Research article

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Manipulating the surface-enhanced Raman spectroscopy (SERS) activity and plasmon-driven catalytic efficiency by the control of Ag NP/graphene layers under optical excitation

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Abstract: Highly efficient plasmon-driven catalysis and excellent surface-enhanced Raman spectroscopy (SERS) performance are proportional to the square of the local electromagnetic field (hot spot). However, a proven way to realize the enhancement in intensity and density of “hot spot” still needs to be investigated. Here, we report on multilayered Ag nanoparticle (Ag NP)/graphene coupled to an underlying Cu film system (MAGNP-CuF) which can be used as an effective SERS substrates realizing ultra-sensitive detection for toxic molecules and in situ monitoring the plasmon-driven reaction for p-nitrothiophenol (PNTp) to p,p'-dimercaptobenzene (DMAB) conversion. The mechanism of ultra-sensitive SERS response and catalytic reaction is investigated via Ag NP/graphene layer-dependent experiments combined with theoretical simulations. The research found that the intensity and density of “hot spot” can be effectively manipulated by the number of plasmonic layers, and the bottom Cu film could also reflect the scattered and excitation beam and would further enhance the Raman signals. Moreover, the MAGNP-CuF exhibits outstanding performance in stability and reproducibility. We believe that this concept of multilayered

plasmonic structures would be widely used not only in the field of SERS but also in the wider research in photocatalysis.

Keywords: hot spot manipulation; multilayered nanostructures; SERS; surface-catalyzed reaction.

1 Introduction

Raman spectroscopy is produced by inelastic scattering caused by molecular vibration, which can provide fingerprint information for molecular diagnosis [1, 2]. However, the inherently scattering intensity of traditional Raman spectrum is very weak. In general, the light intensity is only about 10^{-10} of the incident light intensity, which limits its applied range. Surface-enhanced Raman spectroscopy (SERS) possesses most of the advantages of Raman spectroscopy and provides more abundant information on molecular structure. SERS is a very powerful trace detection tool realizing ultrasensitive, real-time, and in situ detection at the single-molecule level, which has been achieved practical applications in the fields of food safety, medical diagnosis, environmental monitoring, and in situ chemocatalysis monitoring, etc. [3–10]. The generally accepted Raman enhancement mechanism consists of electromagnetic mechanism (EM) and chemical mechanism, while the former is proved to play a leading role. The origin of EM is the surface plasmon resonance (SPR) of metal nanostructures producing electromagnetic field which is localized at the nanoscale forming “hot spots”. Normally, the field strength of the “hot spot” increase with the decrease of the gap size. However, the recent investigations reveal that when the gap size is further reduced to sub-nanometer level, the tunneling current will appear due to the quantum mechanical effect, which

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makes the “hot spot” field strength decrease significantly [11, 12].

Compared to one-dimensional (1D) and two-dimensional (2D) metal structures, the substrates with three-dimensional (3D) nanostructures exhibit much stronger SERS effect and a more uniform SERS signals, which is attributed to the high intensity and density of “hot spot” and promoted proportion of analyte molecules in “hot spots” [13, 14]. Among various 3D nanostructures, multilayered form has received extensive attention both in experiment and theory for some unique characters in the field of plasmon-mediated catalysis and SERS [6, 15–22]. Stephanie Reich et al. demonstrated the excitation of dark modes and the creation of hot electrons benefiting for plasmon-mediated photocatalysis reactions via the multilayered gold nanoparticles [15]. Jian Ye et al. prepared 3D self-assembled gold nanoparticles (Au NP) multilayer structures by the layer-by-layer deposition technique and investigated LSPR sensitivity of the multilayered structures to the change of the environmental refractive index [16]. Yeon et al. proposed a vertical stacking ordered gold nanowire cross structure and successfully realized the detection of Alzheimer’s biomarkers-tau protein and amyloid β [17]. M. Liz-Marzan et al. obtained a multilayered gold nanorod array structure which is highly ordered and perpendicular to the base direction by drop casting method. Note that the array gap would produce standing wave under the excitation of incident light, which is beneficial for the elevation of the density and intensity of “hot spots” [18]. Martin Fischlechner et al. [19] assembled multilayered Au NP onto colloidal spheres to construct SERS sensors and achieved the regulation of resonance wavelength with the number of layers of Au NP changing. This substrate realized a quantitative detection for adenine molecules with a relative standard deviation of 9.44–13.7%. Jianfeng Li et al. constructed multilayered Ag nanostructures and the SERS signal is enhanced with the increase of the number of silver nanocubes in the 1–4 layers [20]. Moreover, they also developed a multilayered SiO_2 shell-wrapped Ag NP structure by simple drop-and-evaporation method, and finally the substrate was used for quantitative analysis of 4-MBA with concentrations 10^{-8} – 10^{-5} M [21]. Baoyuan Man et al. fabricated an Al NP-film system with different Al film layers to elevate the plasmonic coupling intensity. The systems exhibit enormous optical absorption enhancement from ultraviolet to visible range and achieve the highest enhancement factor (EF) in previous reported Al-based structures [6].

