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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**DEVELOPMENT AND OPTIMIZATION OF
PLASMONIC NANOSTRUCTURES FOR
SURFACE-ENHANCED RAMAN SCATTERING**

**QUAN LAM ZHUNG
SCHOOL OF CHEMICAL AND BIOMEDICAL
ENGINEERING**

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ABSTRACT

Surface-enhanced Raman scattering (SERS) exploits the gigantic enhancement of inherently weak Raman signal by intensive interaction of light with molecular species adsorbed on the surface of carefully designed substrate having nanometric roughness. Today, trinitrotoluene (TNT) and melamine contamination are of major concern due to their toxicity and health hazards on human. Therefore, it is important to develop a sensitive and cost-effective probe for the detection of these contaminants in trace amount. First, I designed a cysteine-modified 40 nm gold nanoparticles (AuNP) substrate for selective and sensitive detection of TNT. Due to the formation of Meisenheimer complex upon TNT addition, aggregation between Meisenheimer complex bound AuNP and cysteine-modified AuNP was observed. This study demonstrated that TNT can be detected in 1 nM level with excellent discrimination against other nitro compounds. Second, I introduced a novel silver-coated gold nanostars (Au@AgNS) substrate via seeded-growth method. Au@AgNS with gold to silver ratio of 1:0.25 was demonstrated to achieve the highest intensity through optimization study. By taking advantage of the presence of sharp features and superior optical properties of silver, a detection limit of 1 μ M level of melamine can be obtained.

CHAPTER 1: INTRODUCTION

1.1 Background

Raman spectroscopy has attracted many attentions as a useful spectroscopic technique that observes inelastic Raman scattering of photons from a molecule upon interaction with light. The analysis of inelastically scattered photons provides structure-specific fingerprint of an analyte at the molecular level [1]. This unique feature enables the differentiation of molecule from a mixture of different molecular species. Nonetheless, Raman scattering is naturally very weak as compared to elastic Rayleigh scattering and the Raman cross-section is also much smaller than fluorescence. Therefore, surface-enhanced Raman spectroscopy (SERS) was discovered to address the limitations of Raman spectroscopy.

SERS effect is the result of amplifying Raman signals by several orders of magnitude. The amplification of signals in SERS originates from the electromagnetic interaction of light with metals known as plasmon resonances [2]. Electromagnetic mechanism (EM) and chemical mechanism (CM) are two known mechanisms to describe the SERS phenomenon. The former relies upon the excitation of localized surface plasmon by light on metallic surface or junction between nanostructures often known as hot spots [3]. Whereas, the formation of charge-transfer complexes which enhanced the polarizability of adsorbate contributes to the latter [4]. High sensitivity and selectivity along with highly informative spectra characteristics allows SERS to present tremendous potential for biological and chemical sensing.

Gold and silver nanostructure plays an important role as SERS substrate because of their unique optical properties. These metals are strongly wavelength dependant and provide maximal enhancement under visible and near-infrared light [5]. Silver has sharper resonances and higher refractive index sensitivity but gold is often chosen because of its chemical stability and resistance to oxidation [6]. In addition to material composition, the advantage of metallic nanostructures as SERS substrate lies in the possibility to modify its size and shape by carefully tuning the experimental conditions. By employing different experimental parameters, nanostructure such as gold nanoparticles [7], nanorods [8] and nanostars [9] can be synthesized.

Water and food are two vital components central to all known living systems. In the past, a considerable understanding of the relationship between both water and food qualities towards human health has been developed. Today, water contamination and food adulteration are of major concern for both developing and developed countries due to their toxicity and health hazards on human. Traces of trinitrotoluene (TNT), an explosive widely used in military and industrial applications is found to be present in water in the area where it was used [10]. At the same time, intentional adulteration with melamine to increase the protein content of milk products by unethical manufacturers is also on a rise [11]. As the excessive intake of TNT and melamine jeopardize human health, monitoring the level of both chemicals become very urgent for public health and food safety.

1.2 Project Aims

As a major source of hazardous water pollution, TNT detection becomes a new research focus in recent years. Different analytical methods have been reported but none is ideal due to drawbacks such as complex instrumentation, time-consuming procedure and necessity of tagging. Due to its high sensitivity and cost-effective approach, SERS has attracted a great deal of attention. In my first experiment, I detail the work of Dasary's group with the following objectives:

- Design a cysteine-modified 40 nm gold nanoparticles based SERS substrate for TNT detection with improved stability.
- Improve the sensitivity of the SERS substrate using 785 nm laser.
- Distinguish the selectivity of the SERS substrate for TNT over other nitro compounds.

Food adulteration with melamine presents a major concern and there is a growing need for simpler, quicker and sensitive method to monitor melamine. Recently, the fabrication of gold nanostars has been driven by the superior SERS properties they exhibited owing to their sharp tips and multiple edges. By taking advantage of the superior optical properties of silver, silver coating has shown to be a promising method to enhance the SERS activity of various metallic nanostructures. With that in mind, my second experiment's objectives are to:

- Design a silver-coated gold nanostars based SERS substrate through seeded-growth method.
- Optimize the SERS enhancement of the SERS substrate.
- Evaluate and improve the detection limit of melamine using the SERS substrate.

CHAPTER 2: LITERATURE REVIEW

2.1 Surface-Enhanced Raman Scattering Substrate

A surface-enhanced Raman scattering (SERS) substrate is generally refers to any plasmon resonance supporting structure that will produce suitable Raman amplifications. Good SERS substrates are substrates that support the strongest plasmon resonance and provide the largest enhancement. In this respect, substrates can be distinguished between those that provide a relatively uniform enhancement and those with large variations. The latter exhibit some highly localized positions of very high enhancement and it is particularly suited for single molecule detection. Nonetheless, the former should be preferred for its reproducibility in various applications.

2.1.1 Gold Nanoparticles

Gold nanoparticles (AuNP) play an important role as SERS substrate due to its unique optical properties. Turkevich et al. first reported the synthesis of AuNP through a bottom-up method [7]. In their study, gold (III) chloride was reduced via reducing agent in the form of sodium citrate under rapid stirring which caused gold ions to be reduced to neutral gold atoms and subsequently grew to form AuNP. The presence of negatively-charged citrate ions which adsorbed onto AuNP's surface introduced a surface charge that repelled individual AuNP and prevented them from aggregating. Moreover, they showed that in order to synthesize larger AuNP, less amount of sodium citrate is added. In addition to their fascinating optical properties, AuNP can

be functionalised with specific ligands that favourably interact with target molecule present in a complex mixture.

