TRACE ANALYSIS OF BIOLOGICAL COMPOUNDS BY SURFACE ENHANCED RAMAN SCATTERING (SERS) SPECTROSCOPY

By

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Abstract: Surface Enhanced Raman Scattering (SERS) spectroscopy is a sensitive analytical technique, which extends its applications to the detection of substances at low concentration levels. SERS spectroscopy measurements were performed on Adenine and Rhodamine 6G on silver and gold colloids to evaluate the linearity at low analyte concentrations and at different time intervals. The maximum intensity for Adenine was observed at Raman shifts of 728 cm^{-1} and 1318 cm^{-1} and the maximum intensity for R6G was observed at Raman shifts of 1352 cm^{-1} and 1519 cm^{-1}. These SERS measurements were compared with solution absorbance measurements at 650 nm and 900 nm. Raman enhancement factors have also been evaluated for each compound. The results of these studies demonstrate that SERS techniques provide high sensitivity and have the potential to detect a range of compounds in biological samples.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	Degree centigrade
µL	Micro liters
µM	Micro molar
µm	Micro meter
a.u	Atomic units
Ag	Silver
APTES	Aminopropyl triethoxy silane
Au	Gold
CCD	Charge couple device
Cm	Centimeter
Cu	Copper
CW laser	Continues wave laser
$HAuCl_4$	Tetrachloroaurate
HCl	Hydrochloric acid
HNO_3	Nitric acid
In	Indium
K	Potassium
KHz	Kilo Hertz
Li	Lithium
LOD	Limit of detection
LOQ	Limit of quantification

mL	Milli liter
mM	Milli Molar
mW	Milli Watts
NaCl	Sodium chloride
NIR	Near Infrared
PMT	Photomultiplier tube
ppb	Parts per billion
ppm	Parts per million
Pt	Platinum
R6G	Rhodamine 6 G
Rh	Rhodium
SERS	Surface Enhanced Raman Scattering
SNR	Signal to Noise Ratio
TERS	Tip Enhanced Raman Spectroscopy
UV-VIS	Ultraviolet-visible
V	Volts

1.0 INTRODUCTION

1.1 Raman Scattering:

Indian physicist C. V. Raman reported the Raman Effect[1] in 1929 for which he received the Nobel Prize in the year 1930. Raman spectroscopy is a spectroscopic technique that is based on inelastic scattering, or Raman scattering of monochromatic light. Raman scattering occurs through changes in vibrational and rotational states of a molecule. The rotational shifts are often small and difficult to observe, so Raman scattering is usually observed through vibrational level transitions only.

If a photon of light interacts with a molecule, the photon may be absorbed or scattered. For the absorption process, the photon should meet two conditions. First, the energy of the incident photon should be equal to the energy difference between two states in the molecule, which is called resonance. Second, the transition between the two states causes a change in the dipole moment of a molecule. For photons that are not absorbed, they may be scattered. In molecular scattering, the photons do not need to be resonant with energy transitions of the molecule. A nonresonant photon can excite the molecule to a virtual excited state that is short-lived and the energy of the photon will be quickly re-radiated. If this re-radiated photon has the same energy (frequency) and wavelength as that of the incident photon, then the photon is said to be elastically scattered and this process is called Rayleigh scattering. Light scattering can be obtained by a variety of mechanisms, but only a small fraction, that is less than one photon in 10^6, scatters with energy that is different (usually with lower energy) from the incident photon. Scattering

of these photons is said to be inelastic and this phenomenon is called Raman scattering. In inelastic scattering processes, some energy of the incident photon is exchanged with the molecule, and some part of the energy of the photon is either lost or gained. These energy differences are equal to vibrational and rotational energies of the molecule. If some energy is transferred from the photon to the molecule, then the scattered photon will have lower energy than the incident photon and this phenomenon is called Stokes Raman scattering. If some energy is transferred from the molecule to the photon, then the scattered photon will have higher energy than the incident photon. This is called anti-stokes Raman scattering. The spectrum of the Raman lines is a fingerprint of the molecule, and its intensity is proportional to the number of scattering molecules.

Generally, spontaneous Raman scattering is very weak and the weak inelastically scattered light must be separated from the intense Rayleigh scattered laser light. Previously detectors like photomultiplier tubes (PMTs) were used for Raman measurements, but it took long acquisition times to obtain full spectra. Recently charge coupled device (CCD) detectors have been used to decrease acquisition times and increase the speed of Raman spectroscopy measurements.

1.2 Surface Enhanced Raman Scattering:

Surface enhanced Raman scattering[2] (SERS) was first observed by Fleischman and coworkers in 1974. They observed SERS of pyridine molecules which were adsorbed on an electrochemically roughened silver electrode[3]. Further studies showed that the Raman effect for molecules is greatly enhanced when they are adsorbed onto rough silver or gold surfaces. Surface Enhanced Raman Scattering (SERS) Spectroscopy increases the Raman scattering signal by tens of thousands to even trillions of times to allow the measurement of chemically selective vibrational information[4] from small sample volumes. The SERS intensity can be greatly enhanced when it is close to a rough metal surface. This enhancement allows Raman spectroscopy to be a sensitive analytical technique. SERS is observed for molecules found close to silver or gold nanoparticles because of surface plasmon resonance[5]. Other metals may be used, but usually with a lower enhancement.

SERS is believed to be based on two mechanisms[6]. The first is an enhancement in the electromagnetic field[7] produced at the surface of the metal due to optical excitation. When the wavelength of the incident light is close to the plasma wavelength of the metal, conduction electrons in the metal surface are excited into a surface electronic excited state called a surface plasmon[8]. Molecules adsorbed onto or near the metal surface[9] may be located near a large electromagnetic field localized on the metal. Vibrational modes in the molecule that are normal to the metal surface are most strongly enhanced in SERS spectra. The second enhancement mechanism is chemical enhancement which is caused by the formation of a charge-transfer complex between the metal surface and the

adsorbed molecule. The electronic transitions of many charge transfer complexes are in the visible region of the electromagnetic spectrum, so that resonance enhancement can occur using visible wavelength lasers. The factors that influence the chemical enhancement mechanism are the bonding interaction between the surface metal atoms and the adsorbed molecule, metal surface structures and the orientation and the coverage of the adsorbed molecule.

SERS can be used to study monolayers of materials adsorbed on metals, including metallic electrodes. Surfaces other than electrodes can also be used. The most popular metal surfaces include colloids[10], metal films[11,12] on dielectric substrates and arrays of metal particles bound to metal or dielectric colloids through short linkages. The SERS method is useful for aqueous samples[13] and has strong potential for analysis of biological samples. Analytical applications of SERS include immunoassay[14], pollutant detection and pharmaceutical[15,16] or biological applications.[17,18,19,20]

The SERS effect has been observed with many molecules and with a number of metals like Cu, Ag, Au, Li, Na, K, In, Pt and Rh. Among these metals, Ag and Au are the most studied and the most efficient SERS metals. The shape and size[21] of the metal nanostructures[22] affect the absorbance and scattering processes which further affects the strength of the enhancement. Particles or structures of definite sizes are used in SERS measurements because particles that are too small lose their electrical conductance and cannot enhance the field and particles that are too large allow the excitation of multipoles that can reduce the overall efficiency of the enhancement.

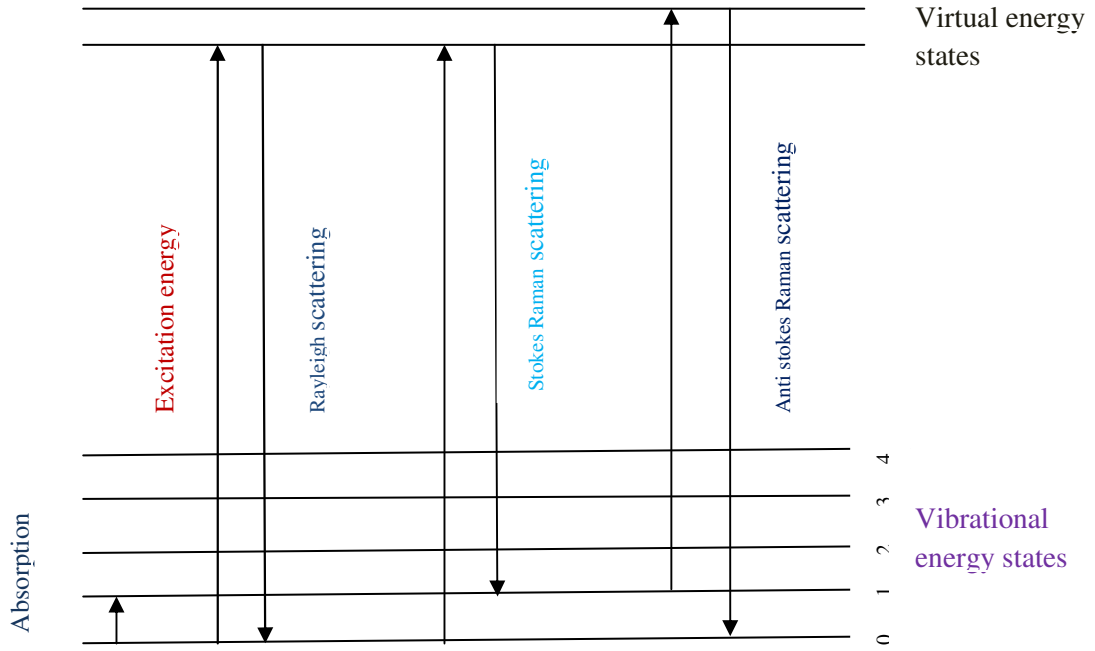

Figure (1) Different possibilities of visual light scattering

The goal of this research is to evaluate the analytical performance of SERS techniques for the trace analytical measurements of biological compounds on gold and silver colloids. In these studies, the measurements are focused on Adenine and a model compound, Rhodamine 6 G. The Raman and SERS spectra of Adenine,[23,24] and R6G[25] have already been reported by several authors. Watanabe *et al.*[26] have reported SERS spectra of Adenine using tip enhanced Raman spectroscopy (TERS). Materny *et al.*[27] have reported SERS spectra of Adenine on silver sols (with sodium citrate activator). In that work, the various concentrations of Adenine in presence of a silver sol were measured to study the concentration dependence. In the current study, SERS spectra of Adenine and Rhodamine 6 G have been measured to study the linear concentration dependence and Raman signal enhancement on both silver and gold colloids using NaCl as an activator.

1.2.1 Raman signal Enhancement:-

Miller *et al.*[28] have studied the Raman signal enhancement of imidazole on gold colloids. To estimate the SERS enhancement, the intensity of the SERS signal is compared with the normal Raman signal. This estimation can be done only for compounds which show features in both the normal and enhanced Raman spectra. The SERS enhancement is calculated as the ratio of Raman intensities normalized by analyte concentration in samples with and without the presence of gold or silver colloids.

$$E_{SERS} = \frac{I_{SERS}}{C_{SERS}} \frac{C_{Normal}}{I_{Normal}} \quad \text{.....(1)}$$

where,

- E_{SERS} is the Raman signal enhancement.
- C_{SERS} is the concentration of analyte in solution with gold/silver nanoparticles (Molarity).
- C_{Normal} is the concentration of analyte in solution without gold/silver nanoparticles (Molarity).
- I_{SERS} is the intensity of SERS signal (counts)
- I_{Normal} is the intensity of Raman signal (counts)

1.3 UV Vis Spectrophotometry:

A spectrophotometer uses light in the visible, near ultraviolet (UV) and near infrared (NIR) ranges to determine the absorbance or transmittance of light by the sample. A beam of light from a visible and/or UV light source is separated into its component wavelengths by a prism or diffraction grating. The instrument measures the intensity of light passing through a sample, and compares it to the intensity of light before it passes through the sample. The absorbance or transmittance of light is proportional to the absorber concentration. This relationship is described by the Beer-Lambert Law.

Beer-Lambert Law:

Beer's law[29] states that the intensity of a beam of parallel monochromatic radiation decreases exponentially as it passes through a medium of homogenous thickness. It can also be stated that the absorbance is proportional to the thickness (pathlength) of the absorber solution.

There are several ways in which the law can be expressed,

$$A = \alpha\, lc \quad \text{..... (2)}$$

$$T = I_1 / I_0 = 10^{-A} = 10^{-\alpha l c} \quad \text{.....(3)}$$

where,

$$A = -\log_{10} (I_1 / I_0) \quad \text{..... (4)}$$

where,

- A is the absorbance of the sample
- I_0 is the intensity of the incident light
- I_1 is the intensity after passing through the material

- l is the distance that the light travels through the material (the path length)
- c is the concentration of absorbing species in the material
- α is the absorption coefficient or the molar absorptivity of the absorber ($L \cdot mol^{-1} \cdot cm^{-1}$)
- λ is the wavelength of the light
- T is the transmittance

The basic parts of a UV-Vis spectrophotometer are a light source (incandescent bulb or deuterium arc lamp), a holder for a sample, a monochromator (to separate different wavelengths of light) and a detector (a photodiode array or charge coupled device (CCD)). This spectrophotometer is either a single beam or double beam. In the single beam design, the absorbance is measured by passing all the light through the sample and again the absorbance of a blank solution is measured separately. In the double beam design, the light is split into two beams where one beam is passed through the sample and the other beam is passed through a reference sample material.

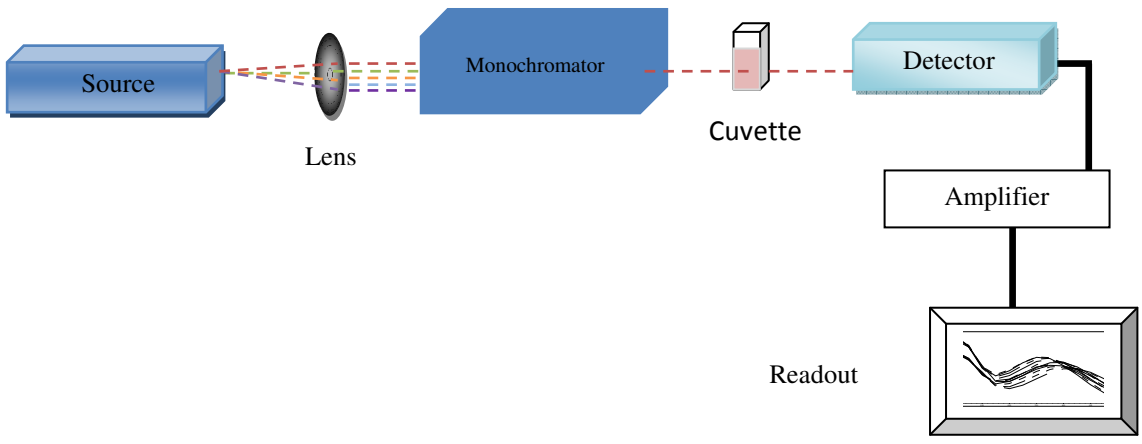

Figure (2): Schematic diagram of UV-Visible Spectrophotometer

1.4 Laser diodes:

Laser diodes[30] convert electrical power into a laser output. Laser diodes are often coupled to optical fibers to deliver the laser radiation. A laser diode is formed from a p-n junction and powered by electrical current. In these lasers, holes are injected from the p-doped material and the electrons are injected from the n-doped semiconductor material. When these holes and electrons recombine, they produce spontaneous emission. When the electrons and holes can remain close to one another without recombining, a photon can stimulate the recombination with energy equal to the recombination energy. This generates another photon with the same frequency as that of first photon and in the same direction. At the injection region these stimulated emissions cause gain in the optical wave and the wave increases by increasing the number of electrons and holes across the junction.

1.5 Optical Fiber:

Optical fibers[31,32] are glass or plastic fibers that allow the transmission of light over long distances with low loss and without any electromagnetic interference. Optical fibers transmit the light by the process of total internal reflection. The use of optical fiber probes[33] in SERS spectroscopy can avoid some problems associated with sample contamination that can occur during sample collection and transportation.

In these studies, two optical fibers are used where one is used for the transmission of light source (laser) to the sample and the other is used to transmit the scattered light to the spectrometer.

1.6 Charge Coupled Device (CCD):

Charge Coupled Devices (CCDs) are light detectors that are silicon based integrated circuits which consists of an array of pixels. CCDs[34] convert light energy in the form of photons into an electronic charge and the electrons are produced by the interaction of photons with the semiconductor material. When a photon from an ultraviolet, visible, or infrared source strikes the semiconductor, free electrons are produced and a hole is created by the absence of an electron in the semiconductor material. During measurements, the charges are accumulated and stored at collector electrodes. At the end of the measurement period, the accumulated charge is measured and is proportional to the light intensity at the pixel.

1.7 Signal-to-noise ratio (SNR):

Noise is the undesired interference with target information (signal) at the detector. Noise causes either random interference or systematic interference.

