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Abstract. Surface-enhanced Raman spectroscopy (SERS) has emerged as one of the thrust research areas that could find potential applications in bio and chemical sensing. We developed colloidal SERS active substrate with excellent sensitivity and high reproducibility using silver nanocube (AgNC) synthesized via the solvothermal method. Finite-difference time-domain simulation was carried out in detail to visualize dipole generation in the nanocube during localized surface plasmon resonance and to locate the respective hot spots in AgNC responsible for the huge Raman enhancement. The prediction is verified by the SERS analysis of the synthesized nanocubes using Rhodamine 6G molecule. An excellent sensitivity with a detection limit of 10^{-17} M and a very high enhancement factor of 1.2×10^8 confirms the “hot spots” in the nanocube. SERS activity is also carried out for crystal violet and for food adulterant Sudan I molecule. Finally, label-free DNA detection is performed to demonstrate the versatility of SERS as a potential biosensor. © 2016 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: [10.1117/1.JNP.10.026020](https://doi.org/10.1117/1.JNP.10.026020)]

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1 Introduction

Recent advances in surface-enhanced Raman spectroscopy (SERS)^{1,2} offer exquisite sensitivity that makes SERS an attractive tool for ultrasensitive analysis.³ In SERS, localized surface plasmons in metal nanostructures⁴ enhance Raman scattering cross section of the molecule adsorbed on it. Researchers associated the SERS intensity to a combination of two effects—electromagnetic (EM) enhancement, where Raman signal intensity is boosted due to plasmon effects especially in anisotropic metal nanostructures with sharp edges, and chemical enhancement (CE), where the polarizability of adsorbed molecules is modified. Even though CE contributes to total SERS enhancement, EM enhancement succeeds as superior.

SERS is one of the few truly nanoscale effects⁵ and is strongly linked to unique properties of metal nanostructures.⁶ The dominant contribution to enhanced Raman signal of SERS up to 12

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orders of magnitude is accounted to the localized surface plasmon property of metallic nanostructures. These localized surface plasmons are coherent oscillations of metal electrons in resonance⁷ with incident light at certain frequency and which is in visible range for coinage metal nanoparticles such as Ag, Au, and Cu.⁸ The near-field enhancement occurs in the vicinity of metal nanoparticle during resonance, and its magnitude is extremely dependent on the geometry and size of the metal nanoparticles. The EM field in the gap region of a pair of strongly coupled nanoparticles and in sharp corners of nanocubes or triangles can be drastically amplified large enough for single-molecule detection.^{2,9} Although the “hot spot” phenomenon has been extensively investigated both from theoretical and experimental points of view, it remains a mysterious, feebly understood topic. Lack of large-scale SERS-active substrates with high density of hot spots yielding huge enhancement is the main obstruction to the development of SERS technique for practical applications. Substantive explorations have been made to achieve rough metal surface,¹⁰ metal nanocolloids,¹¹ and well-ordered patterned structures^{12–14} as SERS substrates that have been fabricated by various methods and techniques.¹⁵

Despite the progress, synthesis of uniform reproducible SERS-active substrates that can yield strong SERS signals in an easy way is still in demand. Albeit of its nonuniformity, colloidal-based SERS substrates uphold its position for easiness in preparation and for the analysis of biological samples in colloidal form itself. Even though silver nanocube (AgNC)-based SERS active substrates have been synthesized via solvothermal method earlier,^{16,17} we could achieve a detection limit of 10^{-17} M and an enhancement factor of 1.2×10^8 , which is three orders higher compared to the reported value¹⁷ of 2.96×10^5 . We herein report the way of dipole generation and electric field enhancement on AgNCs during surface plasmon resonance through finite-difference time-domain (FDTD) method simulation. Finally, label-free detection of DNA is carried out using the synthesized nanocube by SERS.

2 Materials and Methods

2.1 Experiment

Uniform-sized AgNCs were synthesized via the solvothermal method following previous reports.¹⁶ Briefly, 20 ml ethylene glycol (EG) solution of 20 mM NaCl was vigorously stirred for 1 h after addition of 0.8 g of polyvinylpyrrolidone. The mixed solution was added drop-wise into 20 ml of a magnetically stirred EG solution of AgNO₃ (0.15 M) for 1 h. Afterward, the solution was transferred into 100 ml teflon-lined autoclave and was kept at 160°C for 12 h in an electric oven. NaCl acts as nucleation seed for the growth of nanocubes. Finally, the products were centrifuged with acetone and deionized water at 8000 rpm for 30 min. The supernatants were removed and this process was repeated three times in order to improve the purity of the silver nanocolloids. The final samples were preserved in deionized water prior to microstructural and spectroscopic characterization.

