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Fabrication of SERS substrate using nanoporous anodic alumina template decorated by silver nanoparticles

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ABSTRACT

SERS substrate is fabricated using nanoporous anodic alumina membrane decorated by silver nanoparticles, which are self assembled on the surface as well as inside the pores to form a network structure of aligned cylindrical vertical wells creating artificial roughness on the surface of the membrane. When electromagnetic radiation falls on the rough surface, a strong electric field is created due to plasmon excitation on sharp edges of the network structure or at interparticle spacing known as 'hot spots'. This enhanced electric field produces remarkable SERS effect from Raman active R6G molecules attached to silver nanoparticles when excited by a laser source.

1. Introduction

Surface Enhanced Raman Scattering (SERS) has been studied both theoretically [1] and experimentally [2] for nearly last three decades as it is a well known tool for the detection and characterization of very low concentration of molecules in the chemical and biological samples [3]. As Raman signal from the molecules is very weak due to its low scattering cross section and high background fluorescence [4], various techniques are used to get a detectable signal. Among various factors to increase the intensity of the signal, chemical enhancement ($\sim$0 to 10^2) and electromagnetic (EM) enhancement ($\sim 10^4$ to 10^{12}) are very important [5]. The chemical enhancement arises due to charge transfer from metal to the molecule under consideration but the EM enhancement arises from the optical excitation of Surface Plasmon Resonance (SPR) of the metal nanoparticles. When an external EM field is applied on the nanoparticles, the charges get separated (the nanoparticles are polarized) which generate an enormous electric field around the nanoparticles [6]. The generated electric field is highly influenced by the aggregation of nanoparticles; interparticle spacing and the environment [7]. This electric field also enhances the Raman signals from the molecules adsorbed at the surface. As the SERS effect is enhanced remarkably on the rough surfaces [8], it is worth to investigate the fabrication of SERS substrates using ultra fine metal nanoparticles where the roughness can be controlled by the size of nanoparticles, interparticle separation and the nature of aggregation.

Although, SERS effect is known for nearly three decades, search for the suitable substrate is still under active area of research. Different materials and the various structures are investigated to use as SERS substrate. In most cases, silver, gold, or copper nanoparticles are used to fabricate the SERS substrate because of its tunable SPR peak [9]. Several efforts have been given to prepare SERS substrate by depositing thermally evaporated silver nanoparticles on different substrates like glass [10], Anodic Alumina Oxide (AAO) [11,12], porous silicon [13], aluminum foil [14]. Besides these, other methods such as magnetron sputtering [15], Electron Beam Lithography (EBL) [16], and Focused Ion Beam (FIB) [17] or electro-deposition [18] are also being used to fabricate the suitable SERS substrate. In most cases, the main disadvantage is the poor control of the size of nanoparticles, interparticle separation and the aggregation of nanoparticles, which are the key factors in controlling the plasmon resonance responsible for the electric field enhancement. Therefore, although, considerable research articles on the fabrication of SERS substrates have already been published, the precise control of the surface roughness with the size distribution of nanoparticles and interparticles spacing through chemical route is relatively unexplored [19].

In this Letter, we report a simple technique to fabricate the SERS substrate in which surface roughness has been precisely controlled with the nanoparticles aggregation. Here, nanoporous AAO membrane has been decorated by silver nanoparticles with different degrees of aggregation just tailoring the amount of silver nitrate (SN) only. The SPR peak was tuned to cover across a wide range of 350–460 nm to overlap with a 457.9 nm excitation line emitted from the laser source. The as-prepared substrates are highly stable, reproducible and cost effective with high SERS enhancement efficiency (EF). We have used Rhodamine 6G (R6G)

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as the probing molecule to study the SERS performance of as fabricated substrates.