2.1.2 Gold Nanostars

Recently, the fabrication of gold nanostars (AuNS) has been driven by the interest on the LSPR response of metal nanoparticles, in particular for those with morphologies involving sharp tips and edges, where light can be highly concentrated. Two different strategies can be employed in AuNS synthesis which is seeded-growth [9, 12-16] and one-pot methods [17-21]. In the seeded-growth technique, pre-formed gold seeds act as nucleation points on which additional gold ions will be deposited for subsequent growth, usually leading to monodisperse particles with different sizes. On the other hand, in non-seeded method, nuclei evolve in situ to form gold seeds and subsequently bigger particles, through the direct addition of gold ions. However, controlling the formation of nuclei is complicated and often leads to wider distributions of particle size and shape.

Gold nanostars (AuNS) are commonly synthesized with the presence of surfactants such as cetyltrimethylammonium bromide (CTAB) [12, 15, 17, 18, 20], bis-(*p*-sulfonatophenyl) phenylphosphine dihydrate dipotassium (BSPP) [21], sodium dodecyl sulphate (SDS) [13] and poly-(vinylpyrrolidone) (PVP) [9, 14, 16, 19] in both fabrication methods. Chen et al. [17] and Sau et al. [12] were the first two groups to report the synthesis of AuNS using one-pot and seeded-growth method. Unfortunately, their studies were limited by low yield of the morphology of interest and polydisperse particles. Subsequently, a high yield synthesis procedure was detailed in Kumar's

protocol [9] and further improved by Khoury's group [14]. Through seeded-growth method, they showed that the size and morphology of AuNS can be controlled by adjusting the amount of gold seeds. Furthermore, owing to their tunable plasmon, multiple sharp edges and strongly enhanced electromagnetic field localized around its tips, AuNS exhibited superior SERS properties [22].

2.2 Trinitrotoluene

Trinitrotoluene (TNT) is a nitro-aromatic compound first prepared in 1863 by German chemist Julius Wilbrand with the formula $C_7H_5N_3O_6$ [23]. This yellow-coloured, odourless solid is best known as a useful explosive with convenient handling properties and it is used as a reagent in chemical synthesis too. TNT does not occur naturally in the environment. It is prepared in laboratory through nitration process by combining toluene with a mixture of nitric and sulphuric acids via electrophilic substitutions with nitronium ion (NO_2^+) [24].

Originally used as a yellow dye, TNT's potential as an explosive was not appreciated because it was difficult to detonate and less powerful as compared to other alternatives. However, it is one of the most widely used explosives currently in military application which is mainly found in landmines and bombs. As compared to other more sensitive explosives such as nitroglycerin, TNT is preferred due to its insensitivity to shock and friction which reduces the risk of accidental detonation. Apart from being used as pure explosive, TNT can be safely combined with other explosives to form binary mixtures such as cyclotols and octols owing to its unique property where it melts far below the temperature at which it will spontaneously

detonate [25]. In addition to military application, TNT is used in industrial applications as well such as mining, underwater blasting and production of dyestuffs.

Contamination of soil and groundwater with TNT is a major concern due to its significant detrimental effect not only to the environment but to human as well. TNT is poisonous and classified as toxic at concentration above 2 ppb [26]. It can absorb through the skin causing skin irritation with a bright yellow-orange colour. People who are exposed to TNT over a prolonged period tend to experience anaemia and abnormal liver functions. Animal studies indicated that inhalation or ingestion of high levels of TNT may cause spleen, blood, immune system, and reproductive damage [27].

2.2.1 Detection Methods and Limitations

Given the widespread use of TNT formulation, the detection of TNT is crucial for national security, human health and environmental cleaning [28]. Hence, it is important to develop highly sensitive and cost-effective probe for the detection of TNT especially in trace amount. With all this in mind, gas chromatography coupled with mass spectrometry (GC-MS) [29], thermal neutron analysis (TNA) [30] and ion mobility spectrometry (IMS) [31] has been proposed as suitable methods for the detection and quantification of TNT.

The use of GC-MS was presented by Hakansson et al. to observe both positive and negative low mass ions of explosives [29]. In their project, the explosives were vaporized into different abundant ions. Possible identities of the most abundant ions showed in the spectra were related to the properties of individual explosive. The

absence of abundant NO^+ and NO_2^- ions for TNT indicated that TNT does not function as a GC-MS matrix. Vourvopoulos et al. employed TNA technique to identify the elements present in explosives [30]. In their study, neutrons was utilised to impact the explosives which resulted in the emission of characteristic γ -rays that acts as the fingerprints of the elements. By analysing the number of γ -rays emitted, they identified the elements contained within the explosives by comparing the measured value to a given standard value.

Besides GC-MS and TNA techniques, the use of IMS was reported by Eiceman et al. to analyse the properties of gaseous ions found in explosives [31]. In their report, the explosive's vapour was first converted to product ions in the reaction region followed by ions separation and mobility characterization in the drift region. Since the ions of TNT have distinctive mobility or drift times, the fingerprint of TNT was obtained. While all the above-mentioned methods offer advantages, none is ideal due to certain features such as lack of portability, susceptibility to false positives owing to environmental contaminants, and false negative readings due to certain interfering compounds [32].

Nowadays, optical detection techniques based on the design of fluorometric and colorimetric assays have attracted a great deal of attention in the detection of TNT. In particular, the wide variety of fluorescent and dye groups, the simple instrumentation required when using colorimetric probes and the low detection limit reached when employing fluorescence probes make the optical approach largely appealing.

Fluorescence Resonance Energy Transfer (FRET)-based mechanism was proposed by Gao et al. for the sensitive detection of TNT [33]. On this occasion, the surface of silica nanoparticles was functionalised with both a fluorophore and amines. As TNT-amine Meisenheimer complexes formed in close proximity to the fluorophore, fluorescence quenching happened through FRET mechanism with TNT detection limit of 1 nM. In addition, Xia et al. reported another FRET system consisting of gold nanorod (AuNR) and quantum dots (QD) for turn-on fluorescent sensing of TNT [34]. They showed that TNT molecules replaced the QD on the preformed AuNR-QD assembly by forming TNT-amine Meisenheimer complexes. Thus, the FRET was switched off with the limit of detection for TNT was as low as 0.1 nM. Unfortunately, as FRET method identify TNT though the use of fluorescence tag, the need for tagging makes it difficult as real life sensor.

Driven by the need, Jiang et al. reported a simple yet sensitive method based on the colour change of cysteamine-functionalised AuNP induced by the donor-acceptor interaction between TNT and primary amines [35]. The detection limit achieved was 0.5 pM. However, the major drawback to the use of colorimetric assays is that they lack selectivity. Recently, SERS has been used as popular technique to detect trace amount of TNT. Dasary et al. presented a highly selective and sensitive SERS probe for TNT recognition in aqueous solution [36]. They showed that the formation of Meisenheimer complex between TNT and cysteine-conjugated AuNP leads to plasmonic coupling between nanoparticles in close proximity. As a result, the intensity of Raman signals was significantly enhanced, resulting in a detection limit of 2 pM.

