

Development of SERS Spectroscopy for Routine and Rapid Identification of *Escherichia Coli* and *Listeria Monocytogenes* on Silver Colloidal Nanoparticles¹

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ABSTRACT

SERS spectra were collected to explore its potential for rapid and routine identification of *E. coli* and *L. monocytogenes* cultures. Ratios of SERS peaks from K₃PO₄ were used to evaluate the reproducibility, stability, and binding effectiveness of citrate-reduced silver colloids over batch and storage process. Notably, lifetime of silver colloidal nanoparticles was much longer than that of silver nanoparticle island films. Mixtures of different batches of bacterial cultures with colloids revealed that, despite of large variations in relative intensities and positions of SERS bands, intense and unique bands at 712 and 390 cm⁻¹ were consistently observed in *E. coli* and *L. monocytogenes* cultures, respectively. Two specific bands were then used to develop simple algorithms for identification, with a 100% success. As scanning one spectrum took 5~6 min and a minimum of 50 μL colloid/culture mixture was used, silver colloidal nanoparticle based SERS could be used in routine and rapid screening of bacteria in a large scale.

Keywords: SERS, colloid nanoparticles, algorithm, bacteria identification, food safety

1 INTRODUCTION

Rapid, accurate, and preferably routine methods for the identification of foodborne bacteria are increasingly important, because of bio- / agro-terrorism threats, public health concerns, and economic loss. Conventional detection of bacteria is time-consuming, requiring 5-7 days, and is not sufficiently rapid to assure the safety of ready-to-eat food products [1]. Antibody- / nucleic acid-based systems and amplification-based methods have been developed as viable tools to detect bacteria [1,2]. However, these immunoassays have encountered some problems, such as false negative/false positive identification and slow speeds (>50 min). Fast microbial detection requires minimal sample preparation, permits the routine analysis of large number of samples with negligible reagent costs, and is easy to operate. Surface-enhance Raman scattering (SERS) spectroscopy is an alternative approach, not only it has been used to obtain highly structural information, but also it is lack of interference from water.

To better utilize SERS enhancement, different types of gold and silver substrates have been developed, in the forms of metal colloids, roughened electrodes, and vacuum deposited metal/nanoparticle island films [3-6]. Among them, silver colloids have been extensively used, because of following advantages: (1) silver colloids can be easily and inexpensively prepared; (2) silver nanoparticles are suspended in solutions and can slow their oxidation process; (3) silver colloids provide homogeneous binding sites; and (4) sampling method of silver colloids directly mixed with incubated cultures can reduce the possibility of bacterial cross-contamination.

Like other substrates, there are still major problems associated with the reproducibility, stability, and bending effectiveness of silver colloids, as silver nanoparticles might vary from batch to batch in particle size, particle shape, particle size distribution, and aggregation, and tend to aggregate and to precipitate in solutions over time. Such drawbacks seem to make SERS technique unsuitable for qualitative and quantitative applications.

SERS studies of bacteria adsorbed on fresh silver colloids have been reported, revealing its potential in fingerprinting characterization of bacterial structure [5,6]. Although different bacteria could be clearly distinguished with multivariate statistical technique, the models were developed from only one of three colloidal batches [5]. Probably, it indicates the challenge of applying multivariate data analysis to SERS spectra.

Objectives of this work were: (1) to assess the binding effectiveness of citrate-reduced silver colloids over batches and their stability over storage period by using tripotassium phosphate (K₃PO₄) as the analyte, (2) to explore characteristic SERS bands of *E. coli* and *L. monocytogenes* culture, and (3) to develop simple and universal algorithms for their identification. The ultimate goal is to develop SERS technique for rapid and routine detection of *E. coli* and *L. monocytogenes* for public safety and security.

2 EXPERIMENTAL

2.1 Chemical Reagents and Glass Tubes

Chemical reagents (silver nitrate, >99%; trisodium citrate, > 99%; tripotassium phosphate, > 98%) and Tryptic Soy Broth (TSB) were purchased from Sigma-Aldrich (St.