The signal to noise ratio (SNR)[35] is defined as the ratio of the signal power (desired signal) to the background noise (which interferes with the signal).

$$SNR = P_{signal} / P_{noise} \quad \text{..... (5)}$$

Where, P is the average power.

Both signal and noise are measured with the same system. A high SNR indicates that the background noise is low and the signal is relatively intense.

Background noise can be caused by external/environmental factors such as background light, electrical signals and variations in temperature and humidity. The noise caused by external/environmental factors can be reduced or eliminated by controlling the experimental parameters and measurement environment.

1.8 Calibration curves:

In these studies, the calibration curve[36] method is used to estimate the smallest concentration of a substance that can be detected in an unknown sample. A calibration curve is a plot of the analytical signal versus concentration. A series of standard samples with known analyte concentration is prepared. Measurements of these samples produce a series of measurement responses. Using these responses, a calibration curve of the responses is plotted as a function of the analyte concentration.

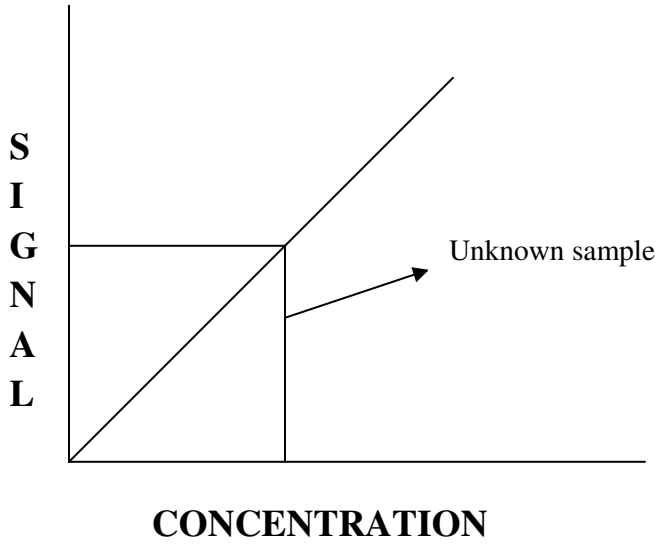

Figure (3) Unknown sample detection from Calibration curve

For most analyses, a plot of the response versus concentration gives a linear relationship, where the best fit line is determined by linear regression analysis.

1.9 Linear regression analysis:

Regression analysis gives the relationship between the dependent variable and the independent variable. A simple equation for linear regression analysis[37] is

$$\mathbf{Y = mX + C} \quad \ldots\ldots (6)$$

where,

m is the slope (sensitivity),

C is the Y-intercept,

X is the independent variable (concentration) and

Y is the dependent variable (signal).

1.10 Limit of Detection (LOD):

The International Union of Pure and Applied Chemistry (IUPAC) defines the LOD[38] as "the concentration, C_L, or the quantity, q_L, is derived from the smallest measure, X_L , that can be detected with reasonable certainty for a given analytical procedure." The value of X_L is given by the equation

$$X_L = X_{bi} + k\, s_{bi} / S \quad \ldots\ldots (7)$$

where,

X_{bi} is the measure of blank measures

s_{bi} is the standard deviation of blank measures

k is the numerical factor (chosen according to the confidence level desired) and

S is the slope of the calibration curve.

The analyte concentration of the blank is zero, so equation (7) becomes

$$X_L = 0 + k\, s_{bi} / S$$

$$X_L = k\, s_{bi} / S \quad \text{.....} \quad (8)$$

Generally, the LOD is the smallest amount of analyte that gives a signal that is different from the blank. For these studies, a value of k=3 (or a signal to noise ratio of 3) is used to define the limit of detection (LOD).

2.0 EXPERIMENTAL

2.1 Instrumental:

A Raman spectrometer consists of four major components:

1) Excitation source (generally continuous-wave CW laser)
2) Sample illumination and collection system (often these are coupled with fiber optics to the excitation source)
3) Wavelength selector
4) Detection and computer control/processing system.

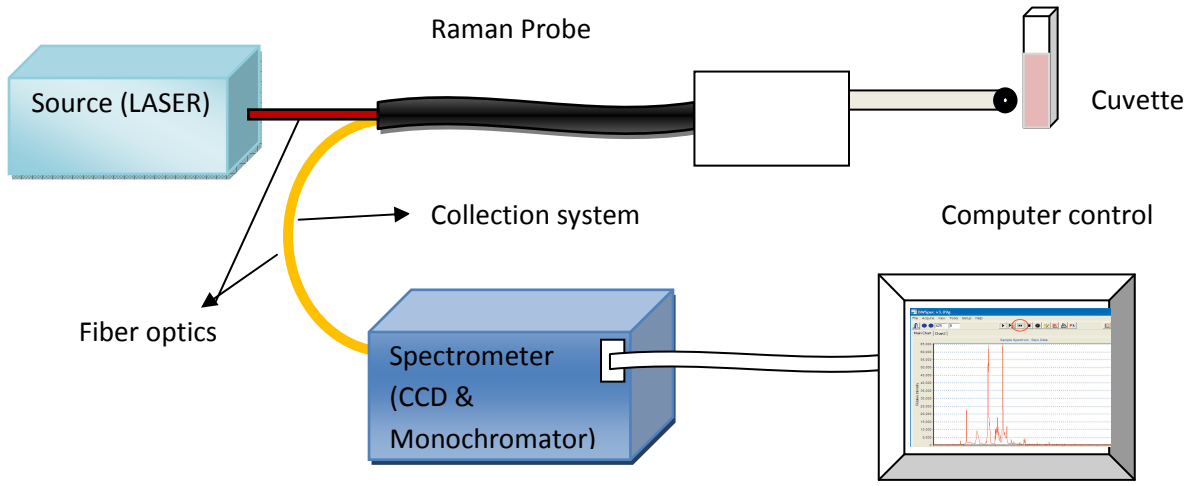

Figure (4) Schematic diagram of SERS Spectroscopy (Red Laser)

2.1.1 Excitation source:-

In the early SERS studies, a mercury lamp was used as an excitation source for Raman spectroscopy. Later on lasers were used that provided light at a single wavelength with enough power to produce Raman scattering. Lasers are good excitation sources for Raman spectroscopy, because:

a) Single lines from lasers can easily produce 1-2 W of power.
b) Laser beams are highly monochromatic, and weaker lines can be eliminated by filters or pre-monochromators.
c) Laser beams are collimated, and can be focused on small samples.
d) Lasers are available over a wide wavelength range from the UV to the NIR.

In these studies, a laser operating at 785 nm (red laser) has been used to perform SERS measurements on different metal substrates.

2.1.2 Red laser:-

Specifications for the laser source used in the SERS measurements are as follows:

MODEL ----BWF1-785-450

WAVELENGTH ---- 785 nm

MODE ---- Multimode

FIBER SIZE --- 100 µm @ N.A. 0.22

TEMPERATURE SETTING --- 23 °C

OPERATING VOLTAGE ---- AC 100-240 V

OUTPUT POWER ---- 0-280 mW

2.1.3 Detector and Monochromator:-

The spectrometer used in these studies contains both detector (CCD) and monochromator in a single unit.

Figure (5) Spectrometer from R 2001 Series

The specifications for the spectrometer used in SERS measurements are as follows:

MODEL ---- BTC11E

OPERATING TEMPERATURE ---- 15-30 °C

DETECTOR ---- TE cooled 2048-element linear silicon CCD array

OF CCD ELEMENTS ----2048 @ 14 µm x 200 µm per element

INTEGRATION TIME ---- 9(min) – 65535 (max) milliseconds

DIGITIZER RESOLUTION ---- 16 bit (65,535:1)

DIGITIZER SPEED ---- 250 KHz (maximum)

2.2 Chemical:

For the SERS measurements, gold and silver colloids are the most popularly used metals. Many authors have published different methods of preparation of colloids/substrates for SERS measurements. In these measurements, sodium chloride (10% NaCl) is used as an activator. Prior to the preparation of colloids/substrates, all the glassware should be thoroughly cleaned in aqua regia (3 parts of HCl and 1 part of HNO_3) and rinsed with triply distilled water and oven dried.

2.2.1 Colloidal Preparation:

2.2.1a Gold colloids:-

The gold colloids were prepared from a method described by Grabar *et al*.[39] After preparation, all of the colloids were stored at room temperature and were generally used within 1-2 months. To prepare the gold colloids, 500 mL of distilled water was placed in 1L round bottom flask, 50 mg of $HAuCl_4$ (Gold Tetrachloroaurate) was added and heated to boiling with vigorous stirring. A 7.5 mL volume of 1% sodium citrate was added and the solution turned to blue within 25 seconds. The final color of the solution changes to red-violet in about 70 seconds. After boiling the solution for 10 more minutes, the heat was removed and the stirring was continued for another 15 minutes.

The particle size of the colloids can be decreased or increased by adding larger or smaller amounts of sodium citrate respectively.

2.2.1b Silver colloids:-

The silver colloid was prepared by a method reported by Lee and Meisel[40]. After preparation, all of the colloids were stored at room temperature and were generally used within 1-2 months. To prepare the silver colloids, 500 mL of distilled water was placed in a 1L round bottom flask and heated to around 45 ºC and 5.3 mL of 0.106 N silver nitrate was added. The solution was heated to almost boiling (~96 ºC) and 10 mL of 1% sodium citrate dihydrate solution was added. The solution was allowed to simmer gently for 90 minutes. After this period, the heat was removed and the solution was allowed to cool with constant stirring.

2.2.2 Substrate Preparation:-

To prepare glass substrates[41], glass slides were washed with aqua regia (3 parts of HCl and 1 part of HNO_3) and washed several times with anhydrous ethanol. Then the glass slides were placed in 10 % Aminopropyltriethoxysilane (APTES) solution for 15 min. Due to this process, an amine terminated surface was created for the binding of the metal nanoparticles onto the glass slide surface. After 15 minutes, the APTES-coated glass slides were washed again with anhydrous ethanol for several times to remove excess of the APTES solution and then the glass slides were placed in an oven at 120 ºC for 3 hours to dry. After drying, the glass slides were placed in colloidal solutions of gold or silver suspensions and left for about 12 hours. Once the metal nanoparticles were bound onto the surface, these glass slides were again washed with anhydrous ethanol and stored in plastic containers either in ethanol or water for later use.

2.2.3 Activator Preparation:-

10% NaCl was used as an activator. For the preparation of this solution, 10 grams of NaCl was weighed and dissolved in 100 mL of water.

3.0 RESULTS AND DISCUSSION

In this section, the results of SERS studies are reported for Adenine and Rhodamine 6 G. For Adenine, SERS spectra on both silver and gold colloids were obtained at different intervals of time at 10 seconds integration time. Calibration curves were performed to check the linear dependence at low concentrations and to determine the detection limits. Results obtained using both silver and gold colloids are compared. Enhancement of Raman spectra and absorbance spectra for Adenine at different concentrations has been studied on both silver and gold colloids.

For rhodamine 6 G, SERS spectra on both silver and gold colloids were collected at different intervals of time at 10 seconds integration time. Calibration curves were performed to check the linear dependence at low concentrations and to determine the detection limits. The SERS Enhancement and the absorbance spectra for rhodamine 6G at different concentrations were studied on silver colloids.

SERS spectra for mixtures of Adenine and rhodamine 6 G at different concentrations and different time intervals at 10 seconds integration time have also been studied on silver colloids.

All the spectra that are reported in these studies are corrected for the background and the blank.

3.1 Calibration of Raman Spectra:

All the spectral data obtained in this work are aquired as counts at individual pixels. These pixel numbers are converted to wavenumbers using Raman shift frequency standards based on reported peaks for naphthalene.[42,43] For converting pixel numbers to wavenumbers, a plot was generated using the frequency standards of naphthalene against pixel numbers. The slope and the Y-intercept from a linear regression of the data were used to convert the pixel numbers to Raman shift.

Figure (6) shows the SERS spectra of Naphthalene (solid), Table (1) shows the frequency standards of Naphthalene for pixel numbers at maximum intensities, and figure (7) shows the calibration curve for frequency standards of naphthalene.

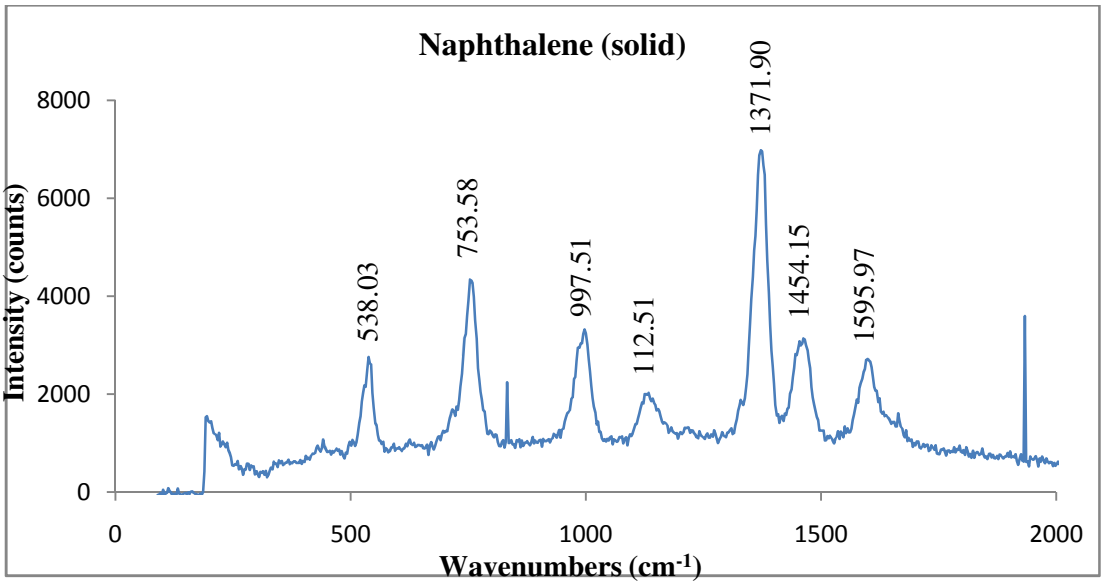

Figure (6) Raman spectrum of Naphthalene

Pixel number	Raman shift (cm^{-1})
158	513.8
234	763.8
320	1021.6
452	1382.2
484	1464.5
532	1576.6

Table (1) Frequency standards of Naphthalene at corresponding pixel numbers

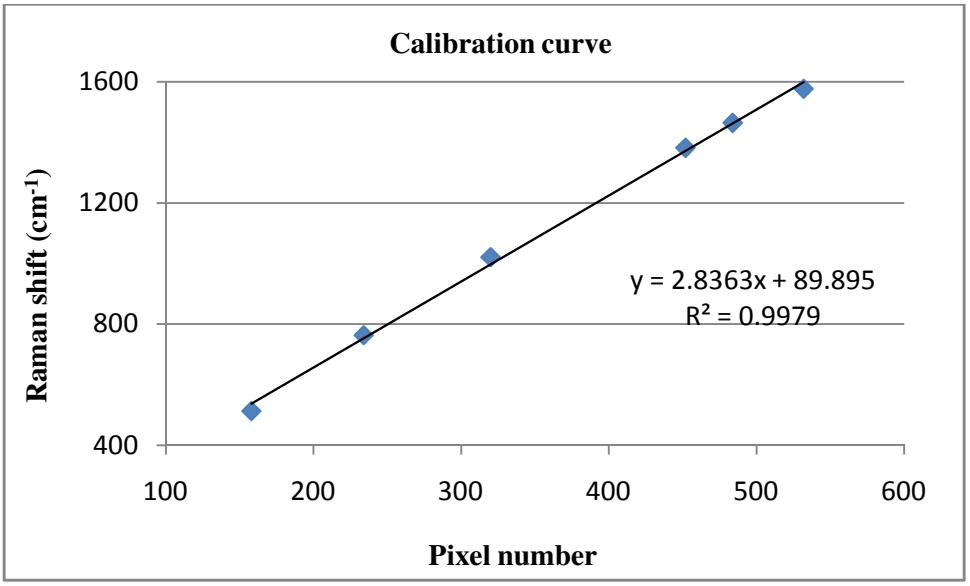

Figure (7) Calibration curve for frequency standards of Naphthalene

3.2 Adenine:

SERS measurements on Adenine were performed on both silver and gold colloids to study the linearity and the enhancement effects. Many experiments were performed to find the linearity and to check the reproducibility for Adenine on both silver and gold colloids. Linear concentration dependence for different compounds like glucose[44], Cresyl Fast Violet (CFV)[45] and amino acid glutamate[46] has been reported by many authors.