2.3 Melamine

In 1834, German chemist Justus von Liebig first invented an organic compound called melamine with the formula $C_3H_6N_6$ [37]. This colourless, crystalline substance is slightly water-soluble and mainly used as a material to make plastics. In early production, melamine was prepared using calcium cyanamide as a starting material [38]. However, nowadays most industrial manufacturers employ urea to produce melamine in a two-step reaction.

Melamine is widely combined with formaldehyde to produce melamine resin, a synthetic polymer that is durable and heat tolerant owing to its highly stable structure. Moreover, due to the release of nitrogen gas, melamine resin is fire retardant when burned [39]. Therefore, it is used in the production of floor tiles, kitchenware and whiteboards. Furthermore, as a thermoset plastic, melamine resin can be easily moulded into desired shape when exposed to extreme heat, which makes it suitable for other industrial applications. In addition, the use of melamine as fertilizer was once suggested because of its high nitrogen content [40]. However, the high cost of manufacturing and slow nitrogen mineralization process has made melamine impractical for use as a fertilizer.

In September 2008, food adulteration with melamine became a subject of health concerns when it was determined to be the cause of death in children. At that time, it was discovered that milk producers in China had intentionally added melamine to milk products such as infant formula in order to raise the nitrogen content, thereby making their products appear more nutritious. Those adulterated milk products were sold not

only to consumers in China but to other countries as well. However, the melamine content in those products was greater than what had been considered to be safe levels. Animal studies revealed that melamine, when combined with cyanuric acid will form crystals that can give rise to kidney stones which potentially causes kidney failure and in some cases, death [41]. As a result, in November 2008, a safety limit of 1 ppm for infant formula has been established by U.S. Food and Drug Administration for the ingestion of melamine [42, 43].

2.3.1 Detection Methods and Limitations

The intentional adulteration of milk products with melamine has been a mounting concern due to its harmful effect towards the health of human especially on children. Since melamine is not a substance that should be present in food at any level, trace amount of melamine in foodstuffs has to be routinely monitored. The most common methods utilized for melamine detection were by gas chromatography with mass spectrometry (GC-MS) [44] and high-performance liquid chromatography with mass spectrometry (HPLC-MS) [45].

Kababick's group presented a method for the screening of melamine using GC-MS [44]. In their study, melamine from dry protein materials was first extracted followed by derivatization process. The sample was analysed and the collected spectral peaks was compared with a blank spike. They showed that melamine can be detected at a low level of 100 ppb. Beside GC-MS method, Filigenzi et al. developed HPLC-MS for melamine analysis [45]. They first extracted melamine through homogenization of kidney tissue. The homogenate was then concentrated and fortified

with isotope-labelled analogue of melamine. After detection, the collected positive sample was quantified and compared with standard method. The limit of detection for melamine using this method was 50 ppb.

Besides the popular GC-MS and HPLC-MS screening methods, a number of novel analytical methods have been attempted. Campbell et al. reported the use of matrix-assisted laser desorption ionization (MALDI) technique to analyse melamine [46]. Their result indicated that $(M+H)^+$ ions was observed for melamine under positive ion conditions. As an excellent alternative to HPLC, Cook et al. presented a sensitive capillary zone electrophoresis (CZE) method for melamine detection [47]. Using a low background electrolyte pH, components in melamine are separated according to their charge/ionic radius ratio and a detection limit of 100 ppb was achieved. Additionally, other methods including near-infrared spectroscopy (NIR) [48] and enzyme-linked immunosorbent assay (ELISA) [49] have been with both techniques established a melamine detection limit of 1 ppm.

Although these methods show high sensitivity, they nonetheless involve sophisticated and time-consuming procedures, especially in terms of sample preparation and subsequent data analysis. In addition, they are not cost-effective screening tools for large numbers of samples typically encountered in food ingredients. Therefore, it is important to develop simpler, quicker, cost-effective and sensitive methods for melamine detection in food systems.

Gold and silver nanoparticles have been used for colorimetric detection due to their unique optical properties which strongly depend on the surrounding dielectric

medium, inter-particle distance, size and shape. Ai's group [50] and Li's group [51] and have both reported the detection of melamine based on melamine-induced colour change of label-free AuNP. The amines groups of melamine molecule are demonstrated to be the key factor to induce AuNP aggregation. These proposed methods can be easily observed by naked eye without the aid of any advanced instrument. However, the major drawback to the use of colorimetric assays as routine food products screening is that they lack selectivity. In order to improve selectivity, Liang's group [43] and Lou's group [52] developed AuNP that have been tailored with cysteamine and 4-mercaptopyridine for the detection of melamine.

Nowadays, SERS has been widely used for evaluating food safety and quality without destroying the sample. Carefully designed SERS substrates have been developed to exploit the dramatic increase in the Raman scattering efficiency of molecules adsorbed to rough metallic surfaces. He et al. [53] and Liu's group [42] employed Klarite, a commercially available substrate for the SERS detection of melamine. However, this commercial substrate does not have very high SERS enhancement. Recently, melamine detection using SERS substrates such as gold [54, 55] and silver [56, 57] nanoparticles have been developed. Their studies have demonstrated that SERS substrate with suitable optimization is highly effective to screen trace melamine.

CHAPTER 3: CYSTEINE-MODIFIED GOLD NANOPARTICLES FOR SELECTIVE AND SENSITIVE TRINITROTOLUENE DETECTION

3.1 Materials

Gold (III) chloride trihydrate (HAuCl_4), sodium citrate tribasic dihydrate, hydroxylammonium chloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$), L-cysteine, 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT), 2-nitrophenol (NP) and nitrobenzene (NB) were purchased from Sigma-Aldrich. All of the chemicals were used as received. Deionized (DI) water purified using Sartorius AG arium system with resistivity of $18.2 \text{ M}\Omega\cdot\text{cm}$ was used in all experiments. The glassware used was cleaned thoroughly with *aqua regia* solution and rinsed with copious amount of DI water prior to use.

3.2 Methodology

3.2.1 Gold Nanoparticles Synthesis

In this experiment, gold nanoparticles (AuNP) with average diameter of 20 nm were synthesized by direct reduction of HAuCl_4 using sodium citrate as reducing agent as well as stabilizing agent. First, 5 mg of HAuCl_4 was dissolved in 50 ml of DI water and the solution was boiled under vigorous stirring. Upon boiling, 5 ml of 38.8 mM sodium citrate was quickly injected to the solution. The solution was allowed to boil

for another 30 minutes. Finally, the solution was cooled down and stored in refrigerator at 4°C for further use.

