

Enhanced Raman Scattering of TNT on nanoparticles substrates:
Ag, Au and Au/Ag Bimetallic colloids prepared by reduction with
sodium citrate and hydroxylamine hydrochloride

by

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ABSTRACT

Surface-Enhanced Raman Scattering (SERS) provides extremely high sensitivity, due to increase in the Raman cross-sections of intrinsically very weak scattering events making these events comparable or even better than photon emission efficiencies found in fluorescence processes. The observation of vibrational spectra of adsorbed species on surfaces by SERS is one of the most incisive analytical methods for chemical and biochemical detection and analysis. The metallic nanoparticles that make SERS possible are of fundamental interest since they possess unique size-dependent properties (optical, electrical, mechanical, chemical, magnetic, etc.). These properties are quite different from the bulk and the atomic state. Bimetallic nanoparticles are of particular interest since they combine the advantages of the individual monometallic counterparts. Metal colloids have become the most commonly used nanostructures for SERS. Gold, silver and Ag/Au bimetallic alloys colloids have been synthesized by chemical reduction methods and have been used for detecting TNT in solution with high sensitivity and high molecular specificity. The study focused on metallic nanoparticles within size range in the 50-100 nm. The nanoparticles were characterized using UV-VIS spectroscopy, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Raman Spectroscopy. The detection of TNT was conducted via an indirect method that involved the alkaline hydrolysis of TNT in presence of a strong base (NaOH). This method offers the advantage of generating reaction products that provided enhanced detection in the presence of roughened surfaces (SERS). The detection of TNT degradation

products was evaluated on gold, silver and silver/gold bimetallic colloids using excitation wavelengths of 785 and 532 nm. The results revealed an increase in the intensity of the vibrational signals, attributed to the SERS spectra of degradation products that resulted from the alkaline hydrolysis of TNT. Bands associated to NO₂ out-of-plane bending modes at ca. 820 and 850 cm⁻¹ and NO₂ stretching modes at 1300-1370 cm⁻¹ were detected.

RESUMEN

Espectroscopia Raman de señal realzada por superficies (SERS, por sus siglas en inglés) provee alta sensibilidad, debido al aumento de área de sección eficaz Raman de esta manera haciendo que procesos de dispersión intrínsecamente muy débiles comparen o sean superiores a eficiencias de emisiones fotónicas encontradas en procesos de fluorescencia. La observación de espectros vibracionales de especies adsorbidas en superficies mediante SERS es uno de los métodos analíticos más incisivos para detección y análisis químico y bioquímico. Nanopartículas metálicas que hacen SERS posible son de especial interés ya que poseen características y propiedades únicas que dependen del tamaño de su tamaño (ópticas, eléctricas, mecánicas, químicas, magnéticas, etc.). Estas propiedades son diferentes del grueso o del estado atómico del material. Las nanopartículas bimetálicas son de especial interés ya que combinan las ventajas de sus contrapartes metálicas individuales. Los coloides metálicos se han convertido en las nanoestructuras de uso general para SERS. Coloides de oro y de plata y de las aleaciones bimetálicas Au-Ag han sido sintetizados por métodos de reducción química y se han utilizado para detectar TNT en solución con alta sensibilidad y la especificidad molecular. El presente estudio se centra en nanopartículas coloidales con un tamaño que oscila entre 50-100 nanómetros. Las nanopartículas coloidales con caracterizadas utilizando técnicas como Espectroscopia UV-VIS, Microscopio de Rastreo de Electrones (SEM), Microscopio de Transmisión de Electrones (TEM) y Espectroscopia Raman. La detección de TNT se llevó a cabo por un método indirecto que

consistió en la hidrólisis alcalina del TNT en presencia de una base fuerte (NaOH). Este método ofrece la ventaja de generar productos de reacción que proveen un alto aumento de la señal Raman gracias a la presencia de superficies rugosas (SERS). La detección de los productos de reacción del TNT fueron evaluados utilizando como soportes coloides de Au, Ag y dopados Au/Ag, usando como longitudes de onda de excitación de 785 y 532 nm. Los resultados se evalúan en el incremento de la señal Raman de los modos vibracionales mas importantes de los productos de degradación de la hidrólisis alcalina del TNT, se observa los modos amplificados de torsión fuera del plano del grupo NO₂ a 820 y 850 cm⁻¹ y el modo de estiramiento del NO₂ a 1300-1370 cm⁻¹.

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*This work is dedicated to the memory of my mother –
I know she would have been proud.*

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I want to thank God for blessing me with the gift of life and health, for providing me faith, hope, and making me a better person each day. Without him this important goal wouldn't be achieved.

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CHAPTER I

INTRODUCTION

In 1928, Sir C.V. Raman documented the phenomenon of inelastic light scattering [1]. Radiation scattered by molecules contains photons with the same frequency as the incident radiation, but also contain photons with frequency shifted. This effect is very weak: approximately one photon out of a million (0.0001%) will scatter from the sample at a wavelength slightly shifted from the original wavelengths [2-4]. Surface Enhanced Raman Spectroscopy (SERS) involves enhancing Raman signal from target molecules adsorbed on roughened metal surfaces [5-6]. Recent advances have made this a versatile technique having diverse applications not only in analytical science but also in biomedicine [7], environmental monitoring [2,4,8] and analysis of explosives [5]. The attractiveness of this technology lies in its potential of providing in the near future, single particle detection capability. The process is nondestructive, sensitive, fast and reproducible [9].

The size of the nanoparticles prepared by chemical methods is generally governed by factors such as the ratio of the capping agent to the reducing agent [10], the nature of the reducing agent and the capping agent [11-12], the temperature of the reaction and the rate of addition of the reducing agent [9]. The possibility of utilizing colloidal dispersions of Ag or Au in aqueous solutions as a method for enhancing Raman scattering was first demonstrated by Creighton and co-workers in 1979 [13]. They showed that the magnitude of the Raman

scattered signal can be significantly enhanced when the scattering event takes place on or near a roughened or irregular noble-metal substrate. Strong electromagnetic fields are generated when the localized surface plasmon resonance (LSPR) of nanoscale roughness features, on silver, gold or copper substrates is excited by visible light. When the Raman scattering is subjected to these augmented electromagnetic fields, the magnitude of the induced dipole increases, and accordingly, the intensity of the inelastic scattering increases. This enhanced scattering process is known as Surface Enhanced Raman Scattering (SERS) a term that emphasizes the key role of the noble metal substrate in the phenomenon. SERS is commonly used to enhance Raman scattering intensities by up to 6 orders of magnitude. In certain cases, though, the SERS enhancement can be enormous [14-17].

Experimentally, it was found that the most intense SERS events were obtained from molecules adsorbed to silver and gold surfaces, although almost comparable intensities have also been observed for many other metal surfaces such as copper, lithium, sodium, potassium, aluminum, or indium [18-19]. The characterization of organic compounds by means of SERS is very attractive because, in contrast to IR spectroscopy, the method can be applied to Raman Spectroscopy detection in aqueous solutions [16-21]. Several procedures have been proposed to prepare a suitable SERS-active support. Very often, silver electrodes or silver and gold colloids have been used because the enhancement factor can be very high for probe lasers working around 450 to 785 nm. For SERS, the most frequently used silver and gold colloids and silver/gold alloy

colloids are produced either by reduction of silver nitrate or chloroauric acid with sodium citrate, the so-called Lee-Meisel [22] method, or by reduction of silver nitrate or chloroauric acid with sodium borohydride following the Creighton procedure [19]. Aside from these, other reducing methods have also been reported for preparing SERS-active silver, gold and gold/silver colloids. Despite the apparent simplicity of the published procedures, the preparation of a highly active SERS colloid is not a trivial subject. Citrate reduction of silver requires addition of the reducing agent to the silver nitrate solution during vigorous stirring and refluxing. For the Creighton silver colloid, a solution of sodium borohydride is mixed with a solution of silver nitrate during intensive stirring in an ice-cold bath. If due care is not exercised during silver colloid synthesis, the colloid aggregates, eventually precipitating the metal. This leads to changing properties of the colloids and thus also to varying enhancement factors. In this work, we report a method for preparing stable, highly SERS-active silver and gold colloids and gold/silver alloys colloids by reduction of silver nitrate and chloroauric acid with hydroxylamine hydrochloride and sodium citrate. The colloids produced were characterized by UV-VIS spectroscopy, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and validated for SERS. The SERS activity of these colloids was tested using TNT as target analyte.

CHAPTER II

THEORY

Emerging as a powerful analytical tool which extends the possibilities of surface vibration spectroscopy, SERS can solve a vast array of chemical problems. The technique is based on the unique properties of nanosized particles, which possess unique size-dependent characteristics, different from the bulk or the atomic state. These particles are known as clusters, colloids, sols, nanoparticles and nanocrystals.

2.1 Stability of Colloidal Solutions

In the absence of suitable stabilizing agents, colloidal particles would be attracted to each other by van der Waals forces, ultimately resulting in the coagulation and precipitation of the sol due to the repulsion barrier between the approaching particles. There are two methods for stabilization, electrostatic and steric. Electrostatic stabilization involves the use of charged capping agents such as sodium citrate [23]. Citrate capped nanoparticles are negatively charged and attract positively charged counter-cations from the solution which results in the formation of a diffuse electrical double layer and consequently a Columbic repulsion between the particles. As long as the electric potential associated with the double layer is high, electrostatic repulsion between the particles will prevent agglomeration. Changes in temperature may cause a sensitive double layer and alter the ionic strength of the solution. The ionic strength can increase by the

addition of salt causing compression of the double layer and shortening the range of repulsion. Charge reduction on the colloid by the addition of a neutral strongly binding adsorbate displaces the absorbed citrate anions and would result in agglomeration (Figures 1 and 2).

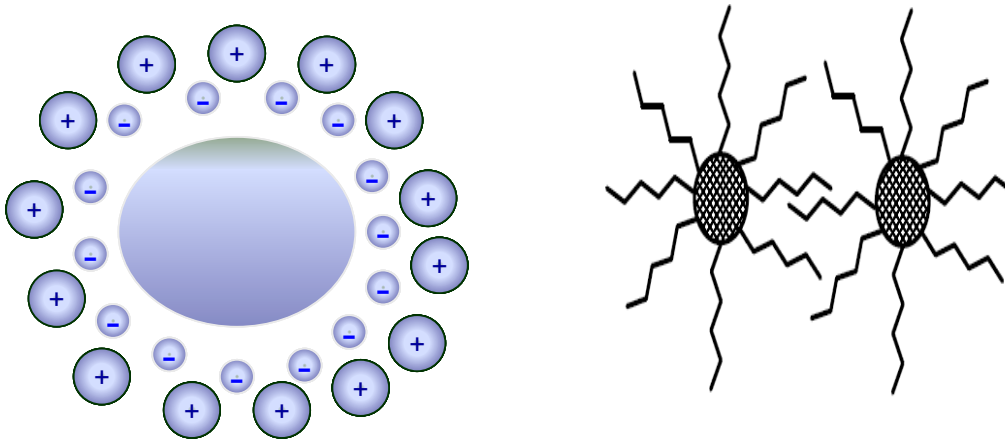


Figure 1. Schematic representation of the stabilization forces in colloidal particles.

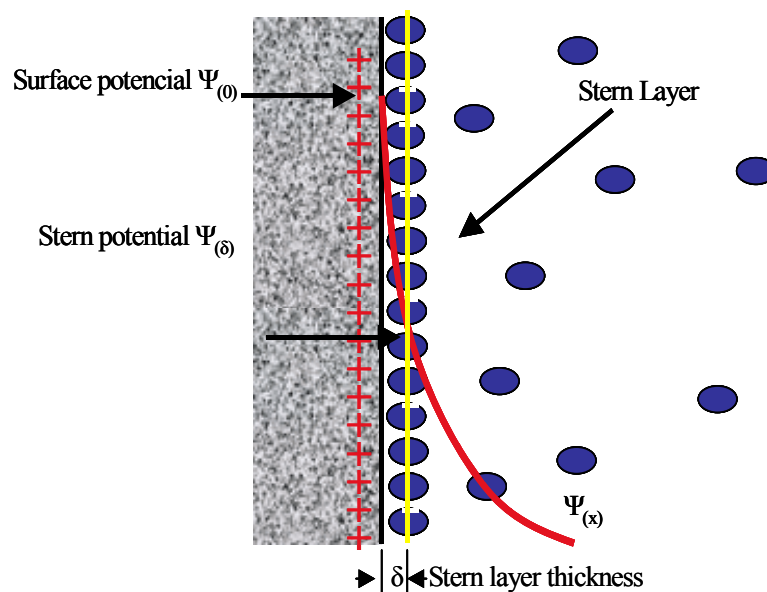


Figure 2. Illustration of Stern double layer.