2. Experimental

NaOH and AAO membrane (diameter 13 mm) of pore diameters 100 nm are purchased from s. d. fine – Chem Limited (Mumbai, India) and Whatman International Ltd. (Maidstone, England) respectively. SN, ammonium solution (28%) and formic acid were purchased from Merck India Limited (Mumbai, India). Methanol (spectroscopic grade) and R6G were purchased from Sigma–Aldrich Corporation (Bangalore, India). To prepare the SERS substrate, aqueous solution of SN is added to the excess amount of NaOH. The gray color precipitate is washed several times with distilled water to remove the un-reacted NaOH or SN if any and filtered. The filtrate is taken in a beaker and 5 ml of the distilled water is added to it and stirred. A grayish colloidal solution is obtained. Ammonium solution is added to the gray solution drop wise while stirring until a transparent solution due to formation of silver ammonium complex ($[Ag(NH_3)_2]^{+}OH$) is obtained. One pre-heated (~390 K) porous AAO membrane is dipped into the solution and desired amount of formic acid is then added to the beaker containing the membrane. Silver nanoparticles are produced upon reduction of the complex by formic acid on both surfaces of the AAO membrane. The membrane containing silver nanoparticles is washed several times with distilled water and dried in a vacuum oven at temperature 333 K. Amount of SN and ammonium hydroxide to form the silver complex for four substrates and amount of formic acid added to the silver complex to reduce to silver are summarized in Table 1.

Table 1
Amount of SN taken in 5 ml of water, corresponding amount of ammonium hydroxide used to form silver complex for four substrates and amount of formic acid added to the silver complex to reduce.

$AgNO_3$ taken (g)	Amount of NH_4OH (μl)	Formic acid added (μl)
0.1	200	400
0.2	400	–
0.3	600	–
0.4	800	–

Optical, morphological and microstructural characterizations of the as-prepared substrates have been carried out. The silver nanoparticles decorated AAO membranes are used to take the absorption spectra with a Cary 5000 (Varian, Mumbai, India) spectrophotometer in the transmission mode with a virgin AAO membrane as reference. R6G is taken in the methanol with a concentration of 10^{-4} M. Five microliters of the solution is spread on one surface of a substrate covering the whole area with a micropipette. After the evaporation of methanol, Micro-Raman measurement was performed on a Jobin Yvon HR 800 micro-Raman spectrometer with 457.9 nm excitation from a 20 mW air-cooled argon ion laser. The laser power at the sample position was about 8 μW. The data acquisition time and the number of accumulations were 1s and 10, respectively. Silver nanoparticles decorated AAO membrane was used to study the morphology and the structures by a Scanning Electron Microscope (JEOL, JSM6700F, Tokyo, Japan). The substrate was ground in a mortar, mixed with acetone using ultrasonication and one drop was taken on a TEM grid to study the microstructures of the silver nanoparticles using a Transmission Electron Microscopy (TEM, JEM-2010, Tokyo, Japan) with an Electron Diffraction facility at 200KV.

3. Results and discussion

The AAO membranes were decorated by silver nanoparticles following the method as described earlier. The amount of ammonium solution added is very important as addition of less amount of ammonium solution may keep some amount of silver oxide