Next, larger AuNP were synthesized through seeded-growth method using $\text{NH}_2\text{OH}.\text{HCl}$ as reducing agent and sodium citrate as stabilizing agent. AuNP with average diameter of 40 nm were prepared using the freshly-prepared 20 nm AuNP as seeds. In the synthesizing process, 12 ml of 20 nm AuNP was added to 150 ml of DI water followed by 1.2 ml of 200 mM $\text{NH}_2\text{OH}.\text{HCl}$ under vigorous stirring. Then, 3 mM of HAuCl_4 dissolved in 12 ml of DI water was added dropwise to the growth solution. After the reaction was complete, 20 ml of 10 mM sodium citrate was added to the solution for 10 minutes. Figure 1 illustrates the schematic of 20 nm and 40 nm AuNP fabrication.

Figure 1: Schematic illustration of the synthesis of (a) 20 nm and (b) 40 nm AuNP

3.2.2 Gold Nanoparticles Surface Modification

For the selective detection of TNT, the surface of the citrate-stabilized AuNP was modified with L-cysteine. The freshly-prepared 40 nm AuNP and 1 μM of L-cysteine were mixed in 9:1 volume ratio under gentle stirring for 1 hour. After that, the solution was centrifuged at 5000 rpm for 10 minutes to remove the excess cysteine. The cysteine-modified AuNP was then added with 1 μM of TNT in methanol and gently stirred for 1 hour. Figure 2 illustrates the schematic of cysteine modification and Meisenheimer complex formation.

Figure 2: Schematic illustration of cysteine modification on AuNP and Meisenheimer complex formation upon TNT addition

3.2.3 Selectivity and Sensitivity Detection

For sensitivity detection, the 40 nm cysteine-modified AuNP was added with TNT with concentration range from 1 μM to 1 nM under gentle stirring. On the other hand, 1 μM of DNT, NP and NB was added individually to the modified AuNP for selectivity analysis. As a control experiment, TNT was added with AuNP without cysteine modification.

3.2.4 Characterization and Measurement

UV-visible (UV-Vis) absorption spectra were recorded using a Shimadzu UV2501 spectrophotometer. Scanning electron microscope (SEM) images were obtained using JEOL JSM-6700F scanning electron microscope. SERS spectra were acquired using Renishaw inVia Raman microscope as shown in Figure 3 with laser wavelength of 785 nm. The laser was focused onto sample solution using a 50X objective lens. Single scan with an integration time of 10 seconds at 100% laser power was performed for each spectra acquisition.

Figure 3: Renishaw inVia Raman microscope instrumentation

3.3 Results and Discussion

3.3.1 Gold Nanoparticles Synthesis

The process of AuNP formation involved the interplay of three steps which is nucleation, growth and peptization. In the first method, it is observed that the colour of HAuCl_4 solution is pale yellow initially due to the presence of charge-transfer-to-solvent (CTTS) band of AuCl_4^- [58]. Upon addition of sodium citrate, the CTTS band disappeared and the solution changed from yellow to colourless in few minutes. Eventually the solution turned wine red after 20 minutes. According to UV-Vis spectrum shown in Figure 4(a), the synthesized AuNP have a peak at wavelength of 524 nm which corresponds to average diameter of 20 nm in size which was supported by SEM image in Figure 4(b). Since the peak width in the spectrum was narrow, the synthesized AuNP had comparable size. Experiments showed that there is a negative correlation between the concentration of sodium citrate and the size of the AuNP. It is observed that when less amount of sodium citrate is added, the synthesized AuNP were larger.

Figure 4: (a) UV-Vis spectrum and (b) SEM image of 20 nm AuNP

In the second method, the colour of the solution which contained the 20 nm AuNP as seeds remained wine red even after the addition of $\text{NH}_2\text{OH}\cdot\text{HCl}$. However, as HAuCl_4 was added dropwise to the solution, the colour of the solution changed to pale red gradually. A control experiment in which the 20 nm AuNP solution added with HAuCl_4 only showed no change to the colour of the solution. This proved that $\text{NH}_2\text{OH}\cdot\text{HCl}$ acted as reducing agent in reducing the Au^{3+} ions into gold atoms. The formed gold atoms deposited on the surface of gold seeds and it started to grow larger. From the UV-Vis spectrum shown in Figure 5(a), the synthesized AuNP have a peak at wavelength of 535 nm which corresponds to an average diameter of 40 nm in size which was supported by SEM image in Figure 5(b). Experiments showed that the seeded-growth method is better in synthesizing larger AuNP as compared to the one-pot synthesis method. This is because in the presence of gold seeds as template, secondary nucleation was prevented and the synthesized large AuNP will have a fairly uniform size.

Figure 5: (a) UV-Vis spectrum and (b) SEM image of 40 nm AuNP

3.3.2 Gold Nanoparticles Surface Modification

In my study, I have used cysteine to modify the surface of AuNP for the selective detection of TNT. Cysteine is an amino acid with three functional groups which is the amine ($-\text{NH}_2$), carboxyl ($-\text{COOH}$) and sulfhydryl ($-\text{SH}$) group. Upon the addition of cysteine, the surface of the AuNP was modified through the formation of Au-S covalent bond with cysteine due to the strong binding affinity of $-\text{SH}$ group towards gold. Since cysteine remains in zwitterionic form, cysteine-modified AuNP are known to aggregate through electrostatic and hydrogen bonding interaction at higher concentrations as shown in Figure 6(a).

However, in my experiment, the addition of cysteine did not change the colour of the solution and UV-Vis spectrum of the cysteine-modified AuNP in Figure 7(a, ii) remained the same. This indicates that there was no aggregation observed when 9:1 volume ratio of 40 nm AuNP and 1 μM of cysteine were stirred. SEM image in Figure 7(b), also confirmed it. When lower concentration of cysteine was used, the attractive force through electrostatic and hydrogen bonding interaction is believed to be weak as compared to the repulsive force exerted by the citrate ions on the surface of adjacent AuNP as shown in Figure 6(b). Hence, the resultant cysteine-modified AuNP was stable.

Figure 6: Schematic illustration of AuNP under (a) high and (b) low cysteine concentration

Figure 7: (a) UV-Vis spectra of 40 nm AuNP with (i) 0; (ii) 1; (iii) 10; (iv) 100 μM of cysteine and (b) SEM image of 1 μM cysteine-modified 40 nm AuNP

3.3.3 Trinitrotoluene Detection

The detection is based on the fact that in the presence of TNT, cysteine-modified AuNP will form a Meisenheimer complex [59]. Due to the strong electron withdrawing effect of the nitro ($-\text{NO}_2$) group, the benzene ring of TNT becomes electron-deficient. At the same time, the $-\text{NH}_2$ group of cysteine possesses a lone pair of electrons which makes it electron-rich. As a result, the $-\text{NH}_2$ group donates its lone pair electrons to the benzene ring and a Meisenheimer complex is formed as shown in Figure 8(a). In addition, as shown in UV-Vis spectra in Figure 9(a, iii), due to electrostatic and hydrogen bonding interaction between Meisenheimer complex bound AuNP and cysteine-modified AuNP, they undergo aggregation. This aggregation phenomenon is illustrated in Figure 8(b). Hence, several hot spots were formed and provided significant Raman signal enhancement through electromagnetic field enhancements when irradiated by 785 nm laser.

