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A rapid and simple chemical method for the preparation of Ag colloids for surface-enhanced Raman spectroscopy using the Ag mirror reaction

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Abstract

Colloidal silver (Ag) nanoparticles (AgNP) have been widely used for surface-enhanced Raman spectroscopy (SERS) applications. We report a simple, rapid and effective method to prepare AgNP colloids for SERS using the classic organic chemistry Ag mirror reaction with Tollens' reagent. The AgNP colloid prepared with this process was characterized using SEM, and the reaction conditions further optimized using SERS measurements. It was found that Ag mirror reaction conditions that included 20 mM AgNO3, 5 min reaction time, and 0.5 M glucose produced AgNP colloids with an average size of 319.1 nm (s.d ±128.1). These AgNP colloids exhibited a significant SERS response when adenine was used as the reporter molecule. The usefulness of these new AgNP colloids was demonstrated by detecting the nucleotides adenosine 5′-monophosphate (AMP), guanosine 5′-monophosphate (GMP), cytidine 5′-monophosphate (CMP), and uridine 5′-monophosphate (UMP). A detection limit of 500 nM for AMP was achieved with the as-prepared AgNP colloid. The bacterium Mycoplasma pneumoniae was also easily detected in laboratory culture with these SERS substrates. These findings attest to the applicability of this AgNP colloid for the sensitive and specific detection of both small biomolecules and microorganisms.

Graphical Abstract

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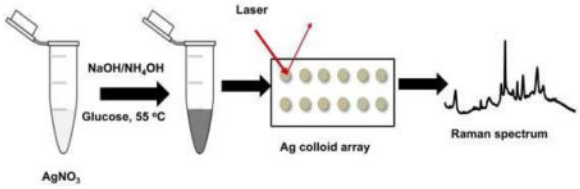

Introduction

Surface-enhanced Raman spectroscopy (SERS)[1–3] is a powerful molecular spectroscopic technique that can provide chemical and structural information about various biomolecules such as DNA, RNA, viruses, bacteria, proteins, nucleotides, and enzymes.[4–6] The SERS effect was originally observed in the 1970′s using free-electron metals such as Ag, Au, or Cu.[2, 7–10] Historically, SERS substrates have been classified in one of two categories: metal colloids or structural solid substrates.[11, 12] In the case of metal colloids, Ag or Au nanostructures are normally prepared in the solution phase and then centrifuged for various applications. Metal colloids include Ag or Au metal colloids, core-shell nanoparticles,[13] nanorods, nanostars, and magnetic nanoparticles with Ag or Au.[13, 14] Solid/planar substrates include silver or gold nanostructures on glass,[15] polymer nanofibers,[16] filter papers,[17] film over nanospheres,[18] anodic aluminum oxide,[19] graphene oxide,[20] and silicon,[21] among others.[22]

Nobel metal nanostructures are commonly used for SERS applications due to the strong localized surface plasmonic properties in these elements.[2, 23] The possibility of using Ag or Au colloids for SERS was demonstrated early.[24] Metal colloids possess various advantages as SERS substrates, including stability, tunability, sensitivity, and specificity.[23] Generally, metal colloids are prepared either with sodium citrate (i.e., the Lee-Meisel method)[25, 26] or sodium borohydride (i.e. the Creighton method)[24] due to the simple preparation methods and large SERS enhancements that arise from these nanoparticles. To meet different experimental requirements, metal nanoparticles can also be prepared using various capping agents such as citrate,[27] polyvinylpyrrolidone (PVP),[28] or cetyl trimethylammonium bromide (CTAB).[29]

With the Lee-Meisel nanofabrication method, it is common to observe Raman bands arising from citrate or other capping agents; these signals frequently interfere with the desired analyte vibrational modes.[30] With NaBH4 as the reducing agent, it is difficult to avoid metal precipitation and nanoparticle aggregation during colloidal synthesis.[31] In addition, solutionphase metal colloids can suffer from low spectral reproducibility due to aggregation. Nanoparticle interferences and aggregation can have been reduced, but not eliminated, by adjusting the pH and addition of stabilizers.[32] For example, Ren and coworkers utilized iodide-modified citrate colloids for detection of DNA bases.[33] In this case, it proved necessary to modify the surface of the metal colloids to avoid signals from the capping/ reducing agents.

While in some cases aggregation is an unwelcome disadvantage, in other cases aggregating agents such as NaCl or MgSO4 are specifically employed with metal colloids in order to

produce localized hot spots that promote high SERS enhancements.[33, 34] In these cases, the various, and sometimes competing, requirements of the metal colloid nanofabrication process can add complexity to the synthetic procedures needed to produce high SERS enhancements. In order to employ SERS for practical applications, it is necessary to develop a simple but effective chemical method for preparation of Ag colloids with reproducible nanoparticle distribution and high sensitivity.

In this report, we demonstrate an efficient method of preparing Ag nanoparticle (AgNP) colloids for SERS using the classical Ag mirror reaction with glucose as a reducing agent. This preparation procedure is simple and reproducible. Moreover, the prepared AgNP colloids can be used immediately for SERS applications without any aggregating agents or surface-modification. The as-prepared AgNP colloid was characterized with SEM images, and the reaction conditions optimized using the SERS intensity of 100 μM aqueous adenine. The performance of the AgNP colloid for SERS applications was evaluated by detecting the concentration-dependence of aqueous mononucleotides. Finally, the biochemical relevance of these AgNP colloids was demonstrated by the detection of bacterial (Mycoplasma pneumoniae) samples in culture. In this case, the freshly prepared colloid was centrifuged with an appropriate amount of mycoplasma samples to encapsulate the organisms with Ag nanoparticles. These results demonstrated that Raman signals from microorganisms are also greatly enhanced due to the simple adsorption of nanoparticles on their surface.

Experimental methods

AgNO3 was purchased from J.T. Baker (Phillipsburg, NJ). Glucose was obtained from Acros Organics (Phillipsburg, NJ). NaOH and high precision microscope cover glass slides (22 × 22 mm) were purchased from Fisher (Hampton, NH). NH4OH, NaCl, formaldehyde, MeOH, aluminum (Al) foil, and the mononucleotides adenosine 5′-monophosphate (AMP), guanosine 5′monophosphate (GMP), cytidine 5′-monophosphate (CMP) and uridine 5′-monophosphate (UMP), were obtained from Sigma Chemical Co. (St. Louis, MO). Adenine was purchased from Alfa Aesar (Ward Hill, MA). Eppendorf tubes (1.5 mL) were obtained from Eppendorf North America (Hauppauge, NY). All chemicals were reagent grade. Ultra-pure water (Milli-Q, 18.2 ML cm) was used to prepare aqueous solutions of the adenine and mononucleotides.