Louis, MO, USA) and Becton, Dickinson and Company (Sparks, MD, USA), respectively, and used without further purification. Disposable glass tubes (6 mm D. x 50 mm L.) were supplied by Fisher Scientific (Suwanee, GA, USA).

2.2 Silver Colloidal Preparation

During six-month period, 8 batches of citrate-reduced silver colloidal suspensions were prepared by the Lee and Meisel procedure [7]. Briefly, a clean 250 mL beaker containing 100 mL of distilled water and the magnetic stirring bar was heated to approximately 45 °C, at which point 18 mg of silver nitrate was added. With further heating and stirring, the solution was brought to boiling point and 2 mL of 1% (w/v) trisodium citrate was introduced. The solutions were boiled for ~15 min with continuous stirring, and formation of silver colloidal nanoparticles was noticed by the gradual color change from a colorless to a gray/green solution. The beaker was taken off the heat, cooled to and kept at room temperature.

2.3 Bacterial Culture Preparation

Over 14 batches of *Escherichia coli* (*E. coli* ATCC 25922) and *Listeria monocytogenes* strains (*L. monocytogenes* ATCC 13932) were incubated in TSB growth media at 37 °C and 35 °C, respectively, for overnight. This growth procedure routinely yielded a culture containing ~10⁹ colony forming units (CFU)/ml of respective bacterium at stationary phase.

2.4 SERS Spectral Collection

SERS spectra were collected on a FT-Raman module for Nicolet 670 FT-IR bench (Madison, WI, USA) using an DTGS KBr detector and XT-KBr beamsplitter. Glass tube containing the analyte / colloid mixture was illuminated using the 1064-nm Nd:YAG excitation laser. Raman scatter was accumulated using 180° reflective mode with 1 W of laser power and 256 scans at 8 cm⁻¹ resolution. All spectra were smoothed with Savitzky-Golay function of 2 polynomial and 11 points by the use of Grams/32 (Version 7.0, Galactic Industries Corp., Salem, NH, USA).

For one bacteria or K₃PO₄ solution against one batch colloidal suspension, two measurements were taken. Immediately after mixing two solutions at a volume ratio of 1:1, glass tube was harshly shaken 5 times and then kept untouched for 10 min before the subsequent SERS measurements.

3 RESULTS AND DISCUSSION

3.1 Reproducibility, Stability, and Binding Effectiveness of Colloidal Nanoparticles over Batch and Storage

In addition to measure the UV-visible spectra of silver colloids in the pure state for the examination of reproducibility and stability, probe compounds such as dyes have been introduced into colloidal solutions to assess their binding effectiveness [8]. In this study, K₃PO₄ was selected as an analyte because it is much smaller than the silver colloidal nanoparticles, has simple structure and characteristic P-O vibrations.

Figure 1 (a) and (b) show SERS spectra of fresh silver colloidal suspension and an aqueous K₃PO₄ in the region of 1700-100 cm⁻¹. It can be seen that, besides the 215 cm⁻¹ band, there are no other significant SERS peaks arising from decomposed chemical residuals and water in silver colloids. Meanwhile, K₃PO₄ solution exhibits three intense SERS-active P-O bands at 1086, 922, and 564 cm⁻¹. As a comparison, FT-Raman spectrum of K₃PO₄ in solid state is presented in Fig. 1 (c) and it is dominated by a very strong band near 922 cm⁻¹, which arises from the symmetric P-O stretching mode (ν_1). Therefore, intense and separated 922 cm⁻¹ SERS peak could be assigned to ν_1 P-O mode and further used to assess the binding effectiveness of silver colloidal nanoparticles.

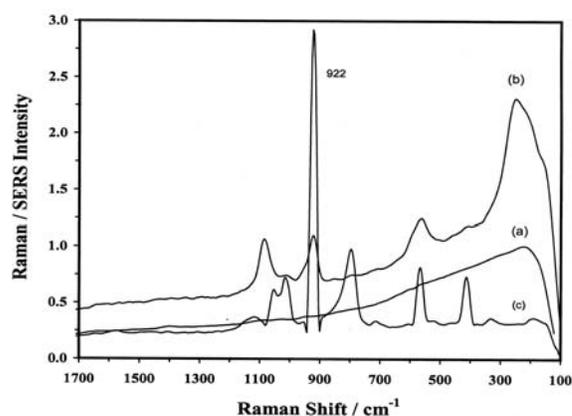


Figure 1: (a) SERS spectrum of fresh silver nanoparticle colloid; (b) SERS spectrum of K₃PO₄ solution at final concentration of 5.0 x 10⁻³ M; and (C) FT-Raman spectrum of K₃PO₄ in solid state.