As previously mentioned that cysteine-modified AuNP are known to aggregate at higher concentration of cysteine, however in my experiments, the formation of Meisenheimer complex upon TNT addition helps the cysteine-modified AuNP to aggregate even at low concentration of Meisenheimer complex. This indicates that Meisenheimer complex bound AuNP has strong ability to form aggregate with normal cysteine-modified AuNP in comparison to the formation of aggregate by cysteine-modified AuNP with itself. SEM images in Figure 9(b) and 9(c) showed that the stability of AuNP did not alter even after the modification by 1 μM of cysteine. However, after the addition of 1 μM of TNT, AuNP aggregation was clearly seen in Figure 9(d).

Figure 8: Schematic illustration of (a) Meisenheimer complex formation and (b) Meisenheimer complex-induced aggregation

Figure 9: UV-Vis spectra of (a) (i) 40 nm AuNP; (ii) Cysteine-modified AuNP; (iii) Cysteine-modified AuNP upon 1 μ M TNT addition and SEM images of (b) 40 nm AuNP; (c) Cysteine-modified AuNP; (d) Cysteine-modified AuNP with TNT

From the SERS spectrum shown in Figure 10(a), it clearly showed a band appears around 2900 cm^{-1} which correspond to the formation of Meisenheimer complex [36]. It is due to the NH_2^+ symmetric stretch, C-H stretching and CH_2 asymmetric stretching. In addition, the SERS spectrum exhibited several prominent TNT peaks. A strong Raman bands at 1026 cm^{-1} is due to CH_3 deformation. Peaks at 1360 cm^{-1} and 1533 cm^{-1} are due to NO_2 symmetric and asymmetric stretching vibration respectively. Peak at 1615 cm^{-1} is due to C=C aromatic stretching vibration. While, the weak Raman band at 790 cm^{-1} is due to C-H out-of-plane bend. These peaks correspond to the molecular fingerprint of TNT [60]. On the other hand, the SERS spectrum showed that the intensity of TNT bands are stronger than the intensity of Meisenheimer complex band, this indicates that the formation of Meisenheimer complexes are significantly low as compared to the TNT concentration used.

It is observed that the aggregation results in a substantial shift in the plasmon band energy to longer wavelength and a red-to-blue colour change. Since the AuNP are in close proximity, plasmonic coupling between nanoparticles results in huge local electromagnetic field enhancements in these confined junctions [61]. Hence, TNT helps to generate hot spots through aggregation of cysteine-modified AuNP. However, when TNT was added to AuNP solution without cysteine modification, no colour change was observed and UV-Vis experiment showed that there is no aggregation. As a result, hot spot has not been generated and no SERS signal was observed from AuNP solution upon TNT addition as shown in Figure 10(b). This clearly shows that cysteine-modified AuNP is a must to generate hot spots in the presence of TNT.

Figure 10: SERS spectra of 1 μM TNT using 40 nm AuNP (a) with cysteine modification and (b) without cysteine modification

3.3.4 Selectivity Detection

In order to prove that the cysteine-modified AuNP probe is highly selective, we replaced TNT with other nitro-aromatic compounds which are DNT, NP and NB. In the experiment, the addition of DNT, NP and NB did not change the colour of the cysteine-modified AuNP solution and UV-Vis spectra as shown in Figure 11(a, ii-iv) remained the same. This indicates that there was no aggregation observed since there is no Meisenheimer complex formed and it is proved by the SEM images in Figure 11(b) and 11(c). However, in the presence of TNT, cysteine-modified AuNP will aggregate as shown by SEM image in Figure 11(d). In the case of DNT, it is probably due to the lack of nitro group in the benzene ring which made DNT only partially electron-deficient. Since the amine group from cysteine cannot donate its lone pair electrons to the benzene ring, DNT may not form Meisenheimer complex. As a result, aggregation of cysteine-modified AuNP is prevented. The same phenomenon applies to NP and NB. Therefore, cysteine-modified AuNP probe showed excellent selectivity over nitro-aromatic compounds like DNT, NP and NB.

Figure 11: UV-Vis spectra of (a) (i) 40 nm AuNP; cysteine-modified AuNP upon addition of 1 μ M (ii) DNT; (iii) NP; (iv) NB; (v) TNT and SEM images of cysteine-modified AuNP with (b) DNT; (c) NP; (d) TNT

Since cysteine-modified AuNP showed excellent selectivity over DNT, NP and NB, there was no SERS signal observed for the three nitro-aromatic compounds as shown in Figure 12(b), 12(c) and 12(d) as opposed to TNT in Figure 12(a). As mentioned previously, aggregation due to the formation of Meisenheimer complex will result in a substantial shift in the plasmon band energy to longer wavelength and a red-to-blue colour change. However, since DNT, NP and NB do not help in generating hot spots though aggregation of the cysteine-modified AuNP, plasmonic coupling between nanoparticles did not happen. Hence, there is no local electromagnetic field enhancement and no SERS signal was observed from AuNP solution. This clearly shows that cysteine-modified AuNP only generate hot spots in the presence of TNT.

Figure 12: SERS spectra of 1 μ M (a) TNT; (b) DNT; (c) NP and (d) NB using cysteine-modified 40 nm AuNP

3.3.5 Sensitivity Detection

Apart from highly selective, sensitivity is another important feature of SERS probe. As shown in Figure 13, the SERS intensity at 1026 cm^{-1} is highly sensitive to the concentration of TNT. The experimental results demonstrated that the detection limit of the SERS probe is as low as 1 nM of TNT. At low concentration of TNT, the SERS signal was weak because of the low number of TNT molecules adsorbed on the cysteine-modified AuNP surface. However, when the concentration of TNT was increased, the number of hot spots generated through aggregation also increased. As a result, the SERS intensity was greatly enhanced. This is because there is more TNT molecules present on the AuNP surface and eventually it achieved optimum intensity at monolayer coverage. After that, the SERS intensity started to decrease. It may be due to the fact that with further increase in concentration of the TNT, multiple layers are formed and SERS signal decrease in intensity.

