of Stokes line. Thus, Stokes scattering is mostly measured in Raman spectroscopy.<sup>3,4</sup>

## 1.1.1 Raman Selection Rules

(a)

Raman scattering and infrared absorption are both results from the quantized vibrational changes and their spectra resemble to each other with intensity differences. In contrast to IR spectroscopy, Raman spectra of aqueous samples can be obtained without having water interference. Additionally, glass substrate can be

used because it is also Raman inactive. These two complementary techniques differ from each other by selection rules, which make some molecules IR-active and the other ones Raman-active. For a molecule to be IR-active there must be a net dipole moment change during vibration motions. For instances, asymmetric stretching of  $CO_2$  has net dipole moment, which is the result of strong band in the IR spectrum of  $CO_2$  at 2350 cm<sup>-1</sup>. On the other hand, symmetric stretching of the same molecule is not IR-active due to the lack of permanent dipole.<sup>5</sup>

For a molecule to be Raman-active, the polarizability of the molecule must change during the vibration motions. It is a momentary distortion of electrons (temporarily polarized), which creates an induced dipole that disappears upon relaxation. Thus, in contrast to IR, symmetric stretching of  $CO_2$  is Raman active because of change in its polarizability by incoming radiation.<sup>3</sup>

Raman spectroscopy offers easy/simple instrumentation with a fast measurement time. It is a non-destructive technique. Furthermore, water has low Raman scattering which enables measurement of aqueous samples.<sup>4,6,7</sup> Despites its advantages, it has smaller cross section  $(10^{-29}-10^{-32} \text{ cm}^2)$  compared to fluorescence  $(10^{-16} \text{ cm}^2)$ .<sup>8-10</sup> The low intensity of Raman spectra, low intensity of excitation sources and the sensitivity of the detector have blocked the applications of Raman spectrometry for many years. The discovery of surface enhanced Raman scattering brings a new sight to Raman scattering by increasing its sensitivity, which becomes useful in many research areas.<sup>11</sup>

#### **1.2** Surface Enhanced Raman Spectroscopy (SERS)

SERS is quite useful for the analysis or detection of various analytes at very low concentrations (~ $10^{-7}$  M and less) as a result of enhanced signals, which have low sensitivity in Raman signals. First example of SERS was performed by Fleischmann *et al.* in 1974 for the analysis of pyridine, which adsorbed onto a roughened silver electrode. The Raman intensity of pyridine was enhanced by  $10^{5}$ - $10^{6}$  factors. At that time, it was thought that the large surface area on the roughened metal caused that enhancement because there was more space on the surface for more pyridine molecules to adsorb.<sup>12</sup> Additionally, Van Duyne *et al.*<sup>13</sup> and Creighton *et al.*<sup>14</sup> found out that there should be some enhancement mechanism behind this dramatic

increase in Raman signal. These mechanisms will be discussed in detail in the following sections. Besides, the molecules having lone pair of electrons and  $\pi$ -electron clouds (double bond) have strong SERS because of the strong electromagnetic field due to the oscillations of electrons. These molecules are aromatic compounds having nitrogen and oxygen group and carboxylic acids.<sup>15</sup> Although SERS is a very powerful surface technique and has many applications, it has few drawbacks. Firstly, the enhancement is limited to metal substrates such as Ag, Au and Cu which have extinctions (absorption and scattering) at visible and near IR regions where Raman measurements mostly occur. Ag and Au are more commonly used than Cu because they have better air stability.<sup>16</sup> Secondly, the enhancement mechanisms behind SERS are not fully understood. Last but not least, it is difficult to produce a perfect substrate that satisfies all the needs.<sup>17</sup>

#### **1.2.1 SERS Enhancement Mechanisms**

Typically there are two suggested enhancement mechanisms, which are electromagnetic (primary) enhancement and chemical enhancement. The first one is caused by the surface plasmon resonance generated on the roughened metal surface and the second one is the result of the analyte chemisorption on a substrate.<sup>18</sup>

#### **1.2.1.1 Electromagnetic Mechanism (EM)**

The electromagnetic enhancement of Raman scattering is directly related with surface plasmons (electromagnetic waves), which are generated by valence electron oscillations of metal at metal-dielectric interface. If these surface plasmons are confined into nanosized metal structures, they are called as localized surface plasmons (LSPs) and their excitation is mostly utilized in SERS.<sup>19-21</sup> Localized surface plasmon resonance (LSPR) is the excitation of surface plasmons by the incoming radiation. Upon irradiation of metallic clusters at their LSPRs, the adsorbed molecules on particular metallic structures, which are close to the surface are affected from the resulting electromagnetic field that causes polarization of the molecule. This polarization leads to a new plasmon surface field which couples with Raman scattered photon. Hence, the intensity of Raman signals increases.<sup>8,22</sup> The EM neither depends on the nanoparticle-molecule interactions on the surface nor adsorption type of the molecule. It is related with the distance between the molecule

and the nanoparticle and the size of the nanoparticles. Very small nanoparticles (2 nm) do not exhibit Raman signal enhancement.<sup>15,23,24</sup> Electromagnetic enhancements (in log scale) of dimer versus isolated single Ag nanoparticles (NP) were compared in Figure 2.<sup>25</sup> It shows that single nanoparticles do not contribute very much to the signal enhancement, whereas at highly localized regions (hot spots) in the junctions between two particles, very high enhancement factors emerge. A very close spacing between nanoparticles (hot spots) is favorable for large enhancements. When Raman-active molecules are between these hot spots, their Raman signals increase dramatically with an enhancement factor of  $10^9$ - $10^{11}$ . Silver nanoparticles are plasmonically more active than gold nanoparticles. However, gold nanoparticles (Au NPs) are more inert than silver nanoparticles. Hence, the choice of the convenient metallic nanoparticle depends on the type of analysis that is going to be performed by SERS.<sup>26</sup> Besides, aggregation of nanoparticles affects the enhancement in Raman signals. For instance enhancement in Raman signal of benzenethiol molecule differs from each other with respect to the aggregated Au colloids with different diameters. 46 nm particles give larger enhancements than either 146 nm or 21 nm particles.<sup>27</sup> Moreover, many researchers have reported that anions can enhance Raman scattering by the anion induced aggregation of metal nanoparticles. Nie and coworkers have reported that the addition of chloride ions to silver colloid allows single molecule detection of Rhodamine 6G (R6G) by increasing electromagnetic field. Nie and co-workers also studied the relationship between excitation wavelength and nanoparticle size. They used 488, 568, 647 nm laser lines to excite LSPR of silver nanoparticles at different sizes (7 nm, 140 nm, and 190-200 nm) and concluded that size of the nanoparticles are related with the excitation wavelength as a result of the different intensities of SERS signals. Thus, enhancement from the nanoparticles depends on the excitation wavelength because of the different LSPR values of different sized nanoparticles. As the size of nanoparticles increases, electromagnetic enhancement increases to a certain limit.<sup>27-32</sup>