Mycoplasma pneumoniae samples for SERS analysis were prepared as follows. A total of 30 mL of Mycoplasma pneumoniae strain M129 was grown in SP4 broth at 37°C for 2–3 days until color change. The average CFU was 5×108 CFU/mL. The bacteria were harvested by centrifuging at 10,000×g for 20 min at 4°C. The pellet was washed twice with 1 mL of 0.85% NaCl and harvested at 10,000×g for 5 min at 4°C. The final pellet was re-suspended in 1 mL 0.85% NaCl and passed through 22G1 1/2 needle to disperse the aggregates. The bacteria were then fixed with 4% formaldehyde by adding 1 mL of 8% formaldehyde that was prepared in 0.85% NaCl to the suspension. The average final bacteria concentration was 7.5 × 109 CFU/mL. The fixed bacteria were stored at 4°C until tested.

SERS spectra were acquired using a Renishaw (Hoffman Estates, IL) inVia confocal Raman microscope system using 785 nm excitation. The SERS spectra were acquired in the range

of 1700–600 cm−1 using a 20× objective, with an integration time of 1 and 2s for adenine and Mycoplasma pneumoniae samples, respectively, with a laser power of 1.46 mW in synchronous scanning mode. For mononucleotides, the integration time was 1s with a laser power 4.47 mW.

To obtain the SERS spectra of the analytes, the freshly prepared AgNP colloid was centrifuged; 20 μL of the analyte solution was added to the precipitate, and the mixture incubated. After 15 min, 5 μL aliquots were removed by pipette and deposited on a glass slide covered with an Al foil. Prior to sample deposition, the Al foil covered glass slide was first washed with MeOH and air dried. After deposition on the substrate, the sample locations were dried in a dricycler for 1–2 hours at room temperature. A minimum of three spectra were collected from different locations at each individual sample location.

Scanning Electron Microscopy (SEM) images were acquired with an FEI (Hillsboro, OR) Quanta FEG 650 Scanning Electron Microscope under high-vacuum. Prior to imaging, the samples were prepared with a Au-Pd sputter coating to promote surface conductivity and reduce charging artifacts. An accelerating voltage of 20 kV was used for most images.

Results and discussion

Preparation and characterization of Ag colloids

Several groups have used the Ag mirror reaction to attach Ag nanoparticles (AgNPs) to various solid substrates such as glass,[15, 35] filter paper,[36] silicon,[37] copper,[35] and polymer optical fibers.[38] In terms of SERS preparation methods, the Ag mirror reaction is advantageous due to its low cost, and is adaptable to the size and shape of the underlying substrates. Although previous publications described applications of the Ag mirror reaction, most of these studies have focused on the preparation of solid Ag nanostructured substrates and did not focus on the use of the Ag colloids for SERS applications. In this current work, we adapt this reaction to prepare AgNP colloids and show its applicability to SERS. The reaction we describe has the following advantages: 1) small volumes (1–10 mL) of Ag colloid can be freshly prepared, 2) the reaction is simple, and the entire nanofabrication process can be completed within 30 min, 3) inorganic or organic colloidal aggregating agents are not needed, and 4) the reaction can be carried out under well-controlled conditions, such as temperature, time and pH.

We employed a general procedure for preparation of AgNP colloids using the traditional Ag mirror reaction, as follows. Tollens' reagent was prepared in an ice bath at 4°C in a 1.5 mL Eppendorf tube. In this procedure, 500 μL of varying concentrations (10–50 mM) of AgNO3 was mixed with 10 μL of 1 M NaOH and 20 μL of concentrated NH4OH. When varying the concentration of AgNO3, the volume of NaOH and NH4OH was also adjusted to ensure that the brown Ag2O precipitate that formed on reaction of AgNO3 and NaOH was just dissolved. The sample was then placed in a 55°C water bath, followed by addition of the glucose solution. In this step, 500 μL of varying concentrations (0.1–0.5 M) of glucose was added to the solution to produce the Ag colloid. After 5 min reaction time, the Ag colloid that formed in the Eppendorf tube was centrifuged for 5 min at 10,000 μg at 4°C. To

optimize the reaction parameters, 100 μM aqueous adenine was used as a standard reporter molecule for SERS measurements.[39–41]

We optimized these general reaction conditions by studying the effect of the concentration of AgNO3, reaction time, concentration of reducing agent (glucose), and concentration of added base. We first studied the effect of AgNO3. Fig. 1 shows SEM images of the Ag colloid that resulted from sample preparation using different concentrations of AgNO3. It is evident from Fig. 1 (top row, images a-c) that low concentrations (1 mM) of AgNO3 produce a low number density, nonuniform distribution of AgNPs. On the other hand, high concentrations of AgNO3 (50 mM) yields larger numbers of nanoparticles, however with high levels of aggregation (Fig. 1, bottom row, images g-i). However, 20 mM AgNO3 gives good numbers of nanoparticles of an appropriate size with a reproducible morphology (Fig. 1, middle row, images d-f). Ag nanoparticle sizes were calculated from these SEM images, with an average value of 319.1 nm (s.d ±128.1), for the optimized AgNO3 concentration of 20 mM in Fig. 1, images d-f. We note that the sizes of the AgNPs produced by this Ag mirror reaction are larger than nanoparticles produced by other solution-based synthesis procedures, e.g., the Lee-Meisel or Creighton methods. However, those methods generally need to create colloidal aggregates of nanoparticles for maximum SERS enhancements. In our case, it is not necessary to add an exogenous aggregating agent to create colloidal aggregates for SERS measurements. For reproducible SERS results, it is necessary only to co-deposit the AgNP colloids with the analytes on a planar substrate, as described below.