Figure 2 depicts the storage time-dependent binding effectiveness of silver colloidal nanoparticles with K₃PO₄ solutions, by using the ratio of SERS intensity at 922 cm⁻¹ against one at 850 cm⁻¹, at which wavenumber no SERS peak was observed. The value of I_{922}/I_{850} tends to decrease slightly after 60-day storage, suggesting that the binding capability of silver colloidal nanoparticles with K₃PO₄ is obviously unaffected during this period. Even at 90th day storage, binding effectiveness is reduced by only 12%. Compared to 1-2 week lifetime of silver nanoparticle island films via thermal vacuum deposition [4], lifetime of silver colloidal nanoparticles is much longer.

Meanwhile, I_{922}/I_{850} values both from eight fresh batches (Day 1) and from seven replicate measurements of one fresh batch were inserted at the most left in Fig. 2. Apparently,

variations of binding effectiveness of silver colloids over batches even within one batch are greater than those of colloids over the storage, with relative standard deviation (RSD) of 8.7% and 3.5% for the batch process and for the storage process, respectively. This result is very close to the best reported RSD of 6.6% between the citrate-stabilized borohydride-reduced silver colloids prepared using the flow system [8], indicating that using batch process can fabricate reproducible citrate-reduced silver colloidal nanoparticles.

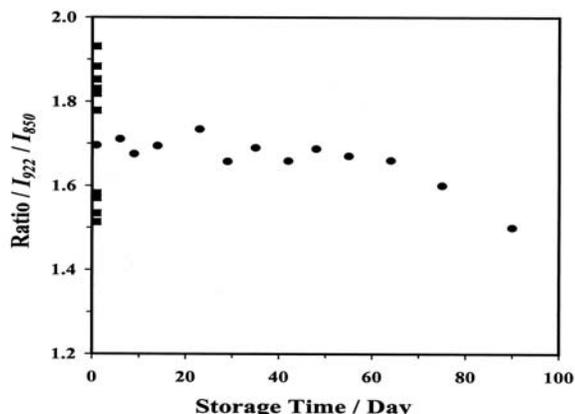


Figure 2: Storage time-dependent binding effectiveness of silver colloidal nanoparticles with K_3PO_4 solutions (final concentration: 5.0×10^{-3} M) by using the I_{922}/I_{850} values (●). I_{922}/I_{850} ratios (■) from both fresh colloidal batches and repeated measurements of one fresh colloid were inserted at the most left for comparison.

3.2 SERS Characteristic of *E. Coli* and *L. Monocytogenes* Cultures

Figure 3 shows typical SERS spectra from mixtures of different batches of *E. coli* cultures and silver colloidal suspensions, in which (a) and (b) compares the spectra of same batch of *E. coli* in two batches of colloids while (c) and (d) presents those of two batches of *E. coli* in one batch of silver colloid. Expectedly, there are large variations in both relative intensity and position of SERS-active bands from one *E. coli* solution to another, because of subtle changes among silver colloidal batches and unpredicted metabolic process between bacterial batches. Since *E. coli* cultures consist of numerous species, which pre-existed in growth media and were produced as the byproducts during the bacterial growth, clear understanding of the origins of SERS-active bands is difficult. Despite of fluctuations in relative intensity and position of SERS bands over both *E. coli* and silver colloidal batches, these SERS spectra do show several common bands. Among them, the 712 cm^{-1} band is quite interesting, because it is always appearing and is the strongest and sharpest.