The maximum intensity for the Adenine molecule was found at 0 minutes after the sample preparation. In each experiment, certain conditions like integration time (10 seconds), the activator (10% NaCl) and sample preparation (2700 µL of silver/gold colloids + 300 µL of sample solution + 150 µL of activator) were maintained constant. The graphs were plotted as Intensity (counts) versus Wavenumber (cm^{-1}). Maximum intensity for Adenine was shown at two Raman shifts (728 cm^{-1} and 1318 cm^{-1}). The Adenine stock solution was prepared by dissolving Adenine (solid) in distilled water with mild heating, and a series of dilutions were made by using distilled water.

3.2.1 Normal Raman spectra:

Normal Raman spectra for Adenine were taken for different forms (solid and solution) of Adenine. For these measurements, only the sample (solid/solution) was used without any colloids or activator. The integration time for solid sample was 10 seconds and for the Adenine solution was 60 seconds. A Normal Raman spectrum for pure distilled water was also obtained to use as a blank. In figures (8) and (9), the normal Raman spectra of Adenine solid sample and Adenine solution are presented. The normal

Raman spectrum is needed to calculate the Raman enhancement of peaks in the SERS spectra for Adenine.

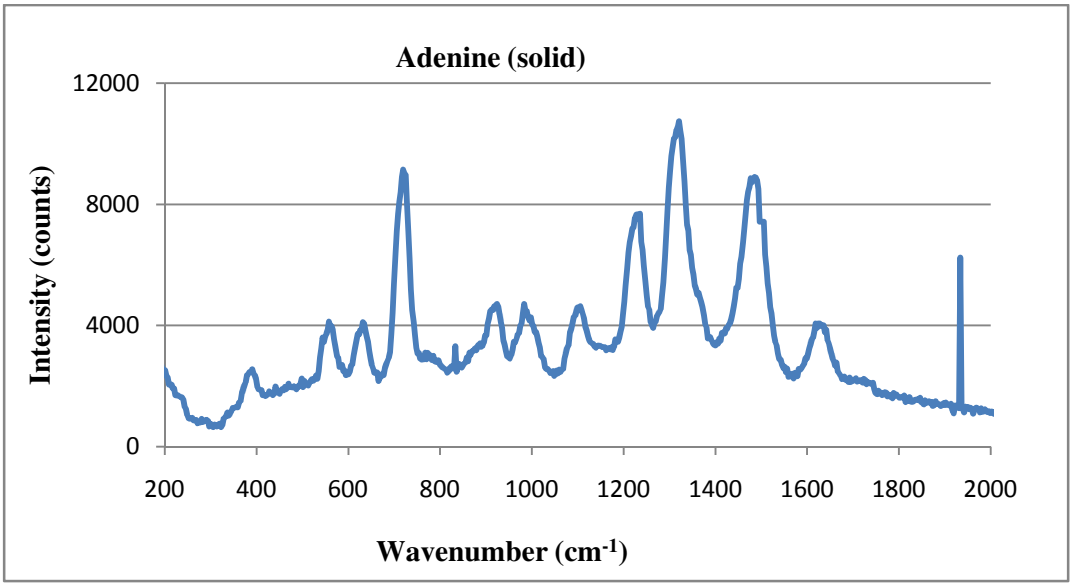

Figure (8): Raman spectrum of Adenine (solid form)

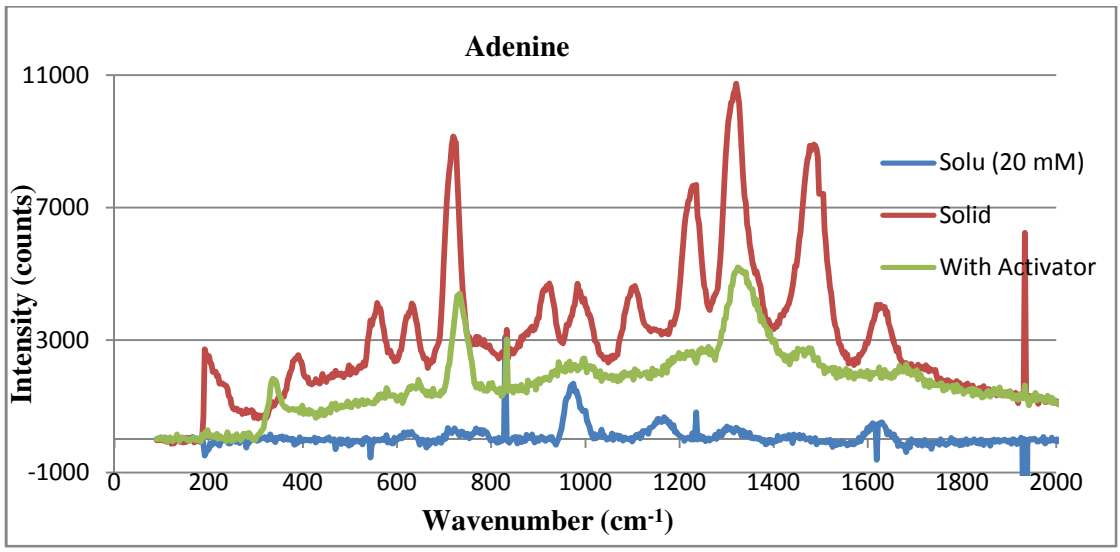

Figure (9): Raman and SERS spectra of Adenine for different forms

3.2.2 Silver Colloids:

Measurements were performed using 2700 µL of silver colloids + 300 µL of sample solution + 150 µL of activator as a sample preparation. SERS spectra at different concentrations (ranging from 10 mM to 0.1 µM) and different time intervals (0, 5 and 10 minutes) were taken to find the maximum intensity and best linearity. Figure (10) shows the maximum intensity at 728 cm^{-1} and 1318 cm^{-1} for Adenine at 0 minutes after sample preparation. The maximum intensity at 728 cm^{-1} arises from the ring breathing mode and maximum intensity at 1318 cm^{-1} arises from the bending and stretching motions of the Adenine molecule[27]. Figure (11) shows linearity can be obtained for concentrations ranging from 1 µM to 0.1 µM.

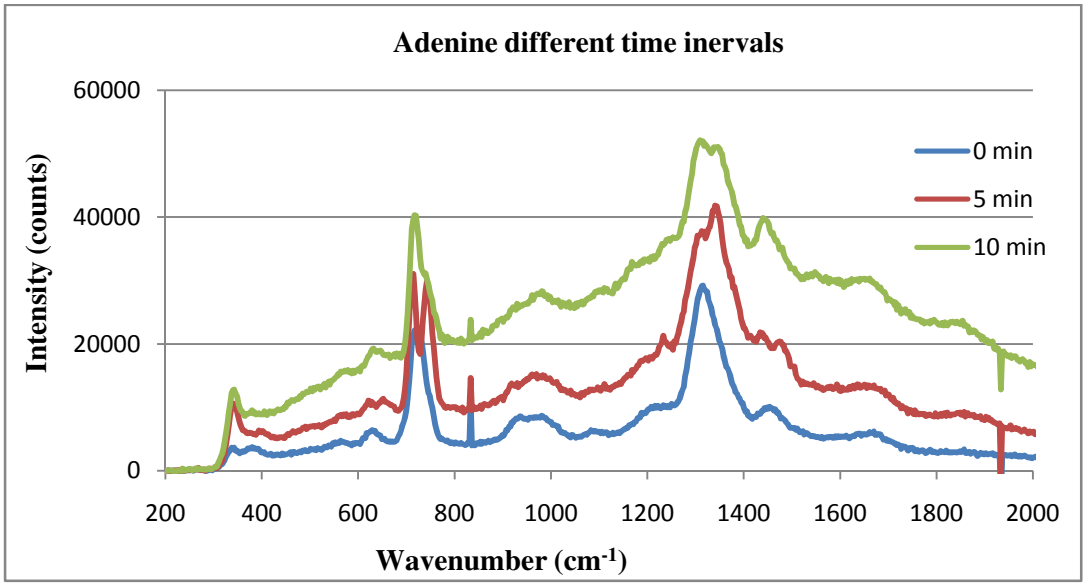

Figure (10): SERS spectra of Adenine (10µM) at different time intervals

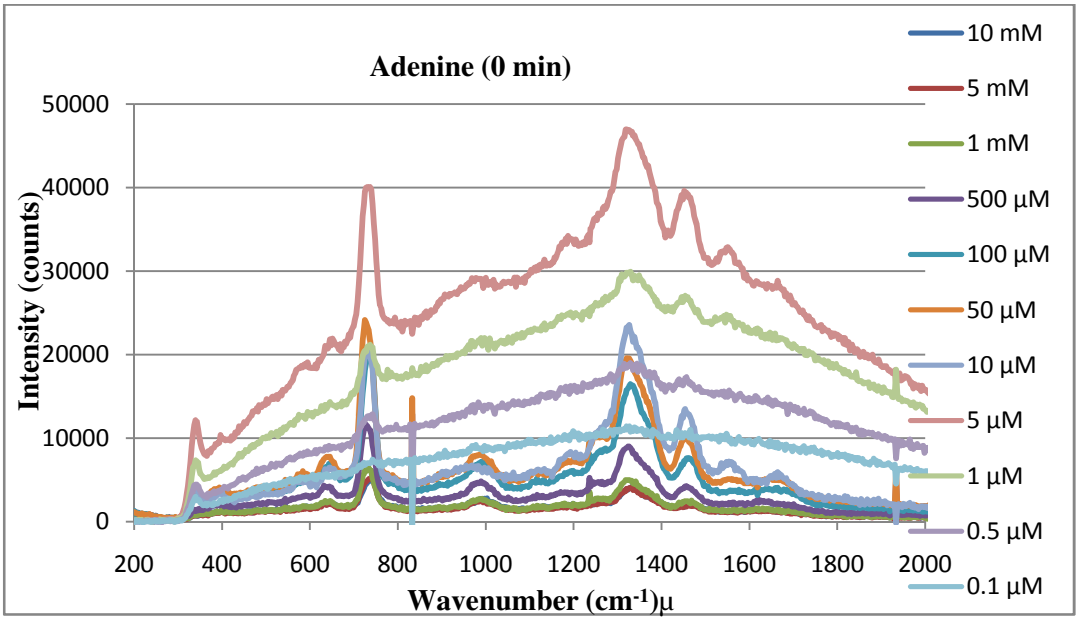

Figure (11): SERS spectra of Adenine (0 minutes) at different concentrations

3.2.2a Calibration curves:

From the data in Figure (11), it can be concluded that linearity can be obtained from concentrations ranging from 1 μM to 0.1 μM, so for different concentrations like 0.9 μM, 0.7 μM, 0.5 μM, 0.3 μM and 0.1 μM, the SERS spectra were taken at 0 minutes after sample preparation with 10 seconds integration time. Graphs were plotted using the SERS intensities at two wavenumbers (peak 1-728 cm^{-1} and peak 2- 1318 cm^{-1}) against the concentration. Table (2) shows the intensities after the subtraction from background and blank at two wavenumbers for different concentrations and Figures (12) and (13) show the linear plots.

Concentration (µM)	Intensity (counts)	
	728 cm^{-1}	**1318 cm^{-1}**
0.1	131.33	438.475
0.3	777	1080.125
0.5	1397.5	2049.125
0.7	2047.833	2842.625
0.9	2494.917	3705.125

Table (2): Maximum intensities of Adenine on Ag colloids at different concentrations

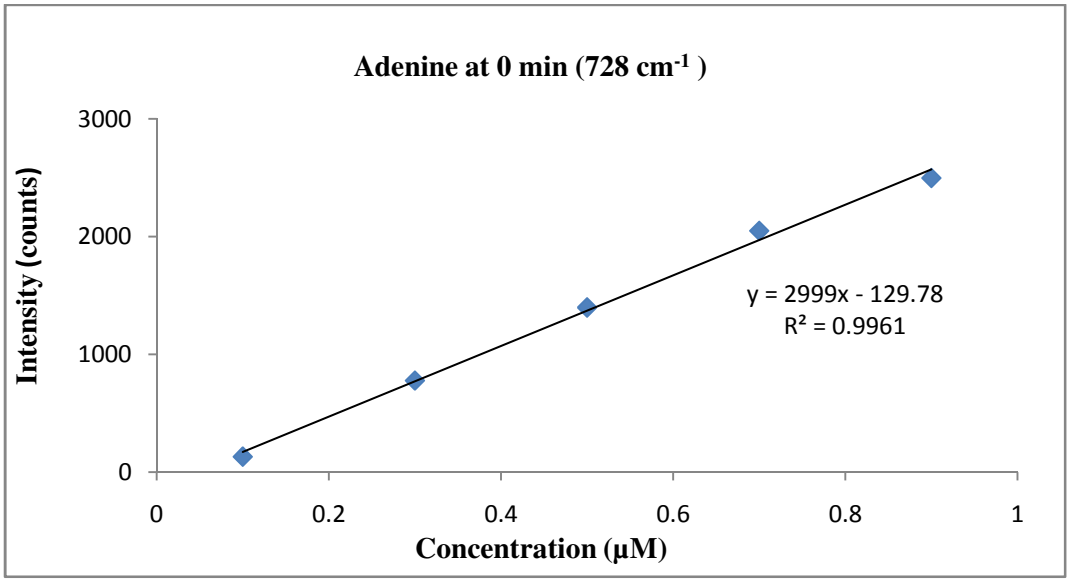

Figure (12): Calibration curve of Adenine (728 cm^{-1}) on Ag colloids

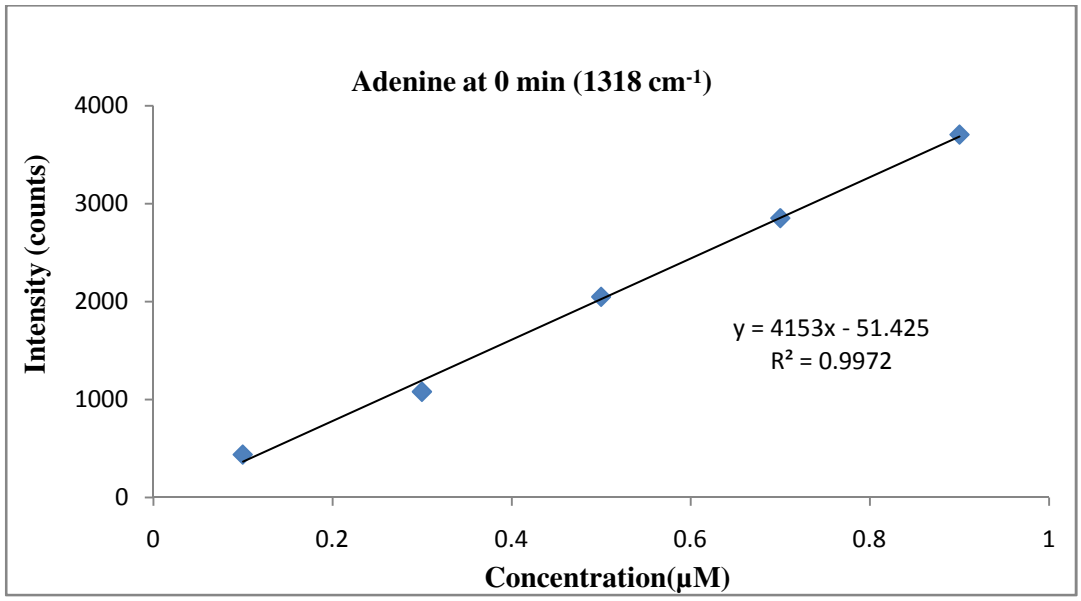

Figure (13): Calibration curve of Adenine (1318 cm^{-1}) on Ag colloids

3.2.2b Growth curve with Absorbance spectra:-

For different concentrations of Adenine, both SERS spectra and absorbance values were taken at 0 minutes and 5 minutes after sample preparation. Table (3) shows the absorbance values at different wavelengths. Figure (14) shows the absorbance spectra at different wavelengths. Absorbance values at 650 nm and 900 nm were recorded and compared with the observed SERS enhancement.

Concentration	Time	400nm	520nm	700nm	800nm	900nm
10 mM	0 min	3.57590	2.37830	2.84300	2.55960	1.89680
10 mM	5 min	2.99090	2.12290	2.50670	2.49850	2.04390
5 mM	0 min	3.62690	2.40750	2.85480	2.61050	1.95430
5 min	5 min	3.04410	2.14440	2.51270	2.53340	2.10420
1 mM	0 min	3.57390	2.36440	2.78300	2.66520	2.07160
1 mM	5 min	3.05820	2.13070	2.43000	2.54810	2.19710
0.5 mM	0 min	3.45740	2.31700	2.70230	2.64650	2.09060
0.5 mM	5 min	3.00550	2.09730	2.35570	2.52990	2.21230
0.1 mM	0 min	3.61540	2.32840	2.58800	2.67730	2.19580
0.1 mM	5 min	3.05690	2.12350	2.22540	2.50510	2.28980
0.05 mM	0 min	3.59580	2.33810	2.48550	2.66760	2.24190
0.05 mM	5 min	3.00480	2.14130	2.09910	2.44450	2.31890

Table (3): Absorbance values of Adenine at 0 and 5 minutes at different concentrations.