**Figure 2.** Electromagnetic enhancement factors (in log scale) of dimers (left and middle) and isolated single (right) silver nanoparticles. Copyright © 2000, American Physical Society. Reprinted with permission from ref (25).<sup>25</sup>

#### 1.2.1.2 Chemical Mechanism (CM)

The chemical enhancement is due to a charge transfer between the metal and the adsorbed molecule. It has less contribution than EM to the Raman signal because it requires direct contact (at atomic levels) of the molecule and metal surface.<sup>21,33</sup> CM contributes to the signal enhancement only 10 or 100. This small contribution becomes effective in combination with EM enhancement by multiplication which is totally  $10^4$ - $10^7$ . The enhancement factor (EF) is calculated using the following formula:<sup>22,33</sup>

$$EF = (I_{SERS} x C_{ORD}) / (I_{ORD} x C_{SERS})$$

where; ORD: Ordinary Raman, I: Intensity, C: Concentration<sup>24</sup>

The CM enhancement is not well understood but it provides important information such as the mechanism of interaction with the nanoparticles and the orientation of the adsorbate with respect to the surface. When there is a shift in SER spectrum compared to the corresponding Raman spectrum, this is attributed to the chemical enhancement mechanism.<sup>34</sup> In some cases EM cannot justify the enhancement mechanism but CM enhancement plays major role. For instance, the enhancement in

 $N_2$  and CO signals are different from each other by 200 times.<sup>22</sup> This difference is due to CM because their polarizabilities are similar and EM is chemically non-selective. Moreover, Campion *et al.* showed that 30 times enhancement of pyromellitic dianhydride adsorbed on flattened Cu (111) surface caused by CM because the electromagnetic contributions of the surface is small (flat surface) and well-understood.<sup>35</sup>

#### 1.2.2 SERS Substrates

It is important in SERS to have versatile substrates. An effective substrate requires some properties which are mainly high enhancement factor, inertness, reproducibility of the signal from that surface, and ease of preparation. Researchers have been trying to find out optimal SERS substrate for many years. Mostly employed substrates are Ag, Au and Cu colloidal nanoparticles since they have tunable LSPR values ranging from visible to near-IR regions and higher electromagnetic enhancement compared to other metals which is shown in Figure 3.<sup>16</sup>



**Figure 3.** Tunable LSPRs of Cu, Au and Ag NPs. Copyright © 2012, Elsevier. Reprinted with permission from ref (16).<sup>16</sup>

There have been various types of substrates used for SERS which are roughened (by REDOX process) metal electrodes,<sup>36-39</sup> colloidal nanoparticles with desired shapes and sizes,<sup>40-49</sup> and SERS active metal surfaces with controllable roughness and thicknesses by lithographic (electron beam) techniques.<sup>19,50-54</sup> Although, colloidal nanoparticles are easily prepared and mostly applied, they usually aggregate after analyte addition.<sup>26,51,55-58</sup> This avoids reproducibility of SER spectra. Therefore,

solid substrates (silicon wafer, glass, ITO coated glass etc...) with immobilized nanoparticles are highly preferred to address this type of aggregation problem.<sup>59-61</sup> To that end, in order to fabricate solid substrate-nanoparticle assemblies, Langmuir Blodgett<sup>62-66</sup> and Layer by Layer<sup>67-70</sup> techniques have been extensively used.

### **1.2.2.1 Fabrication Methods of SERS Substrates**

*Langmuir Blodgett (LB) Films:* LB technique is used in the formation of onedimensional ordered monolayers on water surface which are then moved onto solid substrates.<sup>71</sup> LB film deposition scheme can be seen in Figure 4. There are four steps in general. At first step, organic ligand is functionalized with alkyl chains for their organization on air/water interface. Then, a small portion of organic ligand is dispersed on nanoparticle (aqueous sub phase) surface. After that, an interfacial coordination reaction happens between the nanoparticles in the aqueous sub phase and the organic ligands at air/water interface. Lastly, the ultrathin coordination film is transferred to a flat solid substrate by immersing the substrate to the aqueous sub phase.<sup>72</sup> The advantages of these technique are: formation of uniform ultra-thin nanoparticle film with controllable individual layer thickness, controllable electrical and optical properties of nanoparticle and large scale production of films with low cost.<sup>73</sup> Despite its advantages since LB films are defective and LB is not a simple and not a versatile technique, layer by layer (LbL) film deposition technique becoming more commonly used.<sup>74</sup>



Figure 4. Langmuir Blodgett Technique.

LbL films: After the pioneering studies of R. K. Iler<sup>75</sup> in which multi-layers of oppositely charged microparticles were fabricated, Gero Decher and co-workers<sup>76</sup> introduced the formation of multilayer films using oppositely charged polymers (polyelectrolyte (PE)). The LbL deposition of polyelectrolytes and inorganic nanoparticles began very soon after the development of these films with the aim of producing hybrid films with controllable optical, mechanical and electronic

features.<sup>77</sup> LbL technique enables deposition at room temperature with various layer thicknesses by controlling the parameters such as pH, amount of the salt, concentration and molecular weight of polyelectrolyte and type of the solvent. Various substrates such as glass, silicon wafers, quartz, and ITO coated glass can be used for various applications. LbL ultrathin films are used in various applications and areas such as biomaterials, optics, drug delivery systems, tissue regeneration, dental materials and contact lenses. Multilayer films with nanoparticles are used in fuel cells, photodetectors and water treatment devices.<sup>78</sup> Layer by layer technique is summarized in Figure 5.<sup>79</sup>

In LbL technique a charged substrate is immersed into an oppositely charged polyelectrolyte solution which neutralizes the surface charge and makes it reversely charged and then the substrate is dipped into second polyelectrolyte solution which neutralizes the surface charge again and then makes it oppositely charged. These steps continue until desired thickness of multilayers is reached. Instead of polyelectrolytes, other charged materials can be used in LbL film system. Although the main driving force is electrostatic, there are also other forces such as Van der Waals, hydrogen bonding, and charge transfer interaction used for LbL technique.<sup>80</sup>



Figure 5. Layer by Layer Technique.

### 1.3 Polyelectrolytes

Polyelectrolytes are charged polymer molecules that have ionizable groups when dissolved in water. Natural polyelectrolytes are proteins, polysaccharides and DNA.