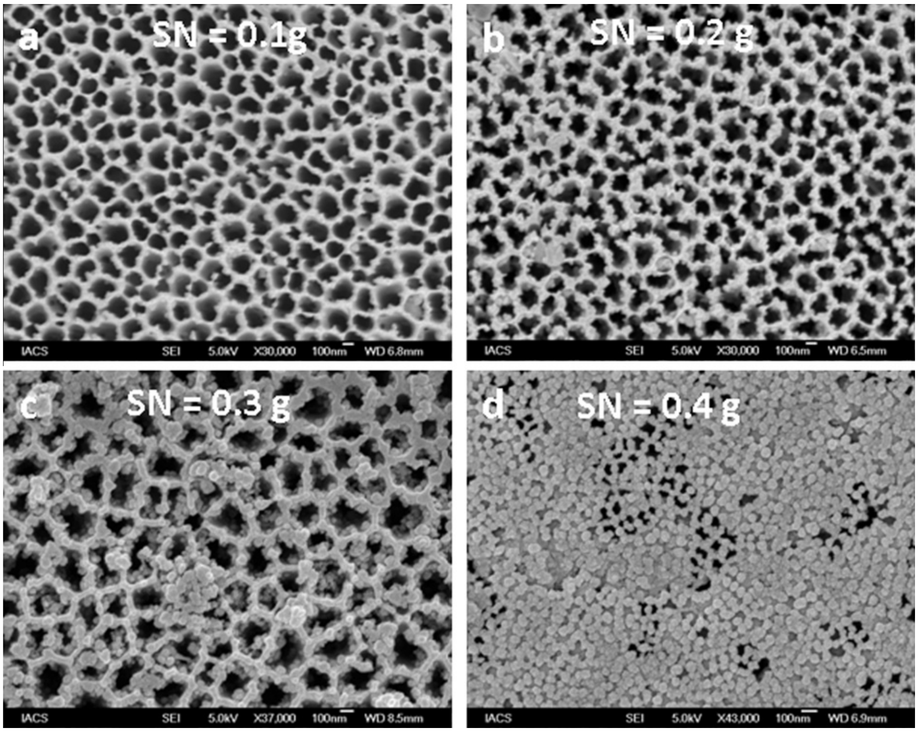

Fig. 1. (a)–(d) are surface morphology of the AAO membranes decorated with 0.1, 0.2, 0.3 and 0.4 g of SN, respectively. It is seen from the images that particles size as well as the roughness increases with the increase in SN due to the aggregation of the silver nanoparticles. However, for SN amount 0.4 g, the effective roughness decreases due to filling of AAO membrane pores.

intact while addition of excess amount may increase the basicity of the solution, which may lead to the dissociation of the AAO membrane. So, an optimum amount of ammonium solution is added. However, the amount of formic acid only controls the reduction process. Formic acid being a weak acid, cannot dissociate the membrane in the time span of the experiment. Hence, we kept the amount of formic acid constant in our as-prepared substrates to study the effect of degree of aggregation of silver nanoparticles on the AAO membrane varying only the amount of SN. For the solution containing 0.1 g of SN, silver started to deposit in the form of nanoparticles on the surface of the AAO membrane after reduction, keeping the pores open as shown in Fig. 1a. For higher SN content (0.2 g) silver nanoparticles are self assembled to form a network structure as shown in Fig. 1b, which creates an artificial roughness on the surface of the membrane. The effect is remarkable for SN content 0.3 g, when a large number of silver nanoparticles are formed on the surface to form an assembly of vertical wells creating a large surface roughness as shown in Fig. 1c. If the SN content is further increased to 0.4 g, the pores of the AAO membrane starts to fill up thereby reducing the effective surface roughness (Fig. 1d). Transmission Electron Microscopy (TEM) was performed to investigate size distribution of the nanoparticles and their aggregation. Fig. 2 shows the TEM image of the substrate containing 0.3 g of SN from which it is seen that most of the nanoparticles are 5–10 nm in diameter, however, some bigger size nanoparticles which are nothing but the aggregation of smaller size nanoparticles are also observed (indicated by an arrow in the Fig. 2). The Selected Area Electron Diffraction (SAED) pattern of the silver nanoparticles is shown in the inset of Fig. 2. The circular rings obtained from the silver nanocrystals corresponding to (1 1 1), (2 0 0), (2 2 0), (3 1 1) planes of silver, clearly indicates the all possible orientation of the silver nanoparticles.

The shape, size and the aggregation of nanoparticles strongly affect the efficiency of the SERS enhancement because these factors influence SPR absorption spectra. The definition of the plasmon does not hold for very small nanoparticles as well as very large nanoparticles because of the fact that in case of very small nanoparticles number of electrons are not sufficient for collective excitation [20] and for very large nanoparticles multipoles [21] which are non-radiative in nature are excited. As only the dipole transition leads to Raman scattering, the higher-order transitions will cause a decrease in the overall efficiency of the enhancement. So, proper tuning of the SPR peak with suitable selection of the nanoparticles size and their aggregation is the primary criterion for nanoparticles based SERS substrates. Njoki et al. reported a size dependant SERS enchantment and revealed a critical size [22]. The electric field generated around the nanoparticles becomes more intense at the sharp edges or between two nanoparticles separated by nanometric distances as investigated by Koh et al. by Electron Energy Loss Spectroscopy (EELS) [23], typically known as 'hot spots'. When two nanoparticles in the aggregate are very close to each other, the generated electric field around each nanoparticle is coupled and their plasmon peaks become broad due to hybridization of the plasmons. The basic physics behind the plasmon hybridization is that the interaction between the primitive plasmons of individual components give two energy levels, one anti-bonding (w+) orbital and the other bonding (w−) orbital. The separation between the bonding and anti-bonding energy levels depends both on the size as well as the distance between the nanoparticles. The splitting of the bonding and anti-bonding energy levels causes the peak broadening due to the hybridization.