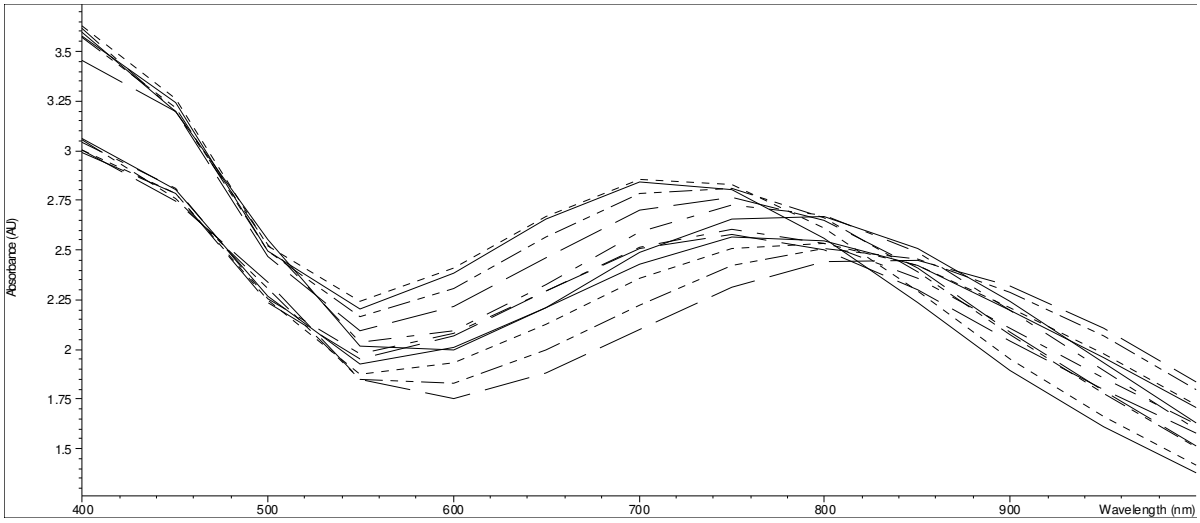

Figure (14): Absorbance spectra of Adenine at different concentrations.

Table (4) shows the absorbance values at 0 and 5 minutes after sample preparation at 650 nm. Figure (15) shows the plot for Absorbance values at 0 and 5 minutes after sample preparation at 650 nm against the log values for concentration (µM). There is a significant increase in the absorbance values after 10 µM concentration of Adenine.

Concentration (µM)	0 min	5 min
0.1	1.9379	1.2989
0.5	1.9072	1.3416
1	1.8905	1.3703
5	1.9063	1.4185
10	1.978	1.572
50	2.2106	1.8817
100	2.3285	1.9959
500	2.4643	2.1289
1000	2.5645	2.207
5000	2.6661	2.296
10000	2.6537	2.2959

Table (4): Absorbance values of Adenine at 0 and 5 minutes at 650 nm

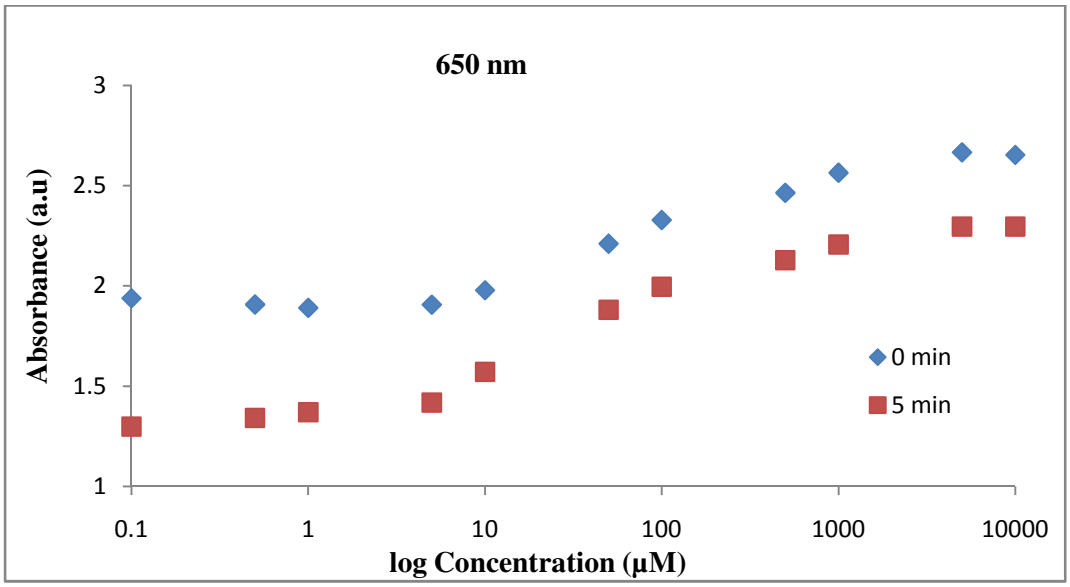

Figure (15): Absorbance values of Adenine Vs log concentration at 650 nm

Table (5) shows the absorbance values at 0 and 5 minutes at 900 nm. Figure (16) shows the plot for absorbance values at 0 and 5 minutes after sample preparation at 900 nm against the log values for concentration (µM). For a time of 5 minutes, there was an increase in absorbance at 10 µM.

Concentration (µM)	0 min	5 min
0.1	2.0454	1.2842
0.5	2.0336	1.3556
1	2.022	1.412
5	2.077	1.6186
10	2.2115	1.9893
50	1.9328	2.11
100	1.8924	2.0658
500	1.7888	1.977
1000	1.7757	1.9561
5000	1.6602	1.8559
10000	1.6121	1.7974

Table (5): Absorbance values of Adenine at 0 and 5 minutes at 900 nm

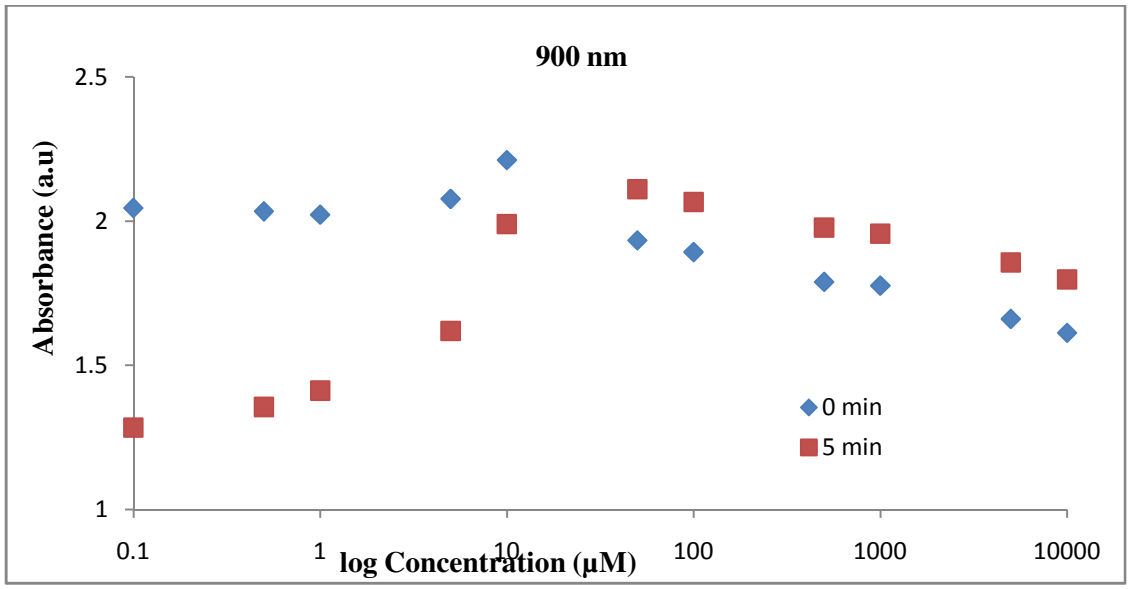

Figure (16): Absorbance values of Adenine Vs log concentration at 900 nm

Table (6) shows the SERS enhancement values at two wavenumbers for different concentrations. Figures (17) and (18) show the plots SERS enhancement against concentration. The SERS enhancement at these two wavenumbers decreases at 10 µM. The maximum enhancement observed is approximately 2 x 10^6 and is in agreement with values reported for Adenine and other compounds[27].

It is observed that the absorbance and the SERS enhancement effect both change significantly at 10 µM. This same trend has been reported in studies of the SERS response from imidazole on gold nanoparticles.[28]

Cocentration (µM)	Enhancement	
	728 cm^{-1}	1318 cm^{-1}
10000	165.6296291	83.5042
5000	374.6114731	171.225
1000	2506.724756	1081.451
500	9346.4052	3957.978
100	83971.31419	39011.29
50	199102.9769	89023.45
10	841780.6801	532734.6
5	1970108.926	923496.9
1	2633042.839	1688061
0.5	2775599.12	2022661
0.1	2380973.123	2737981

Table (6): Enhancement values of Adenine at different concentrations

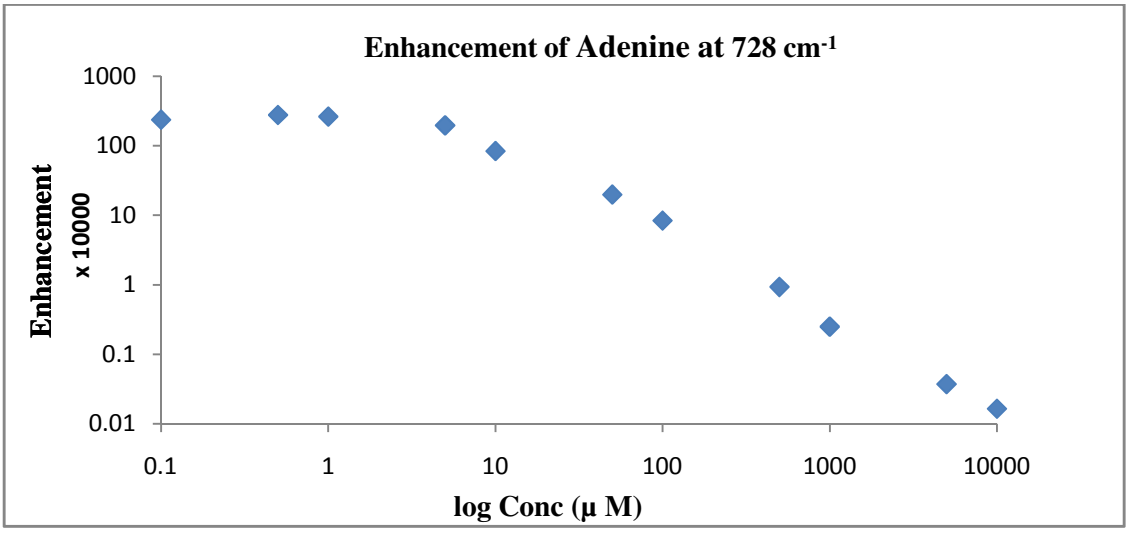

Figure (17): Enhancement of Adenine (728 cm^{-1}) Vs log Concentrations

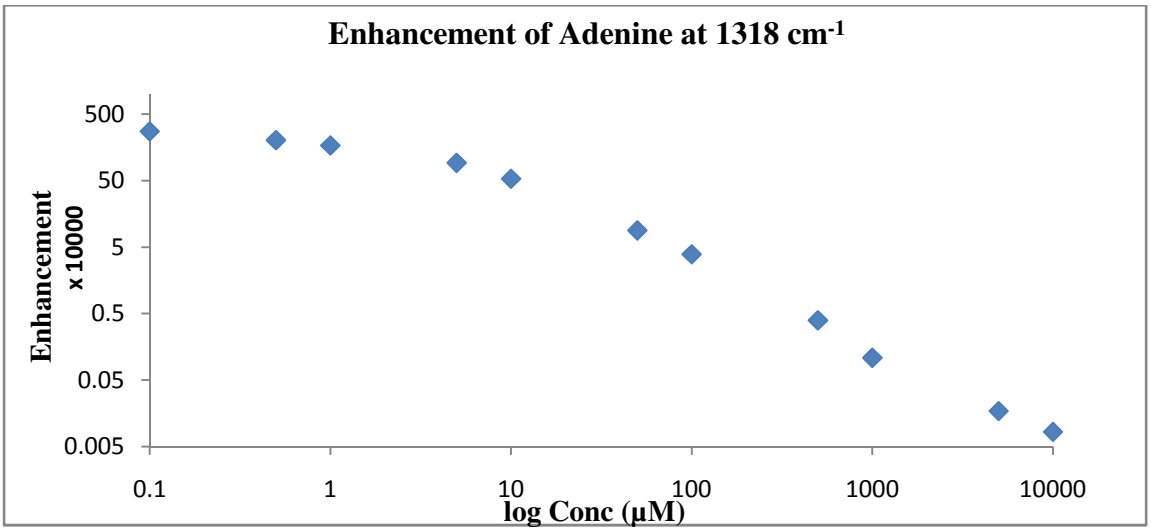

Figure (18): Enhancement of Adenine (1318 cm $^{-1}$) Vs log Concentrations

3.2.3 Gold Colloids:

Measurements of Adenine were performed using 2700 µL of gold colloids + 300 µL of sample solution + 150 µL of activator as a sample preparation. SERS spectra at different concentrations (ranging from 10 mM to 0.1 µM) and different time intervals (0, 5 and 10 minutes) were collected to find the maximum intensity and best linearity. According to Figures (19) and (20), the maximum SERS intensity for Adenine is observed at 0 minutes after sample preparation and the linearity can be obtained for concentrations ranging from 1 µM to 0.1 µM.

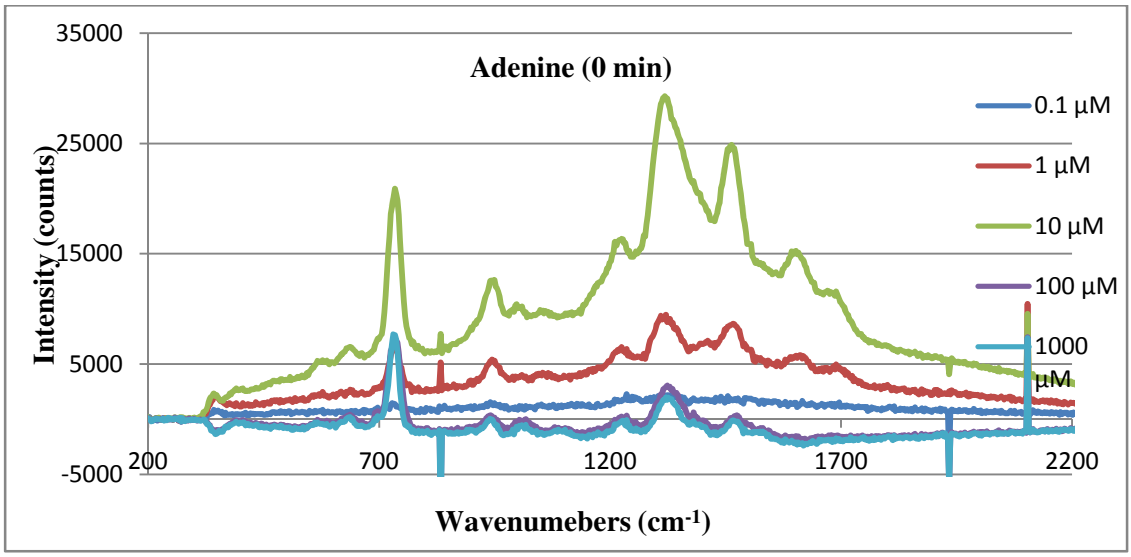

Figure (19): Different concentrations of Adenine on gold at 0 minutes

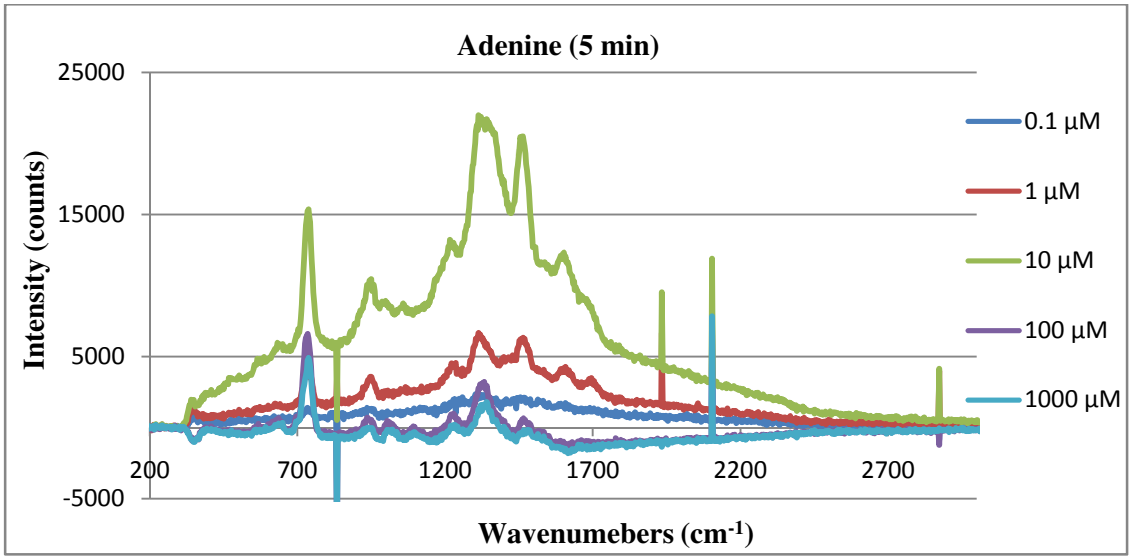

Figure (20): Different concentrations of Adenine on gold colloids at 5 minutes

3.2.3a Calibration curves:-

From Figure (20), it is observed that linearity can be obtained from Adenine concentrations ranging from 1 µM to 0.1 µM, so for different concentrations of 0.9 µM, 0.7 µM, 0.5 µM, 0.3 µM and 0.1 µM, the SERS spectra were measured at 0 minutes after sample preparation with 10 seconds integration time. Graphs were plotted using intensities at two Raman shifts (728 cm^{-1} and 1318 cm^{-1}) against the Adenine concentration. Table (7) shows the intensities at two wavenumbers for different concentrations and Figures (21) and (22) show the linear plots.