When polyelectrolytes are dissolved in water, they break into charged polymer groups. Polyelectrolytes are divided into two groups which are strong and weak based on their degree of ionization. According to the type of their charges, they are called as polyanion, polycation, polyacids or polybases.<sup>81</sup> Strong polyelectrolyte is fully ionized (or charged) regardless of pH of the solution. Some examples of synthetic strong polyelectrolytes are poly(styrenesulfonate)(PSS), Poly(3,4ethylenedioxythiophene) (PEDOT), and poly(diallyldimethylammonium chloride) (PDADMAC).<sup>82-85</sup> A weak polyelectrolyte, by contrast, has pH-dependent ionization (partially ionized) which depends on pKa or pKb values (at which half of the ionizable groups are dissociated). Some examples of synthetic weak polyelectrolytes polyethyleneimine are; (branched/linear)(PEI), poly(L-lysine)(PLYS), poly(aniline)(PANI), poly(L-glutamic acid). polyamidoamine (PAMAM) poly(methacrylic acid) (PMMA).83-90 The most common weak polyelectrolyte pair in PEM formation is poly(allyamine hydrochloride) (PAH)/ poly (acrylic acid) (PAA). For instance, different conformations (extended coil/flattened or random coil/loopy) can be obtained with the use of PAH and PAA polyelectrolytes at different pHs and the thicknesses of multilayers can be controlled. Articles which are related with pH-dependent LbL formation suggest that strong polyelectrolytes have low thickness values (10Å for a bilayer) whereas weak polyelectrolytes have large thickness values as a result of change in degrees of ionization values with respect to pH (80Å for a bilayer) (as shown in Figure 6).<sup>80,91</sup>



(a)PAH 6.5/PAA 6.5



free COOH groups

(b) PAH 2.5/PAA 2.5 (c) PAH 7.5/PAA 3.5



Figure 6. (PAH/PAA) LbL films at various pHs.

pKa values of PAH and PAA are 8.8 and 6.5 respectively. However, the  $pK_a$  of a weak polyelectrolyte film is sensitive to its local ionic environment and can shift notably from its solution value. For instance,  $pK_a$  of PAA shifts to near 3.0 when assembled into multilayer because of the complexation with positively charged polybase favors dissociation of the polyacid PAA. A large shift in  $pK_a$  for polybases occurs when the solution pH is much different than the  $pK_a$  of the polybase.<sup>91</sup>

When pHs of both PAH and PAA polyelectrolytes are 6.5 (Figure 6a), PAH and PAA becomes fully charged like strong polyelectrolytes and this makes film thickness thin as a result of effective ionic crosslinking of polyelectrolyte multilayers (theoretically 10Å for a bilayer).<sup>92</sup>

When pHs of both PAH and PAA are 2.5 (Figure 6b), PAA is only 20-30% ionized and PAH is fully charged. Unbalanced ionization degrees of the polymers leave many free carboxylic acid functional groups on PAA chain. For the neutralization of adsorbed cationic binding site at PAH chain, PAA chain forms many loops and tails because it has low anionic charge density. Very thin third layer of PAH is enough to equilibrate the low charge of PAA such that the free carboxylic acid groups lie on the previously adsorbed PAA layer diffuse into PAH outermost layer. This results in intermediate film thickness (theoretically 40Å for a bilayer).<sup>92</sup>

When pH of PAH is 7.5 and PAA is 3.5 (Figure 6c), the ionization degree of PAA is very low and PAH is partially charged (less than fully ionized). The structure of the film resembles to the one shown in Figure 6b for the first bilayer (loop and tail structure) but it differs from it by the high ionization degree of PAA groups in PAH solution at pH 7.5. As a result of this, the amount of adsorbed PAH increases which causes thicker films (theoretically 80Å for a bilayer)<sup>92</sup> with interpenetrated PAH layers into PAA chains having loop and tails. This film will be investigated more in the results and discussion part.<sup>80,93</sup>

### 1.4 Nanoparticles

The term nano is derived from "nanos" which means dwarf in Greek and it is used as denoting a factor of  $10^{-9}$  (one billionth). The nanoparticles (<100 nm in size) have special properties such as; better electrical and heat conductivity, improved or hindered aggregation, and enhanced catalytic activity compared to their bulk

counterparts due to their large surface area and quantum size effect.<sup>94-97</sup> Different shaped nanoparticles have many applications in biomedical areas such as diagnosis and therapy of diseases (especially cancer), imaging, drug delivery and in SERS because of their optical properties like surface plasmon resonance.<sup>95</sup> Various anticancer drugs both kill healthy and non-healthy cells. In order to increase the efficiency of these drugs, targeted nanoparticles can be used without giving harm to the healthy cells.<sup>98,99</sup> Gold nanoparticles offer well-established surface characteristics and simple chemistry for straightforward modifications. For example, it is possible to conjugate targeting group drugs, DNA/RNA, smart polymers (piezoelectric & thermoresponsive materials, shape memory alloys) and charged molecules on the surface of gold nanoparticles (Figure 7).<sup>100</sup>



Figure 7. Possible surface modifications of Au NPs.

Among various nanoparticle types, gold nanoparticles have appealing features such as biocompatibility, non-cytotoxicity, chemically inertness and ease of functionalization. After the first synthesis of colloidal gold by Michael Faraday in 1857,<sup>101</sup> synthesis of gold nanoparticles has been improved and well-documented with different size, shape and morphologies during the last decade. There are many synthesis protocols for gold nanoparticles including physical (microwave irradiation), chemical (reduction of HAuCl<sub>4</sub>) and biological (using fungi and bacteria as nanofactories) methods. Chemical methods are the primary choices for most biomedical applications.<sup>102-106</sup> Besides gold nanoparticles, these well-known strategies yield gold nanospheres, nanowires, nanorods and nanocages.<sup>107-110</sup> Designing strategies and controlling the shape and size of the gold nanoparticles have great impact and importance on its physical properties. For instance, increase in the particle size causes a red-shift (higher wavelength) in the absorption spectrum.<sup>111</sup> Furthermore, changing shape of the particle to a nanorod pushes the "absorption maximum" to near-IR region of the electromagnetic spectrum.<sup>96</sup>

#### 1.4.1 Synthesis of Gold Nanoparticles

In a typical synthetic procedure, chloroauric acid (HAuCl<sub>4</sub>) is reduced to Au<sup>0</sup> by using some well-known reducing agents (e.g., sodium borohydride, sodium citrate dihydrate etc.) while gold solution is boiling. In a well-established *Turkevich method*,<sup>112</sup> sodium citrate is used as both reducing and capping agent. The color of the solution changes from yellow to wine red, which indicates the formation of gold nanoparticles. Murphy's and El-Sayed's procedures are the other well-established procedures for the synthesis of gold nanoparticles using Mie theory<sup>113,114</sup> with seed-mediated growth.<sup>115,116</sup> The developments of Au NPs from the invention of electron microscope (1932) to 2011 are shown in Figure 8. Improvements in chemistry and physics (optics and microscopy) provide better control of nanoparticle properties such as size and shape.<sup>117</sup>



**Figure 8.** Golden Timeline Copyright © 2013, American Physical Society. Reprinted with permission from ref (117).<sup>117</sup>