Fig. 3 shows the UV–vis absorption spectra for four substrates from which it is seen that the plasmon band of silver nanoparticles splits into several peaks. For 0.1 g of SN substrate, the splitting is not so prominent but the peak is broad ranging from approximately 350 to 422 nm, however, for 0.3 g of SN substrate, splitting of the plasmon band into sharp peaks is observed and the SPR peak is red shifted up to nearly 460 nm. As mentioned above, silver nanoparticles are self assembled to form a network structure of vertically aligned cylindrical wells creating sharp edges, which produce an artificial roughness on the surface of the AAO membrane. The splitting of the plasmon band for 0.1 g of SN substrate is due to hybridization of the primitive plasmons of self assembled silver nanoparticles. The effect increases as amount of SN increases and for 0.3 g of SN substrate, the band splits into several intense peaks. This is due to the fact that with increasing SN amount, roughness on the surface increases and for 0.3 g of SN substrate the splitting is remarkable with formation of intense peaks due to strong enhancement of the electric field produced on the sharp edges where silver nanoparticles are self assembled. The broadening of SPR peak or the increase in the absorption cross section in the order of 0.1 g < 0.2 g < 0.3 g of SN substrates gradually overlaps

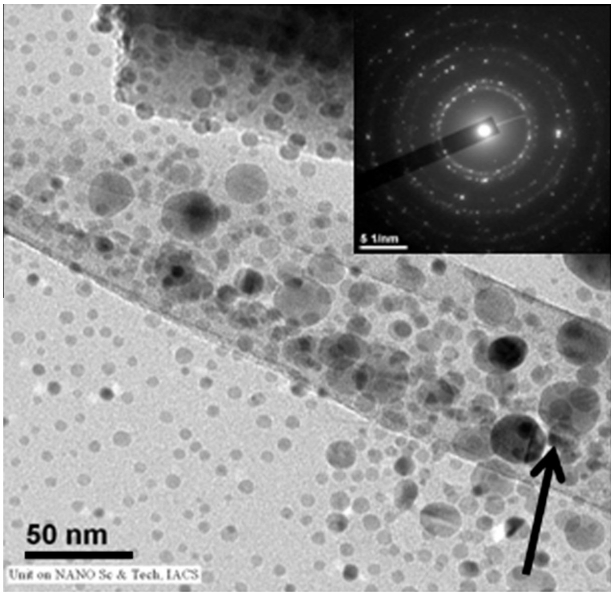

Fig. 2. (a) Transmission Electron Micrograph (TEM) image of the substrate with 0.3 g of SN to investigate the size distribution of the nanoparticles. It shows average size of the nanoparticles as 5–10 nm with some larger particles (15–20 nm). Inset in the image is the Selected Area Electron Diffraction (SAED) pattern of the nanoparticles.

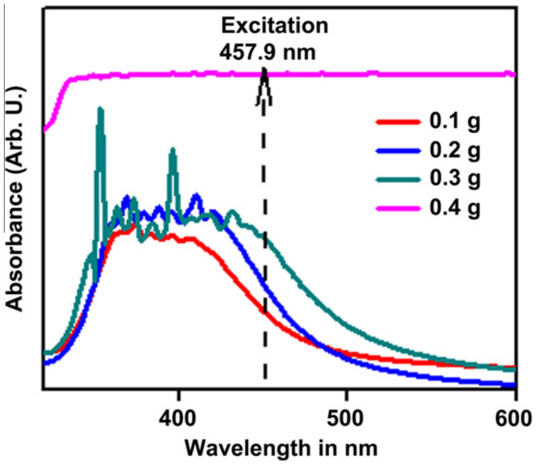

Fig. 3. The absorption spectra recorded from four SERS substrates. The variation in the absorption spectra with SN is prominent. The broadening as well as intensity of the SPR peaks in the absorption spectra increases with increase in SN up to 0.3 g. However, for 0.4 g of SN the substrate becomes opaque.