Representative SERS spectra of three *L. monocytogenes* batches in different silver colloids are shown in Fig. 4, in which (a) and (b) compares the spectra of one *L. monocytogenes* culture in two colloidal batches whereas (c)

and (d) compares those of two *L. monocytogenes* batches in one colloidal suspension. Like the observation in Fig. 3, there are large differences in both relative intensity and position of SERS bands within *L. monocytogenes* cultures. Close examination of SERS spectra yields a 390 cm^{-1} band that is common, the strongest, and the best separated, indicating that this 390 cm^{-1} band might be useful for the identification of *L. monocytogenes* suspensions.

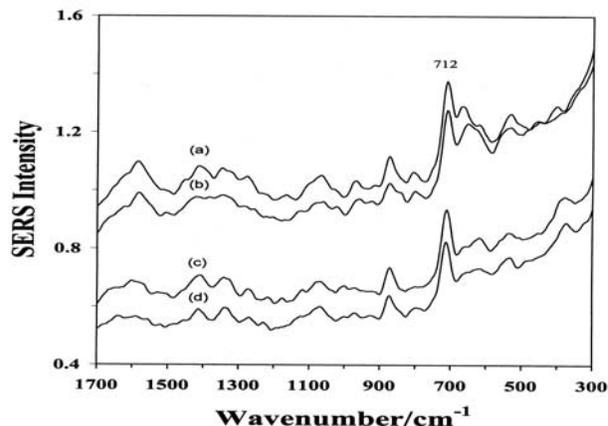


Figure 3: Representative SERS spectra of *E. coli* cultures, (a) and (b) represents the same batch of *E. coli* in two batches of silver colloids while (c) and (d) compares two batches of *E. coli* in one batch of silver colloid.

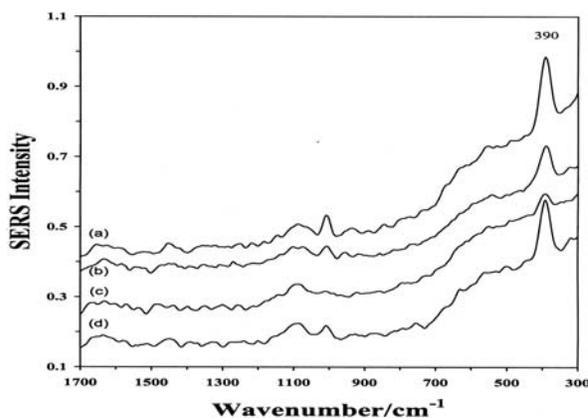


Figure 4: Representative SERS spectra of *L. monocytogenes* cultures, (a) and (b) represents the same batch of *L. monocytogenes* in two batches of silver colloids while (c) and (d) compares two batches of *L. monocytogenes* in one batch of silver colloid.

Most striking differences of SERS spectra between *E. coli* and *L. monocytogenes* cultures are the appearance of prominent SERS peaks in the *E. coli*. Such distinctions could reflect the genetic, structural or metabolic differences of two very distinctive microorganisms, *E. coli* is in *Enterobacteriaceae* family and *L. monocytogenes* in *Listeriaceae* family. Therefore, intense and well-isolated 712 and 390 cm^{-1} SERS peaks could be used to identify *E. coli* suspensions from *L. monocytogenes* ones.

3.3 Identification of *E. Coli* and *L. Monocytogenes* from Simple Algorithms

It is of interest to examine whether *E. coli* and *L. monocytogenes* cultures could be identified from the perspective unique bands. As discussed above and reported by others [5], SERS bands vary greatly even for one analyte in different silver colloidal batches. Therefore, in this study, one bacterial culture was mixed with at least two batches of silver colloidal suspensions that have stored at different periods. Figure 5 shows the ratio of I_{712} / I_{730} (horizon) against one of I_{390} / I_{352} for 18 batches of *E. coli* cultures, 14 batches of *L. monocytogenes* cultures, and silver colloidal suspensions.