#### 1.5 Nanoparticle Embedded Polyelectrolyte Films

Due to the different properties of nanoparticles from their bulk counterparts, nanoparticles can find applications in areas such as photoelectronics, magnetism, catalysis and sensing.<sup>118-124</sup> For that reason, fixing nanoparticles on a solid surface homogeneously is important and also challenging. Mixing of nanoparticles with polymers mechanically is one of the simple fabrication methods but the films are not very homogeneous and the process is generally not very reproducible. Another technique is spin coating of polymer-nanoparticle mixture but it is difficult to form very thin films using this method.<sup>125-127</sup> New assembly techniques are necessary to create advanced materials with outstanding properties for various applications.<sup>128</sup> For that reason, Langmuir Blodgett (LB) films with controlled thicknesses and organization of components (at molecular level) have been emerged. Additionally, LB films have been used as organic conductors and diodes because LB technique allows control of molecular distances and orientation of molecules which is an important feature for electrical conductivity.<sup>129</sup> Another application of LB films is using them as sensors. For instance, polypyrrole molecule is deposited on selected surfaces by LB technique for the detection of ammonia.<sup>130</sup>

Besides LB films, layer by layer deposition allows fine control of the film properties on various substrates by combining different species such as nanoparticles, nanosheets, nanoshells, nanowires with polymers.<sup>131-133</sup> Dai et al. prepared a nanoparticle containing polymer film surface in order to use it as catalyst and antimicrobial coating. With this approach, a new way to prepare nanoparticles has emerged; nanoparticles can be distributed in/on the surface homogeneously and polymers avoid the aggregation of nanoparticles.<sup>134</sup> Jiang *et al.* investigated 4-(dimethylamino)-pyridine (DMAP) stabilized Au NPs between oppositely-charged polyelectrolyte (PAH/PSS) layers. The results show that stable thin films can be obtained with controllable optical properties.<sup>135</sup> In a similar work, Au NPs and polyelectrolytes were fabricated with spin-assisted LbL assembly with different number of bilayers. Films have red-shifted plasmon resonances because of the difference in local dielectric field as compared to nanoparticle solution. Tunable plasmon resonances are beneficial for sensing applications.<sup>136</sup> Another example for LbL film preparation was using semiconductor NPs such as HgTe for photoelectronic applications.<sup>137,138</sup>



**Figure 9.** Number of SERS papers as a function of time. Copyright  $\bigcirc$  2012, Elsevier. Reprinted with permission from ref (16).<sup>16</sup>

### 1.6 Applications of SERS

SERS is mostly employed in catalysis, chemical analysis, electronics, sensors, diagnostic & imaging, and biological systems. The number of publications related with SERS is increasing in each year as shown in Figure 9.<sup>16</sup> The reason behind this is that SERS is an important tool for chemical and biological systems in order to characterize trace amount of adsorbed species as in the case of sensor applications without giving any harm to the species.<sup>139-155</sup>

### **1.6.1 PE-NP Films for SERS Applications**

Nanoparticle embedded polyelectrolyte (LbL) films can also be used in SERS applications. Although there are not many applications, these types of films are important for SERS applications and are listed below.<sup>156-158</sup>

Zeng *et al.*<sup>156</sup> used triangular silver nanoparticles with a comparison of spherical ones for their PDDA-NP self-assembled films as a SERS substrate for Rhodamine 6G (R6G) molecule. Silver triangle nanoparticles are interesting because of their tunable surface plasmon resonance (SPR) values from visible to near infrared range and the formation of hot spots at the triangular tips. According to electromagnetic enhancement theory, triangular nanoparticles which have stronger absorbance than spherical ones at the excitation wavelength should yield stronger enhancement in SERS.<sup>25,49,159,160</sup> However, SERS signal of R6G molecules from spherical nanoparticles inside the film is larger than that of triangular ones indicated that there

have to be other parameters affecting the enhancement mechanism. The fact is that, the aggregation of spherical nanoparticles which is a leading factor for strong SERS effect and it is more effective when spherical ones are used.<sup>25,161</sup>

The other example of SERS substrate was prepared by LbL PE-NP multilayer film method. Abalde-Cela *et al.*<sup>157</sup> was prepared exponentially grown LbL film having 30 bilayers of (PDADMAC/PAA) and then it was incorporated with Ag nanoparticles for the detection of environmentally significant dioxins having detection limit of  $10^{-8}$  M for the first time.



Figure 10. LbL grown (PE/NP)<sub>n</sub> film preparation with desired number of layers (a).
SER spectra of LbL grown PE/NP film with 1, 3, 5, 7 and 11 layers (b). Copyright © 2005,
American Chemical Society. Reprinted with permission from ref (158)<sup>158</sup>.

The other example is the study of Hu *et al.*<sup>158</sup> where gold nanorods were used with PSS alternatively for the assembly of LbL multilayered films in order to probe 4-aminothiophenol molecule (4-ATP). The preparation of LbL film (Fig. 10a) and

SER spectra of different number of layers for the probe molecule (Fig. 10b) are shown. As the number of nanorod layers increases, SERS signal of 4-ATP molecule also increases.

#### 1.7 Aim of the Study

In this study, the aim is to prepare two different kinds of SERS substrates which are composed of oppositely charged polyelectrolytes incorporated with Au NPs. PAH and PAA polyelectrolytes are chosen for the LbL growth. Since, they are weak polyelectrolytes; the film growth can be controlled with pH. According to the different thicknesses of the films at different pH values, they are classified as linear or exponentially grown films. The effect of the type of polyelectrolyte films, Au NP deposition times and layer number of polyelectrolytes on SERS signals of 1.0 x 10<sup>-5</sup> M BT will be investigated. For the characterization of polyelectrolyte films; UV-vis and AFM measurements will be performed.
### **CHAPTER 2**

### **EXPERIMENTAL PART**

### 2.1 Materials

PAH ( $M_w = 58,000$ ) (Cat. No: 283223) PAA ( $M_w = 1,800$ ) (Cat. No: 323667), gold (III) chloride hydrate (99.999%)(Cat. No: 254169) and trisodium citrate dihydrate (Cat. No: W302600) were purchased from Sigma-Aldrich. The structures of PAH and PAA are given in Figure 11. Tris (hydroxymethyl) aminomethane hydrochloride (Tris HCl) buffer was purchased from Scharlau. Benzenethiol was purchased from SAFC. Ethanol was purchased from J.T. Baker. All chemicals were used as received without any purification.



Figure 11. Chemical structures of PAH (a) and PAA (b).