2.2 Preparation of Metal Nanoparticles

Several transition metals have been stabilized in colloidal form by reducing the corresponding metal ions. The procedures involve the use of reduction agents such as sodium borohydride, superhydride, trisodium citrate, hydrazine, ascorbic acid and tartaric acid. Nucleation and growth are two closely interlinked processes that depend markedly on the nature of the reduction and capping agents. Strong reducing agents as borohydrides and superhydrides induce the formation of nucleation centers which then grow into small clusters whose final size is determined by the nature of the capping agent. Weak agents such as hydrazine, ascorbic acid, tartrate and trisodium citrate exhibit slower reduction process. Early methods of preparation of nanoparticles required aqueous media as well as many applications (particularly biological ones) required them to be water soluble and remain suspended in water with no loss of physical or chemical properties. Among the methods described, the citrate reduction method is most widely used in which the citrate anion acts both as a capping as well as reducing agent at elevated temperatures. In general, the use of a chemical reducing agent has the advantage of feasibility and rapid preparation of the colloidal suspension together with the higher stability of the suspended nanoparticles. This stability is due to the adsorption of the counter ions of the salts employed in the colloid preparation, which confer a high electric charge to the nanoparticles. However, these colloids display several disadvantages such as the existence of impurities resulting from the residual oxidation of species and the counter ions of the employed salts. The advantages of hydroxylamine

hydrochloride reduced silver colloid are seen in its fast preparation at room temperature and its immediate applicability for SERS spectroscopy [23,24].

2.3 Control of Size and Shape of Metal Nanoparticles

A convenient method to increase particle size in a uniform manner is by seed growth. In contrast to other methods which result in high polydispersity and varied shape, this method offers narrow size distribution and near spherical particles. The “seed growth” has the advantage of eliminating nucleation and promoting only the growth process. In this case the metal ions are reduced on the surface of pre-formed metal nanoparticles and are then catalyzed. Shape-controlled synthesis of metal nanoparticles is very important since almost all properties of nanoparticles are shape and size dependent [25].

2.4. Preparation of Bimetallic Nanoparticles

Bimetallic systems are of great interest in catalysis and surface science since they possess unique properties different from their constituents. Nanobimetals have received a lot of attention since they combine the inherent advantages of bimetals with their small size, Toshima and co-workers [26] have carried out extensive studies on bimetallic nanoparticles and have proposed three different types of structures: (a) core-shell, (b) cluster-in-cluster, and (c) random alloy. These structures can manifest in the final composition depending on the reaction conditions and nature of the metals constituents. A schematic representation of the proposed structures is given in figure 1.2. Chemical co-reduction methods

are formed by simultaneous reductions of metal salts impregnated on an inert support. These methods have been reported in both aqueous and organic media. The citrate reduction method has been employed successfully to prepare Au-Ag, Au-Pt and Pd-Pt bimetallic particles. The successive reduction method has been used extensively to prepare core-shell structured bimetals. Turkevich and Kim have used the citrate stabilized Pd nanoparticles as seeds to deposit Au in the presence of a weak reducing agent such as NH_2OH [27].

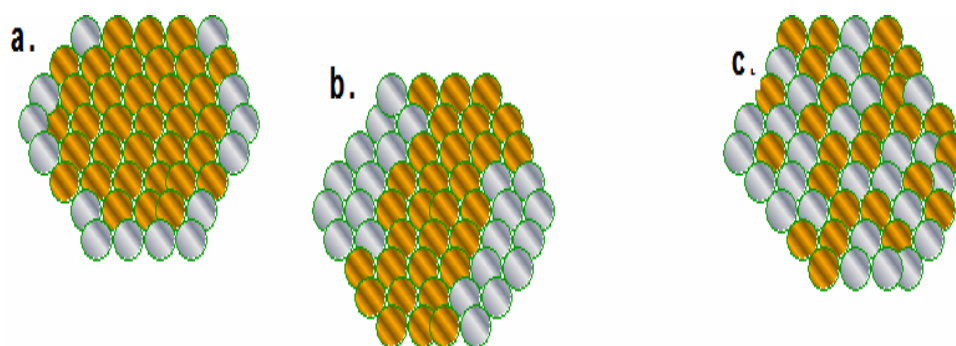


Figure 3. Representation of the various structures proposed for bimetallic clusters. (a) core-shell, (b) cluster-in-cluster and (c) random alloy. (Adapted from ref. 24).

2.5 Properties of Nanoparticles

The criteria for generation of a surface plasmon depend on the excitation frequency and the dielectric constants of the surface. Light causes excitation among the metallic electron and fulfills the necessary surface plasmon requirements by having dielectric constants with a large negative real component and a small imaginary component. Therefore, Ag and Au have become the substrates of choice for SERS experiments. The real component of Ag dielectric

constant gives an optimized local field for small spherical Ag particles with radii in the 10-100 nm range, when using visible excitation, making it a popular SERS substrate. In predicting the optimal SERS enhancement, the importance of the morphology of the metal particles on the substrate's surface cannot be underestimated. The shape and radius of curvature of each metal particle on the surface govern the relationship between scattering intensity and the distance of the adsorbate from the surface, as well as the overall enhancement. Some of the properties such as optical, electronic, chemical, magnetic and mechanical are affected by these size dependent effects [25].

2.5.1 Optical Properties

The optical properties of nanoparticles are strikingly different from that of the bulk. Certain colloidal metals exhibit strong absorption bands in the visible region and are therefore intensely colored. Among the possible noble metals with applications in SERS (Ag, Au, and Cu), silver is the more universal substrate for several reasons: broad plasmon resonance in the visible-near infrared region, high stability and ease of preparation. The absorption band, which is induced by an interacting electromagnetic field, is referred to as the "*surface plasmon*", and appears in the absorption spectrum due to the collective coherent oscillation of the conduction band electrons occupying energy states just above the Fermi level. The position of the surface plasmon depends on several factors among which particle size and shape as well as nature of the surrounding medium are the most important parameters. In general, the surface plasmon values of Ag, Au

and Cu having particles size below 20 nm, lie close to 400 nm, 520 nm and 570 nm respectively.

For dilute colloidal solutions containing spherical nanoparticles of sizes smaller than the wavelength of absorbing light, the scattering cross-section and higher order electric and magnetic multipole oscillations can be neglected and only the dipole absorption of the Mie equation (1), contributes to the total extinction cross section (C_{ext}) of the nanoparticles. This is given by the following relation:

$$C_{\text{ext}} = 24 \pi^2 R^3 \epsilon_m^{3/2} / \lambda * \epsilon_2 / ([\epsilon_1 + 2 \epsilon_m]^2 + \epsilon_2^2) \quad (1)$$

where R is the radius of the nanoparticle, λ is the wavelength of the absorbing radiation, ϵ_m is the dielectric constant of the surrounding medium and ϵ_1 and ϵ_2 are the frequency dependent real and imaginary parts of the dielectric function of the material. For free electron metals, ϵ_2 is fairly constant in the UV-Vis range and a maximum in the absorption (surface plasmon maximum) occurs when $\epsilon_1 = -2 \epsilon_2$. The width and height of the surface plasmon band are determined by ϵ_2 . The surface plasmon is very sensitive to particle size and shape. The former effect manifests itself in the breadth and intensity of the plasmon band. Other investigations have shown, that the plasmon bandwidth has a 1/R dependence, where R refers to the radius of the particle. When the diameter of the particle becomes smaller than the mean free path of the conduction electrons (below approximately 3 nm) there is electron scattering at the particle boundaries resulting in broadening and damping of the surface plasmon. Quantum size effects become important and the changes are referred to as “intrinsic size effects”. In the case of particles larger than 25 nm, there is an increase in the

bandwidth with increase in particle size. The changes are, however, ascribed to retardation effects as well as the involvement of higher order multipoles and are called “extrinsic effects” When the particle shape changes from a sphere to say a spheroid, ellipse or a rod, a profound change in the absorption spectrum is observed. There is a loss of degeneracy of the dipole plasmon, and the plasmon absorption splits into two bands corresponding to the oscillation of free electrons perpendicular to and along the long axis of the particles. These two modes are called the “transverse” and “longitudinal” surface plasmon modes respectively [20,25].

2.5.2 Alloying Properties of Au-Ag Bimetals

The optical absorption spectra of Au-Ag alloy nanoparticles generally exhibit one surface plasmon band whose maximum depends on the alloy composition. Although the values of the free electron plasmon frequency (ω_p) are almost equal, the difference in their frequency location is mainly due to the differing contributions of the interband transitions to the dielectric functions of the two metals. The surface plasmon oscillation in alloy particles is a hybrid resonance resulting from excitations of conduction as well as the d-band electrons [25].

2.5.3 Electrical Properties

Bulk metals possess a partially filled electronic band and can easily conduct electrons due to the availability of a continuum of energy states about the Fermi level. As the size is reduced, the density of states in the valence and conduction bands reduce to such an extent that the electronic properties change drastically.

The quasi-continuous density of states is replaced by discrete energy levels with the average level separation comparable to or larger than the thermal energy [25,26].

2.6 Surface Enhanced Raman Scattering (SERS)

Raman and Krishnan first experimentally observed the phenomenon and reported in their 1928 *Nature* paper that the inelastic scattering effect was characterized by “its feebleness in comparison with the ordinary scattering” [2]. This “feeble” phenomenon is now known as Raman scattering. The change in wavelength that is observed when a photon undergoes Raman scattering is attributed to the excitation (or relaxation) of vibrational modes of a molecule. Because different functional groups have different characteristic vibrational energies, every molecule has a unique Raman spectrum. In accordance with the Raman selection rule, the molecular polarizability changes as the molecular vibrations displace the constituent atoms from their equilibrium positions. The intensity of Raman scattering is proportional to the magnitude of the change in molecular polarizability. Thus, aromatic molecules exhibit more intense Raman scattering than aliphatic molecules. Even so, Raman scattering cross sections are typically 14 orders of magnitude smaller than those of fluorescence; therefore, the Raman signal is still several orders of magnitude weaker than the fluorescence emission in most cases. Because of the inherently small intensity of the Raman signal, the sensitivity limits of available detectors, and the intensity of the excitation sources, the applicability of Raman scattering was restricted for

many years. Creighton and co-workers have demonstrated that colloidal metal solutions of Au, Ag, and Cu that show sharp surface plasmon bands in the visible region are excellent candidates for SERS studies. Requirement of large concentration samples is a main disadvantage of Raman spectroscopy is overcome by SERS. Several theories have been proposed to account for the large increase in intensity of the Raman signal and excellent review articles are available in the literature, though certain aspects are not yet quite understood and are still being investigated. Two primary mechanisms are believed to be responsible for the SERS enhancement: a long-range classical electromagnetic (EM) effect and a short-range chemical (CHEM) effect [25,27].

2.6.1. Electromagnetic Effect (EM)

The EM enhancement mechanism contributes greater than 10^4 times enhancement over normal Raman scattering. In order to understand the EM enhancement, one must consider the size, shape, and material of the surface's nanoscale roughness features. If the correct wavelength of light strikes a metallic roughness feature, the plasma of conduction electrons will oscillate collectively. Because this collective oscillation is localized at the surface of this plasma of electrons, it is known as a localized surface plasmon resonance (LSPR). The LSPR allows the resonant wavelength to be absorbed and scattered, creating large electromagnetic fields around the roughness feature. When the incident light is resonant with the collective modes of oscillation of the metal conduction band electrons there is an enhancement of the electromagnetic field at the

surface at both the incident and Raman scattered wavelengths. This enhancement in turn induces a change in polarizability of the molecule leading to intense Raman signals. For the electromagnetic enhancement effect to be operative, it is not necessary to be in contact with a metal surface [25].