Several research groups have reported the use of the formation of Meisenheimer complex to detect TNT [35, 36]. Although the detection limits are very low to picomolar level, their SERS probes are relatively complex and low in reproducibility. By contrast, the developed cysteine-modified AuNP in my experiment is simple and shows high reproducibility. Moreover, the experimental method is cost-effective without the requirement of sophisticated instrumentations and the need of fluorescent tagging on nanostructures to achieve the required sensitivity. These excellent properties enable the practical application of the SERS probe for on-the-spot detection of TNT at a very low level.

Figure 13: Plot illustration of relationship between Raman intensity at 1026 cm^{-1} and TNT concentration

CHAPTER 4: SILVER-COATED GOLD NANOSTARS FOR MELAMINE DETECTION

4.1 Materials

Gold (III) chloride trihydrate (HAuCl_4), sodium citrate tribasic dihydrate, hydrochloric acid (HCl), sodium hydroxide (NaOH), cetyltrimethylammonium bromide (CTAB), crystal violet (CV), 2-naphtalenethiol (2-NT) and melamine were purchased from Sigma-Aldrich. Silver nitrate (AgNO_3) was obtained from Strem Chemicals while L-ascorbic acid (L-AA) was acquired from Tokyo Chemical Industry. All of the chemicals were used as received. Deionized (DI) water purified using Sartorius AG arium system with resistivity of $18.2 \text{ M}\Omega\cdot\text{cm}$ was used in all experiments. The glassware used was cleaned thoroughly with *aqua regia* solution and rinsed with copious amount of DI water prior to use.

4.2 Methodology

4.2.1 Gold Nanoparticles Synthesis

Gold nanoparticles (AuNP) with average diameter of 14 nm were synthesized in this experiment by direct reduction of HAuCl_4 using sodium citrate as reducing agent as well as stabilizing agent. First, 60 mg of HAuCl_4 was dissolved in 400 ml of DI water and the solution was boiled under vigorous stirring. Upon boiling, 4 ml of 170 mM sodium citrate was quickly injected to the solution. The solution was allowed to

boil for another 30 minutes. Finally, the solution was cooled and stored in refrigerator at 4°C for further use.

4.2.2 Gold Nanostars Synthesis

Here, gold nanostars (AuNS) were synthesized through seeded-growth method with L-AA as reducing agent. First, 2 ml of 25 mM HAuCl₄ was added to 98 ml of DI water and the solution was gently stirred. After that, the growth solution was added with 200 µl of 1 M HCl followed by 1 ml of freshly-prepared 14 nm AuNP as seed. Then, 400 µl of 10 mM AgNO₃ was added into the mixture and the solution vigorously stirred for 2 minutes. Next, 1 ml of 100 mM L-AA was pipetted into the mixture under vigorous stirring for 2 minutes. Finally, 8 ml of the solution was added with 500 µl of 10 mM of CTAB and it was vigorously stirred for 3 minutes.

4.2.3 Silver-coated Gold Nanostar Synthesis

First, 100 µl of 5 mM AgNO₃ was added to 8 ml of freshly-prepared AuNS and the mixture was gently stirred. Then, 250 µl of 100 mM L-AA was added into the gently stirred solution. After that, 500 µl of 100 mM NaOH was pipetted into the mixture under vigorous stirring for 2 minutes. Finally, the solution was added with 500 µl of 10 mM of CTAB and it was vigorously stirred for 3 minutes. The synthesis procedure above was repeated by changing the volume of 5 mM AgNO₃ added to 200, 300, 400, 500, 600 and 700 µl. The freshly prepared AuNS and seven different silver-coated gold nanostars (Au@AgNS) solutions were centrifuged at 2500 rpm for 12 minutes. Then, the supernatants were removed and the pellets were re-dispersed with 3

ml of DI water. Figure 14 illustrates the schematic of AuNS and Au@AgNS fabrication.

Figure 14: Schematic illustration of the synthesis of (a) AuNS and (b) Au@AgNS

4.2.4 Intensity Optimization on Crystal Violet and 2-Naphthalenethiol

In this study, AuNS and seven different Au@AgNS solutions were added with 1 mM of CV in 9:1 volume ratio under gentle stirring for 2 hours. The experiment above was repeated by substituting CV with 10 mM 2-NT.

4.2.5 Melamine Study and Limit of Detection

Here, the Au@AgNS with optimized silver amounts was added with melamine at concentration range from 1 mM to 1 μ M under gentle stirring for 2 hours.

4.2.6 Characterization and Measurement

UV-visible (UV-Vis) absorption spectra were recorded using a Shimadzu UV-2501 spectrophotometer. Scanning electron microscopy (SEM) images were obtained using JEOL JSM-6700F scanning electron microscope. Transmission electron microscopy (TEM) observations were conducted on a JEOL JEM-2010 electron microscope. SERS spectra of CV and 2-NT were analysed using Ocean Optics PeakSeeker Raman microscope as shown in Figure 15 with 785 nm laser at 300 mW power and 10 seconds integration time. While, SERS spectra of melamine were acquired using Renishaw inVia Raman microscope with 785 nm laser too. The laser was focused onto sample solution using a 50X objective lens. Single scan with an integration time of 10 seconds at 100% laser power was performed for each spectra acquisition.

Figure 15: Ocean Optic PeakSeeker Raman microscope instrumentation

4.3 Results and Discussion

4.3.1 Gold Nanoparticles and Nanostars Synthesis

In the first experiment, AuNP with average size of 14 nm was prepared based on Turkevich's method through slight modification. It is observed that during the reaction process the colour of HAuCl_4 solution changed from pale yellow to colourless in the space of few minutes after the addition of sodium citrate which act as reducing agent. The solution eventually turned wine red as the AuNP started to form after 20 minutes.

The freshly-prepared 14 nm AuNP was then used as seeds to grow AuNS. It is observed that during the reaction process the colour of the growth mixture changed from pale yellow to dark green instantly after the addition of reducing agent in the form of L-AA. The UV-Vis spectra in Figure 16 illustrated the peaks for both AuNP and AuNS. It is shown that the peak at wavelength of 519 nm corresponds to AuNP with an average diameter of 14 nm in size while the synthesized AuNS produced a red-shifted peak at wavelength of 776 nm.

Figure 16: UV-Vis spectra of (i) 14 nm AuNP and (ii) AuNS

Currently, two different strategies are employed in AuNS synthesis which is seeded-growth and one-pot methods. In seeded-growth technique, pre-formed gold seeds act as nucleation points for subsequent growth. On the other hand, in non-seeded method, nuclei evolve in situ to form gold seeds and subsequently bigger particles. However, AuNS synthesis using one-pot method was limited by its low yield of the morphology of interest and polydisperse particles. In addition, as surfactant-stabilized seeds may not stimulate irregular growth because of their relatively protected surface, citrate-stabilized AuNP was used as seeds to grow AuNS with plasmon peak resonance around 785 nm in this experiment.