### 2.2 Polyelectrolyte Multilayer Formation: LbL Technique

Glass slides were soaked in concentrated sulfuric acid for 85 minutes and rinsed with copious amount of distilled and deionized (DI) water ( $18M\Omega$ -cm Millipore Milli-Q) and dried under nitrogen gas. After that the slides were soaked in 0.25 M sodium hydroxide solution for 10 minutes and rinsed with copious amount of distilled and deionized (DI) water ( $18M\Omega$ -cm Millipore Milli-Q) and dried under nitrogen gas. 10 mM (according to repeating unit M<sub>w</sub>) PAH solution with a pH of

~5.0 was prepared in 10 mM Tris HCl buffer and 10 mM (according to repeating unit M<sub>W</sub>) PAA solution with a pH of ~4.0 was prepared in DI water. For linear grown system, pH of PAH and PAA solution were adjusted to 6.5 by adding sufficient amount of 0.1 or 1.0 M NaOH. For exponentially grown system, pH of PAH solution was adjusted to 7.5 by adding 0.1 or 1.0 M NaOH and pH of PAA solution was adjusted to 3.5 by adding 0.1 or 1.0 M HCl. Negatively charged glass slides were dipped into first positively charged PAH solution, rinsed 2 times (each 1 minute) with 10mM Tris HCl buffer and dried under nitrogen gas. Tris HCl buffer (working range pH: 7-9) was used instead of DI water because it is hard to adjust pH of DI water to 7.5 for PAH solution. Then the slides were dipped into negatively charged PAA solution for 10 minutes, rinsed 2 times (each 1 minute) with DI water and dried under nitrogen gas. Schematic representation of adsorbed Au NPs on (PAH/PAA)<sub>n</sub> multilayer films can be seen in Fig. 12. Polyelectrolyte and rinsing solutions were changed at every 3 bilayers (6 layers) and their pHs were controlled and adjusted again with 0.1 and/or 1.0 M HCl or NaOH if necessary. LbL film deposition continued until the desired number of layers was obtained. Multilayer films having 9, 29, 39, 49 numbers of layers were prepared.



Figure 12. Schematic representation of adsorbed Au NPs on (PAH/PAA)<sub>n</sub> multilayer films.

### 2.3 Synthesis of Au Nanoparticles

All glass wares were cleaned in 3.0 M nitric acid and rinsed with DI water. Spherical gold nanoparticles having LSPR maximum at ~520 nm were synthesized by Turkevich method.<sup>112</sup> A stock solution of  $5.0 \times 10^{-3}$  M gold (III) chloride hydrate was prepared in DI water and stored at dark. An intermediate gold standard solution with a 5.0 x  $10^{-4}$  M was prepared to synthesize Au NP. 1.0% (w/w) sodium citrate solution was prepared as a reducing and capping agent. On a magnetic stirrer, gold solution was heated to boil. 5.0 mL of 1.0% (w/w) sodium citrate solution was added to the boiling solution and after that the solution was stirred and heated 15 minutes more. The color of the solution turned from pale yellow to ruby red which indicates the formation of gold nanoparticles. The formation reaction of gold nanoparticles is shown as follows:<sup>162,163</sup>

2HAuCl<sub>4</sub>+3C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>(Citric acid)  $\rightarrow$ 2Au+3C<sub>5</sub>H<sub>6</sub>O<sub>5</sub>(3-ketoglutaric acid)+8HCl+ 3CO<sub>2</sub>

### 2.4 Coating of Polyelectrolyte Films with Au NPs

Multilayers were dipped into 15.0 mL Au NP solution with 2, 6 or 12 hour deposition times. After the Au NP deposition step, the films were rinsed with DI water. Before SERS measurements, the prepared SERS substrates were dipped into  $1.0 \times 10^{-5}$  M benzenethiol solution for 2 hours.

### 2.5 Characterization Techniques

*Ellipsometry:* The LbL film growth of polyelectrolyte multilayers (up to 100 nm) was followed by PHE-102 ellipsometer.

*UV-vis Spectroscopy:* For the LSPR measurements of Au nanoparticles, Varian Cary 100 Bio UV-Vis Spectrometer was used.

*Dynamic Light Scattering (DLS) and Zeta Potential:* Hydrodynamic size and zeta potential of Au nanoparticles were measured using Malvern Zetasizer Nano-ZS. Average hydrodynamic size of Au nanoparticles was acquired by DLS technique. Zeta potential data were gathered from electrophoretic mobility values using Smoluchowski approximation.

*Atomic Force Microscopy (AFM):* The roughness, thickness and 2D/3D images of the multilayers with and without gold were measured using Veeco MultiMode V instrument in tapping mode at METU Central Laboratory.

*Raman Spectroscopy:* Horiba LABRAM Raman spectrometer at Chemistry Department, Bilkent University was used to record the SER spectra of the analyte molecules. The instrument calibration was checked with with silicon wafer before each measurement. 632 nm He-Ne excitation laser operated at 2.50 mW power. Slit size of 200  $\mu$ m and confocal hole size of 1100  $\mu$ m were used. Data were collected using 50x objective (spot size ~1), 600 g/mm grating with a resolution of 2-3 cm<sup>-1</sup> with 25s acquisition time and each spectrum was taken 4 times and then averaged. For SERS mapping, Bruker Senterra Raman spectrometer at Restoration Department, METU was used with a 785 nm laser operated at 1 mW power and a resolution of 9-10 cm<sup>-1</sup>. 30x25 data points (total surface area: 120x88 $\mu$ m<sup>2</sup>) were collected with lens using 5 s integration time and the signal on each point averaged twice. The baseline is substracted from the Raman spectra using Origin program. The same instrument with 100 s integration time and 2 accumulations was used to get SER spectra for the prepared substrates without the probe molcule.

### **CHAPTER 3**

### **RESULTS AND DISCUSSION**

In this part, characterization of spherical gold nanoparticles (Au NPs) and Au NPabsorbed linear and exponentially grown multilayer films of  $(PAH/PAA)_n$  as SERS substrates are discussed.

### 3.1 Characterization of Spherical Au NPs

Gold nanoparticles have characteristic optical properties in the visible region of the electromagnetic spectrum because of localized surface plasmon resonances (LSPR) of their free electrons.<sup>164</sup> The extinction spectra of Au nanospheres are calculated by Mie theory and nanospheres which are smaller than 100 nm have LSPR peaks at the green part of the visible region.<sup>165</sup> Gold nanoparticles of diameter 3 nm and below are too small to have a plasmon band.<sup>117</sup>

In Fig. 13 LSPR band of gold nanoparticles synthesized in this study using Turkevich Method at ca. 518 nm is shown. This method produces gold colloids with a size of 20 nm  $\pm$  2 nm. The size can be tuned by the amount of reductant (sodium citrate) i.e. higher concentration of sodium citrate relative to gold chloride solution produces smaller nanoparticles down to 12 nm and lower concentration of sodium citrate relative to gold chloride solution produces larger nanoparticles up to 150 nm.<sup>166</sup> For further demonstration of size distribution we performed DLS measurement. The average size of Au NPs was found to be 11.9  $\pm$  0.6 nm (5% RSD, averaged from 6 measurements). For the charge of Au NPs, Zeta potential measurements were performed in which citrate capped Au NPs have -36.8  $\pm$  1.3 (4% RSD, averaged from 6 measurements). These results are consistent with literature values.<sup>167,168</sup> Average hydrodynamic size distribution and zeta potential of gold nanoparticles are given in Figs. 14 and 15, respectively.<sup>112</sup>



Figure 13. UV-Vis spectrum of Au NPs.