Concentration (µM)	Intensity (counts)	
	728 cm^{-1}	1318 cm^{-1}
0.1	840.5125	824.9167
0.3	1829.363	1514.683
0.5	2644.313	2208.083
0.7	3145.713	2657.817
0.9	3760.513	3000.767
1	3615.963	3326.217

Table (7): Intensities for different concentrations of Adenine at 10 seconds integration time

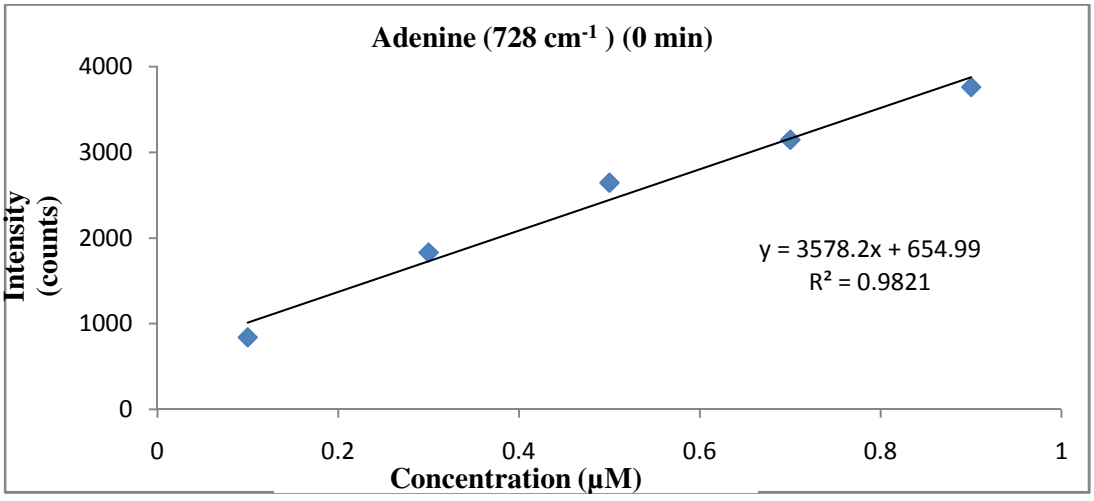

Figure (21): Calibration curve for Adenine (728 cm^{-1}) on Au colloids at 10 seconds integration time

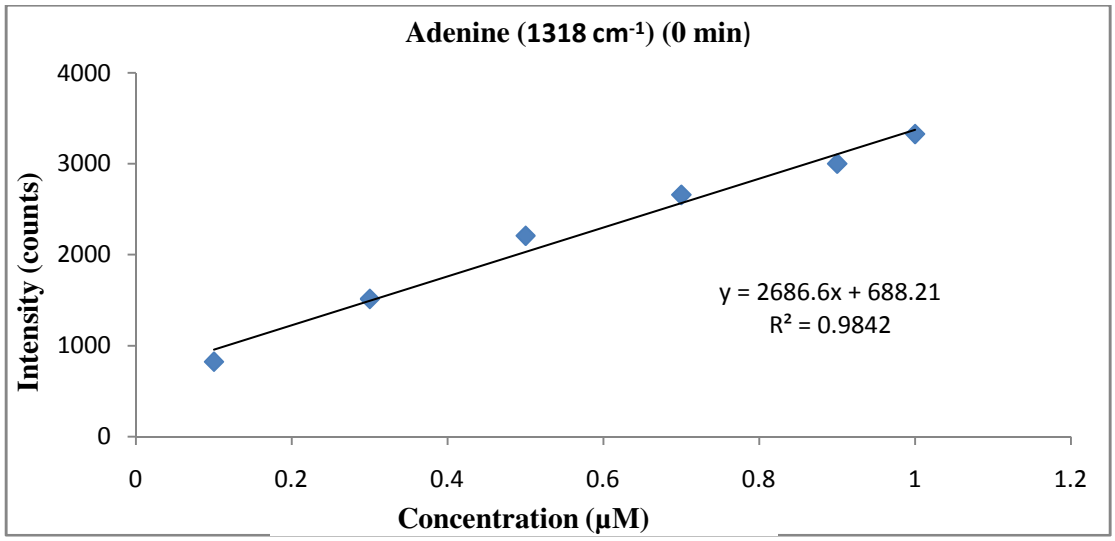

Figure (22): Calibration curve for Adenine (1318 cm^{-1}) on Au colloids at 10 seconds integration time

The integration time was increased to 60 seconds from 10 seconds and the concentrations for checking linearity were reduced, but the other conditions like sample preparation, activator and time interval (0 min) were constant. The Adenine concentrations that were used were 0.1 µM, 0.09 µM, 0.07 µM, 0.05 µM, 0.03 µM and 0.01 µM. Table (8) shows the maximum intensities at different concentrations and figures (23) and (24) show the calibration curves for maximum intensities against the concentration at two different Raman peak positions (728 cm^{-1} and 1318 cm^{-1}).

Concentration (µM)	**Intensity (counts)**	
	728 cm^{-1}	**1318 cm^{-1}**
0.1	3210.861	2178.417
0.09	2970.639	2096.583
0.07	2339.417	1699.75
0.05	1504.889	1208.167
0.03	888.9722	956.8333
0.01	533.3889	610.1667

Table (8): Intensities for different concentrations of Adenine on Au colloids at 60 seconds integration time

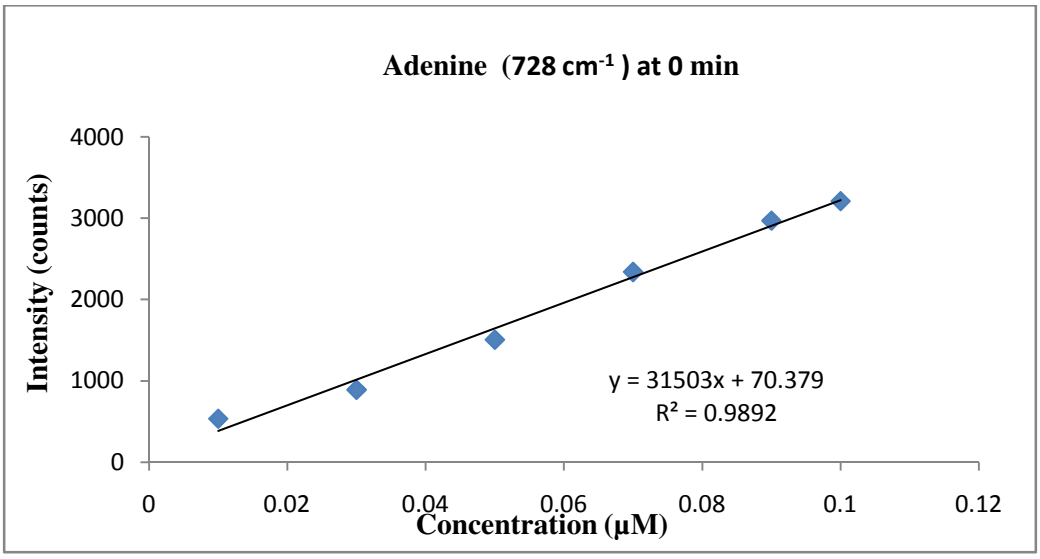

Figure (23): Calibration curve for Adenine (728 cm^{-1}) on Au colloids at 60 seconds integration time

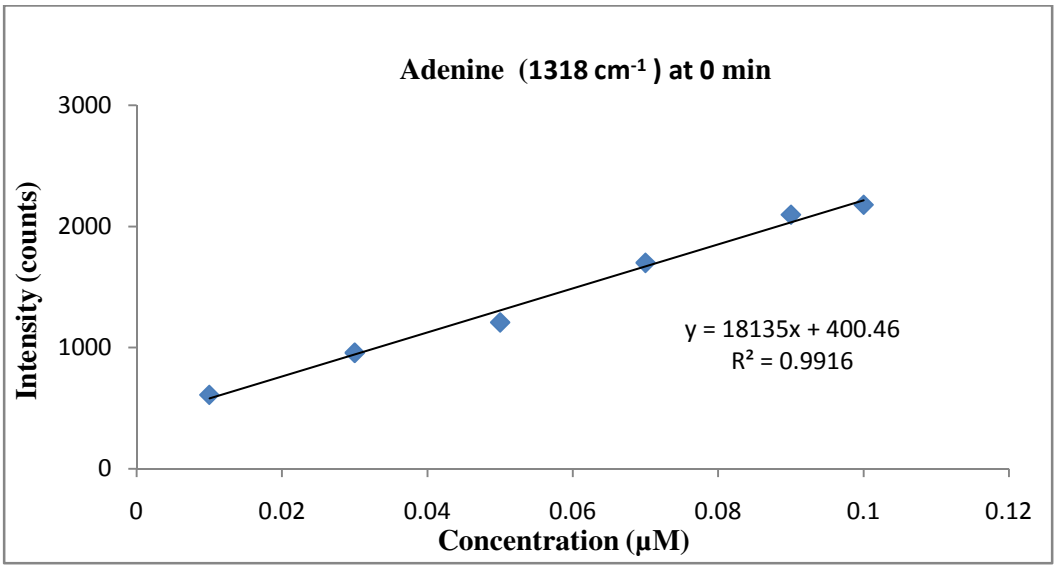

Figure (24): Calibration curve for Adenine (1318 cm^{-1}) on Au colloids at 60 seconds integration time

As the data in Figures (23) and (24) show, the linearity is observed to continue to concentrations between 0.01 µM and 0.1 µM. The limits of detection for Adenine on Au colloids for these conditions are estimated to be below 0.01 µM.

3.2.3b Growth curve with Absorbance spectra:-

For different concentrations of Adenine both SERS spectra and absorbance values were taken at 0 minutes after sample preparation in gold colloids. Table (9) shows the absorbance values at different wavelengths. Figure (25) shows the absorbance spectra at different wavelengths. Absorbance values at 650 nm and 900 nm were measured and compared with the SERS enhancement at different Adenine concentrations in gold colloids.

Concentration	Absorbance (a.u)				
	400nm	**526nm**	**650nm**	**785nm**	**900nm**
10 mM	0.46274	0.57207	0.49713	0.78918	0.18589
5 mM	0.44856	0.56688	0.48529	0.72013	0.13134
1 mM	0.43907	0.55356	0.45670	0.70209	0.10227
500 µM	0.43002	0.54598	0.44356	0.67968	8.3941E-2
100 µM	0.48104	0.60566	0.49905	0.75159	0.15626
50 µM	0.46050	0.58192	0.46641	0.74440	0.17519
10 µM	0.43061	0.54264	0.30432	0.61266	0.39919
5 µM	0.44248	0.55533	0.30463	0.52949	0.44547
1 µM	0.44638	0.55214	0.37580	0.50237	0.44952
0.5 µM	0.40479	0.49647	0.32932	0.41947	0.38972
0.1 µM	0.40975	0.49226	0.35160	0.40545	0.39543
Blank	0.40417	0.47444	0.34028	0.36608	0.36148

Table (9): Absorbance values at different wavelengths.

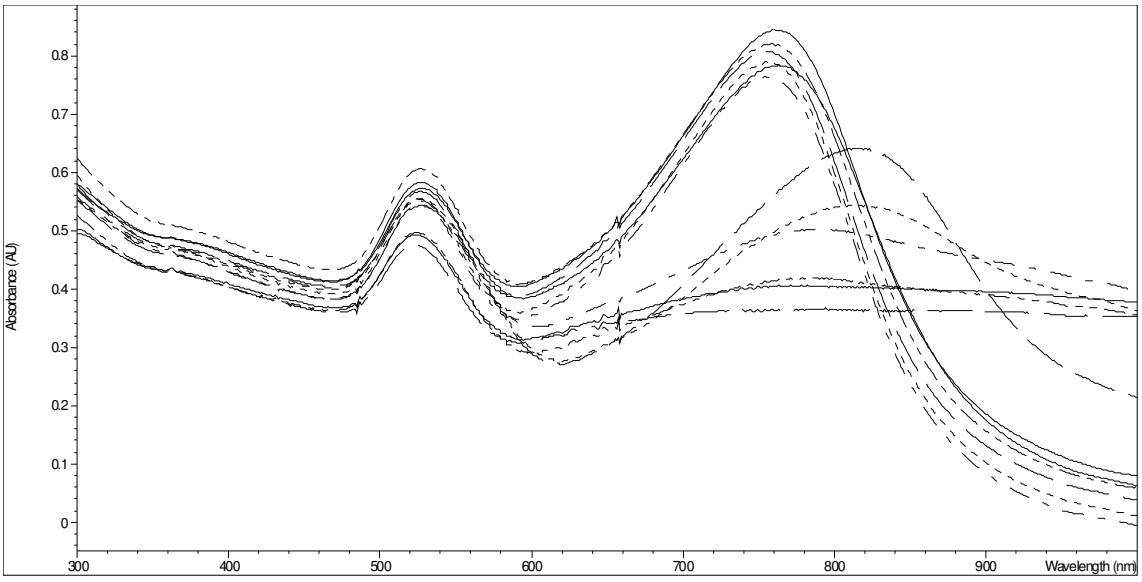

Figure (25): Absorbance spectra of Adenine on gold at different concentrations.

Table (10) shows the absorbance values at 0 minutes at 650 nm and 900 nm. Figures (26) and (27) show the plots for absorbance values at 0 minutes after sample preparation at 650 nm and 900 nm against concentration (µM). The absorbance is observed to change significantly at 650 nm and at 900 nm at an Adenine concentration of 10 µM.