As shown in Figure 17, the reaction between HCl and AgNO₃ resulted in the formation of insoluble AgCl which adsorb onto the gold seeds in the HAuCl₄ growth solution to serve as a nucleation points for the growth of nanostar's tips [62]. After the addition of L-AA, the Au³⁺ ions was rapidly reduced and deposited on the uneven AgCl surface. As a result, the tips were formed and grew until the Au³⁺ ions were completely reduced. The role of HAuCl₄, gold seeds, AgNO₃ and L-AA has been studied previously where modifications in these parameters clearly affect the size and shape of nanostars's tips [22]. In my experiment, by adjusting the amount of gold seeds to be added while keeping others constant, AuNS with plasmon resonance around 785 nm was produced.

Figure 17: Schematic illustration of the synthesis of AuNS

4.3.2 Silver-coated Gold Nanostars Synthesis

In this experiment, AuNS was used as a template to grow Au@AgNS. It is observed that during the reaction process the colour of solution changed from dark green initially to bluish-green and eventually yellowish-green after the addition of AgNO₃ in increasing amounts. Each AuNS solution which contains 1.6 mg of HAuCl₄ was added with 85, 170, 255, 340, 425, 510 and 595 µg of AgNO₃. Correspondingly, these solutions are referred to by their gold to silver (Au:Ag) ratio of 1:0.05, 1:0.10, 1:0.15, 1:0.20, 1:0.25, 1:0.30 and 1:0.35.

The UV-Vis spectra in Figure 18 illustrated the different spectrum for Au@AgNS added with 100 to 500 µl of 5 mM AgNO₃. It is shown as the amount of Ag⁺ ions increased, the peak of the spectrum started to blue-shift accordingly from 776 to 634 nm. At the same time, another peak around 556 nm started to emerge as the spectrum blue-shifted with increased amount of Ag⁺ ions. SEM and TEM images in Figure 20(a, b) and 21(a, b) showed the synthesized AuNS without silver coating. The average core and tip size of AuNS is 60 and 20 nm respectively. While, SEM and TEM images in Figure 20(c, d) and 21(c, d) showed that the silver actually deposited onto the AuNS with the nanostar's tips still preserved.

In the same experiment, the amount of Ag⁺ ions was increased further to study the effect of Ag⁺ ions on the morphology of Au@AgNS. The UV-Vis spectra in Figure 19 illustrated the additional spectrum for Au@AgNS added with 300 to 700 µl of 5 mM AgNO₃. It is shown as the amount of Ag⁺ ions increased, the blue-shifted peak started to merge with the peak at 556 nm until it became a single peak. SEM and

TEM images in Figure 20(e, f) and 21(e, f) showed that the morphology of Au@AgNS had become spherical in shape with the nanostars' tips embedded in silver layer as silver amounts were increased.

Figure 18: UV-Vis spectra of (i) AuNS and Au@AgNS with (ii) 100; (iii) 200; (iv) 300; (v) 400 and (vi) 500 µl of 5 mM AgNO₃

Figure 19: UV-Vis spectra of Au@AgNS with (i) 300; (ii) 400; (iii) 500; (iv) 600 and (v) 700 µl of 5 mM AgNO₃

Figure 20: SEM images of (a, b) AuNS; (c, d) Au@AgNS with Au:Ag ratio of 1:0.25 and (e, f) Au@AgNS with Au:Ag ratio of 1:0.35

Figure 21: TEM images of (a, b) AuNS; (c, d) Au@AgNS with Au:Ag ratio of 1:0.25 and (e, f) Au@AgNS with Au:Ag ratio of 1:0.35

Recently, the fabrication of AuNS has been driven by the interest on its strong optical properties, due to morphologies involving sharp tips and edges, where light can be highly concentrated. In addition, the hybridization of the core and tips of nanostars increase both the excitation cross sections and the localized electromagnetic field, thus creating a hot spot suitable for SERS sensing applications. Silver coating is a well-known method to increase the SERS activity of AuNP by taking advantage of the superior optical properties of silver. In my experiment, by adjusting the amount of silver to be added, Au@AgNS with the desired morphology was produced.

As shown in the Figure 22, in the synthesis of Au@AgNS, L-AA was used as the reducing agent to reduce Ag^+ ions in the AuNS solution. When L-AA was injected to the AuNS solution containing AgNO_3 , instant colour change was not visible which indicates that the reduction process did not take place. However, when a small amount of NaOH was added to the solution, rapid colour change was observed which showed the reduction of Ag^+ ions to silver. This indicates that the solution was not sufficiently basic initially as L-AA favoured to react under basic condition [63].

Figure 22: Schematic illustration of the synthesis of Au@AgNS

4.3.3 Intensity Optimization on Crystal Violet and 2-Naphthalenethiol

In the first part of SERS optimization study, AuNS and seven different Au@AgNS solutions were added with CV, an organic compound mainly used as dye in histological stain. Due to the presence of amine ($-NH_2$) groups, CV easily adsorbed onto the surface of nanostars during incubation. The characteristic peaks observed from the SERS spectra of CV shown in Figure 23(a, b) are ring C–H bending at 1174 cm^{-1} , N–phenyl stretching at 1368 cm^{-1} and ring C–C stretching at 1620 cm^{-1} [64]. On the other hand, in the second part of the experiment, CV was replaced by 2-NT. Owing to the presence of sulfhydryl ($-SH$) group, 2-NT adsorbed strongly to the surface of nanostars through the formation of Au-S bond. The SERS spectra of 2-NT in Figure 24(a, b) was characterized by C-H bending at 1064 cm^{-1} , ring stretching at 1375 cm^{-1} and C-C stretching at 1620 cm^{-1} [65].

The SERS optimization of AuNS and Au@AgNS was investigated and compared using the three prominent SERS peaks of CV and 2-NT discussed above. As shown in Figure 25, the CV intensity for Au@AgNS with gold to silver ratio of 1:0.05 was slightly higher than AuNS. It is suggested that the low amount of silver coating may be insufficient to produce optimum SERS activity. When the amount of silver was increased, the CV intensity enhanced significantly as Au@AgNS with gold to silver ratio of 1:0.25 achieved the highest intensity. However, the CV intensity started to decreased when the gold to silver ratio of Au@AgNS was increased beyond 1:0.25. This result demonstrated that the optimal silver coating on Au@AgNS was found to occur before the nanostars tips were completely embedded under the silver layer. Moreover, same SERS intensity pattern was observed for 2-NT as shown in Figure 26.