The morphological studies of the synthesized nanocolloids were carried out using Zeiss Ultra 55 FESEM (field emission scanning electron microscope). The surface plasmon resonance study was carried out by analyzing the ultraviolet–visible (UV–Vis) absorption spectra of silver nanocolloids acquired with Jasco V 570 UV-Vis-NIR spectrophotometer. Raman measurements were performed on a Horiba Jobin Yvon LabRAM micro-Raman spectrometer with 514.5-nm laser excitation line. The probed area was 1 μ m in diameter and the incident power was 9 mW. All the spectra were obtained at room temperature with an integration time of 5 s. Rhodamine 6G (R6G) molecule was used as a reporter probe for SERS measurement. AgNC-based SERS substrate was fabricated by dropping the solution onto a cleaned silicon substrate and then allowing to dry naturally. Loading probe molecules onto AgNCs was accomplished by dropping 30 μ l of R6G solutions onto the as-prepared substrate and dried naturally. DNA of *Escherichia coli* with a concentration 400 ng/ml and as that of salmon fish with a concentration of 0.1 ng/ml were used for the analysis. DNA uncoiling was achieved in solution, prior to SERS analysis, by heating the DNA solutions in TE buffer [10 $\times$ Tris ethylenediaminetetraacetic acid (EDTA) (TE), 100 mM Tris–Cl (pH 8.0), 10 mM EDTA (pH 8.0), and sterilizing solutions by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid] to 86°C for 10 min. This step was followed

by rapid cooling in an ice bath. The prepared SERS substrates were then immersed in DNA solution for 12 h. Finally, the substrates were taken out and washed carefully with deionized water, followed by drying in N_2 flow.

2.2 Theoretical Modeling

FDTD method is one of the analytical methods for solving Maxwell's equations.^{18,19} The two-dimensional (2-D) FDTD method (Lumerical Solutions Ltd.) was carried out to calculate the electric field distribution in nanocube and three-dimensional (3-D) set up was used for calculating scattering cross section of AgNC. Perfectly matched layers are employed as boundaries along x and y directions. The computational domain consists of a square of grid points with a resolution of $x = y = 0.5$ nm. Grid size is set to be 3 nm for 3-D simulation for capturing the electric field at the nanocubes, especially at the corners. Grids of smaller dimensions indicate that the simulation results are converged. A total field scattering field plane wave source with wavelength corresponding to localised surface plasmon resonance (LSPR) of nanocubes with propagation along z -axis was used for 3-D simulations, and for the 2-D simulation it was along y -axis. The simulation time was set at 200 fs so that the fields decay completely before the termination of simulation.

3 Results and Discussion

Since the number of “hot spot” sites in metal nanostructures is determined by the morphology, it is a key factor in determining the sensitivity of SERS active substrates.²⁰ For this reason, morphological characterization was carried out by FESEM. From Fig. 1(a), it is clear that the AgNCs with an average size of 65 nm were grown during the solvothermal process. Figure 1(b) shows the size distribution of the synthesized nanocubes and confirms that the nanocubes are grown nearly with homogeneous size. It is also confirmed that no shapes other than nanocubes are grown during the solvothermal process.

The localized surface plasmons are coherent dipole oscillations of conduction electrons in metal nanostructures in resonance with incident light of wavelength comparable to size of the nanostructure. The surface plasmon resonance study of AgNC was carried out by analyzing the UV-Vis absorption spectra of solution. The spectrum (Fig. 2) shows more than one resonance peak, because the cube has several distinct symmetries for dipole resonance. The main dipole resonance peak is at 465 nm, and two smaller resonance peaks are observed at 350 and 389 nm as shoulders. Scattering cross section (σ) for a 65-nm AgNC is calculated via Mie theory using Lumerical FDTD simulation software. Figure 2 clearly confirms that the experimental spectra match with predicted spectra.

Fig. 1 (a) FESEM image of silver nanostructures synthesized at 160°C for 12-h solvothermal reaction and (b) particle size distribution of the nanocubes.

Fig. 2 (a) UV-Vis absorption spectra of silver nanocubes, (b) calculated scattering cross section for silver nanocube of size 65 nm by 3D FDTD.