2.6.2. Chemical/ Charge Transfer Effect (CT)

The CHEM enhancement mechanism, now thought to contribute an enhancement factor of 10^2 , asserts that a charge-transfer state is created between the metal and adsorbate molecules. This mechanism is site-specific and analyte dependent. The molecule must be directly adsorbed to the roughened surface in order to experience the CHEM enhancement. The chemical enhancement of the Raman cross section is attributed to the charge transfer between adsorbate and substrate orbital. CTs are therefore dependent on the energy alignment between Fermi energy of the metal and the energy levels of the adsorbate. Though the exact mechanism is still controversial, enhancement of the Raman signal is proposed to be assisted by the presence of active sites for adsorption, which can be generated on the metal surface by different techniques [27].

CHAPTER III

THE PROBLEM

In the post September 11, 2001 world, requires to detect explosives, and chemical and biological agents, at extremely low (trace) levels has been thrust to the forefront in popular media and people's minds. Hardly a day goes by without another suicide attack or car bomb somewhere in the world. The safety of civilized people depends on the capability to detect explosive materials. Yet, even before the realities of terrorist actions were felt even on the previously-thought-to-be-impenetrable soil of the US, nearly all, if not all, known instrumental methods had already been investigated for their applicability to the detection of explosive materials [28,29]. It is necessary to find a rapid and cost-effective solution. For this reason it is essential to selectively detect explosives at ultra low concentration levels. Modern security systems require the development of sensors capable of achieving such selectivity [2]. Surface-enhanced resonance Raman scattering (SERS) is an extremely powerful analytical tool that yields information about bulk explosives. In addition, the molecular structure of the analyte, in the form of a vibrational spectrum, also gives sensitivities comparable to that achieved using fluorescence spectroscopy, with detection limits down to pico and femtomolar concentrations.

The methods used to detect explosives can be subdivided into those that detect vapors or particles emitted from the materials, those that detect dissolved or suspended solids in solutions, and those that probe solid materials. Raman

spectroscopy provides a simple method of detecting and identifying explosives in bulk samples or as crystals under a microscope. The sharp spectra obtained enable the identification of individual components of mixtures without the use of separation procedures. However, the inherent lack of sensitivity of Raman scattering makes it less effective for the identification of low concentrations of target molecules in dispersed samples in solution or the vapor phase [30]. In this study, we show how the technique of SERS, enables the detection of 2,4,6-trinitrotoluene (TNT) at low concentration levels. In SERS, an analyte which contains a chromophore is adsorbed onto a roughened metal (usually silver, gold and silver/gold alloy) and the enhanced Raman scattering recorded from the surface. The wet chemistry provides an inexpensive and versatile approach to metal nanoparticle fabrication. The chemical reduction synthesis method was selected due to his low cost and versatility. Our efforts were focused on understanding and by controlling different properties, as size and shape, by means of different reducing agents. The goals of this study are to prepare and stabilize silver, gold and alloys colloids that can exhibit chemical properties and thermodynamic stability associated to their size.

3.1 Investigated Compounds

3.1.1. 2, 4, 6-Trinitrotoluene (TNT)

TNT (Figure 4) is by far the most important explosive for blasting charges of all weapons. It is very stable, neutral and does not attack metals. TNT is almost insoluble in water, sparingly soluble in alcohol and soluble in benzene and

acetone. The purity grade of the product is determined by its solidification point. The minimum value for military purposes is 80.2 °C.

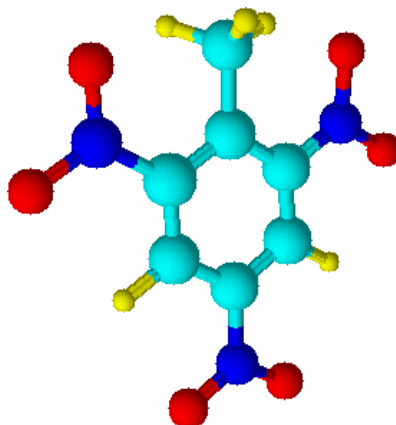


Figure 4. Structure of 2, 4, 6-Trinitrotoluene (TNT).

3.1.3. Adenine

Adenine is a purine also known as 6-aminopurine. Its chemical structure is shown in Figure 5. It is a purine derivative that is one of the five organic bases occurring in the nucleic acids of cells. When adenine is attached to DNA, it forms a bond with another molecule called thymine, a pyrimidine, on the other side of the DNA strand. It is these bonds which give DNA its double-helix structure. The sequence of DNA, or the order in which nucleotides are placed, allows for the diversity among all living organisms. The importance of adenine to RNA is similar to that of DNA.

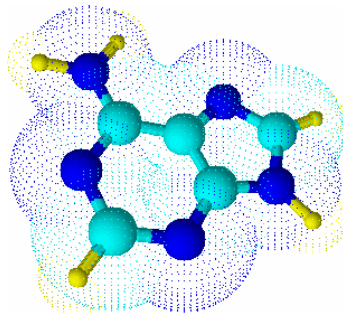


Figure 5. Structure of adenine.

3.1.3. Pyridine

Pyridine (Fig. 6) is a colorless liquid with an unpleasant smell. It can be made from crude coal tar or from other chemicals. Pyridine is used to dissolve other substances. It is also used to make many different products such as medicines, vitamins, food flavorings, paints, dyes, rubber products, adhesives, insecticides, and herbicides. Pyridine can also be formed from the breakdown of many natural materials in the environment.

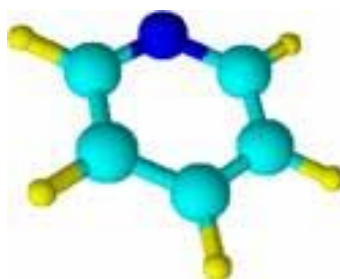


Figure 6. Structure of pyridine.

CHAPTER IV

PREVIOUS WORK

SERS was discovered, though not recognized as such, by Fleischmann et al. in 1974, who observed intense Raman scattering from pyridine adsorbed onto a roughened silver electrode surface from aqueous solution [18]. The motivation for this work was to develop a chemically specific spectroscopic probe which could be used to study electrochemical processes in situ; Fleischmann's approach was to roughen the electrode to increase its surface area and, hence, the number of adsorbed molecules available for study. Jeanmaire and Van Duyne [31] and Albrecht and Creighton [19] recognized independently that the large intensities observed could not be accounted for simply by the increase in the number of scatterers present and proposed that an enhancement of the scattered intensity occurred in the adsorbed state. Interestingly enough, these papers presaged a debate about the SERS mechanism which ran furiously for nearly a decade and about which research is still being conducted. Jeanmaire and Van Duyne tentatively proposed an electric field enhancement mechanism whereas Albrecht and Creighton speculated that resonance Raman scattering from molecular electronic states, broadened by their interaction with the metal surface, might be responsible. As it turned out, they were both right in concept, though not in detail. SERS research accelerated dramatically in the early 1980s with contributions from chemists, physicists and engineers from around the world. It is not hard to see the motivation for such interest. Lee and Meisel proposed the mechanism of

reduction for silver and gold colloids [22]. The colloidal nanoparticle solutions obtained by citrate reduction of gold presented the most negative of potential difference values, in comparison with other reducing agents, and sodium citrate gave the highest stability in these colloids [15]. The stability of the colloids is strongly dependent on the preparation method. Bell and Spence investigated ways to find a suitable medium which would protect the Ag or Au nanoparticles of chemical attack [32]. The general strategy adopted uses of a hydrophilic swelling polymer, which is mixed with the sol to form a viscous solution that is then dried, in the polymer. This methodology can be used to generate SERS-active materials. Graham and Smith [33] showed how the technique of surface enhanced resonance Raman scattering (SERRS), enables the detection of the explosive TNT at low concentration levels. In SERRS, an analyte which contains a chromophore is adsorbed onto a roughened metal (usually silver or gold) and the enhanced Raman scattering recorded from the surface. Hayazawa and collaborators reported the vibrational frequencies of several bands observed for adenine molecules, using silver layer coated to the surface enhanced Raman scattering [34]. In the first part of this study were described the experimental results of the tip-enhanced near-field Raman spectroscopy of single nanocrystals of the DNA base adenine molecules. In the second part, the tip enhanced Raman bands were detected and compared with the bands obtained in conventional SERS.

CHAPTER V

METHODOLOGY

5.1 Reagents and Chemicals

Silver nitrate, trisodium citrate, hydrogen tetrachloroaurate (III) hydrate (99.9% Au), hydroxylamine hydrochloride and sodium chloride were purchased from Sigma Aldrich. 2,4,6-trinitrotoluene (TNT), 98% pure was obtained from ChemService, West Chester, PA. Nanopure® deionized water was used for the preparation of the aqueous solutions.

5.2 Cleansing Method.

Several methods were developed for the preparation of silver colloids by chemical synthesis. Before beginning each synthesis, all glassware was vigorously cleaned using aqua regia, strong acid mixture of HCl:HNO₃ (4:1) which was handled with extreme care as copious amounts of gas were liberated. The use of aqua regia can cause explosions if the acid is stoppered and bad burns occur if it comes into contact with skin. Finally, the glassware was washed several times with triple distilled water.

5.3 Preparation of colloidal suspensions

The preparation of the colloidal suspensions was conducted by two different methods described next.

5.3.1. Preparation of Silver Colloids

5.3.1.1 Citrate –Lee and Meisel Method

In this method developed by Lee and Meisel 100 ml of a 10^{-3} M AgNO_3 aqueous solution was heated to boiling, and then 2.5 ml of a 1% trisodium citrate ($\text{C}_6\text{H}_5\text{O}_7\text{Na}_3$) solution was added to it [22]. The mixture was kept boiling for 1 hour, and then allowed to reach thermal equilibrium at room temperature. The resultant colloidal solution exhibited a dark grey color.

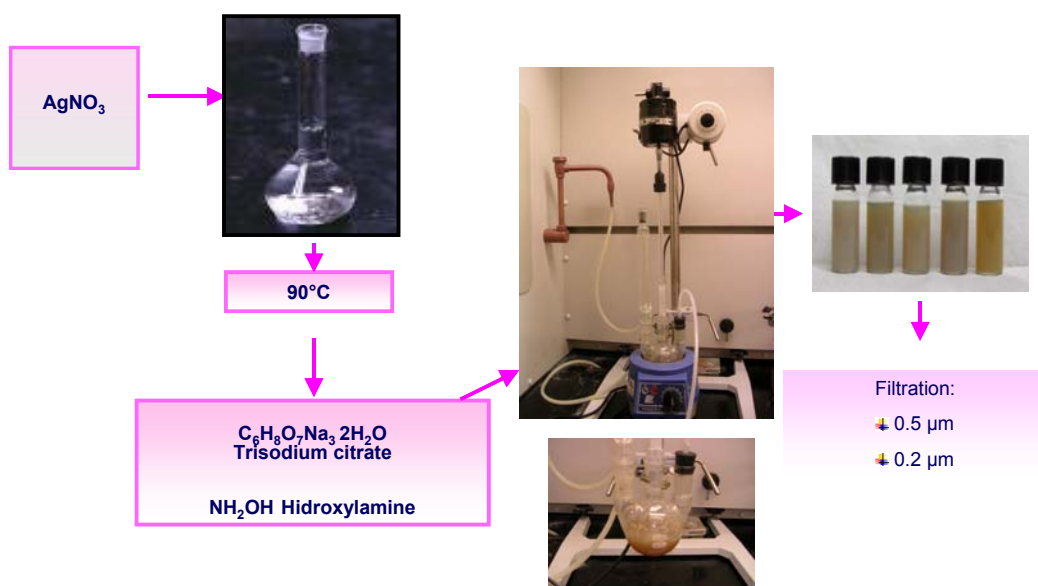


Figure 7. Synthesis of silver colloids a yellow-blue color resulted.