We obtained the SERS spectra of adenine molecules adsorbed onto the Ag colloids prepared using varying concentrations of AgNO3 in the Ag mirror reaction. These reaction conditions corresponded to the images seen in Fig. 1. The SERS spectra of adenine were obtained by dispersing 20 μL of 100 μM adenine onto the AgNP colloid precipitate, followed by a 15-min incubation time. Next, 5 μL of the adenine-AgNP colloid mixture was drop coated on an Al foil covered glass slide for SERS measurements. Fig. 2A shows that the resulting SERS spectra of 100 μM adenine on the AgNP colloids, plotted as a function of the concentration of the AgNO3 added to the reaction between 10–50 mM. The spectra in Fig. 2A exhibit the characteristic adenine peak at 735 cm−1, as previously described.[42, 43] The three most intense SERS peaks in Fig. 2A are: 735 cm−1, attributed to the adenine ring breathing mode; 1330 cm−1, attributed to δ(C2–N3, N1–C2, C5–C6, C5–N7); and 1459 cm−1, attributed to δ(C4–N9, C4–C5, C6–N10, N7–C8, δ(C2–H).[44, 45]

A plot of the intensity of the adenine 735 cm−1 peak as a function of the concentration of added AgNO3 in the Ag mirror reaction is presented in Fig. 2B. The SERS results in Fig. 2B are consistent with the SEM images in Fig. 1, i.e. lower and higher AgNO3 concentrations result in AgNPs that are either few in number, or produce larger numbers of aggregates. In the SERS results, 10 mM AgNO3 produces the lowest measured intensity for adenine, while 50 mM produces enhanced, but not maximal intensity. In contrast, 20 mM AgNO3 produces a suitable SERS substrate in both AgNP numbers and morphology, as well as the maximum SERS response. At 20 mM AgNO3, large (~300 nm) AgNP colloids can be prepared using the Ag mirror reaction for SERS applications. An additional benefit of this method is that it does not need external aggregating agents to obtain AgNPs that provided enhanced SERS signals.

Optimization of reaction parameters for the preparation of Ag colloids

As seen in both Figs. 1 and 2, when the concentration of AgNO3 was less than, or greater than, 20 mM, the SERS response of the resultant AgNPs was not optimized. Therefore, 20 mM AgNO3 was selected as the most favorable AgNO3 concentration for SERS enhancement with the Ag mirror reaction. We chose the Ag mirror reaction as a nanofabrication process because it is suitable for preparation of small amounts of Ag colloid (1–5 mL) in a rapid manner. We investigated the various reaction parameters comprising the Ag mirror reaction to optimize the conditions that resulted in the best AgNPs for SERS response.

First, we investigated the SERS response of the AgNPs produced by the Ag mirror reaction as a function of the reaction time after glucose is added to the Eppendorf tube. Fig. 3 illustrates the time-dependence SERS intensity of the adenine 735 cm−1 band as an indicator of SERS response. In these experiments, the concentration of AgNO3 was fixed at either 20, 30, and 50 mM, the concentration of glucose was 0.5 M, and the reaction time varied between 5–25 min. As the reaction time increased, we empirically observed that a Ag mirror coated the inner surface of the Eppendorf tube, resulting in a decreased amount of Ag nanoparticles in solution, and a decreased SERS intensity. Regardless of the concentration of AgNO3, a 5-min reaction time was optimal to produce soluble Ag colloids, and reduce the formation of a Ag mirror film on the reaction tube walls. Based on these results, we fixed 20 mM AgNO3 and a 5-min reaction time as optimal experimental conditions.

Next, we investigated the concentration of the reducing agent (glucose) that resulted in the optimal formation of colloidal Ag nanoparticles. In these experiments, we held AgNO3 at 20 mM and the reaction time at 5 min, as described above. Fig. 4 shows the results of varying the concentration of glucose added to the reaction tube at 55°C. There was only minor variation of SERS response (measured using the A 735 cm−1 band) as the concentration of glucose varied from 0.1 to 0.5 M. The glucose concentration was held constant at 0.5 M for all subsequent experiments.

Finally, the volumes of NaOH and NH4OH were varied to examine their effect on the measured SERS response. The function of these aqueous ions in the Ag mirror reaction are: 1) NaOH converts the aqueous AgNO3 complex into a brown Ag2O precipitate, and 2) NH4OH dissolves the Ag2O precipitate and forms the aqueous diamine Ag complex Ag(NH3)2OH, which is then reduced by glucose in the final reaction step. To produce the classic Tollens' reagent, 2 equivalents of NH4OH are used for each equivalent of NaOH. In these experiments, we used 20 mM AgNO3, 0.5 M glucose, and 5 min reaction time. We varied the amount of NaOH used in the reaction, while keeping the volume ratio of NaOH:NH4OH at 1:2. As seen in Fig. 5, 50 μL of 1M NaOH and 100 μL of conc. NH4OH produced an increased SERS response, based on the adenine 735 cm−1 reporter band. To evaluate the within-batch reproducibility of the Ag colloid preparation, 100 μM adenine was applied to the freshly prepared Ag colloid, and multiple (9) spots applied to glass slides at separate locations. Multiple spectra were obtained at each location. The peak intensities of the 735 cm−1 band showed a within-batch variability of 9.9% (relative standard deviation).

The between-batch variability of the SERS response was determined by holding all the optimized experimental reaction parameters constant, and then repeating the AgNP substrate preparation protocols three times. As above, 100 μM adenine was applied to each AgNP preparation, and multiple (9) spots applied to glass slides at separate locations. Multiple spectra were obtained at each location. This resulted in a between-batch variability of 10.6% (relative standard deviation).

In summary, the optimal reaction conditions for preparation of Ag colloids using the Ag mirror reaction were: 500 μL of a 20 mM AgNO3 solution in an ice bath at 4°C, to which was added 50 μL of 1 M NaOH. Concentrated NH4OH (100 μL) was then added to the aqueous Ag solution to dissolve the resulting precipitate. Glucose (500 μL at 0.5M) was added into the solution and kept for 3 min at room temperature. Finally, this reaction mixture was placed in a 55°C water bath for 5 min to complete the Ag mirror reaction. These optimized reaction conditions were used to prepare Ag colloids for further SERS investigations.