Figure 14. Hydrodynamic size distributions of Au NPs by Dynamic Light Scattering method for 6 measurements.



Figure 15. Zeta potentials of Au NPs for 6 measurements.

### 3.2 The Multilayer Films of (PAH/PAA)<sub>n</sub>

Oppositely charged weak polyelectrolytes were deposited alternatively by electrostatic interactions using LbL assembly method. Nanoscale control of the film thickness depends on parameters such as the type and molecular weight of the polyelectrolytes, pH and ionic strength.<sup>169-173</sup> Two systems with different growth profiles were investigated throughout this study, i.e. linear and exponentially grown multilayer films.

For pH 3.5/7.5 PAA/PAH multilayers (exponential growth), with PAA ( $pK_a = 3.0$ ), has low ionization, whereas PAH ( $pK_a = 8.8$ ) is partially protonated.<sup>173</sup> After the two layers, deposited PAA becomes fully ionized in PAH solution at pH 7.5 (third layer). Thus, large amount of PAH chains were attracted from the solution to pair up with the previous PAA layer. As the amount of PAH chains is high the outermost PAH layer becomes thick and covers the underlying PAA chains. Highly interpenetrated multilayer structures with thick outmost layer are obtained.<sup>174,175</sup> Although exponential growth mechanism is not well-understood, there are some mechanisms suggested to explain and understand the growth. Initially, it was stated that increase in the film roughness should be responsible for such a behavior but thicknesses up to micrometers cannot be explained only with roughness phenomenon.<sup>175</sup> Later, Elbert and co-workers<sup>174</sup> suggested that the exponential growth is the result of the complexation of oppositely charged polyelectrolytes on the film. For instance in one example, poly(L-lysine) (PLL) / hyaluronic acid (HA) film formation was analyzed. It was observed that, PLL (polycation) diffuses "in" and "out" of the film in the course of growth. Diffused out PLL chains form complexes with polyanion HA at the outer layer of the film. Thus, the thickness of the outmost layer is directly related with the amount of PLL chains which diffuse out in the presence of HA. This build-up process was also studied by another group in which fluorescent molecules were used in order further to clarify the mechanism.<sup>175</sup> Fluorescent dye conjugated polyelectrolytes, which was monitored via confocal laser scanning microscopy, clearly showed "in" and "out" diffusion process.

For pH 6.5/6.5 PAA/PAH (linear growth) a zipped and smooth structure with interpenetrated layers of neighboring polyelectrolytes is obtained as a result of fully

charged polyelectrolyte pairs. Each polyelectrolyte deposition step overcompensates the charge of the previous polyelectrolyte.  $^{77,80,175-177}$  Fig. 16 shows the general chemical structure of aligned polyelectrolytes, consisting of a repeating carbon backbone with amine (NH<sub>2</sub>/ NH<sub>3</sub><sup>+</sup>) and carboxyl (COOH/COO<sup>-</sup>) functional groups for PAH and PAA respectively.<sup>178</sup>



Figure 16. Functional groups of PAH (pH= 6.5), PAA (pH= 4.0) polyelectrolytes.

### 3.3 Multilayer Growth

Growth of polyelectrolyte films was followed by ellipsometer, by measuring the thicknesses of the film after each layer deposition. Layer growth of exponentially grown (PAH/PAA)<sub>n</sub> film is shown in Fig. 17. Note that the exponentially grown film thickness reaches up to 100 nm for 12 layers (data not shown) as can be understood from the exponential increase of the line. Due to the instrument limitations, the film thickness upper than 100 nm could not be measured.



Figure 17. Thickness values of the exponential grown (PAH/PAA)<sub>n</sub> multilayer films..

# 3.4 UV-vis Spectroscopy of Citrate Capped Au NPs on (PAH/PAA)<sub>n</sub> Films with Different Deposition Periods for Exponentially and Linear Grown Systems

UV-vis spectra of absorbed Au NPs on (PAH/PAA)<sub>n</sub> films with various deposition periods were measured. A red shift is observed when Au NPs are absorbed into the films compared to Au NPs in solution. Au NPs in the films have different dielectric properties as a result of their strong interactions with polyelectrolyte layers and with each other. These interactions are responsible for collective oscillations of electrons and thus surface plasmon resonances (SPR) of Au NPs. A red shift of SPR is an expected phenomenon when Au NPs approach to each other due to polyelectrolyte-Au NP interactions. Furthermore, increasing the number of polyelectrolyte layers causes a change in interactions of Au NPs with each other and polyelectrolyte layers and causes change in Au NPs LSPR band with more red shift in SPR.<sup>136</sup> Optical properties of nanoparticles are sensitive to size, shape, concentration, agglomeration and so on. Kelly et al. carried out a theoretical study and discussed the influence of size, shape and dielectric environment on the optical properties of Ag NPs. They have reported that SPR shifted to red when the shape of a spherical nanoparticle became flattened or increased in size as a result of increased electromagnetic effects.28

Fig. 18a represents the absorbance of citrate capped Au NPs having 12 h deposition time as a function of number of layers for exponentially grown films. Increasing nfrom 9 to 29 resulted in increased plasmon resonance with a red shift in the absorption peak. For other layers (n = 39 and 49) plasmon resonance of Au nanoparticle can be also seen but n = 39 has a decrease in absorption. It may be because of a defect on the surface of the film. For 6 h and 2 h Au NP deposition times, absorbance of Au NPs (Figs. 18b and 18c) were lower (except n = 9) as compared to 12 h deposition time.



Figure 18. UV-vis absorption spectra of Au NPs absorbed on exponentially grown  $(PAH/PAA)_n$  multilayer films with increasing number of layers (L). Deposition time of Au NPs (a) 12h (b) 6 h (c) 2 h.



Figure 19. UV-vis absorption spectra of Au NPs absorbed on linear grown (PAH/PAA)<sub>n</sub> multilayer films with increasing number of layers. Deposition time of Au NPs (a) 12h (b) 6 h (c) 2 h.