5.3.1.2 Hydroxylamine – Leopold and Lendl Method

The colloid was prepared by dissolving 0.017 mg of silver nitrate (AgNO_3) in 90 ml water. A sample of 0.021 mg of hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) was dissolved in 5 ml water and 4.5 ml of 0.1 M sodium hydroxide was added to

it. The mixture was immediately added to the AgNO_3 solution, and grey-brown solution was obtained in a few seconds. [22,23]

5.3.2. Preparation of Gold Colloids

Gold colloid was prepared by adding 0.1 ml of HAuCl_4 solution (1% w/v in water) to 40 mL of triply distilled water. Then, 1 ml of the trisodium citrate ($\text{C}_6\text{H}_5\text{O}_7\text{Na}_3$) solution (1% w/v) was added dropwise while the solution was stirred. The resulting mixture was boiled for 5 minutes to produce a colloidal dispersion with a dark-red color. [6, 11-12]



Figure 8. Synthesis of gold colloids a violet color resulted.

5.3.3. Preparation of Silver/gold Colloids

5.3.3.1 Silver/gold colloids: Method of Rivas et al.

For the Ag-coated Au colloids, different aliquots (between 1 and 8 ml) of a 1 mM AgNO_3 solution were added dropwise to 20 ml of a preformed gold sol. After the

addition of an appropriate aliquot of sodium citrate solution, the mixture was boiled and stirred for an hour [35].

5.4. Preparation of 2,4,6-Trinitrotoluene Solutions

The detection of TNT was conducted via an indirect method that involved the alkaline hydrolysis of TNT in presence of a strong base, such as sodium hydroxide (NaOH). This method offered the advantage of generating reaction products that provided enhanced detection in the SERS experiments. A sample of 0.0229g of TNT was weighed and added to 100 mL of high purity water. A final concentration of 1×10^{-2} M was obtained. Molar concentrations of 1×10^{-3} , 1×10^{-4} , 1×10^{-5} , 1×10^{-6} , 1×10^{-7} , 1×10^{-9} , 1×10^{-10} , 1×10^{-12} M solutions were obtained by successive dilutions of the stock solution.

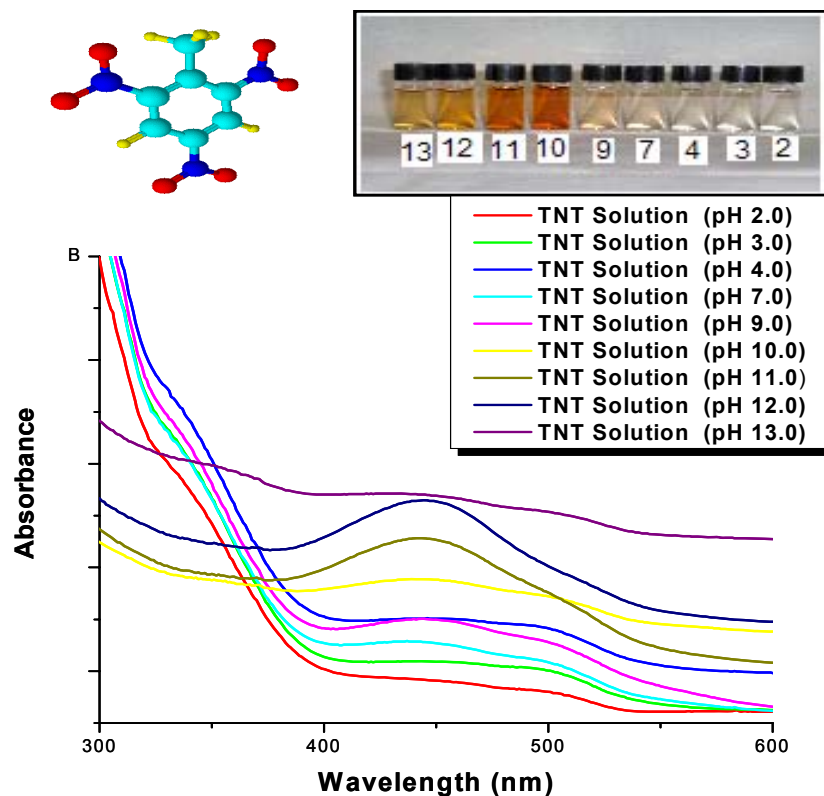


Figure 9. UV TNT Spectrum at different pH values

The pH dependence of Raman signal from colloids was also studied as shown in Figure 9. It was established that decreasing the pH results in enhancement of Raman spectrum. A decrease in the width and change in relative intensity of the SERS bands was also noticed. This is probably due to the progressive transition from a highly covered surface SERS spectrum to a less covered area.

5.5 Sample Preparation for Analysis by SERS

For SERS analysis, 800 μL of the silver colloids, 100 μL of the sample to be analyzed (TNT or adenine or pyridine), and 100 μL of the sodium chloride solution (NaCl 0.6 M) were combined in a vortex vial. The pH of the solution was

adjusted to 13 with a dilute of sodium hydroxide (NaOH) solution. The solution was then stirred in a mini vortex stirrer for a period of 30 seconds. Then, a small volume of this sample was removed by means of a capillary tube (1.6 x 90 mm) for the SERS experiments.

5.6 Instrumentation

The preparation of the nanoparticles was characterized with a Varian Cary-100 UV-visible double beam spectrophotometer. The morphology of the particles was examined using a JEOL JSM 6460LV Scanning Electron Microscope (SEM). Vibrational analysis was conducted using a RM2000 Renishaw Raman Microspectrometer equipped with a Leica microscope with a 10X objective. (Fig. 10)



Figure 10. Renishaw Raman Microspectrometer (RM2000) with Leica objectives with 10x magnification was used.

The TNT detection was achieved at a wavelength of 532 and 785 nm, and laser power of 0.01 mW. The excitation source was a VERDI-6 diode-pumped 532 nm

green laser from Coherent, Inc. with a variable output power of up to 6 W. The Raman spectra were collected in the range of 100 - 3500 wavenumbers (cm^{-1}), with an integration time of 10 seconds per scan. The Raman spectrum was the result of the average of 3 scans.

CHAPTER VI

RESULTS AND DISCUSSION

6.1 UV-VIS, SEM and TEM Results

The method of preparation of the colloidal solutions was optimized to have the maximum SERS effect on TNT vibrational signatures. The concentration of the reducing agent, the addition rate of the reducing agent, and the stirring rate were evaluated in the previous context.

Prior to UV-VIS analysis, the colloidal solution was prepared by transferring 1 μ L of the colloidal suspension and 3 mL of deionized water in a 1 cm quartz cuvette. The UV-VIS spectra of the colloidal suspensions were acquired from 200 nm to 800 nm.

The shapes and sizes of these particles are better characterized using electron scanning probe microscopy. Colloidal preparations yielded particles that were approximately spherical, as can be observed from High Resolution-Transmission Electron Microscope (HR-TEM) images shown in Fig. 11.

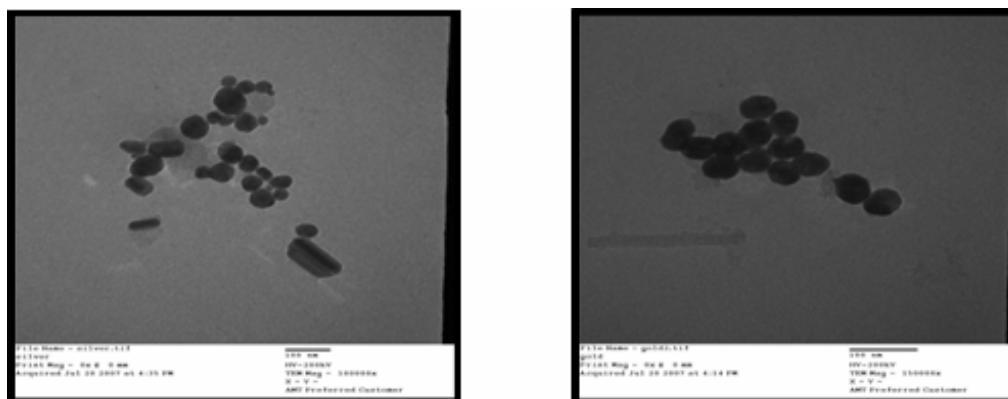


Figure 11. TEM images of gold (left) and silver (right) colloids reduced with sodium citrate.

The average size and size distribution nanoparticles in the silver colloidal are dependent on increase in temperature. The temperature necessary to maintain steady growth of nanoparticles, as the size distribution sharpens, is very important for the electromagnetic enhancement at the surface. Colloids in aqueous solution, prepared by chemical methods, tend to form clusters because of their complicated double layer structure. Colloids have much higher morphological heterogeneity, since they are integrated by nanoparticles with a large spectrum of sizes and shapes. This correlates with the broader plasmon resonance extinction band, demonstrating the existence of small particles.

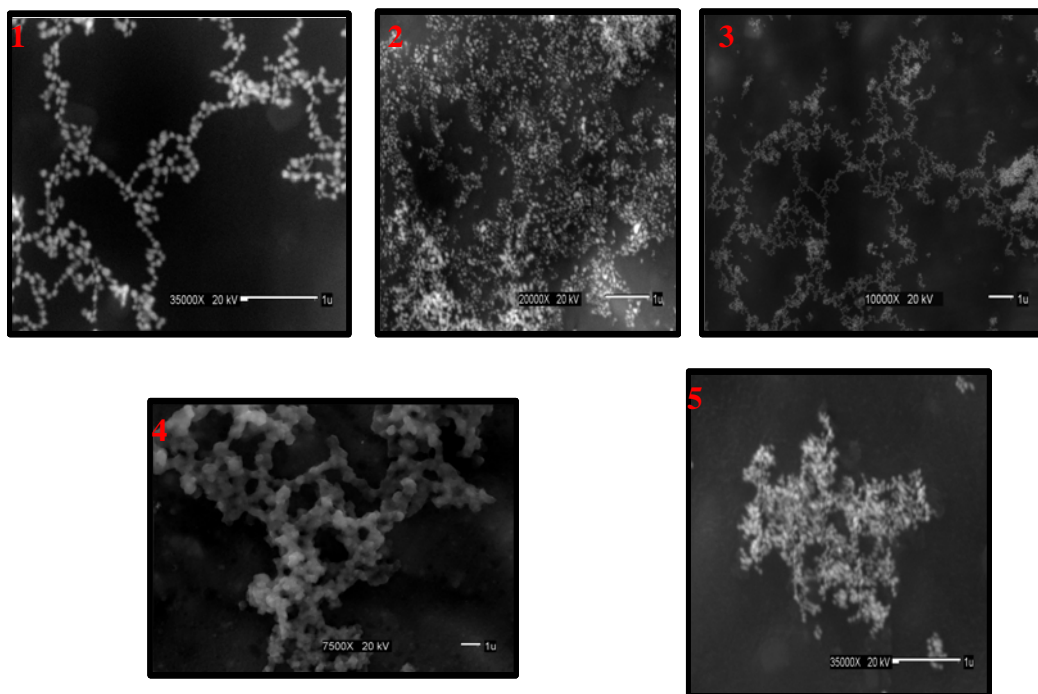


Figure 12. SEM images of the silver colloids reduced with: (1, 2) citrate sodium; (3) gold colloids reduced with citrate sodium; (4) silver colloids reduced with hydroxylamine; and (5) gold colloids reduced with hydroxylamine.