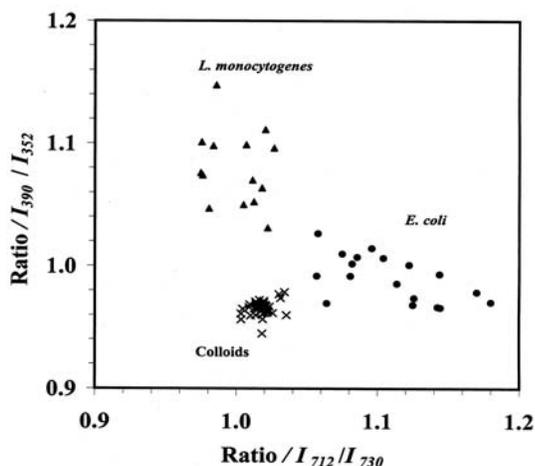


Figure 5: Plot of the ratio of I_{712} / I_{730} (horizon) versus one of I_{390} / I_{352} (vertical) for *E. coli* cultures (●), *L. monocytogenes* cultures (▲), and silver colloids (x).

Fig. 5 reveals an excellent separation and indicates that the *E. coli* and *L. monocytogenes* cultures can be correctly identified from each other, by the ratio of I_{712} / I_{730} greater than 1.1 and the ratio of I_{390} / I_{352} larger than 1.0, respectively. *E. coli* and *L. monocytogenes* cultures display more scatter distribution than silver colloids, probably due to a number of factors, such as the variations of chemical components and heterogeneities over batches of bacterial cultures and colloidal suspensions. Meanwhile, close disposition of silver colloids suggests the reproducibility, consistency, and stability over batch and storage.

Although multivariate data analysis of SERS spectra has been attempted to discriminate different bacteria, SERS data from only one batch of colloid were used to develop the models [5]. Clearly, it is impractical and might lose some information. Consequently, development of ratio algorithms utilizing the unique SERS bands is simple and can be universal applied for fast, accurate, and routine screening of *E. coli* and *L. monocytogenes* cultures on a variety of silver colloidal suspensions over batches and storages.

4 CONCLUSIONS

This study demonstrates the potential of SERS technique for rapid and routine identification of *E. coli* and *L. monocytogenes* cultures adsorbed on silver colloidal nanoparticles. To overcome poor reproducibility of SERS signal of biological analyte, spectra of mixing various batches of both cultures and colloids were examined. Particularly, characteristic bands at 712 and 390 cm^{-1} , consistently appeared and had the strongest intensity, were identified from *E. coli* and *L. monocytogenes* cultures, respectively. Two unique bands were then used to develop simple algorithms for the identification of *E. coli* and *L. monocytogenes* cultures with a 100% success. This is the first time to report the characteristic SERS bands of *E. coli* and *L. monocytogenes* cultures and to develop simple and universal algorithms for bacterial detection from the respective exclusive SERS.

To assess the reproducibility and stability of citrate-reduced silver colloids over batch / storage process and their binding effectiveness, ratio of SERS-active P-O band at 922 cm^{-1} in K_3PO_4 aqueous solutions were used. RSD of 8.7% from I_{922} / I_{850} ratios of K_3PO_4 solutions against different colloidal batches was very close to the best reported RSD of 6.6% using the flow system [8], suggesting that batch process can produce reproducible citrate-reduced silver colloidal nanoparticles.

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REFERENCES

- [1] T. Ryser and E. H. Marth, Ed., "Listeria, Listeriosis, and Food Safety", 2nd Edition, Marcel Dekker, New York, 225-261, 1999.
- [2] T. Geng, J. Uknalis, S.-I. Tu and A. K. Bhunia, *Sensors*, **6**, 796, 2006.
- [3] M. Moskovits, *Rev. Mod. Phys.* **57**, 783, 1985.
- [4] H. Li and B. M. Cullum, *Appl. Spectrosc.* **59**, 410, 2005.
- [5] R. M. Jarvis and R. Goodacre, *Anal. Chem.* **76**, 40, 2004.
- [6] M. Laucks, A. Sengupta, K. Junge, E. Davis and B. Swanson, *Appl. Spectrosc.* **59**, 1222, 2005.
- [7] P. C. Lee and D. Meisel, *J. Phys. Chem.*, **86**, 3391 1982.
- [8] R. Keir, D. Sadler, and W. E. Smith, *Appl. Spectrosc.* **56**, 551, 2002.

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