Fig. 3 Schematic representation of generation of dipoles (a) at the edges and (b) at the corners of cubic structure.

When EM radiation is incident on metal nanostructures, electric dipoles are fashioned onto the confined nanostructure surface and are oscillated with a natural frequency termed as plasmon frequency. Mainly, two different ways of charge accumulation are possible in AgNC, one along edges and the other across diagonal of cubes. The diagonals can be body diagonals or face diagonals. The two possible dipole generations in AgNCs are schematically represented in Fig. 3. The variation in position of LSPR in accordance with morphology can be explained on the basis of Mie theory. According to Mie theory, the extinction spectrum for an arbitrary shape is given by²¹

$$E(\lambda) = \frac{24\pi^2 N a^3 \epsilon_{\text{out}}^{3/2}}{\lambda \ln(10)} \left[\frac{\epsilon_i(\lambda)}{\epsilon_r(\lambda) + \chi \epsilon_{\text{out}}^2 + \epsilon_i^2(\lambda)} \right], \quad (1)$$

where ϵ_r and ϵ_i are the real and imaginary components of the metal dielectric function, respectively, and ϵ_{out} is the dielectric constant of the external environment. Here, χ is the shape factor that accounts for metal nanostructures with various geometries and $\chi = 2$ represents the case for spherical shape. ϵ_r is a function of wavelength and, hence, the position of LSPR is determined by the situation at which $\epsilon_r = \chi \epsilon_{\text{out}}$. The broadening in measured absorption spectra is due to inhomogeneous size distribution of nanostructures. In fact, position of surface plasmon is determined by the magnitude of restoring force offered by the positive ion core in metal nanostructures. In the case of charge accumulated across diagonals, the charge separation will be large. Hence, surface plasmon resonance corresponding to dipoles created on diagonally opposite corners is red shifted with that corresponding to the edges. Figure 2 clearly confirms that the experimental spectra match with predicted spectra.

2-D FDTD method (Lumerical Solutions Ltd.) was employed to locate “hot spot” sites in the synthesized nanocubes during LSPR. The excitation wavelength is selected in accordance with

Fig. 4 Electric field intensity distribution (a) at the corners of AgNC of size 65 nm, (b) surrounding spherical silver nanoparticles of size 65 nm, and (c) at cross-diagonal corners of nanocube of size 65 nm.

the theory that maximum electric field enhancement is induced only on excitation with a wavelength corresponding to localized surface plasmon resonance.⁷ Figure 4(a) visualizes the “hot spot” sites appearing at the corners of nanocube, and Fig. 4(b) shows the field enhancement around spherical silver nanoparticles. Figures 4(a) and 4(b) confirm the huge enhancement in electric field at sharp corners of nanocube as compared with spherical shape. It is also observed that the electric field enhancement occurs across the diagonal of cube [Fig. 4(c)]. These results clearly support the predicted dipole generation in AgNC. Hence, the three peaks are assigned as follows: the peaks at 350 nm arise from one edge of the cube being positively charged while the opposite side is negatively charged. The LSPR peaks observed at 389 and 465 nm are assigned to dipoles generated across the face and body diagonals of the cube, respectively.

FDTD analysis provides the information that the synthesized nanocubes generate “hot spot sites” at the corners. But the field enhancement does not have large dependence on edge length, since hot spot sites are generated at the corners and field intensity is mainly affected by the variation in angles between edges. Even though the size distribution is not homogenous in nature, the prepared AgNCs are excellent candidates for SERS active substrate with good uniformity.

So as to demonstrate competency in SERS activity of the synthesized nanocube colloids, SERS spectra were recorded with R6G as the test molecule with concentration varying from high to the detection limit. SERS studies of R6G were conducted under surface-enhanced resonance Raman conditions with an excitation laser of wavelength 514 nm near the peak absorption wavelength of R6G (~ 532 nm) and are shown in Fig. 5. Figures 5(a) and 5(b) show the representative Raman measurement of R6G molecule in methanol with a concentration of 4.5×10^{-7} M on bare glass substrate and on AgNC-based SERS substrate, respectively. Figure 5 clearly demonstrates the capability of AgNC as SERS active substrate. The measured spectral characteristics of R6G on the nanocube surfaces include 611, 773, 1011, 1184, 1311, 1363, 1568, and 1650 cm^{-1} . The 611, 773, and 1184 cm^{-1} modes are associated with C—C—C ring in-plane, out-of-plane bending, and C—C stretching vibrations, respectively; the 1363,

Fig. 5 (a) Normal Raman spectra of R6G molecule and (b) SERS spectra of 4.5×10^{-7} M R6G using AgNC as SERS active substrates. The inset shows the intensity variation of prominent peak, 611 cm^{-1} of R6G as a function of concentration.