UV-Vis spectra of Au NPs deposited on linear grown films with 12 h deposition time were measured (Figure 19a). As the number of layer increases from 9 to 29, the LSPR peak of Au NPs became narrower and the maxima of the peak increased. The broader peak for n = 9 proves the greater size of Au NPs with a red shift of the absorption maxima. The agglomeration of Au NPs can be seen from AFM images in Fig. 26. For 6 h and 2 h deposition times (Figures 19b and 19c), absorption of Au NPs decreases which is a result of the low coverage of the film with Au NPs.

### 3.5 AFM Measurements

### 3.5.1 AFM of Exponential System

First, 2D and 3D AFM images of  $(PAH/PAA)_n (n = 9,29)$  multilayers were studied (Figs. 20 and 21). As seen in these figures, the surface roughness increased as the number of layers deposited at the surface increased. Note that the roughness (R<sub>a</sub>) values were recorded from  $25x25 \ \mu\text{m}^2$  images. For n = 29 two scan sizes were given for the better representation of loop-rich conformation of this layer. The reason for increasing surface roughness can be explained by: i) increased surface area and amount of deposited particles on the surface by increase in number of layers and /or ii) loop and tail structure of exponential films as the number of layer increases. Since the thickness and roughness values of  $(PAH/PAA)_{29}$  are high to measure using AFM, the layers higher than n = 29 were not characterized by AFM.



Figure 20. 2D (a) and 3D (b) images of (PAH/ PAA)<sub>9</sub> exponential system without Au NPs.



**Figure 21.** 2D (a) and 3D (b) images of (PAH/ PAA)<sub>29</sub> exponential system *without Au NPs*. The loop and tail structure of (PAH/PAA)<sub>29</sub> can be seen for both small (5x5μm<sup>2</sup>) and large (25x25μm<sup>2</sup>) surface area.

We then analyzed the surface morphology of the films having Au NPs (Deposition time=12 h) (Figs. 22 and 23). The roughness value of (PAH/PAA)<sub>29</sub> with Au NP was lower than that of the (PAH/PAA)<sub>29</sub> without Au NPs. This may be because Au NPs fill the loose multilayer structure of the exponentially grown films resulting in lower surface roughness. Similar phenomenon was not observed for 9L film. This is probably due to stratified structure of the 9L film than that of 29L resulting in absorption of Au NPs at the surface rather than absorption by the multilayer matrix as observed in 29L films.



Figure 22. 2D (a) and 3D (b) images of (PAH/ PAA)<sub>9</sub> exponential system with Au NPs.



Figure 23. 2D (a) and 3D (b) images of (PAH/ PAA)<sub>29</sub> exponential system with Au NPs.

### 3.5.2 AFM of Linear System

2D and 3D AFM images of  $(PAH/PAA)_n$  (n = 9, 29) were measured with and without Au NPs (12 h deposition). Roughness and thickness values increased for  $(PAH/PAA)_n$  multilayers as the number of polyelectrolyte layers increased (Figs. 24 and 25) but it is not much significant as compared to exponentially grown system (Figs. 20 and 21).



Figure 24. 2D (a) and 3D (b) images of (PAH/PAA)<sub>9</sub> linear system without Au NPs.



Figure 25. 2D (a) and 3D (b) images of (PAH/PAA)<sub>29</sub> linear system without Au NPs.

Moreover, the roughness values increased for both n = 9 and n = 29 (Figs. 26 and 27) with Au NPs which is similar to exponential 9L film (Fig. 22). 9L film follows a different trend and this is also observed in UV-vis spectrum of linear grown 9L which has it's a broader absorbance peak with a red shift compared to other layers (Fig. 19a). This difference is probably due to tightly packed aggregated Au NPs on the surface which also causes higher SERS signals (discussed later).



Figure 26. 2D (a) and 3D (b) images of (PAH/PAA)<sub>9</sub> linear system with Au NPs.



Figure 27. 2D (a) and 3D (b) images of (PAH/PAA)<sub>29</sub> linear system with Au NPs.

Linear grown films having 39 and 49 layers were also measured. Roughness and thickness values increased as the number of layers increase for both without (Figs. 28 and 29) and with (Figs. 30 and 31) Au NP coated multilayer films.



Figure 28. 2D (a) and 3D (b) images of (PAH/PAA)<sub>39</sub> linear system without Au NPs.



Figure 29. 2D (a) and 3D (b) images of (PAH/PAA)<sub>49</sub> linear system without Au NPs.



Figure 30. 2D (a) and 3D (b) images of (PAH/PAA)<sub>39</sub> linear system with Au NPs.



Figure 31. 2D (a) and 3D (b) images of (PAH/PAA)<sub>49</sub> linear system with Au NPs.

### 3.6 SERS of Citrate Capped Au NPs Containing (PAH/PAA)<sub>n</sub> Films with Different Deposition Periods for Exponentially and Linear Grown Systems

SER spectra of  $1.0 \times 10^{-5}$  M BT molecule on linear and exponentially grown films with Au NPs were measured. The effects of the type of PE films (linear or exponentially grown), the number of polyelectrolyte layers and depositon times of Au NPs on SERS signals were investigated. SER spectrum of BT molecule showed

characteristic vibrational features at Raman lines of 423 cm<sup>-1</sup>, 693 cm<sup>-1</sup>, 999 cm<sup>-1</sup>, 1024 cm<sup>-1</sup>, 1075 cm<sup>-1</sup> and 1575 cm<sup>-1</sup>. These lines correspond to C-S stretching mode, C-H out-of -plane deformation mode, C–C–C in-plane ring-breathing mode, in-plane C–H bending mode, in-plane ring-breathing mode coupled with the C–S stretching mode and the C–C stretching mode bonds of the molecule, respectively.<sup>19</sup>

For exponentially grown films, 12 h (Fig. 32a), 6 h (Fig. 32b), 2 h (Fig. 32c) deposition periods of Au NPs were examined to investigate the effect of number of layers on SERS signals of  $1.0 \times 10^{-5}$  M BT. For 12 h Au NP deposition, the SERS signal increased as the number of layers increased from n = 9 to n = 29. As shown in Fig. 18a, the absorbance of Au NPs increased from n = 9 to n = 29. As the number of Au NPs increases, SERS signals also increase. However, in 2 and 6 h Au NP deposition times, there is not a direct relationship between number of layers, SERS intensity and deposition periods. Exponentially grown systems are reported to be better SERS substrates because Au NPs can interact both with the last layer of the multilayer film and the inside part of the film by the affinity of Au NPs to amine and carboxylic acid groups of polyelectrolytes as a result of the loop-rich structure of the film having high diffusivity. This can allow the assembly of probe molecules onto or inside the film because of larger deposition of Au NPs inside and on the surface of the film (hot spots).<sup>157</sup>



Figure 32. SER spectra of  $1.0 \times 10^{-5}$  M BT molecule on Au NP containing exponentially grown (PAH/PAA)<sub>n</sub> multilayer films with different Au-NP deposition times ((a) 12h (b) 6 h (c) 2 h). Spectra are shifted vertically for clarity.