Concentration (µM)	**Absorbance (a.u)**	
	650 nm	**900 nm**
10000	0.49713	0.18589
5000	0.48529	0.13134
1000	0.4567	0.10227
500	0.44356	0.08394
100	0.49905	0.15626
50	0.46641	0.17519
10	0.30432	0.39919
5	0.30463	0.44547
1	0.3758	0.44952
0.5	0.32932	0.38972
0.1	0.3516	0.39543

Table (10): Absorbance values at 0 minutes at 650 nm and 900 nm

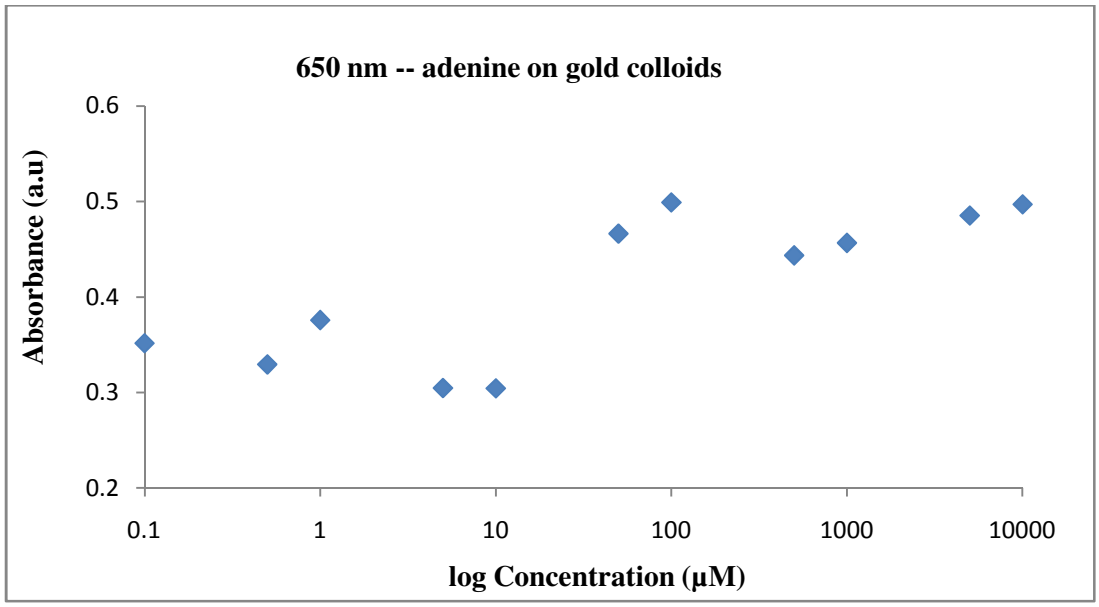

Figure (26): Absorbance of Adenine on Au colloids at 650 nm Vs log concentration

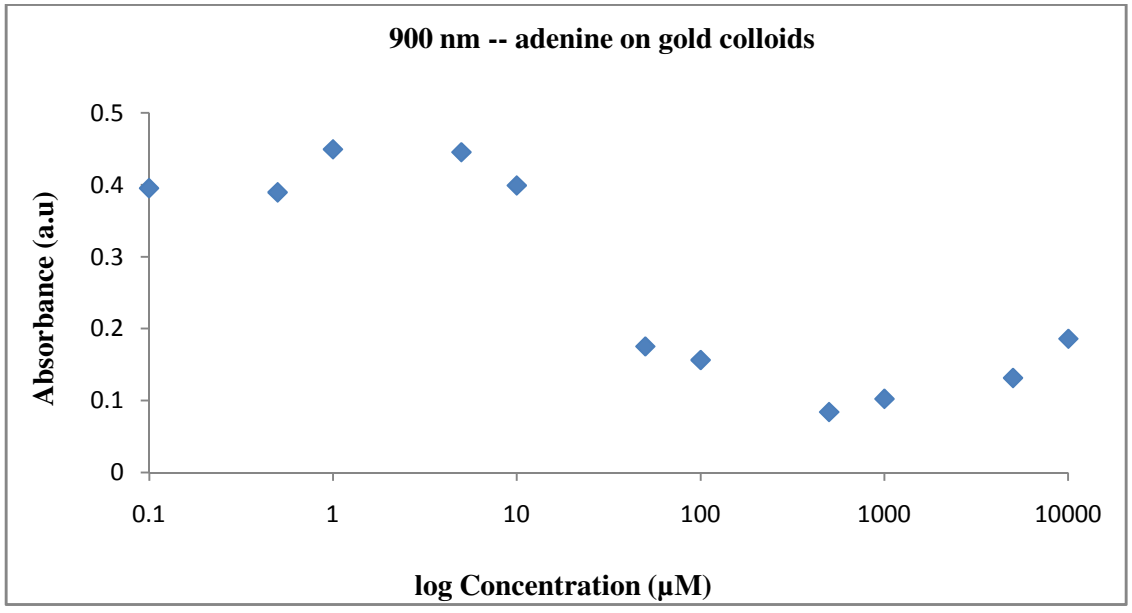

Figure (27): Absorbance of Adenine on Au colloids at 900 nm Vs log concentration

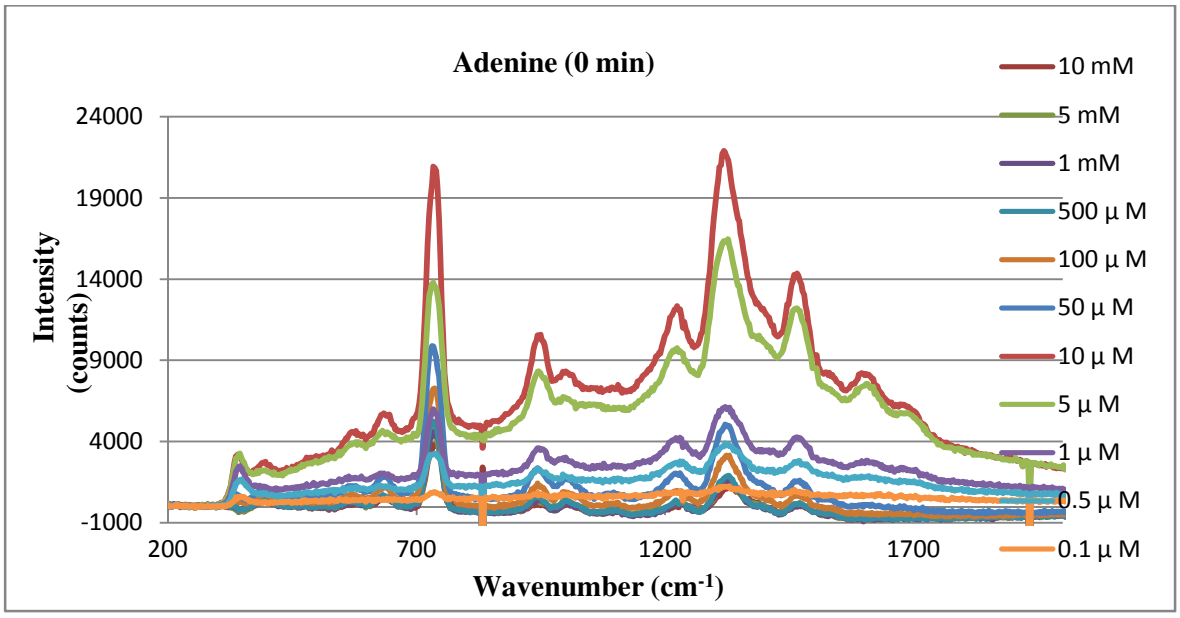

Figure (28): SERS spectra of Adenine on gold at different concentrations

Table (11) shows the SERS enhancement values at two Raman peaks for different Adenine concentrations. Figures (29) and (30) show the plots of the SERS enhancement against Adenine concentration. The SERS enhancement at these two Raman shifts is observed to change significantly at 10 µM.

As these data show, the absorbance and the SERS enhancement effect are both observed to change significantly at 10 µM. These results are similar to the data observed for Adenine on silver colloids. The data are also similar to those reported previously for imidazole on gold colloids my Souza *et al.*[28] In general, an increase in the absorbance at longer wavelengths is an indication of a shift in the surface plasmon absorption due to aggregation of the colloidal nanoparticles. This shift is usually related to an increase in the SERS enhancement

Concentration (µM)	Enhancement	
	728 cm^{-1}	**1318 cm^{-1}**
10000	2275.538	558.6022
5000	542.0764	132.9918
1000	2691.595	753.7465
500	6031.962	1685.821
100	39034.18	11274.7
50	102697.5	30889.78
10	900466.7	411424.4
5	1072933	575473.7
1	2364471	1088127
0.5	2397438	1238307
0.1	2653057	1804865

Table (11): Enhancement values of Adenine on Au colloids at different concentrations

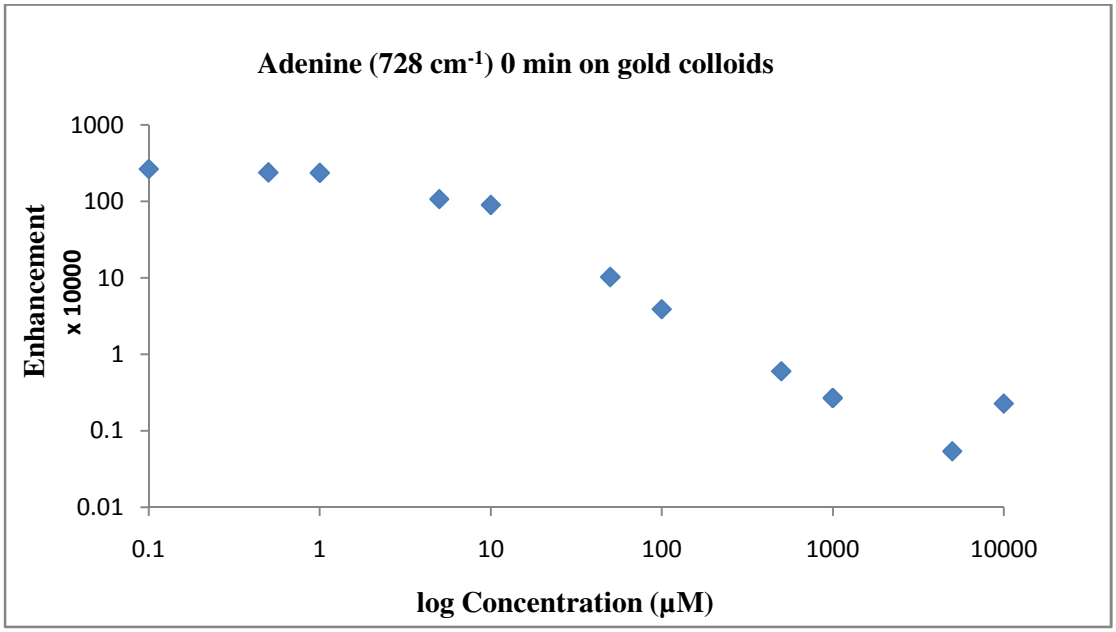

Figure (29): Enhancement values of Adenine (728 cm^{-1}) on Au colloids Vs different log concentrations

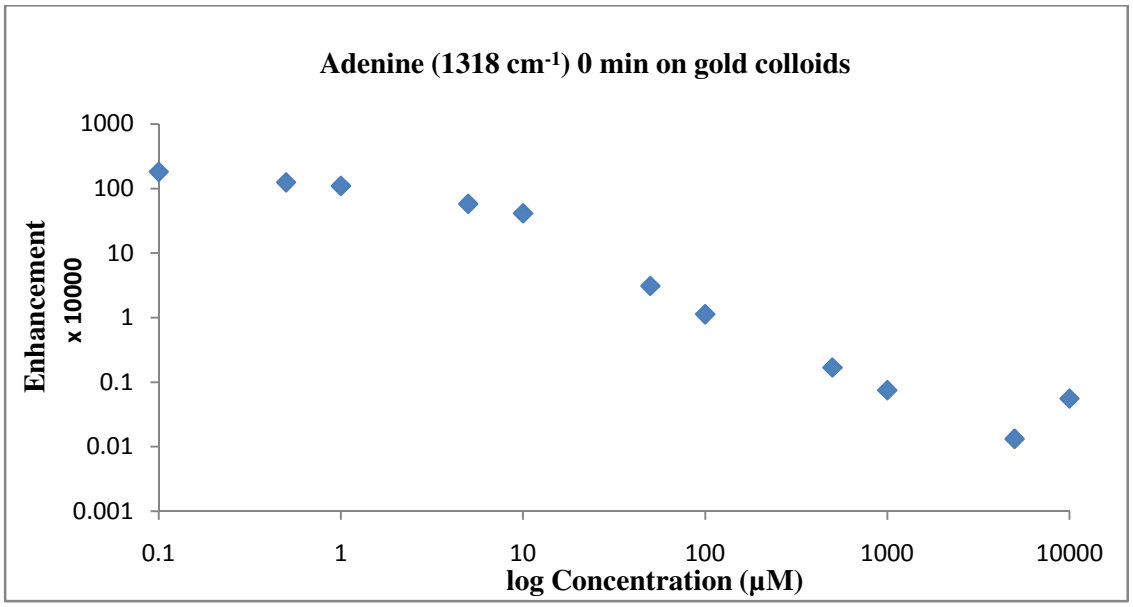

Figure (30): Enhancement values of Adenine (Peak 2) on gold Vs different log concentrations

3.2.4 Comparison of Adenine on both silver and gold colloids:

Table (12) shows the detection limits of Adenine molecule on silver and gold colloids at two Raman shifts (728 cm^{-1} and 1318 cm^{-1}). At these two Raman shifts, the detection limits were approximately equal. Lower detection limits can be possible with an increase in integration time.

Colloids	**Limit Of Detection (LOD)**	
	728 cm^{-1}	**1318 cm^{-1}**
Silver	0.16 µM	0.15 µM
Gold	0.14 µM	0.15 µM

Table (12): LOD values of Adenine on gold and silver colloids

The sensitivity observed in these studies for measurements of Adenine compares favorably to results reported previously by Materny *et al.*[27]

3.3 Rhodamine 6 G:

SERS measurements for Rhodamine 6G[47] were performed on both silver and gold colloids to study the linearity and the enhancement effects. Several experiments were performed to find the linearity and to check the reproducibility for R6G on both silver and gold colloids. The maximum intensity was found at 5 minutes after the sample preparation for silver colloids and at 0 minutes for gold colloids. In each experiment, certain conditions like integration time (10 seconds), the activator (10% NaCl) and sample preparation (2700 µL of silver/gold colloids + 300 µL of sample solution + 150 µL of activator) were maintained constant. The spectra were plotted as Intensity (counts) Vs Wavenumber (cm^{-1}). The maximum intensity for R6G was observed at two Raman peaks (1352 cm^{-1} and 1519 cm^{-1}). The R6G stock solution was prepared by dissolving R6G (solid) in methanol, and the series of dilutions were made from stock solution using methanol.

3.3.1 Normal Raman spectra:

Normal Raman spectra were obtained for the R6G solution. For these measurements, only the sample solution without any colloids or activator was used. The integration time for the R6G solution was 60 seconds. A normal Raman spectrum of pure methanol was also taken to use as a blank. Figure (31) shows the normal Raman spectrum for R6G solution.

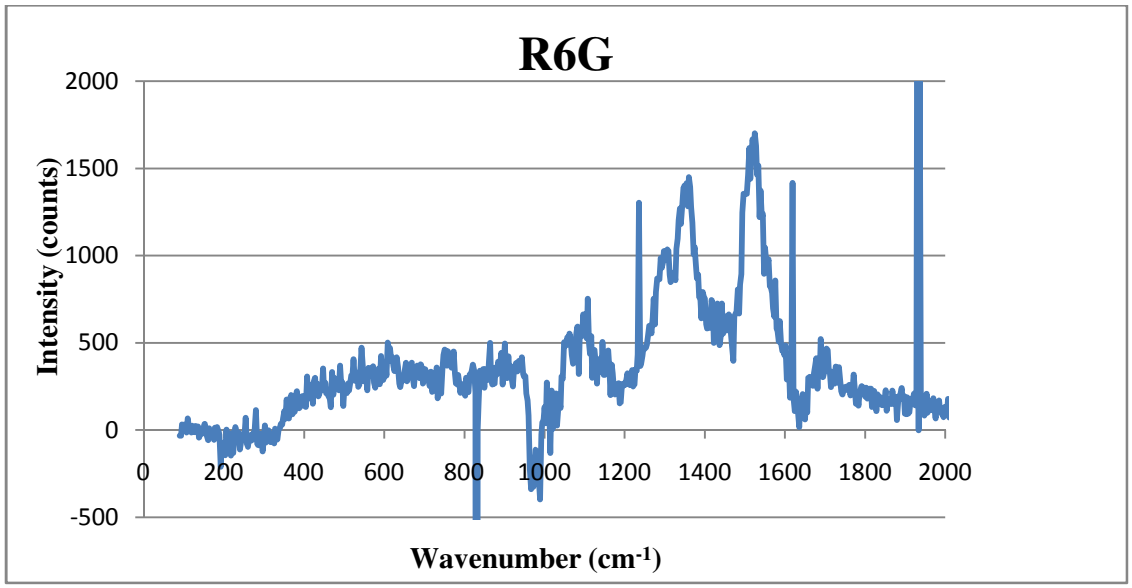

Figure (31): Normal Raman spectrum of R6G

3.3.2 Silver Colloids:

3.3.2a SERS spectra at different concentrations and different time intervals:-

Measurements of R6G were taken using 2700 µL of silver colloids + 300 µL of sample solution + 150 µL of activator as a sample preparation. SERS spectra at different concentrations (ranging from 500 µM to 0.1 µM) and different time intervals (0, 5 and 10 minutes) were taken to find the maximum intensity and best linearity. Figure (32) shows the maximum intensity for R6G at 5 minutes after sample preparation. Figure (33) shows that linearity can be obtained for concentrations ranging from 1µM to 0.1µM.