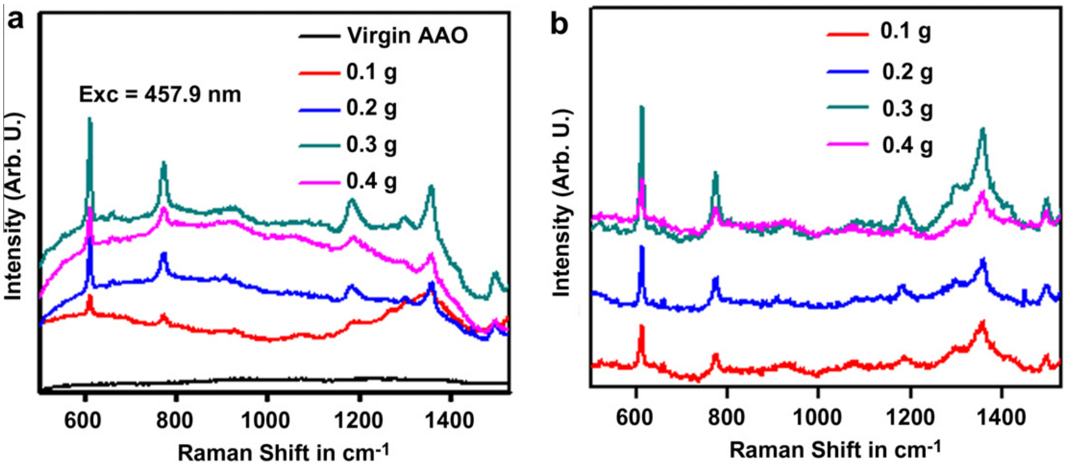

Fig. 4. SERS spectrum of the substrates. (a) The Raman signals recorded from the substrates with its background emission. The intensity of the Raman signals increases with increase in SN. For the substrate with SN amount of 0.4 g the intensity decreases. (b) The Raman signals after subtraction of its background emission.

with the excitation wavelength of laser at 457.9 nm thereby producing ‘hot spots’. However, all the pores of the AAO membrane are covered by silver nanoparticles if the SN amount is increased further to 0.4 g, reducing the effective roughness and making the substrates nearly opaque for transmission of light. Opacity in transmitted light in case of 0.4 g SN substrate results a broad hump in absorption spectra shown in Fig. 3. This phenomenon diminishes the intensity of the Raman signal from the surface adsorbed molecules.

SERS effect of the substrate is investigated using R6G as the Raman active compound (analyte) with a micro-Raman set up. Fig. 4a gives the SERS spectra for the virgin AAO membrane as well as the relative enhancement of the Raman signal for silver nanoparticles decorated AAO membranes creating different degree of roughness. After subtraction of the background emission, the spectra look very sharp and the intense characteristic peaks of R6G are displayed in Fig. 4b. The characteristic peaks of R6G at 612, 773 and 1182 cm^{-1} are associated with C–C–C ring in-plane, C–H out of plane bend mode and C–C stretching vibrations, respectively. The bands at 1360, 1496 and 1642 cm^{-1} are usually assigned to aromatic C–C stretching vibrations of the R6G molecules but none of the Raman lines from methanol at 1035 and 1465 cm^{-1} can be seen in the spectrum. We have noticed that there is a small shift ($\sim$4 to 17 cm^{-1}) in the Raman peak positions relative to its literature values [11]. Niu and his co-workers fabricated a ‘silver nano-particles/sample molecules/silver film system and showed that the spectral shift in the Raman spectra arise due to the interaction of the nanoparticles with the analyte molecules [24]. In the present work, the spectral shift arises due to the interaction between the silver atoms and the carboxyl group of R6G. From Fig. 4a it is seen that the R6G molecule attached to the virgin AAO membrane does not show any Raman signal. The response starts when the silver nanoparticles (0.1 g) are formed on the surface. Intense Raman signals are observed for 0.3 g of SN substrate when an artificial roughness is created by silver nanoparticles self assembled to form a network structure of aligned vertical wells. The Raman Enhancement Efficiency (EF) per molecule is expressed as [25,26],