Ag, Au and Au/Ag colloids prepared at four different concentration of reducing agents show difference in their spectral absorption characteristics, which may be

attributed to morphological differences. The absorption maximum of the colloidal suspensions provides information on the average particle size, whereas its full-width at half-maximum (FWHM) gives an estimate of particle size distribution. Ag colloids prepared with hydroxylamine and citrate had absorption maxima at 450 nm and 420 nm, respectively, showing small differences in average particle sizes and were comparable with previous studies [18,22,23] as seen Figures 13 and 15. The FWHM of these suspensions showed a wide range of particle dispersion. Ag colloids prepared with hydroxylamine showed absorption maxima shifted to longer wavelength, indicating presence of larger average particle sizes which may be due to partial aggregation. Colloidal suspensions of silver particles were of bright yellow-greenish color due to the intense bands about the excitation of the surface plasmon resonance. Plasmon bands of gold and gold/silver colloids were 547 nm and 523 nm, respectively. On the other hand, a FWHM of 65 nm for gold and 44 nm for gold/silver alloy is indicative of colloidal suspensions with modest monodispersity. However, for silver a FWHM of ~ 117 nm indicates poor monodispersed nanoparticles in these metallic suspensions. The shapes and sizes of these particles are better characterized using TEM. Colloidal preparations yield particles that are approximately spherical as seen in Figures 11 and 12. According to TEM images, the particle size of gold nanoparticles are 54 ± 5 and for silver are 60 ± 11 . The average size and size distribution nanoparticles in the silver colloids are dependent on temperature increase. Silver, as demonstrated in previous results is highly polydispersed. The temperature control necessary to maintain steady growth of nanoparticles, as the

size distribution sharpens, is very important in order to achieve a large electromagnetic enhancement at the metallic colloidal surface.

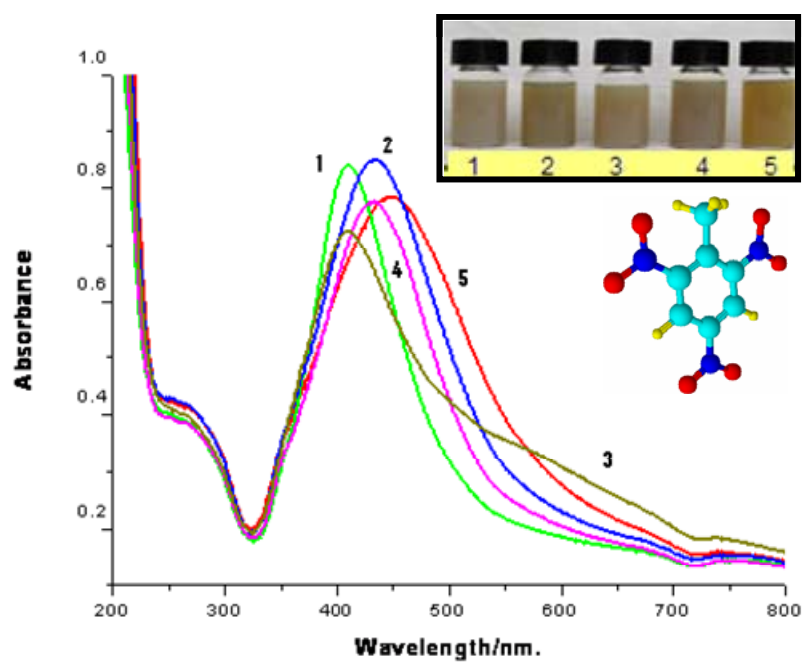


Figure 13. UV-Visible spectra of obtained silver colloids reduced with: (1-4) different concentrations of sodium citrate; and (5) reduced with hydroxylamine.

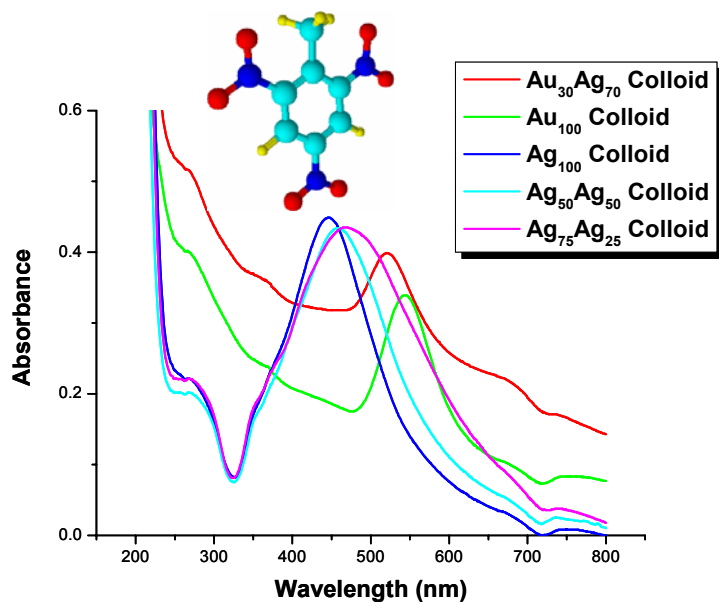


Figure 14. UV-VIS spectra of various colloids: Ag, Au, Ag/Ag and doped Au/Ag.

The surface plasmon oscillation in bimetallic alloy particles is a hybrid resonance resulting from excitations of conduction as well as the d-band electrons. Figure 14 shows the absorption spectra of Ag-coated Au colloids ($Au_{100-x}Ag_x$). The colloids show only an absorption band above 500 nm, which can be exclusively attributed to plasmon resonance of Au particles. When the Ag fraction is increased, a slight downshift in the wavelength of the maximum is observed. The appearance of only one absorption band corresponding to Au indicates that homogeneous mixed colloidal particles of both metals are formed without significant formation of independent particles. The presence of separate Ag and Au resonances in the latter colloid suggests the formation of separate particles in the medium.

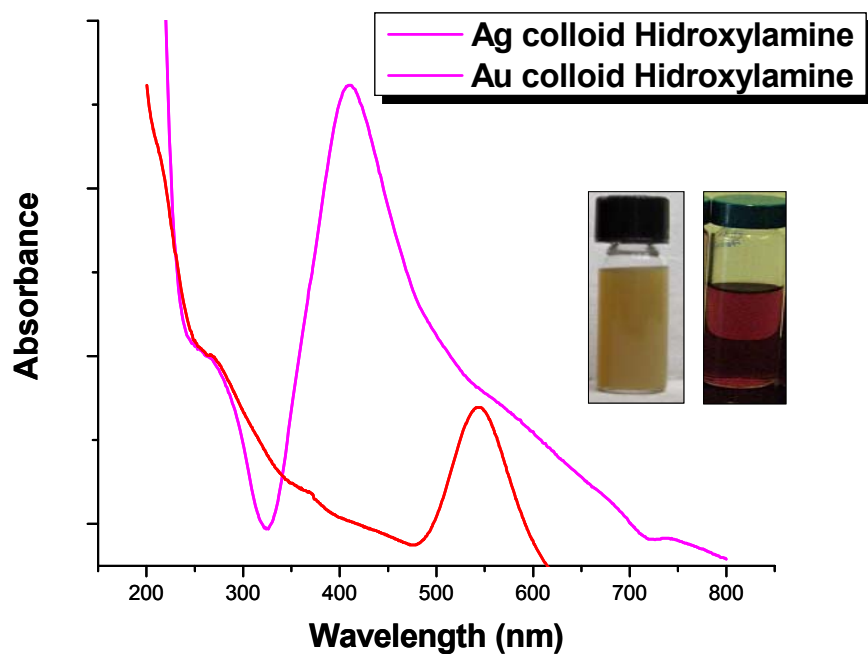


Figure 15. UV spectrum Silver and Gold colloids reduced with hydroxylamine.

A convenient method to increase particle size in a uniform manner is by “seed growth”, seed crystals essentially serve as templates on which further growth of nanoparticles occurs. The method of seed growth has the advantage of eliminating nucleation and promoting only the growth process. In this approach, metal ions are reduced on the surface of pre-formed metal nanoparticles, using weak reducing agents such as citrate, NH_2OH and ascorbic acid. The preformed particles catalyze the reduction of metal ions on their surface. Shape-controlled synthesis of metal nanoparticles is very important since almost all properties of nanoparticles are shape and size-dependent.

6.2 Aggregation

Aggregation is required for obtaining large surface enhancements of Raman signals of analytes adsorbed on the colloidal metallic surface. Various salts can be used for inducing aggregation. Although, the most common ion is Cl^{1-} , the effect of others ions on the aggregation process was investigated. The anions studied were Cl^{1-} , PO_4^{2-} , NO_3^{2-} , SO_4^{2-} and CO_3^{2-} .

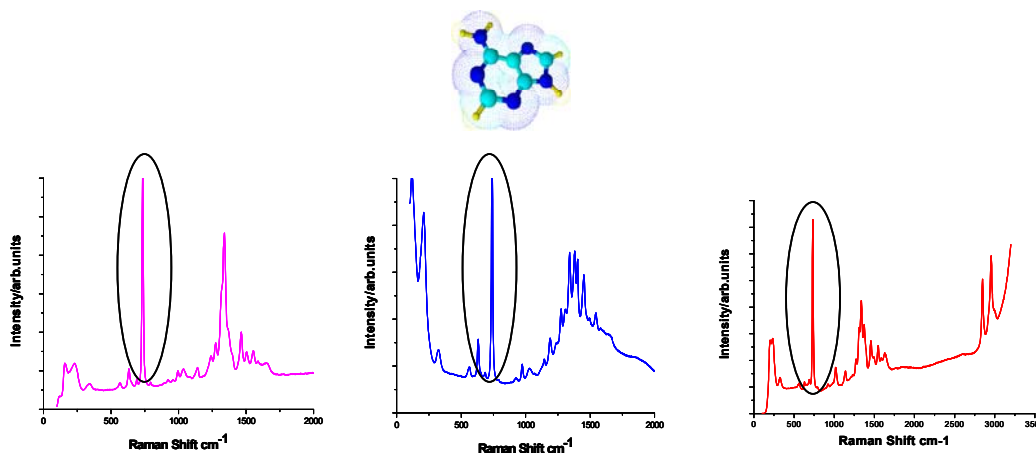


Figure 16. SERS Intensity of the adenine peak (732 cm^{-1}) using silver colloids.

Adenine solutions (10^{-3} M) and Pyridine (10^{-3} M) present characteristic and persistent peaks ca. 732 cm^{-1} and 1000 cm^{-1} , respectively, as seen Figures 16-18, which was used for analyzing the effect of salt on the SERS intensities. In the case of pyridine, a close doublet is observed, the relative intensities of which depend on the orientation of the molecule on the metallic surface. Table II shows these results. It can be clearly observed that the best aggregation is found by using Cl^{1-} .

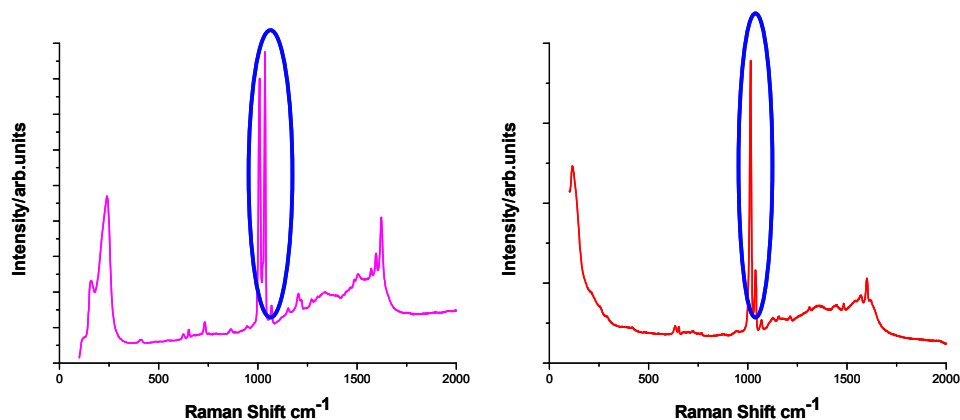


Figure 17. SERS Intensity of the pyridine peaks (1000 cm^{-1}) using silver colloids.