Figure 23: SERS spectra of 1 mM CV for (a) Au@AgNS with Au:Ag ratio of 1:0.25 and (b) AuNS

Figure 24: SERS spectra of 10 mM 2-NT for (a) Au@AgNS with Au:Ag ratio of 1:0.25 and (b) AuNS

Figure 25: SERS intensity of 1 mM CV for AuNS and Au@AgNS with increasing Au:Ag ratio

Figure 26: SERS intensity of 10 mM 2-NT for AuNS and Au@AgNS with increasing Au:Ag ratio

4.3.4 Melamine Study and Limit of Detection

From the SERS optimization study using CV and 2-NT, it was observed that Au@AgNS with gold to silver ratio of 1:0.25 showed the highest SERS intensity as compared to other nanostars. Therefore, I have used Au@AgNS with gold to silver ratio of 1:0.25 as a sensitive SERS probe for melamine detection. Melamine is an organic compound with three amine (-NH_2) groups. Upon the addition of melamine, the melamine easily adsorbed onto the surface of nanostars due to the presence of -NH_2 group. Li's group reasoned that adjacent melamine-coated AuNP could be cross-linked by hydrogen bonding between melamine molecules, thereby inducing AuNP aggregation [51]. However, as shown in Figure 27 in my study, I found that the melamine could not induce the colour change of solution through aggregation of nanostars. This may be due to the fact that the synthesized nanostars were stabilized against aggregation with the presence of CTAB layer which repelled the attraction between adjacent nanostars.

Figure 27: UV-Vis spectra of (i) 10 μM melamine using Au@AgNS with Au:Ag ratio of 1:0.25 and (ii) Au@AgNS with Au:Ag ratio of 1:0.25 only

As shown in Figure 28, prominent melamine peaks was observed when melamine was investigated using Au@AgNS with gold to silver ratio of 1:0.25 as compared to control experiment. The characteristic peaks obtained from the SERS spectra of melamine are ring breathing mode II and I at 707 and 1001 cm^{-1} which involve the in-plane deformation of triazine ring [66]. Sharp tips and edges are essential features of nanostars which contributes stronger enhancement than nanoparticles with comparable size. Moreover, by taking advantages of silver coating, SERS activity is greatly enhanced. In my study, the synthesized Au@AgNS with gold to silver ratio of 1:0.25 exhibited optimal silver coating while retaining its star-like morphology. The hybridization of nanostars' core and tips together with strong optical properties of silver created a hot spot for sensitive melamine detection.

Figure 28: SERS spectra of (a) 10 μM melamine using Au@AgNS with Au:Ag ratio of 1:0.25 and (b) Au@AgNS with Au:Ag ratio of 1:0.25 only

As shown in Figure 29, the SERS intensity at 702 cm^{-1} is highly sensitive to the concentration of melamine. The experimental results demonstrated that the limit of detection for melamine using Au@AgNS with gold to silver ratio of 1:0.25 as SERS probe is $1\text{ }\mu\text{M}$. At low concentration of melamine, the SERS intensity was weak because of the low number of melamine molecules adsorbed on the nanostars' surface. When the concentration of melamine was increased, more hot spots were occupied by melamine molecules, thereby increasing the SERS intensity. Eventually the SERS probe achieved the highest intensity at optimal coverage of melamine molecules. After that, the SERS intensity started to decrease. It may be due to the fact that with further increase in concentration of melamine, multiple layers are formed and leads to decrease in SERS intensity.

The use of commercial SERS substrates in food screening for potential melamine adulteration has been previously reported [42, 53]. Although these substrates show high reproducibility, they are relatively expensive and low in sensitivity. On the other hand, the designed Au@AgNS with optimized gold to silver ratio in my experiment is inexpensive and shows high sensitivity. Furthermore, this SERS probe is cost-effective compared to other bulky instrumentations, which are expensive, non-portable and time-consuming. Owing to the presence of sharp morphologies and superior optical properties of silver coating, the designed SERS probe thus offers potential application for trace melamine detection.

Figure 29: Plot illustration of relationship between Raman intensity at 702 cm^{-1} and melamine concentration

CHAPTER 5: CONCLUSION AND FUTURE WORK

In my first project, I successfully designed a cysteine-modified 40 nm AuNP based SERS substrate for selective and sensitive detection of TNT. Since detection method such as GC-MS or FRET is not ideal as real-time sensor for TNT, SERS nonetheless offers advantages through strong optical properties and tunable surface chemistry. By modifying the surface of AuNP with cysteine through formation of Au-S bond, cysteine-modified AuNP are able to form Meisenheimer complex in the presence of TNT. Due to the interaction between Meisenheimer complex bound AuNP and cysteine-modified AuNP, aggregation-induced colour change happened. As a result, AuNP in close proximity created hot spots that resulted in huge local electromagnetic field enhancement through plasmonic coupling between AuNP. The SERS substrate is stable and able to achieve detection limit at 1 nM of TNT. Moreover, it showed good selectivity over other nitro compounds such as DNT, NB and NP.

In my second project, I successfully introduced a novel Au@AgNS as SERS substrate through seeded-growth method. Subsequently, this novel SERS substrate was characterized and optimized for the sensitive detection of melamine. The fabrication of AuNS has been driven by its unique optical properties due to the presence of sharp tips and edges which exhibit strong SERS enhancement compared to AuNP. By taking advantages of silver coating, the SERS activity of Au@AgNS was further enhanced. Through SERS optimization study, Au@AgNS with gold to silver ratio of 1:0.25 was chosen as it demonstrated the highest intensity when this substrate was investigated

using CV and 2-NT. This is due to the fact that Au@AgNS with gold to silver ratio of 1:0.25 exhibited optimal silver coating while retaining its star-like morphology. The hybridization of nanostars' core and tips together with strong optical properties of silver created a hot spot for sensitive melamine detection. As a result, the limit of detection using Au@AgNS with gold to silver ratio of 1:0.25 as SERS probe is 1 μ M.

In my future work, I would like to study the detection of TNT by designing a cysteine-modified SERS substrate using AuNS. Due to the presence of sharp morphologies, AuNS offers several advantages over AuNP. The hybridization of AuNS's core and tips along with the plasmonic coupling arise from the Meisenheimer complex-induced aggregation generate hot spots which further enhance the SERS activity. Although cysteine-modified AuNP offered creditable sensitivity, the employment of AuNS nonetheless allows better detection sensitivity owing to their superior optical properties.

Furthermore, improvement can be made in the study of Au@AgNS based SERS substrate for melamine detection. As the amine groups of melamine have a weaker affinity towards silver surface of Au@AgNS, it is reasoned that the present of CTAB layer used to stabilize the Au@AgNS may possibly hindered the melamine molecules from tightly adsorb onto the surface of Au@AgNS. As a result, there is no Au@AgNS aggregation induced by hydrogen bonding between melamine-coated Au@AgNS and strong SERS enhancement through plasmonic coupling does not materialized. In order to overcome this drawback, the CTAB layer can be removed or substituted with other capping agents. Last but not least, the SERS substrate can be extended to the sensitive detection of heavy metals, pathogens and pesticides.

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