SERS detection of mononucleotides and Mycoplasma pneumoniae

To assess the potential of these freshly prepared Ag colloids as SERS substrates for detection of biomolecules, we have used the mononucleotides adenosine 5′-monophosphate (AMP), guanosine 5′-monophosphate (GMP), cytidine 5′-monophosphate (CMP) and uridine 5′-monophosphate (UMP) as model systems. Several researchers have previously used Raman and SERS to examine nucleobases and nucleotides.[38, 46, 47] Purine and pyrimidine bases can be easily detected by SERS due to their strong interaction with Ag or Au nanoparticles. However, it is sometimes difficult to obtain intense SERS signals from nucleotides due to their orientation on the metal nanostructures. Fig. 6 shows the SERS spectra of four different nucleotides AMP, CMP, GMP, and UMP obtained using the Ag colloid. SERS spectra of nucleotides can differ based on various factors, including substrate nanomorphology, pH, ionic strength, and concentration. Nevertheless, Fig. 6 shows that we can successfully detect the four mononucleotide bases with the colloids prepared using the Ag mirror reaction. It is worth noting that the spectra of AMP, in particular, in Fig. 6 is very similar to that of the SERS spectra of 2′-deoxyadenosine 5′- monophosphate (dAMP) previously published using citrate-reduced Ag colloids that employed MgSO4 as an aggregating agent.[48]

We have further examined the concentration dependence of the SERS response for one of the nucleotides, AMP. Fig. 7A shows the Ag colloid SERS spectra of AMP over the concentration range 1–100 μM. The spectra have two dominant features, one at 735 cm−1 and one at 1333 cm−1, which are assigned to the ring breathing mode, and the ring stretching mode in adenine, respectively.[49, 50] The spectral features of the AMP spectra are in agreement with previous SERS studies of AMP.[49–51] Fig. 7A shows that in the spectra of AMP, the spectral features of 1 μM AMP can be clearly detected with 785 nm excitation. Fig. 7B shows the linear regression curve for AMP obtained using the intensity of the ring stretching mode at 1333 cm−1 band as a function of concentration. Using the standard LOD criterion of 3 times the noise level in the blank spectra, the estimated

detection limit for AMP calculated from the data in Fig. 7B is 500 nM. This application attests that these AgNP colloids can be used for nucleotide detection with good sensitivity.

In addition to nucleotide detection, the as-prepared Ag colloid was used to detect Mycoplasma pneumoniae. Mycoplasma pneumoniae is a cell wall-less prokaryote which causes respiratory disease in children and young adults. Clinical mycoplasma diagnosis is currently based primarily on serology or nucleic acid amplification.[52, 53] However, serologic detection requires time following infection for an antibody response, and PCR-based based methods are plagued by issues of reliability, standardization, and cost. Therefore, a new, sensitive diagnostic test for mycoplasma is needed.

SERS methods have previously been used to detect Mycoplasma pneumomiae.[54, 55] These studies used nanofabrication methods based on oblique angle vapor deposition to produce Ag nanorod arrays that were employed as the SERS-active substrate. It was shown that multivariate statistical analysis of mycoplasma SERS spectra could reproducibly differentiate multiple mycoplasma strains in culture, as well as in true clinical specimens. Although previous work showed that SERS was a viable platform for rapid and sensitive detection of mycoplasmas, one drawback to the use of Ag nanorod arrays as the SERS-active substrate was that they were timeconsuming and costly to produce. In this current work, we used the colloids obtained from the Ag mirror reaction as the SERS substrate. Freshly prepared Ag colloid was mixed with 200 μL of Mycoplasma pneumoniae, strain M129, obtained from laboratory culture, and centrifuged for 10 min at 10,000 mg. The Ag colloid-mycoplasma mixture was diluted with 20 μL of water; 5 μL of the Ag colloid-mycoplasma samples were drop coated on an Al foil covered glass slide, and the SERS spectra collected.

Fig. 8 shows the SERS spectra of Mycoplasma pneumoniae, strain MP129, obtained from this simplified Ag colloid sample preparation method. Two different sample amounts were applied to the Ag colloids, 200 μL and 50 μL. The most characteristic bands observed in Fig. 8 are at 1590, 1560, 1394, 1237, 1130, 1005, 853, and 711 cm−1. These bands have previously been observed in mycoplasma SERS spectra, and have been assigned to C=C (1590 cm−1); amide III (1241 cm−1); aromatic breathing mode (1005 cm−1), and carbohydrate (711 cm−1).[54, 56, 57] The spectra in Fig. 8 illustrate that a simple sample preparation procedure (i.e, co-mixing of bacteria with Ag colloids, followed by centrifugation) results in strong and characteristic SERS spectra of Mycoplasma pneumoniae.

Conclusions

We have successfully prepared AgNP colloids for SERS applications using the classical Ag mirror reaction. The preparation and detection method described here is simple, efficient, and easy to use. It does not utilize aggregating agents or surface modifiers that introduce background interferences. The entire reaction can be carried out in a 1.5 mL Eppendorf tube in 30 min. The resulting AgNP colloids were characterized by SEM images and SERS measurements. Based on SEM images, it was found that nanoparticles with an average size of 319.1 nm (s.d ±128.1), produced optimum SERS response. The SERS intensity of 100

μM adenine was used to identify the optimum reaction conditions. These were found to be 20 mM AgNO3, 5 min reaction time, and 0.5 M glucose (Figs. 2–4).

The applicability of these AgNP colloids for biomolecule detection was evaluated using aqueous solution-phase mononucleotides as well as the bacterium Mycoplasma pneumoniae. Spectra were obtained of the four nucleotide bases AMP, CMP, GMP, and UMP (Fig. 6) that agreed with previous SERS spectra of these mononucleotides. A plot of the linear dependence of the SERS intensity of AMP with concentration resulted in a limit of detection of 500 nM for AMP (Fig. 7). The SERS spectrum of the bacterium Mycoplasma pneumoniae (Fig. 8) was obtained in a simplified manner by first mixing the bacteria with the Ag colloid mixture in solution, followed by centrifugation. The observed Raman bands in the Ag colloid SERS spectra of Mycoplasma pneumoniae agree with previous SERS studies of mycoplasmas obtained using Ag nanorod substrates. Overall, the process described here for Ag colloid preparation appears to be an easily implemented method for detection of both small molecules and microorganisms using SERS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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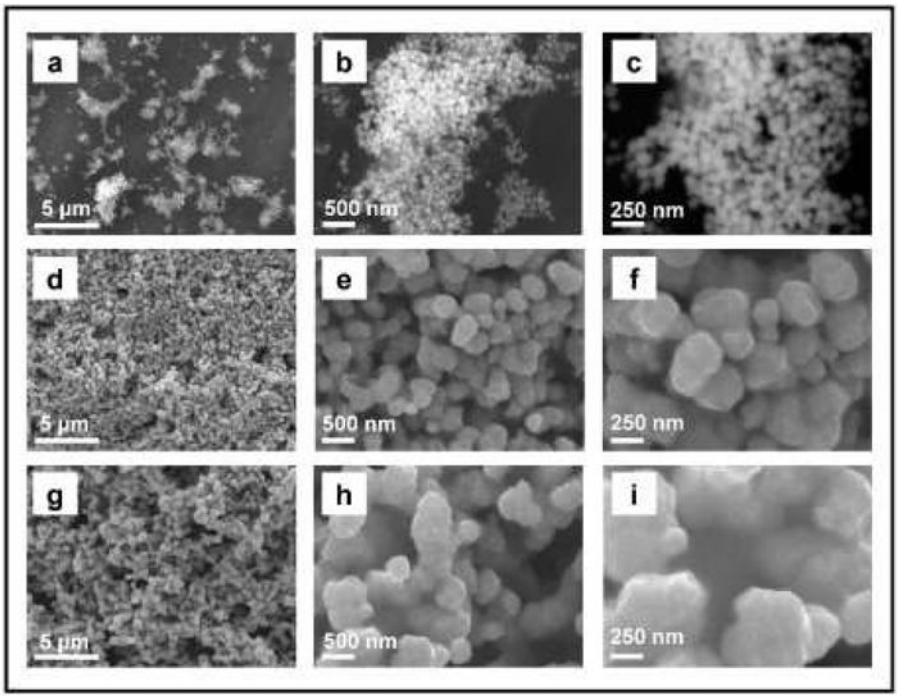