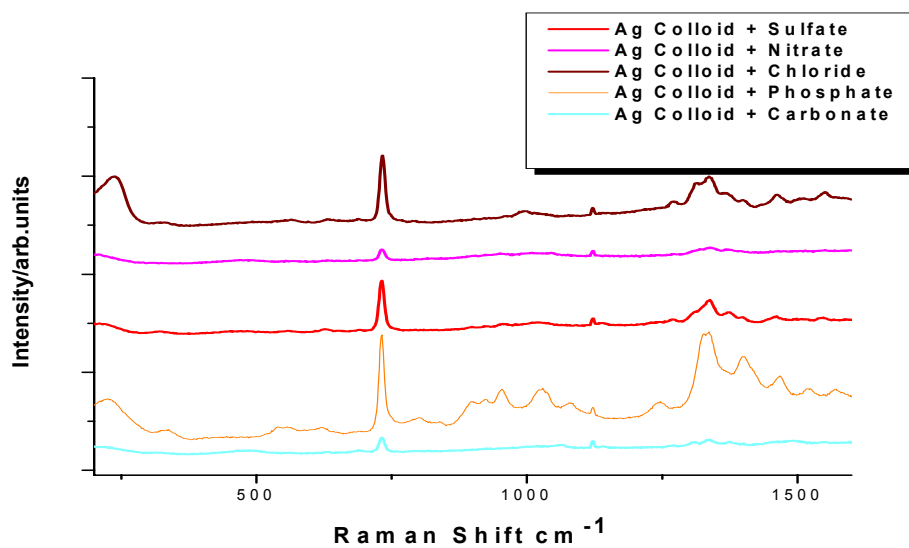


Figure 18. Spectrum of different salts used.

Pyridine and nitrobenzene were selected as target molecules for evaluating SERS activity. These molecules are known to be Raman active as reported in many of the previous works.

Table 1 Intensities of the salts used

ION	SERS Intensity (Counts)
CO_3^{-2}	6660
Cl^{-1}	63010
NO_3^{-1}	45062
SO_4^{-2}	37859
PO_4^{-2}	27599

6.3 TNT detection using silver colloids: Effect of pH conditions

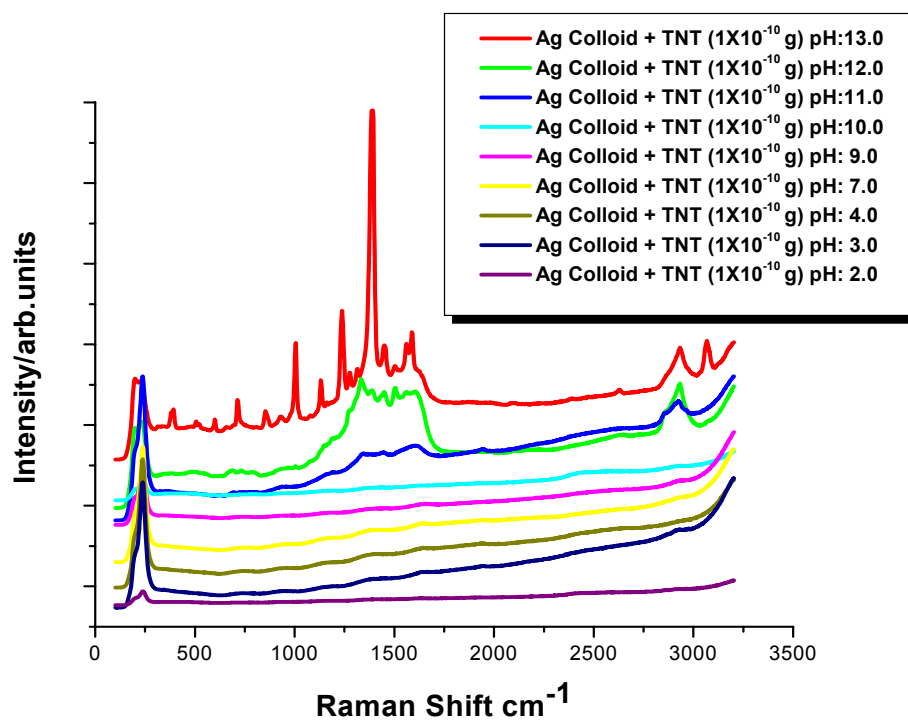


Figure 19. SERS Intensity Silver colloid + TNT (1×10^{-10} g) with different valor of pH

As a means to further increase the enhancement of TNT detection in diluted solutions, we investigated the effect of pH in the SERS spectra of TNT solutions. The study involved the collection of SERS spectra of aqueous TNT solutions (100 $\mu\text{g/mL}$) prepared at different pH values. TNT solutions prepared in a basic environment ($\text{pH} > 12$) produced a colored solution with a yellow to brownish hue as shown in Figure 19. SERS spectrum of the TNT solution at $\text{pH} > 12$ revealed the presence of various intense bands not seen in the samples at a lower pH (Figure 20).

To optimize the adsorption process of the analyte, the pH values of the solution were changed from 2 to 13. The optimum pH was 13 because well defined bands could be detected (Figure 21) at very high pH, as well, shows the SERS spectra of each of the sample components. The importance of the addition of Cl^{-1} as aggregator is emphasized. One important thing is the fact that the symmetric nitro stretch typically found in the normal Raman spectra of TNT ca. 1358 cm^{-1} , was shifted to a 1392 cm^{-1} . This suggests that the modification retains the basic nitroaromatic structure of the molecule, maintaining not only the nitro group but also the aromatic ring breathing (1006 cm^{-1}).

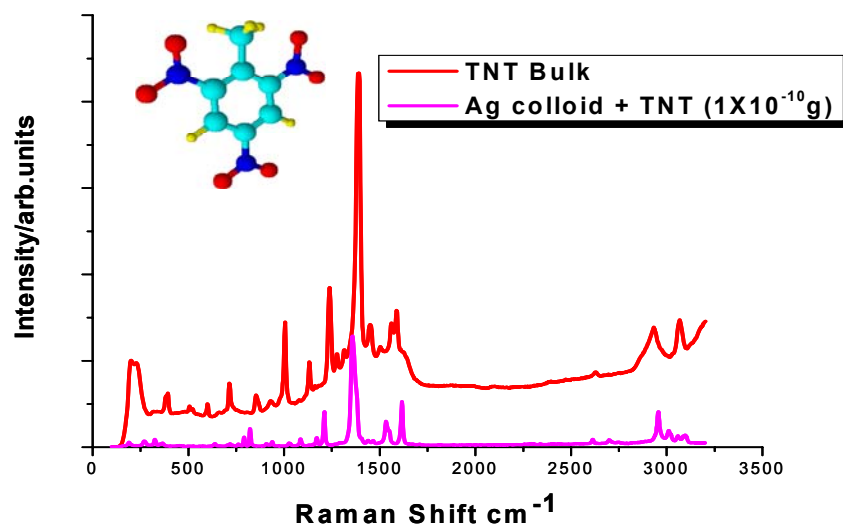


Figure 20. Comparison of Normal Raman spectrum of neat (crystalline) TNT (bottom trace) with SERS spectrum of TNT on a colloidal silver surface at 10^{-10} g TNT + OH^{1-} .

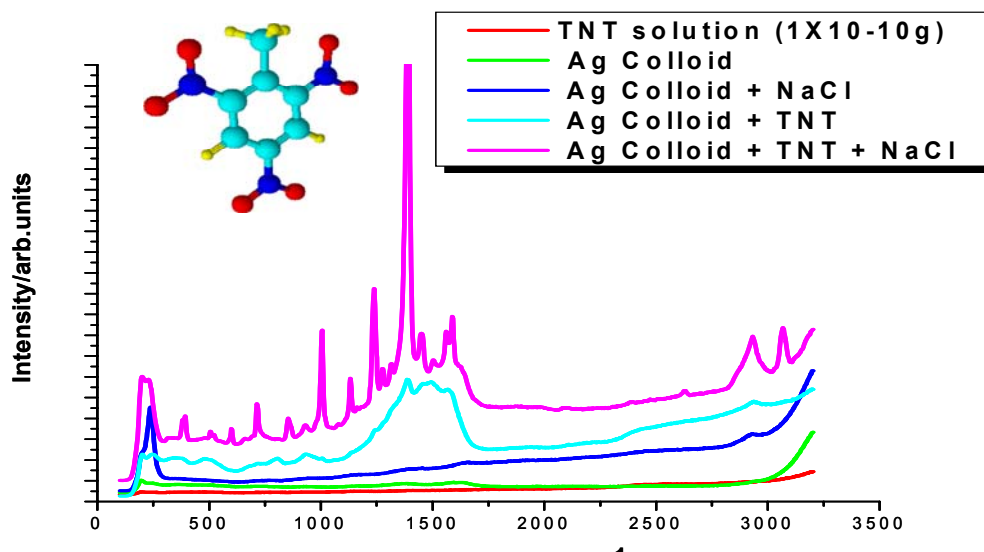


Figure 21. SERS spectra of each individual component in the working SERS mixture typically analyzed.

This spectrum probably corresponds to one of the degradation products reported by Thorn *et.al* [30]. Also, the adsorption process was probably more efficient and intimate at basic pH, allowing a higher enhancement due to the electromagnetic nature, which is related to the roughness of the surface. The pH dependence of Raman signals from colloids was also investigated. It has been established that increase in pH value results in enhancement of Raman spectrum, decrease in bandwidth and change in relative intensity of the bands. This is because of the progressive transition from a highly covered surface SERS spectrum to a less covered one. The differences in the relative intensities of the surface-enhanced bands illustrate selective enhancement resulting from the molecular adsorption geometry on the metal surface.

The appearance of the strong band at about 1006 cm^{-1} (ring breathing mode) in the SERS spectrum is particularly interesting. This vibration is very weak in the "normal" Raman spectrum of TNT [24]. The relatively strong enhancement of this mode in the surface-enhancement spectrum suggests that TNT-modified molecules on colloidal silver are oriented perpendicular to the particle surface (Figure 22). The bands at 1359 and 1204 cm^{-1} (normal Raman) are slightly shifted to higher wavenumbers, and an additional band appears at 1239 cm^{-1} .

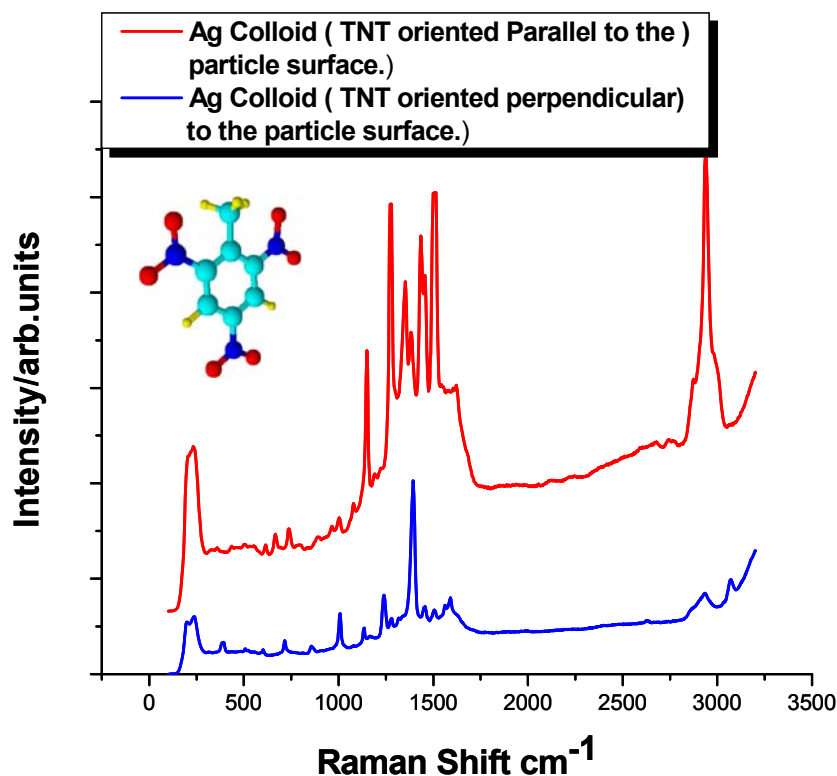


Figure 22. Comparison of SERS spectra on silver colloid (top trace) and gold colloid (bottom trace). In the case of adsorption on gold, spectrum shows evidence of TNT orientation perpendicular to the surface.