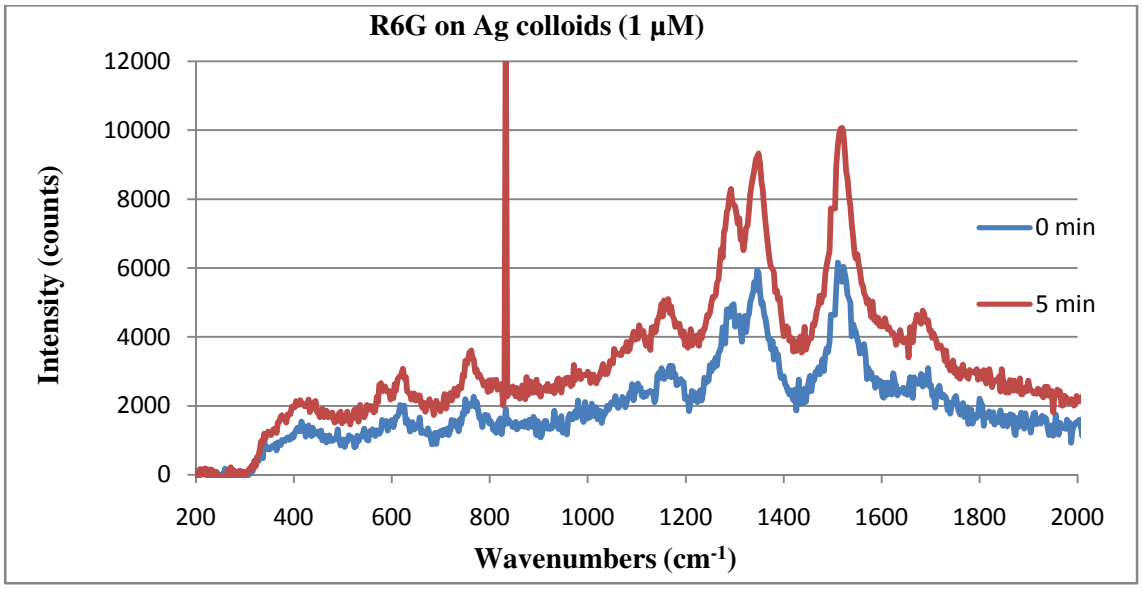

Figure (32): SERS spectra of R6G (1 µM) on silver colloids at 0 and 5 minutes after sample preparation

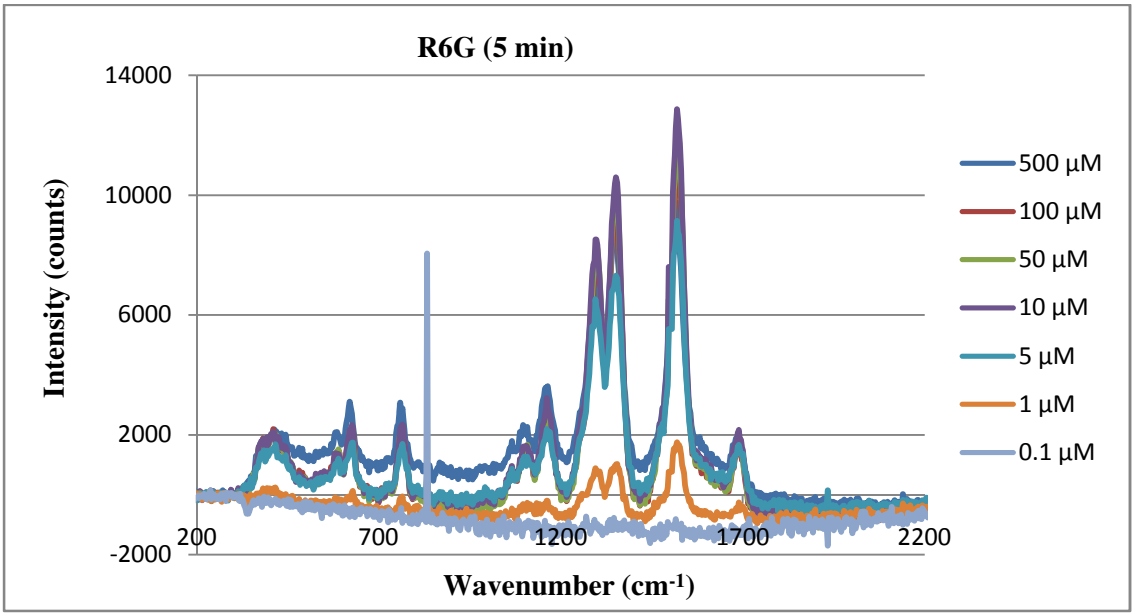

Figure (33): SERS spectra of R6G on silver colloids at different concentrations for 5 minutes

3.3.2b Calibration curves:

From Figure (33), it is observed that linearity can be obtained from concentrations ranging from 1 µM to 0.1 µM, so for concentrations 0.9 µM, 0.7 µM, 0.5 µM, 0.3 µM and 0.1 µM, the SERS spectra were taken at 5 minutes after sample preparation with 10 seconds integration time. Graphs were plotted using maximum intensities at wavenumber (1519 cm^{-1}) against the R6G concentration (µM). Table (13) shows the maximum intensities at different concentrations and Figure (34) shows the linear plot. The LOD for R6G is found to be 0.04 µM.

Concentration (µM)	**Intensity at 1519 cm^{-1} (counts)**
0.9	6480
0.7	4256
0.5	3617
0.3	2073
0.1	619

Table (13): Intensities for R6G on Ag colloids at different concentrations

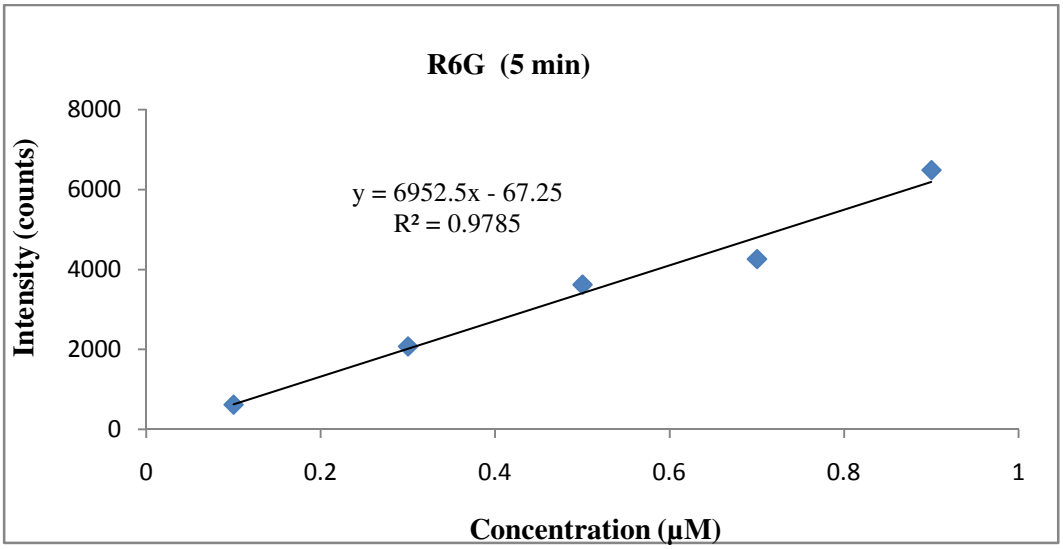

Figure (34): Calibration curve for R6G on Ag colloids at 5 minutes

3.3.2c Growth curve with Absorbance spectra:

For different concentrations of R6G, both SERS spectra and absorbance values were taken at 0 and 5 minutes after sample preparation. Table (14) shows the absorbance values for the R6G samples on silver colloids at different wavelengths. Figure (35) shows the absorbance spectra at different wavelengths. Absorbance values at 650 nm and 900 nm were measured and compared with the SERS enhancement.

Concentration (µM)	400nm	520nm	650nm	780nm	900nm
500 -- 0 min	3.80390	3.56950	2.53040	2.75190	2.13770
500 -- 5 min	3.11940	3.47400	2.14750	2.54260	2.27760
100 -- 0 min	4.00000	2.94130	2.30700	2.86590	2.22610
100 -- 5 min	3.16700	2.56830	1.90760	2.49960	2.36540
50 -- 0 min	4.00000	2.73930	2.14220	2.65220	2.14420
50 -- 5 min	2.89220	2.34010	1.73890	2.19450	2.17390
10 -- 0 min	4.00000	2.44640	2.04220	2.42530	2.18910
10 -- 5 min	2.33710	1.92100	1.52960	1.74510	1.83710
blank -- 0 min	3.52740	2.18120	1.88760	2.03350	2.00400
blank -- 5 min	1.94190	1.55910	1.31210	1.27820	1.26730
5 -- 0 min	3.81440	2.36670	2.00690	2.33400	2.13580
5 -- 5 min	2.20010	1.80880	1.46040	1.60770	1.68380
1 -- 0 min	3.64680	2.30280	1.97520	2.16620	2.04170
1 -- 5 min	2.07900	1.69790	1.39910	1.41960	1.44970
0.5 -- 0 min	3.43230	2.22240	1.92730	2.09670	2.03850
0.5 -- 5 min	2.07650	1.65740	1.38950	1.38820	1.38940
0.1 -- 0min	3.39040	2.19350	1.90080	2.10810	2.04160
0.1 -- 5 min	1.95340	1.58900	1.33800	1.33950	1.34380

Table (14): Absorbance values of R6G on Ag colloids at different concentrations.

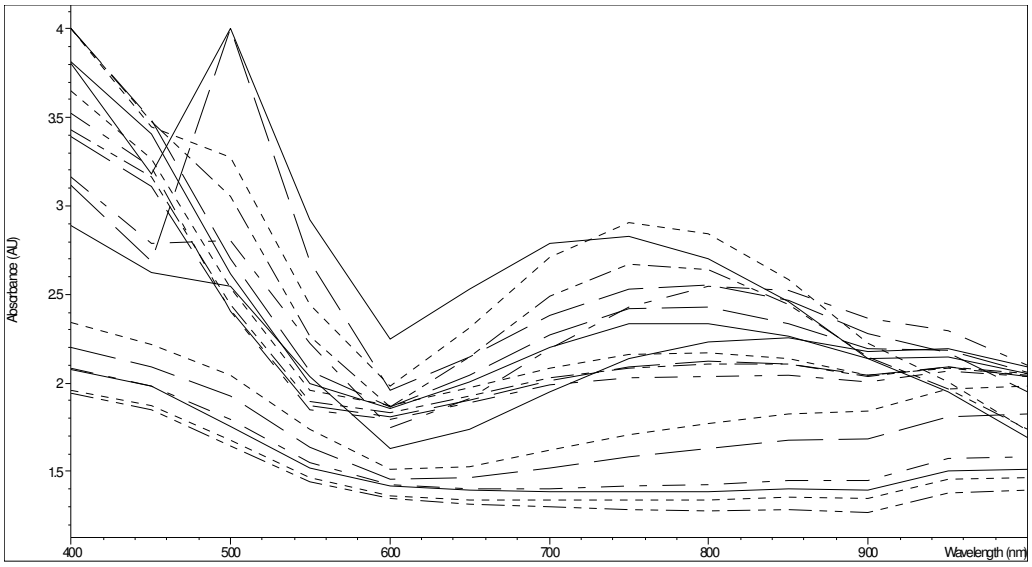

Figure (35): Absorbance spectra of R6G on Ag colloids at different concentrations.

Table (15) shows the absorbance values at 0 and 5 minutes after sample preparation at 650 nm. Figure (36) shows the plot for Absorbance values of R6G on silver colloids at 0 and 5 minutes after sample preparation at 650 nm against the values for concentration (μM). There is a small increase in absorbance values after approximately 5 μM concentration of R6G for 5 minutes after sample preparation.

Concentration (μM)	**650 nm**	
	0 min	**5 min**
0.1	1.9724	1.3793
0.5	1.9694	1.367
1	1.9751	1.3914
5	2.0117	1.4044
10	2.0311	1.5918
50	2.0995	1.6972
100	2.1828	1.7797
500	2.5536	2.1668

Table (15): Absorbance of R6G on Ag colloids at 0 and 5 minutes at 900 nm

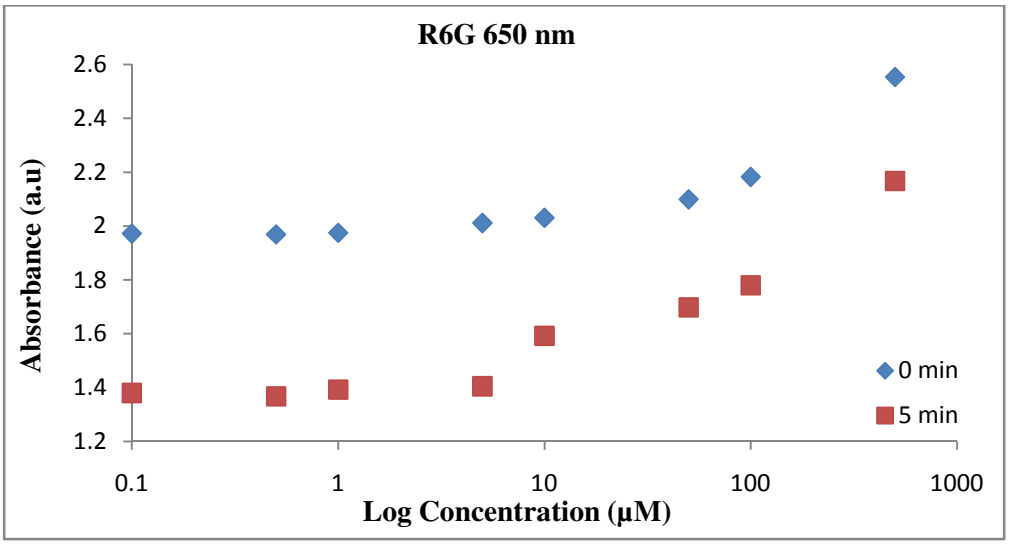

Figure (36): Absorbance values of R6G on Ag colloids Vs log Concentration

Table (16) shows the absorbance values at 0 and 5 minutes after sample preparation at 900 nm. Figure (37) shows the plot for Absorbance values at 0 and 5 minutes after sample preparation at 900 nm against the values for concentration (µM). There is an increase in absorbance values after 5 µM concentration of R6G for 5 minutes after sample preparation. At 0 minutes there was no significant change for different concentrations.

Concentration (µM)	900 nm	
	0 min	5 min
0.1	2.0518	1.3948
0.5	2.0375	1.3545
1	2.0658	1.4375
5	2.1715	1.5566
10	2.1639	1.9354
50	2.1375	2.1417
100	2.1596	2.2558
500	2.1123	2.2772

Table (16): Absorbance values of R6G on Ag colloids at 0 and 5 minutes at 900 nm

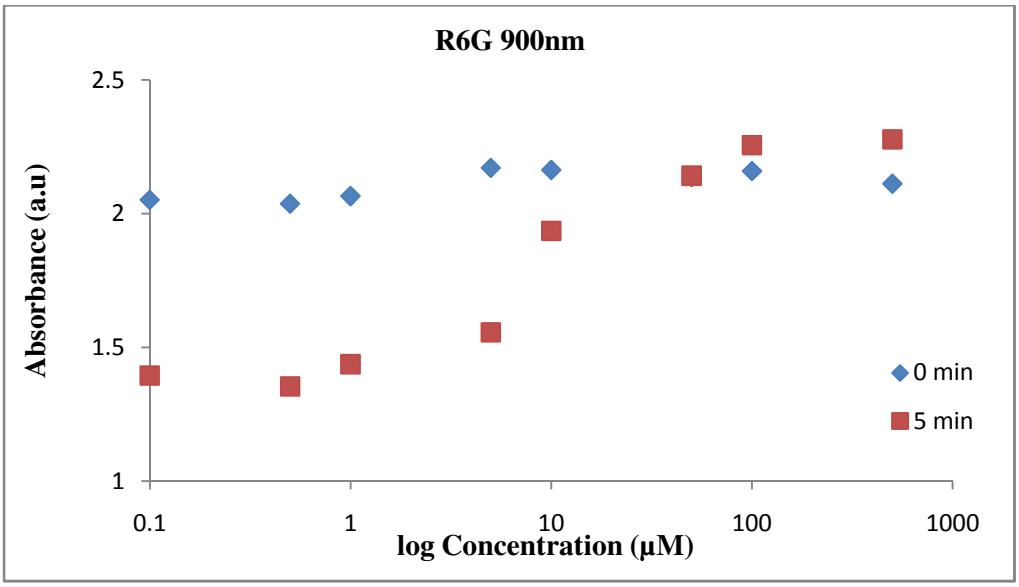

Figure (37): Absorbance values R6G on Ag colloids Vs log Concentration

Table (17) shows the SERS enhancement values at two Raman peaks for different concentrations. Figures (38) and (39) show the plots of SERS enhancement against concentration (µM). The SERS enhancement at these two wavenumbers is observed to change significantly at a R6G concentration of 10µM. As these data show, both the absorbance and the SERS enhancement effect change significantly at 10 µM concentration.