Figure 1.

SEM images of AgNP colloids at different magnifications as follows: scale bar of left column (a-g) 5 µm, middle column (b-h) 500 nm and right column (c-i) 250 nm. AgNP colloid was prepared with varied AgNO3 concentration as follows: top row (a-c) 1 mM AgNO3, middle row (d-f) 20 mM AgNO3, and bottom row (g-I) 50 mM AgNO3.

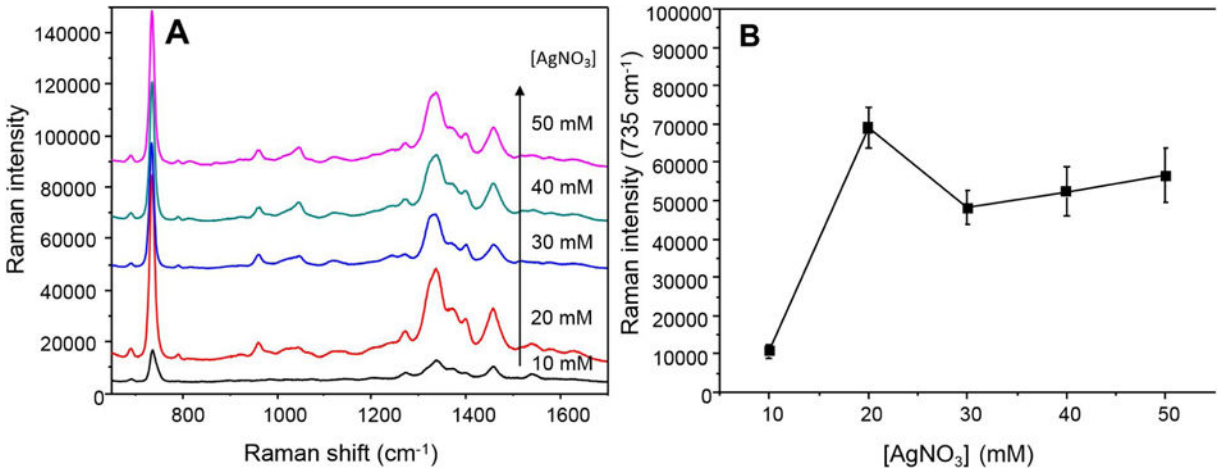


Figure 2.

(A) SERS spectra of 100 μM adenine adsorbed on AgNP colloids prepared using increasing concentrations of AgNO3 between 10 mM–50 mM.

(B) SERS intensity of 735 cm−1 band of 100 μM adenine adsorbed on Ag colloids prepared using increasing concentrations of AgNO3.