Also, when the pH was changed, an enhancement of the out-of-plane modes of the nitro group ($1230\text{-}1270\text{ cm}^{-1}$) and the C-H bonding of methyl group (3000 cm^{-1}) takes place, confirming that the adsorption of modified-TNT (degraded) occurs parallel to the rough surface of the colloid.

Figure 23 shows silver colloids reduced with sodium citrate and how it was possible to reach a detection limit of 10^{-15} g . The spectra show a decrease in the intensity of the vibrational modes of TNT and no shift of the principal bands used for detection.

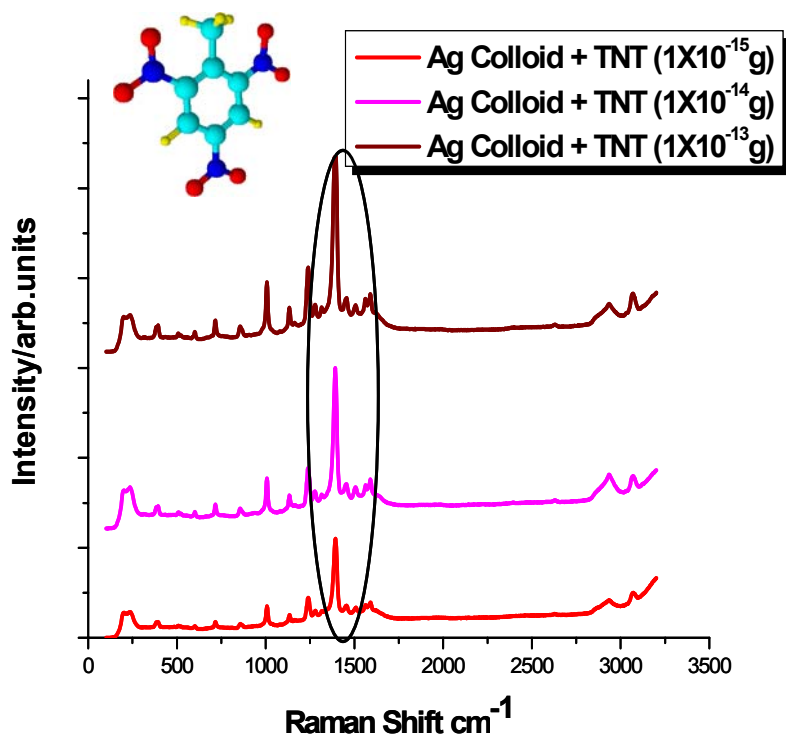


Figure 23. SERS Intensity silver colloids with limit the detection 1×10^{-15} g.

Figure 24 shows the effect of choosing the correct particle size on the SERS spectra obtained. A good particle sizes can be obtained by wet chemistry synthesis by combining all previously discussed parameters.

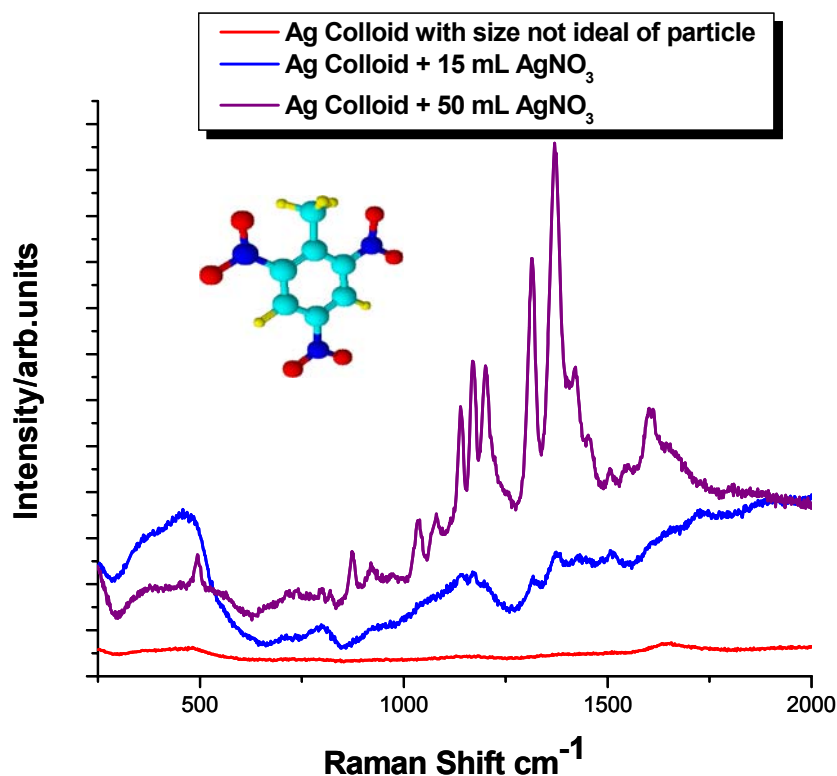


Figure 24. SERS Intensity silver/silver colloid to obtain a particle size of ideal.

6.4 TNT detection using gold and gold/silver colloids

Gold colloids were synthesized and used efficiently to detect TNT. Although vibrational enhancements are lower for gold nanoparticles, these can be tailored to fit into different chemical environments from aqueous suspensions are to some extent more reproducible and stable than the Lee-Meisel citrate-reduced silver colloids. Figure 25 shows the SERS spectra of TNT-modified using gold, gold/silver and silver as SERS substrates. Silver is known to be an excellent substrate for SERS studies while the effect is less pronounced on Au. Combining these two metals may result in new stable substrates for SERS studies.

Particular emphasis is given to the Au-Ag system in the solution phase as well as on a substrate.

Citrate stabilized $\text{Au}_{0.3}\text{-Ag}_{0.7}$ alloy nanoparticles which combine the high SERS activity of Ag with the stability of Au colloid are shown to be ideal substrates for SERS studies. Concentrations as low as 10^{-15} g of TNT can be detected in the presence of Cl^- ions, using an excitation wavelength of 532 nm. The exact mechanism of formation of bimetallic and alloy nanoparticles is not yet clearly understood. Fermi level equilibration studies during the formation of the bimetallic particles may help in the fundamental understanding of bimetal/alloy formation.

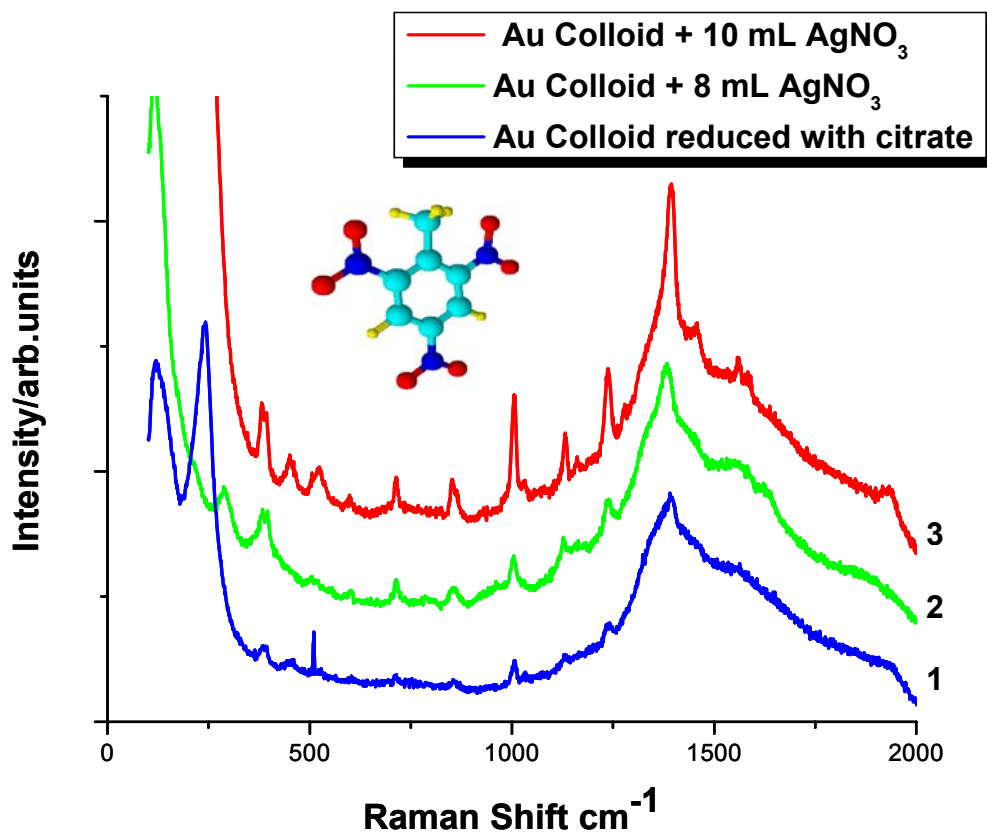


Figure 25. SERS Intensity Au/Ag colloids to obtain ideal particle size: (1) gold colloid; (2) gold colloid reduced with 8 mL AgNO_3 ; (3) gold colloids reduced with 10 mL AgNO_3 .

6.5 TNT detection using Hydroxylamine silver colloids

Citrate reduction of silver requires addition of the reducing agent to the silver nitrate solution during vigorous stirring and refluxing. If due care is not exercised during silver colloid synthesis, the colloid aggregates, eventually precipitating the metal. This leads to changing properties of the silver colloid and thus also to varying enhancement factors, highly SERS-active silver colloids by reduction of silver nitrate with hydroxylamine hydrochloride at alkaline pH and at room temperature. The advantages of the hydroxylamine hydrochloride reduced silver colloid are seen in its fast preparation at room temperature and its immediate applicability for SERS spectroscopy [38-40]. The colloids synthesized with hydroxylamine, they were firsts characterized with adenine, observing the principal band at ~ 760 nm. Then its conditions were optimized for a better adsorption of TNT on the rough surface generated by the colloid. Initially the pH value was changed noticing a similar behavior to the silver colloid reduced with citrate. At basic pH values a better definition of the principal vibrational modes of TNT was observed.

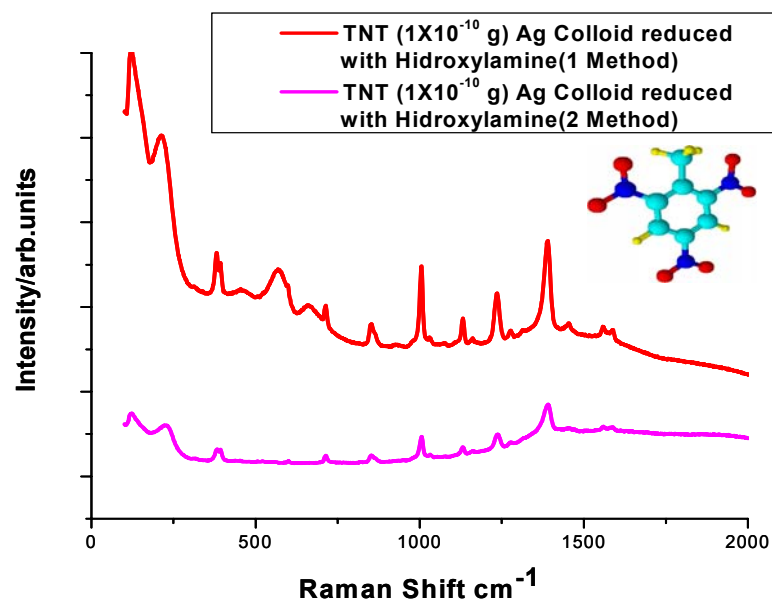


Figure 26. SERS Intensity Silver colloids reduced with hydroxylamine for two different methods.