$$G = \frac{I_{\text{SERS}}}{I_{\text{Ref}}} \cdot \frac{N_{\text{Ref}}}{N_{\text{SERS}}}$$

where I_{SERS} is the enhanced intensity due to SERS effect of the surface adsorbed R6G molecules, I_{Ref} is the spontaneous scattering intensity from the bulk R6G molecules under the laser spot on the virgin AAO membrane, N_{Ref} is the number of molecules excited by the laser on the virgin AAO membrane and N_{SERS} is the number of molecules spread uniformly under the laser spot on the fabricated SERS substrate. Assuming the scattering volume as 1 μm^3 and molar volume of the R6G to be $36.84 \times 10^{-5}\ \text{m}^3$, N_{Ref} is calculated to be approximately 16.34×10^{20}. If five microliters of a 10^{-4} M of R6G in methanol solution is spread on the membrane having a total surface area of about $132.75 \times 10^{-6}\ \text{m}^2$, N_{SERS} comes to be nearly 2.27×10^{15}. The value of G calculated from the SERS peak at 612 cm^{-1} for three substrates are (i) 7.72×10^5 (ii) 8.3×10^5 (iii) 9.1×10^5 and (iv) 8.54×10^5.

It is to be mentioned that the enhancement of Raman signal is the maximum when wavelength of the laser source is comparable to the plasmon excitations. In our case we have used excitation wavelength of 457.9 nm, which is the higher limit of the SPR peaks. Remarkable effect of enhancement in the Raman signal would be obtained if we could use the excitation line around 400 nm by proper choice of the laser source and the optical arrangement. In addition to laser wavelength, the intensity of the Raman signal may be increased using potential strategies such as core–shell nanostructures and tuning the shape (cubic, triangular, rods) of the nanoparticles. Recently, Kumar et al. has reported enhancement of electric fields using core–shell nanostructures with Ag core and Au shell and observed large enchantment of Raman signal for Au:Ag = 0.4 [27]. However, formation of core–shell nanostructures on the AAO membrane by coreducing the mixture of gold and silver complexes and investigation of its SERS activity is underway.

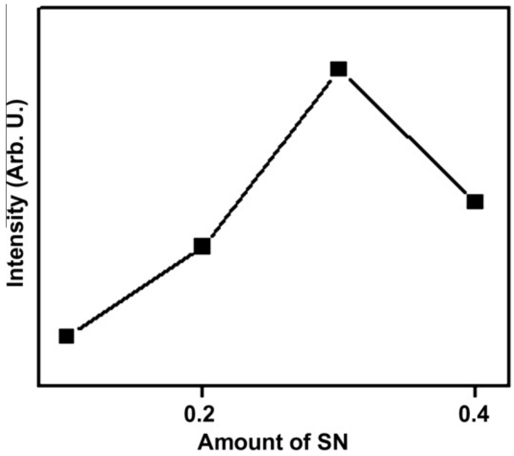

Fig. 5. The graph showing variation in intensity of the Raman signal with amount of SN. The intensity of the SERS signal starts to decrease for SN amount of 0.4 g.

The variation in intensity of the Raman signal of as-prepared substrates with amount of SN used is demonstrated in Fig. 5. It is seen that the intensity of the Raman signal of the surface adsorbed R6G molecules is enhanced with increase in amount of SN. As amount of SN increases, more and more pores are decorated with silver nanoparticles as well as aggregation of nanoparticles takes place. This aggregation of nanoparticles produces large number of 'hot spots' which enhances Raman signals from R6G molecules up to 0.3 g of SN. However, for SN amount of 0.4 g, intensity of the Raman signals decreases due to filling of nanopores of AAO membrane creating a smooth surface.

4. Conclusion

SERS substrate is fabricated using AAO membrane decorated by silver nanoparticles via a simple straightforward chemical route. Silver is deposited in the form of nanoparticles in such a way that a network structure is formed on the surface of AAO membrane to produce an artificial roughness which creates strong enhancement of the electric field. Intense SERS effect is observed due to electric field enhancement at the sharp edges on the surface of the AAO membrane. We believe this technique will be very useful to fabricate the SERS substrates in a controllable way where the electric field enhancement can be controlled in a precise way by tailoring the particle size distribution etc.

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