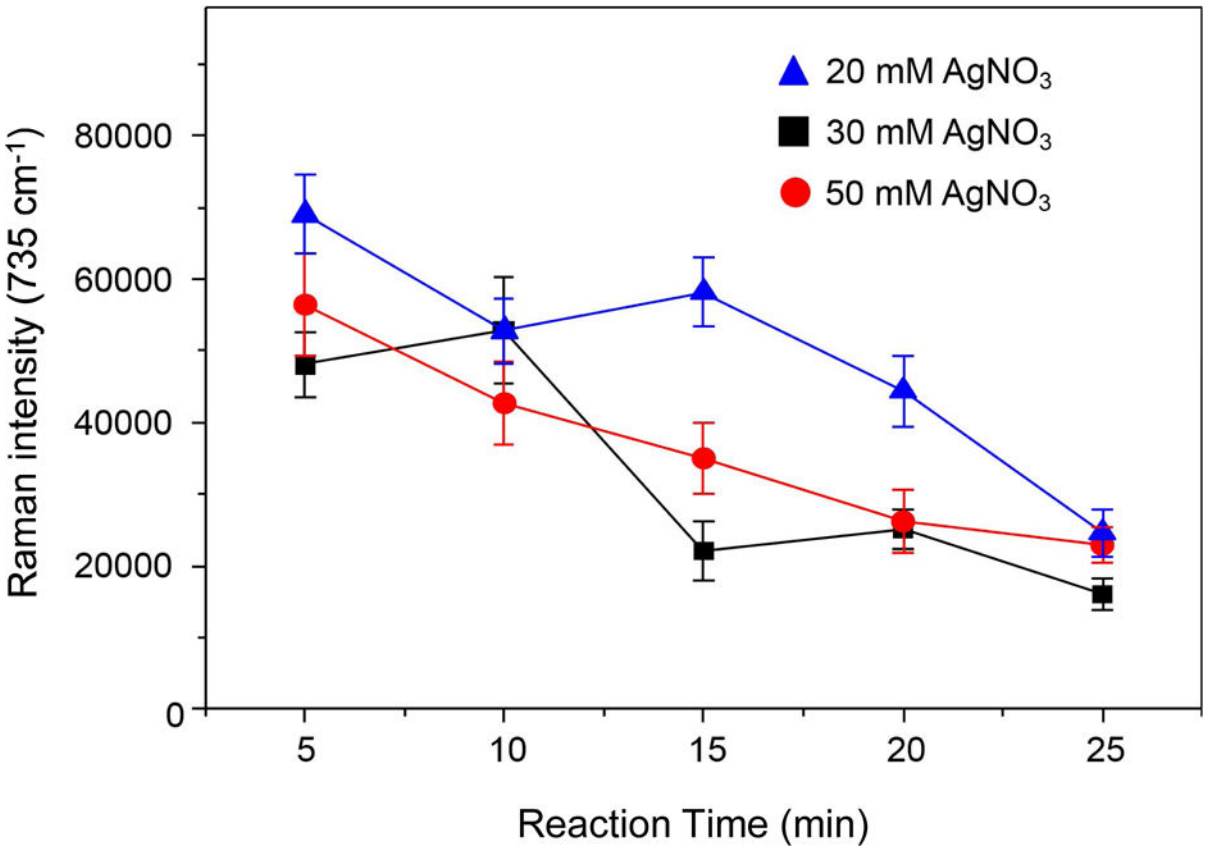


Figure 3.

SERS intensity of 100 μM adenine at 735 cm−1 for increasing Ag mirror reaction times using 20 (▲), 30 (■), and 50 (●) mM AgNO3 concentration.

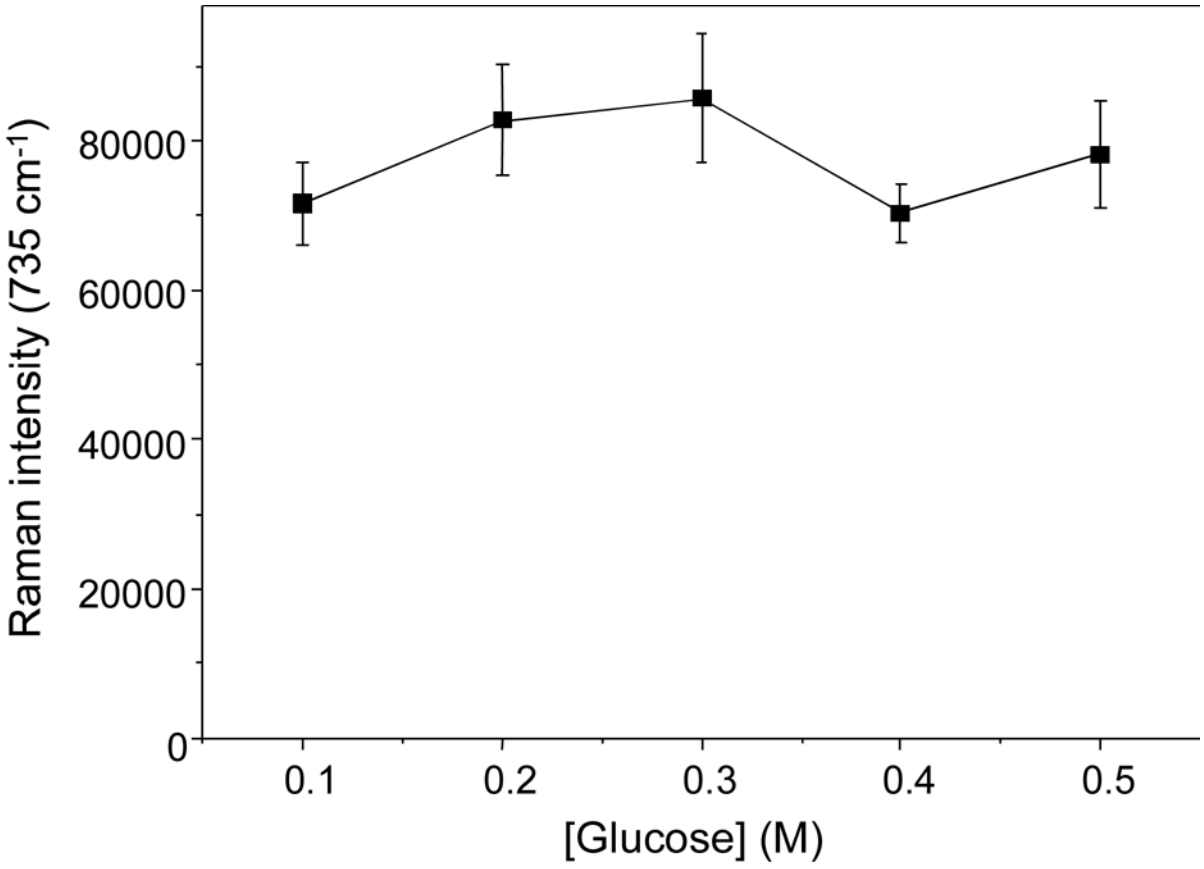


Figure 4.

SERS intensity of 100 μM adenine at 735 cm−1 with increasing concentrations of glucose. In these reactions, the Ag mirror reaction time was held constant at 5 min.