1572 , and 1649 cm^{-1} modes are associated with aromatic C—C stretching vibrations; and 1011 cm^{-1} is associated with the phenyl ring and COOC_2H_5 side group.^{22,23}

The detection limit of our AgNC-based SERS active substrate is determined by repeating the Raman experiment under the same conditions with lower concentrations of R6G. The corresponding intensity variation of prominent peak, 611 cm^{-1} of R6G as a function of concentration, is shown in the inset of Fig. 5. The prominent peaks of R6G were clearly observed even at a concentration of 10^{-17} M and thus the detection limit is found to be 10^{-17} M. The reproducibility and uniformity of nanocube-based SERS substrates are confirmed by repeated SERS measurements on different positions and on different sets of samples. The 2-D color mapping in Fig. 6 shows that different points in the substrate provide the SERS signal with almost the same intensity. The average enhancement factor of the SERS substrate is estimated to be 1.2×10^8 . The earlier reports on SERS activity of sharp AgNCs show that a maximum enhancement factor of 2.96×10^5 is achieved. The economically synthesized, reproducible SERS substrate with a large enhancement factor is expected to be a significant contribution in the field of qualified SERS substrates.

Fig. 6 Variation in Raman intensity of R6G over AgNC SERS substrate.

Fig. 7 SERS spectra of 6×10^{-6} M crystal violet and 3×10^{-6} M Sudan I.

Fig. 8 SERS spectra of uncoiled DNA of (a) salmon fish and (b) *E. coli* using AgNCs synthesized via solvothermal method.

The competence of the fabricated colloid-based SERS substrate can be shown by demonstrating its capability of detecting multianalyte molecule. SERS analysis was again carried out using crystal violet and food adulterant Sudan I as probe molecules. The SERS spectra of 6×10^{-6} M crystal violet and 3×10^{-6} M Sudan I are given in Fig. 7. All the peaks match with the characteristic peaks of crystal violet and Sudan I.²⁴ The peaks at 802, 912, 1172, and 1372 cm^{-1} in the spectra match with characteristic peaks of crystal violet. The identification of Sudan I demonstrates the potential of fabricated AgNC colloid-based SERS substrates in food adulterant detection. Application for food adulterant detection is under study and thus not included in this manuscript.

In order to further demonstrate the potential of AgNC-based SERS substrate as biosensor, we have conducted SERS analysis using double-strand DNA as the target molecule. Barhoumi et al.²⁵ carried out SERS analysis of single-stranded and double-stranded DNA using gold nano-shells. Silicon nanocone arrays²⁶ and citrate-reduced silver colloids²⁷ were also employed for label-free detection of DNA. DNA uncoiling was achieved in solution, prior to SERS analysis by following the procedure of previous reports.²⁵ Figure 8 shows the SERS spectra of DNA of salmon fish and *E. coli* and the peaks at 732, 956, 1331, 1397, and 1454 cm^{-1} are the characteristic peaks of adenine.²⁵ The peaks at 668 cm^{-1} are assigned to breathing mode of guanine, and the peaks at 1367 and 1660 cm^{-1} correspond to characteristic peaks of thymine.^{28,29} Therefore, detection of DNA is successfully performed by colloid-based SERS substrate without any labeling, based on its intrinsic Raman spectra only. Label-free detection of DNA using colloidal substrates synthesized via the solvothermal method explores an economic platform for SERS-based biosensor applications.

4 Conclusion

SERS activity of the AgNCs synthesized via the solvothermal method was successfully investigated and found very competent with high reproducibility and detection limit of 10^{-17} M. SERS activity for crystal violet and for food adulterant Sudan I molecule was also tested, which demonstrates the multianalyte detection capability of the SERS substrate. It is demonstrated that AgNCs are suitable for sensitive label-free DNA detection and are potential candidates for SERS-based biosensors. Localized surface plasmon resonance and EM enhancement in the nanocubes were studied in detail by the FDTD method and “hot spot” sites in AgNCs were located. Based on these results, it can be proposed that AgNCs are a promising SERS substrate for the detection of trace amount of chemical and biochemical analytes.

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