For linear grown system, 12 h, 6 h and 2 h deposition times of Au NPs were examined to understand the effect of number of layers on SERS signals of  $1.0 \times 10^{-5}$  M BT (shown in Figs. 33 and 34). For 12 h and 6 h Au NPs deposition, there is a 10x increase in SERS signals of  $1.0 \times 10^{-5}$  M BT for (PAH/PAA)<sub>9</sub> multilayer film

compared to other layers. This may be because of the formation of hot spots at the Au NP agglomerates. As the deposition time of Au NPs increases, SERS signals increase for 9L films. For the other layers, there are not significant increases in SERS signals as a function of the deposition time of Au NPs.



**Figure 33.** SER spectra of 1.0 x 10<sup>-5</sup> M BT molecule on Au NP containing linear grown (PAH/PAA)<sub>9</sub> multilayer films with different Au-NP deposition times ((a) 12h (b) 6 h (c) 2 h). Spectra are shifted vertically for clarity.



**Figure 34.** SER spectra of  $1.0 \times 10^{-5}$  M BT molecule on Au NP containing linear grown (PAH/PAA)<sub>n</sub> multilayer films with different Au-NP deposition times ((a) 12h (b) 6 h (c) 2 h). Spectra are shifted vertically for clarity.

As can be seen from Fig. 35, the blank signals from linear (PAH/PAA)<sub>29</sub> film (blue) or (PAH/PAA)<sub>29</sub> film coated with Au NPs (black) are negligible compared to SERS signal from BT molecules on the surface of Au nanoparticles containing linear (PAH/PAA)<sub>29</sub> film (red). These measurements were performed in order to show there is no signal coming neither from polyelectrolyte multilayer nor polyelectrolyte multilayer with Au NPs. For the other multilayer films prepared in this study, no Raman signals were measured from multilayered films with and without Au NPs. A peak near 1500 cm<sup>-1</sup> is due to glass emission.



**Figure 35.** SER of linear (PAH/PAA)<sub>29</sub> film with 12h Au NP deposition (black), with Au NP and BT molecule (red) and without Au NP and BT molecule (blue). All spectra were taken using 785 nm laser. Spectra are shifted vertically for clarity.

### 3.6.1 Reproducibility of SERS Signals

SERS signals from different points of substrates for exponentially (Fig. 36) and linear grown (PAH/PAA)<sub>n</sub> films (Fig. 37) were taken and one of them is shown in below for comparison of the signal reproducibility of linear and exponential systems.



Figure 36. SER spactra of  $1.0 \times 10^{-5}$  M BT on exponentially grown (PAH/PAA)<sub>29</sub> films with 12h Au NP deposition.



**Figure 37.** SER spactra of  $1.0 \times 10^{-5}$  BT on linear grown (PAH/PAA)<sub>29</sub> films with 12h Au NP deposition.

## 3.6.2 Enhancements of Raman Signals for Different Concentrations of BT versus Neat Raman Signal

To show the limit of quantitation of BT molecule on Au NP coated multilayered films, Raman spectrum of BT was taken from the concentrated solution (~10 M) as shown in Fig. 38. The SER spectra of BT molecule having concentrations between  $1.0 \times 10^{-5}$  M and  $1.0 \times 10^{-7}$  M are shown in the same figure. The Raman spectrum of 10 M BT is included for comparison purposes. The high sensitivity of the SERS substrates allowed the spectra to be measured at a concentration level of  $1.0 \times 10^{-6}$  M. The quantitation limit achieved was  $1.0 \times 10^{-6}$  M.



**Figure 38**. Raman spectrum of neat BT molecule and SER spectra of 10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup> M BT on linear (PAH/PAA)<sub>29</sub> film with 12h Au NP deposition.

#### 3.7 SERS Maps on Linear and Exponentially Grown Films

Many spectra can be obtained with SERS map tool for a selected area (120x88  $\mu$ m<sup>2</sup>). Spacing between each data point was ~4  $\mu$ m. In Figs. 39, 40, 41 and 42 SER spectra were gathered for 9, 29, 39 and 49 layer expo films, respectively and SER spectra are shown for 9 (Fig. 43) and 29 (Fig. 44) layer linear films, respectively. Each point represents the intensity of the spectrum (with respect to the most intense point) taken from a point on the films. For exponential system the signal intensities deviate from

each other with ca. 40% RSD on 120x88  $\mu$ m surface area except for 39 layer which showed ~80% RSD. This can be related to the high roughness values of exponentially grown films or related with Au NPs that are diffused through the pores of exponentially grown films. On the other hand, for linear system (n = 29), the nanoparticle distribution on the surface was more homogeneous which leads similar signal intensities on the surface with less than 20% RSD for 29 layer (Fig. 44).



**Figure 39.** 3D SERS map of 1.0 x 10<sup>-5</sup> M BT on Au NP containing (12 h deposition time) exponentially grown (PAH/PAA)<sub>9</sub> film.



**Figure 40**. 3D SERS map of 1.0 x 10<sup>-5</sup> M BT on Au NP containing (12 h deposition time) exponentially grown (PAH/PAA)<sub>29</sub> film.



**Figure 41.** 3D SERS map of 1.0 x 10<sup>-5</sup> M BT on Au NP containing (12 h deposition time) exponentially grown (PAH/PAA)<sub>39</sub> film.



Figure 42. 3D SERS map of  $1.0 \times 10^{-5}$  M BT on Au NP containing (12 h deposition time) exponentially grown (PAH/PAA)<sub>49</sub> film.



**Figure 43.** 3D SERS map of of 1.0 x 10<sup>-5</sup> M BT on Au NP containing (12 h deposition time) linear grown (PAH/PAA)<sub>9</sub> film.



**Figure 44.** 3D SERS map of 1.0 x 10<sup>-5</sup> M BT on Au NP containing (12 h deposition time) linear grown (PAH/PAA)<sub>29</sub> film.

### **CHAPTER 4**

### CONCLUSIONS

In this study, linear and exponentially grown films containing Au NPs were prepared as SERS substrates. The important parameter for our systems was pH and the pH of the medium was changed to prepare different film morphologies. Roughness values of exponentially grown systems were larger than linear grown system without Au NPs. Multilayer films containing gold nanoparticles were immersed for 2 h in aqueous solutions containing BT in concentrations ranging from  $10^{-5}$  M down to  $10^{-7}$  M. From the SERS map data, the signals of linear system were more reproducible than (except 9L) exponentially grown systems. The effect of number of layers and deposition times of Au NPs is not significantly different than each other considering enhancement in Raman signals except for 9L films. Both systems showed enhancements for  $1.0 \times 10^{-5}$  M BT molecule with minor differences except linear system of (PAH/PAA)<sub>9</sub> with a large enhancement for all Au NP deposition times.

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