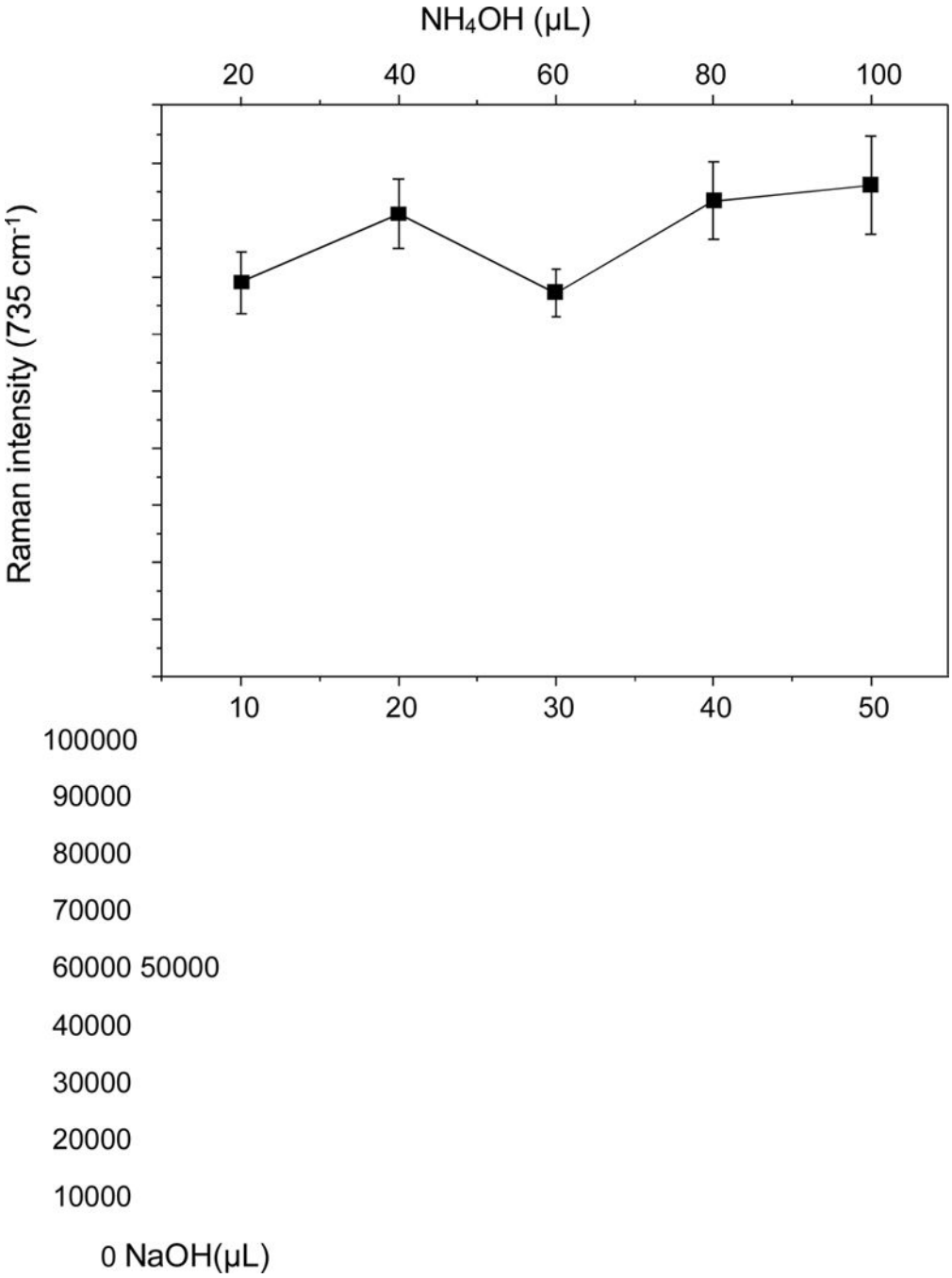


Figure 5.
SERS intensity of 100 μM adenine at 735 cm−1 adsorbed on AgNP colloid prepared using different volumes of NaOH and NH4OH

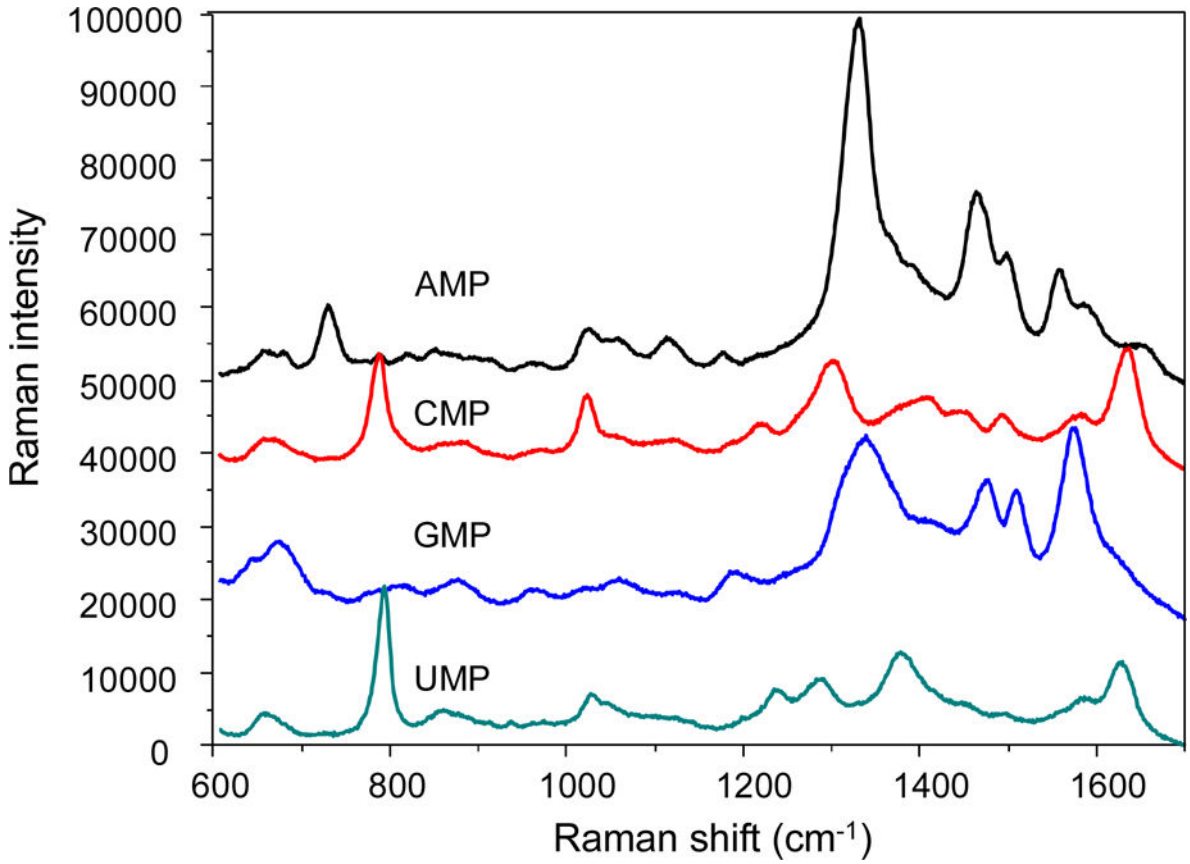


Figure 6.

SERS spectra of the four nucleotides AMP, CMP, GMP, and UMP adsorbed onto the Ag colloid. The concentration of each of the nucleotides applied to the AgNP colloid was 100 μM.