Concentration (µM)	Enhancement	
	1352 cm^{-1}	**1519 cm^{-1}**
500	1055.644786	843.294176
100	6378.11454	5340.40128
50	13411.35698	10944.04832
10	68878.96225	60115.3464
5	93304.6982	83101.5744
1	118318.074	119750.544
0.5	145165.1495	128911.592
0.1	60377.114	215625.032

Table (17): Enhancement of R6G on Ag colloids at different Concentrations (µM)

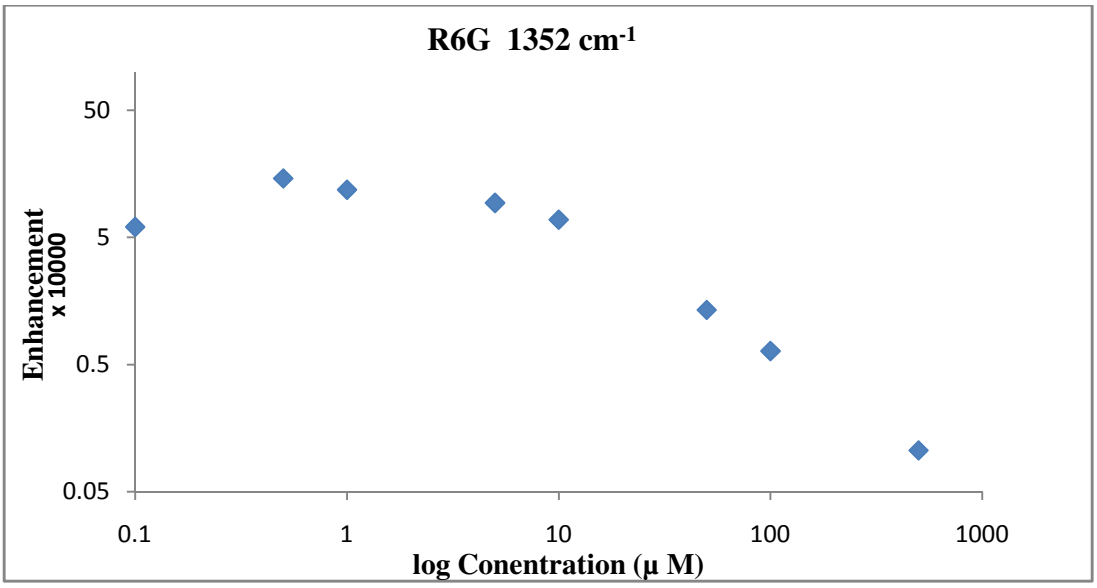

Figure (38): Enhancement of R6G (1352 cm^{-1}) on Ag colloids Vs log Concentration (µM)

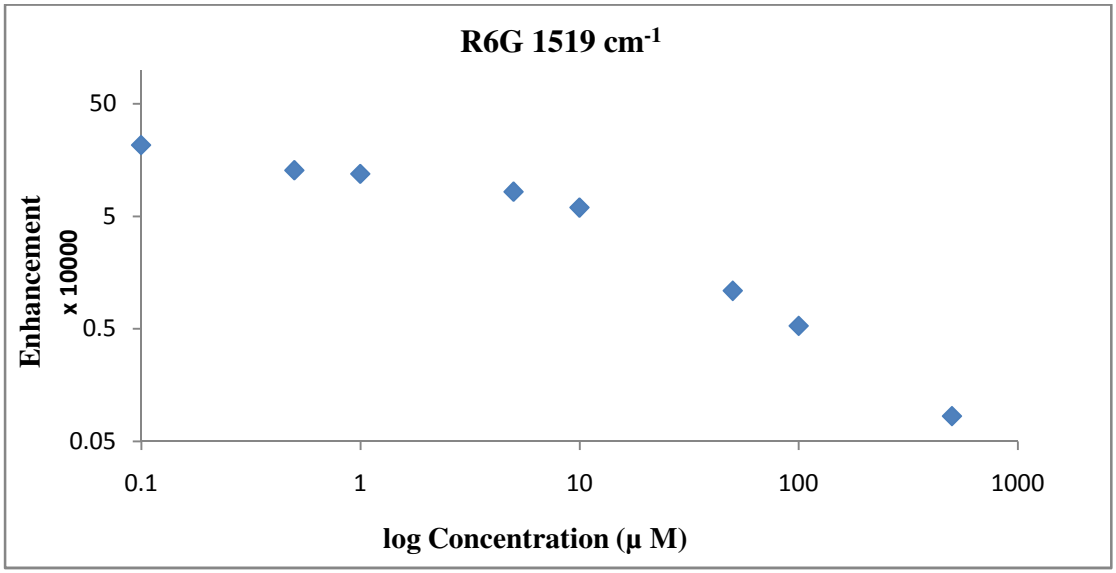

Figure (39): Enhancement of R6G (1519 cm^{-1}) on Ag colloids Vs log Concentration (µM)

The trend of these data is similar to that observed for Adenine studies on silver and gold and also that reported for imidazole on gold colloid.[28]

3.3.3 Gold Colloids:

SERS measurements of R6G were performed on gold colloids for different concentrations ranging from 100 µM to 0.1 µM. Figure (40) shows SERS spectra of R6G at 0 minutes after sample preparation. As expected, higher concentrations show higher intensities. SERS spectra, were also collected (data not shown) for concentrations like 1.0 µM 0.9 µM, 0.7 µM, 0.5 µM, 0.3 µM and 0.1 µM, but no linearity was observed.

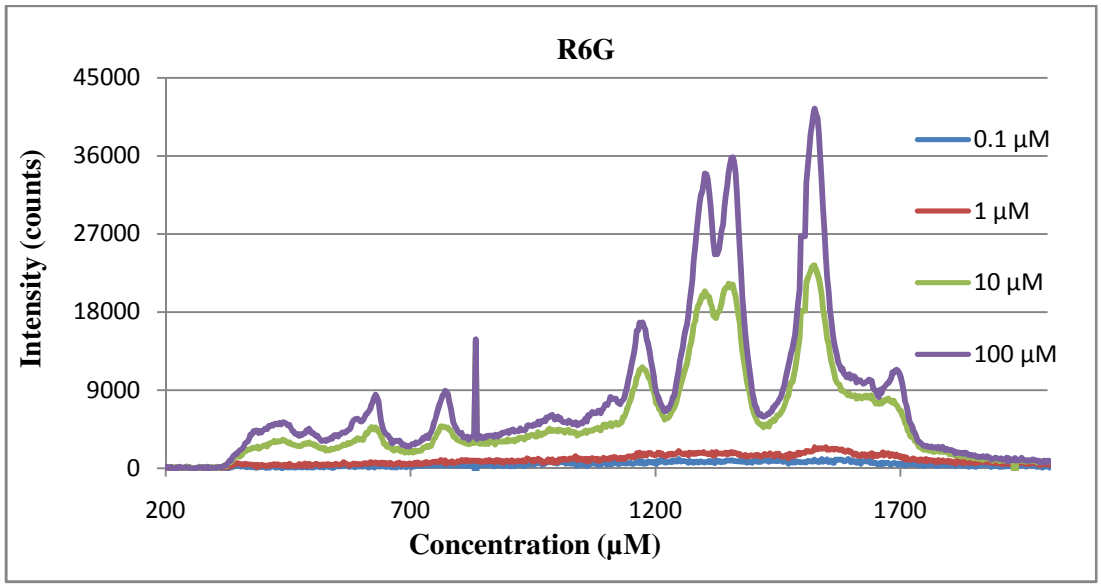

Figure (40): SERS spectra of R6G on Au colloids at different concentrations

3.4 Mixtures:

3.4.1 Silver Colloids:-

It is expected that real samples will contain mixtures of two or more SERS-active compounds. In these studies, preliminary evaluations of the SERS responses of solutions containing mixtures of Adenine and R6G on silver colloids were performed to study the differences in intensities of the compounds in a mixture. For sample preparation, 2700 µL of silver colloid + 150 µL of Adenine solution + 150 µL of R6G solution + 150 µL of activator (10% NaCl) were used. Table (18) shows the different concentrations of Adenine and R6G. Figures (41), (42) and (43) show the spectra for mixtures of Adenine and R6G at 0 minutes, 5 minutes and 10 minutes after sample preparation respectively.

Adenine(µM)	**R6G (µM)**
10	10
10	5
10	1
5	5
5	1
1	1

Table (18): Different concentrations of Adenine and R6G mixtures.

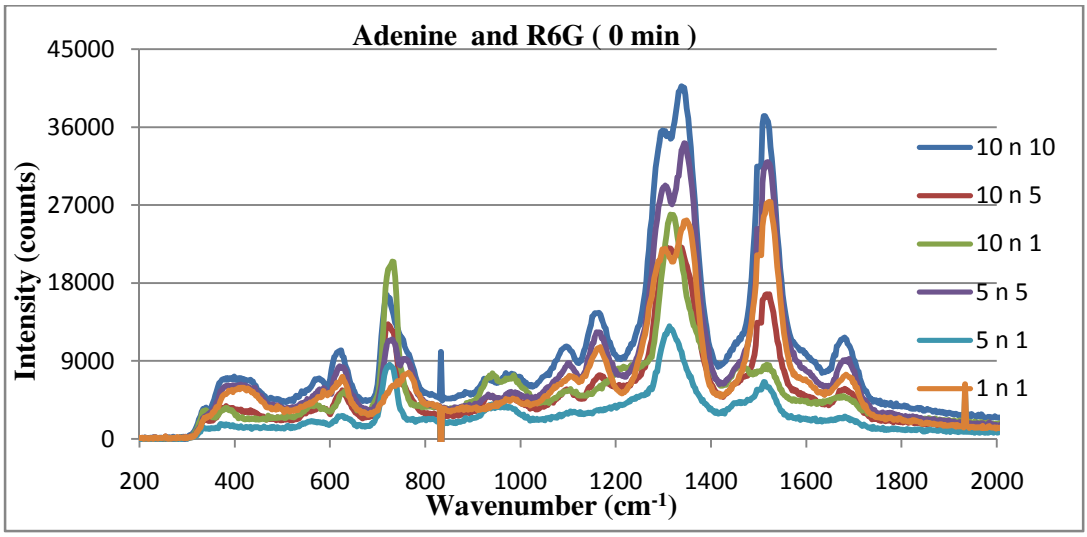

Figure (41): Different concentrations of Adenine and R6G mixtures at 0 minutes

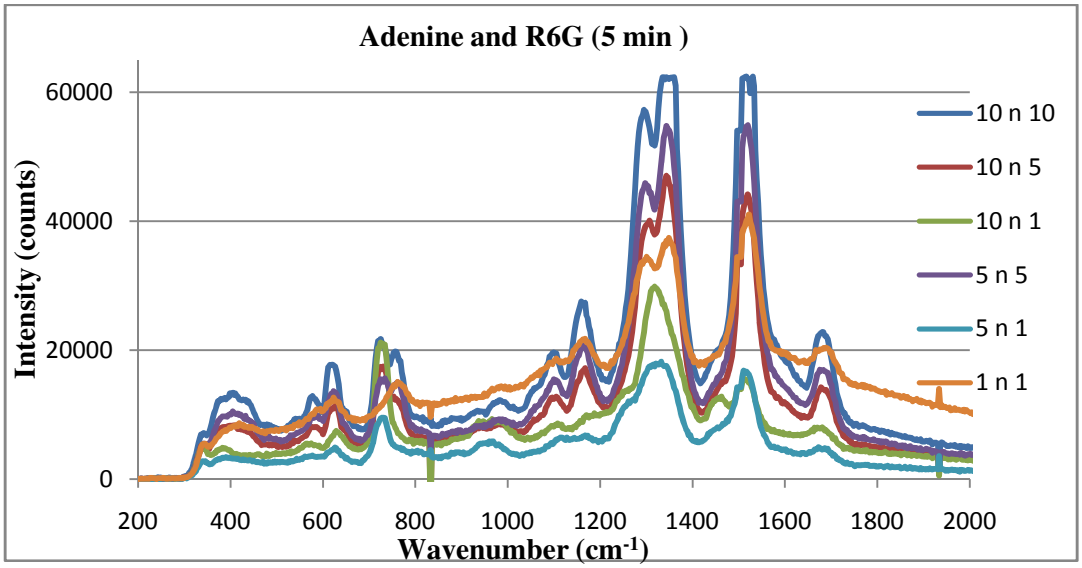

Figure (42): Different concentrations of Adenine and R6G mixtures at 5 minutes

As these preliminary data show, the resulting SERS spectra have features from both Adenine and R6G. In general, the overall intensity is dependent on the concentration of each compound, although the changes in intensity do not always appear to be proportionally related to the concentration.

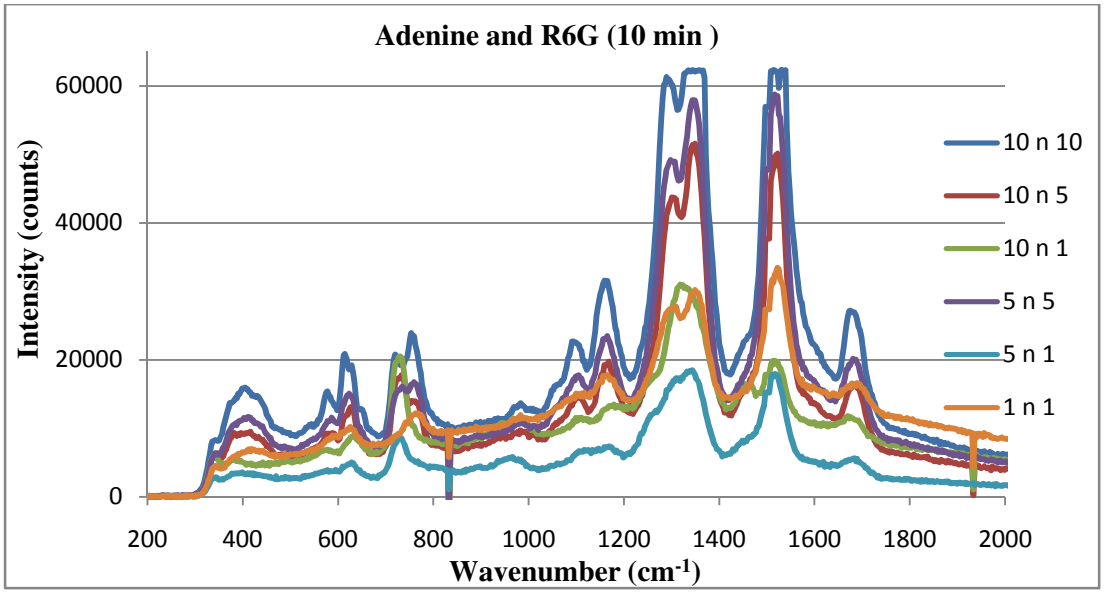

Figure (43): Different concentrations of Adenine and R6G mixtures at 10 minutes

4.0 CONCLUSIONS

The results of these studies have shown that a linear concentration dependence for Adenine on both silver and gold colloids can be obtained in the concentration range 1 μM to 0.1 μM at 10 seconds integration time and at 0 minutes after sample preparation. The absorption of Adenine at 650 nm and 900 nm and the Raman signal enhancement on both silver and gold colloids are observed to change significantly at a concentration 10 μM. SERS measurements of Adenine show that the maximum intensity was obtained at 10 μM.

A linear concentration dependence for R6G on silver colloids could be obtained in the concentration range 1 μM to 0.1 μM at 10 seconds integration time and at 5 minutes after sample preparation. A linear concentration dependence for R6G was not observed on gold colloids. The absorption of R6G at 650 nm and 900 nm and the Raman signal enhancement on silver colloids were both observed to change significantly at a concentration 10 μM. SERS measurements of R6G show that the maximum intensity was obtained at 10 μM.

SERS spectra for mixtures of Adenine and R6G at different concentrations and different time intervals at 10 seconds integration time have been reported. The spectra for these show the spectral features of both Adenine and R6G.

5.0 FUTURE STUDIES

Further studies of SERS techniques using Adenine and R6G should be performed for linear concentration dependence and detection limits on both silver and gold nanoparticles and on the solid substrates modified with immobilized metal nanoparticles.

The linear concentration dependence and the detection limits should be studied for Adenine and R6G at lower concentration levels (below 0.1 μM) by increasing the integration time from 10 seconds to 60 seconds and improving the optical system.

SERS measurements for different mixtures of compounds should be performed at different time intervals and different integration times to evaluate the ability to selectively measure individual compounds in complex mixtures.

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