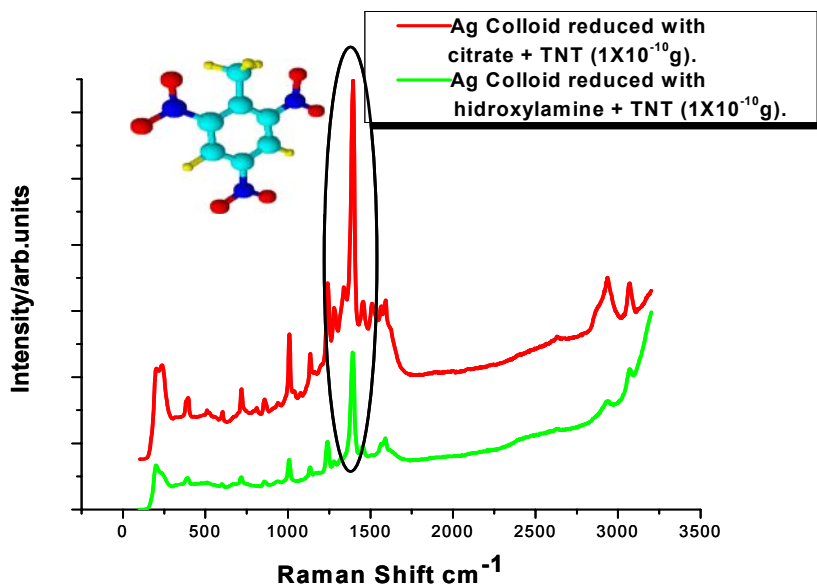


Figure 27. Comparison silver colloids reduced with citrate sodium and hydroxylamine.

Figure 26 shows how the intensity of the principal vibrational modes of TNT in the silver colloid reduced with hydroxylamine changes with the size of the nanoparticles. This was possible varying the addition speed of the reducing agent, same as done in the silver colloid reduced with citrate. The optimum size of the nanoparticle made by the rough surface is between 60-80nm. If the particle size is smaller the principal vibrational modes will still be seen but with least enhancement. Figures 27 and 28 show the enhancement factors of the synthesized colloids. It can be observed a greater enhancement of the TNT signal in the silver colloid reduced with citrate. A possible explanation can be that the citrate is a stronger reducing agent than hydroxylamine, which means there is a greater control in the size of the nanoparticles.

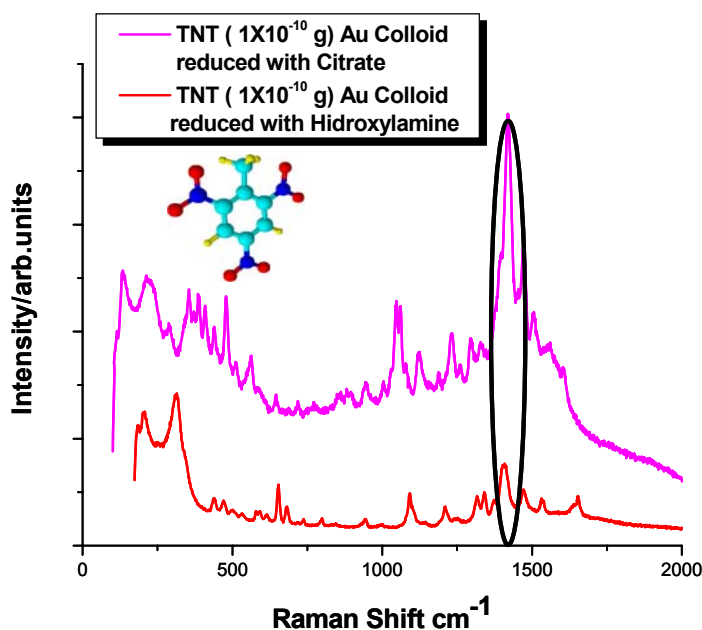


Figure 28. Comparison Gold colloids reduced with citrate sodium and hydroxylamine

Figure 29 shows the SERS spectra of TNT-modified using gold, gold/silver and silver as SERS substrates. Silver is known to be an excellent substrate for SERS studies while the effect is less pronounced on Au. Combining these two metals may result in new stable substrates for SERS studies. Particular emphasis is given to the Au-Ag system in the solution phase as well as on a substrate.

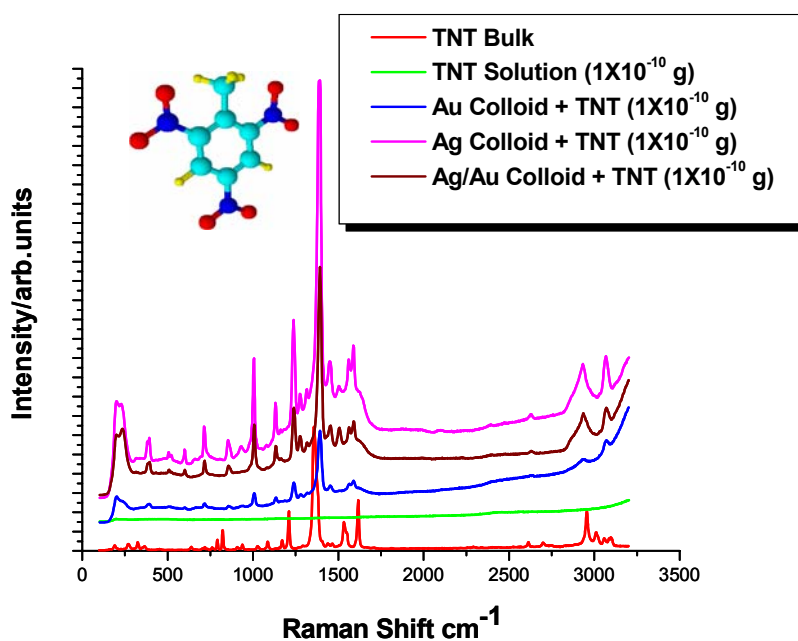


Figure 29 Comparison Silver, Gold and Gold/silver colloids reduced with citrate sodium.

Figure 30 shows a normal Raman spectrum of a saturated solution of TNT in methanol. No methanol signal is observed after subtraction of solvent. TNT can be characterized by 4 principal bands: 1358 cm^{-1} (symmetric nitro stretching), 1551 cm^{-1} (asymmetric nitro stretching), 1212 cm^{-1} (C-H ring bend and in-plane rocking) and 1621 cm^{-1} (2,6-NO₂, asymmetric ring stretch). Since the symmetric nitro stretching is the most intense band it was used for surface enhancement factor (SEF) calculations. SEF were calculated according to Kneipp [24]. Methanol does not present SERS effect for that reason it can be used as an internal standard for estimation of SEF. Tables 2 and 3 show the SEF for the prepared colloids using equation 1. These results confirm that silver citrate colloids are better for the detection of this nitro explosive

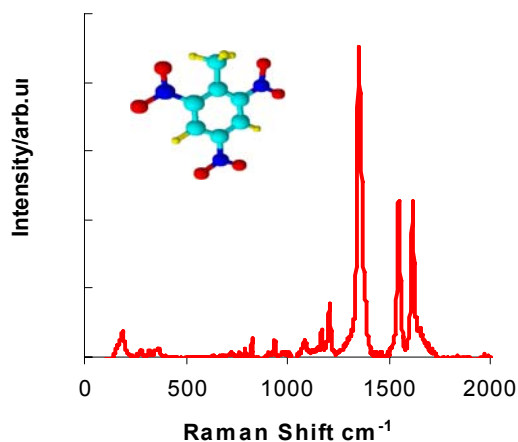


Figure 30 Raman spectra of TNT in methanol at 0.1 M. 0.1 W, accumulations: 3, Integration time: 10 and excitation line of 532 nm

For surface-enhancement, the commonly used Lee-Meisel citrate-reduced silver colloid gives the largest enhancement (Table 2), but some new colloids give a

better stability and reproducibility that is the case of the silver colloid reduced by hydroxylamine.

Table 2. Surface enhancement factors for 2,4,6-TNT degradation products using metallic colloids.

Colloid	SEF
Au colloid	4.82E+10
Au/Ag colloid	7.82E+10
Ag colloid	1.31E+11

Table 3. Comparison of band areas for 2,4,6-TNT using metallic colloids.

Colloid	Reducing Agent	Intensity
Silver	Citrate	129689
Gold	Citrate	19864
Silver	Hydroxylamine	46826
Gold	Hydroxylamine	3729

$$SEF = \frac{I_{NO_2, TNT}}{I_{Methanol}} \times \frac{C_{Methanol}}{C_{TNT}} \quad (2)$$

Figure 31 shows the enhanced factor of synthesized colloids. It can be observed a greater enhancement of the TNT signal in the silver colloid reduced with citrate.

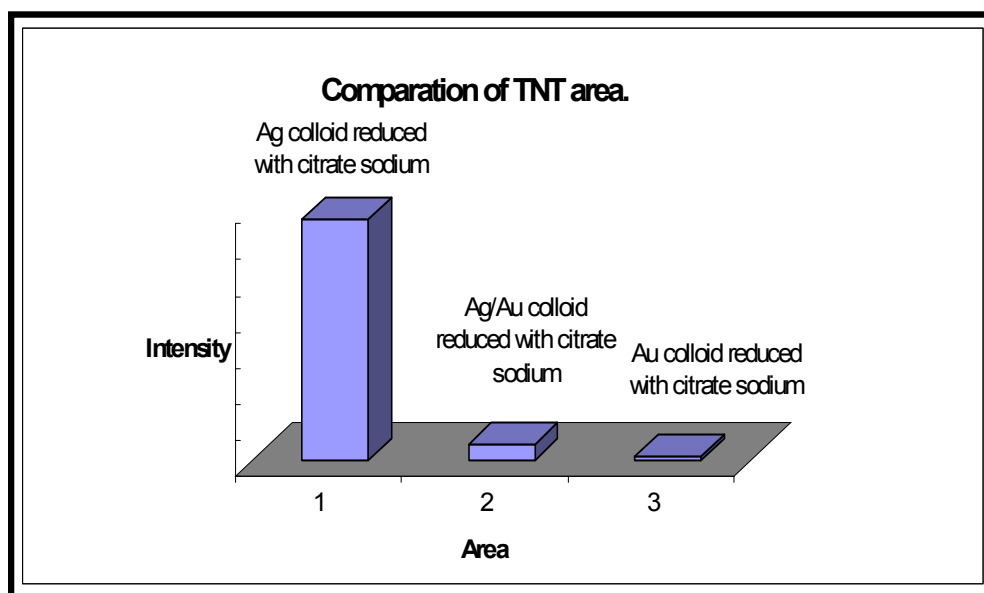


Figure 31. Enhanced of signal Raman for silver colloids reduced with sodium citrate.

CONCLUSIONS

In the present work several methods of preparing silver colloids reduced with citrate sodium and hydroxylamine were studied. By changing several parameters of the synthesis such as stirring rate, speed of addition of the reducing agent and the nature of the reducing agents used, the first in-depth method for detecting of 2,4,6-trinitrotoluene in aqueous solution using SERS was presented. The detection of TNT was conducted via an indirect method that involved the alkaline hydrolysis of TNT. The susceptibility of TNT to basic hydrolysis was advantageous to this project as the species produced in the hydrolysis offered enhanced Raman scattering when combined with SERS active substrates. It was observed that that the proposed methodology provides the user with the flexibility to modify almost all the components to achieve optimum results. At the same time, a slight alteration in any of the parameters (such as surface preparation, sample presentation or equipment settings) may give very different results. Much work is being done to control the morphology of various surfaces, their reproducibility and wider applicability.

Different methods to prepare colloidal nanoparticles of silver, gold and gold-silver using citrate and hydroxylamine and its effect on the enhanced Raman scattering of TNT were also investigated. Our studies revealed that silver colloids prepared with citrate as the reducing agent offered enhanced detection than silver colloids prepared with hydroxylamine. It was demonstrated that at a controlled pH 13 in liquid phase of 2,4,6-trinitrotoluene it was possible to detect the TNT in silver

roughened surfaces (metallic colloids). Enhanced signals due to the presence of the NO₂ out-of-plane bending modes were still detectable at 820 and 850 cm⁻¹ and the NO₂ stretching mode in the 1300-1370 cm⁻¹ region. The aromatic ring breathing mode near 1000 cm⁻¹ was also enhanced.

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