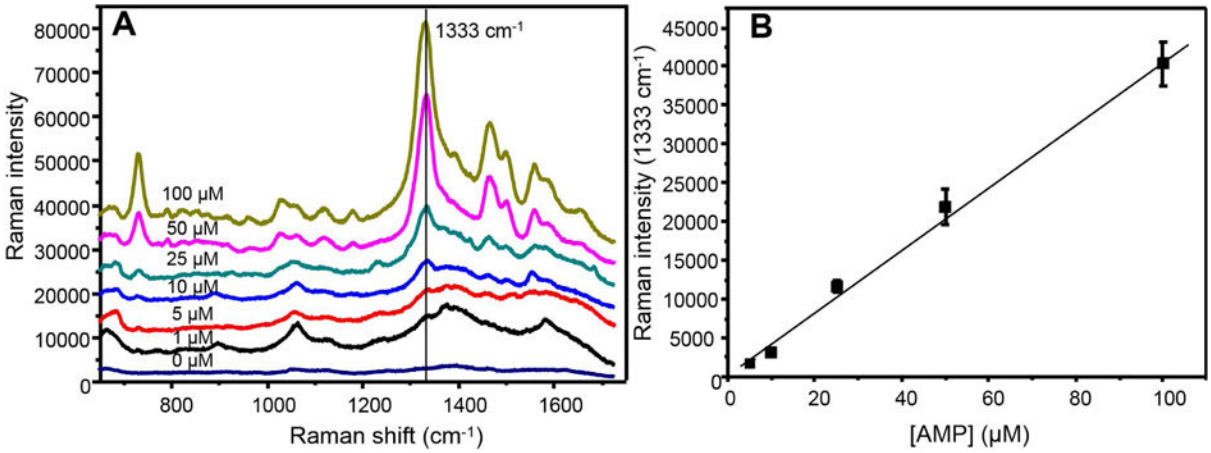


Figure 7.

(A) SERS spectra of AMP adsorbed on the AgNP colloid plotted at different concentrations of AMP.

(B) Intensity of AMP band at 1333 cm-1 plotted as a function of concentration of AMP applied to the AgNP colloid.

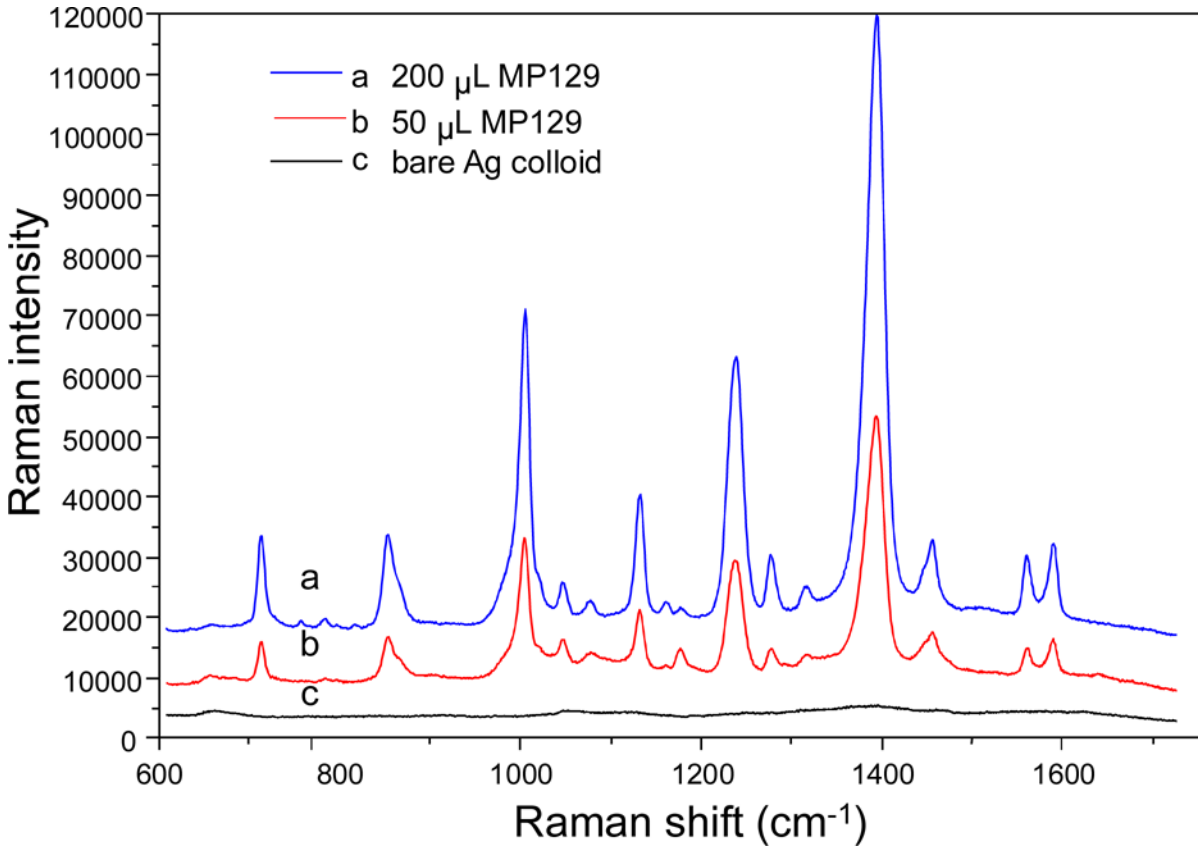
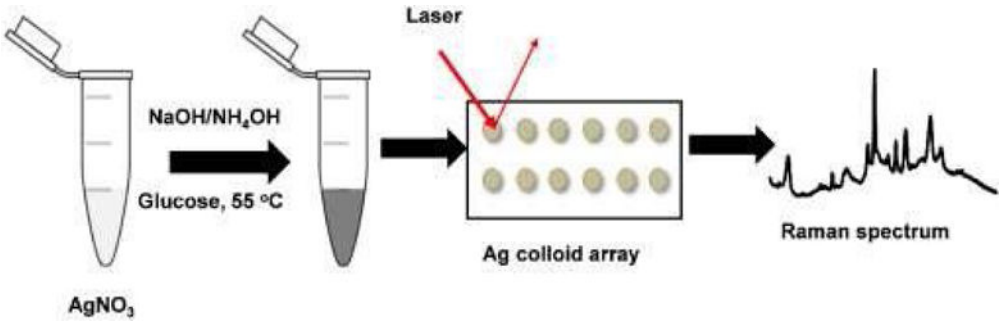


Figure 8.
SERS spectra of (a) bare AgNP colloid and (b and c) Mycoplasma pneumoniae strain MP129 samples adsorbed onto AgNP colloid.



Scheme 1.

Schematic diagram of the preparation of colloids produced by the Ag mirror reaction for SERS analysis.