In general, the 3D multilayered nanostructures should perform a much better SERS effect and potential of

chemocatalysis reactions for the creation of high density “hot spots”. However, there are still some problems with the reported multilayered nanostructures: (1) using pure metal structures, which is unsuitable for the adsorption of analyte molecules; (2) metal nanoparticles prone to aggregation producing charge exchange, which would weaken the “hot spots” intensity; (3) to control the interparticle gap with the molecular chains that interleaves among nanoparticles also influencing on the permeation of analytes into “hot spots” and further affecting the SERS and photocatalytic ability; (4) the close packing of the metal nanoparticles decrease the penetration of the incident light, which would affect the stimulation of the vertical “hot spots”.

In order to overcome these issues, we proposed a very facile way to fabricate a multilayered Ag NP nanostructures spaced by bilayer graphene films coupled to an underlying Cu film (MAGNP-CuF). The MAGNP-CuF featured sandwich-type plasmonic nanostructures possessing highly dense Ag NP nanoarrays are uniformly formed to generate strong and reproducible “hot spots” especially at the graphene gap area. Graphene films have been proved to be an excellent component as SERS or surface-catalyzed substrates for the excellent molecular adsorption ability, atomically flat surface, high molecule affinity, fluorescence quenching effect, etc. [23]. Our systematic study of the layer-dependent effect of MAGNP-CuF on SERS and surface-catalyzed reaction, combined with theoretical simulations, indicates the main mechanism. By way of contrast, an improved EF is observed as the increase of Ag NP/graphene layers, and the MAGNP-CuF realized ultra-low detection limit for toxic molecules. Moreover, the PNTP to DMAB conversion are also carefully studied on MAGNP-CuF. Interestingly, the plasmon-driven, surface-catalyzed reactions is also related closely to the number of Ag NP/graphene layers, and importantly, the six-layer MAGNP-CuF exhibit a considerably higher plasmon-induced reaction efficiency than other MAGNP-CuF structures.

2 Experimental section

2.1 Fabrication of graphene

Copper foil (purity 99.99%, 0.2 mm) was annealed at 1000 °C in an H_2/Ar atmosphere (H_2 50 sccm, Ar 50 sccm) for 1 h and was polished by chemical-mechanical polishing method. Then the copper foil was thoroughly washed after sonication in acetone, alcohol, and DI water, respectively. The chemical vapor deposition process was performed in a tube furnace system. The copper foil was cut into 1×1 cm pieces and

loaded into the quartz tube with the pressure pumped to 10^{-3} Pa. Then CH_4 and H_2 (CH_4 :50 sccm; H_2 :50 sccm) was introduced into the quartz tube and the temperature was increased to 1000 °C. After 30 min growth, the temperature of the quartz tube was quickly cooled down to room temperature in the 50 sccm H_2 atmosphere.

2.2 Fabrication of the MAGNP-CuF SERS substrates

First, Ag NP with 2.5 nm thickness was directly deposited on the graphene/Cu foil substrate through a thermal evaporation method. The deposition rate was about $0.3 \text{ \AA/s}$ under the pressure of 6.5×10^{-5} Pa. In the etching process, the Ag NP plays a role as support for graphene film replacing the PMMA. The $1 \times 1 \text{ cm}$ Ag NP/graphene/Cu foil were placed in 0.1 M aqueous $(\text{NH}_4)_2\text{SO}_4$. After removing the copper foil in the etchant, the Ag NP/graphene film was transferred into DI water to wash away remaining etchants (done three times). Finally, the Ag NP/graphene was transferred to the other Ag NP/graphene/Cu foil substrate. On this basis, the two-layer MAGNP-CuF substrate was obtained. Simply repeating the Ag NP/graphene transfer process, the three-, four-, five- and six-layer nanostructures can be also fabricated.

2.3 Characterization

The scanning electron microscopy (SEM) images of samples were acquired by SEM (Zeiss Gemini Ultra-55). Transmission electron microscopy (TEM, JEM-2100) and high-resolution transmission electron microscopy (HRTEM) were carried out to investigate the characteristics and structures of the samples. The reflectance spectra of MAGNP-CuF were collected by a UV-Vis photometer (UV3600). To investigate the SERS activity of the proposed MAGNP-CuF substrates, a Raman spectrometer (Horiba HR Evolution) equipped with 532 nm wavelength laser was used. The grating was 600 gr/mm, and the spot size was around $1 \text{ }\mu\text{m}$. The objective lens $50\times$ was used and the acquisition time was 4 s. The rhodamine 6G (R6G , 10^{-6} – 10^{-12} M), crystal violet (CV, 10^{-5} – 10^{-10} M), and thiram (10^{-2} – 10^{-6} M) were all detected using the MAGNP-CuF. $2 \text{ }\mu\text{L}$ solution of the analyte molecules were directly dropped on the 3D nanostructure and dried in air. For the plasmon-driven, surface-catalyzed reactions, the MAGNP-CuF substrates were immersed in a 10^{-3} M PNTP ethanol solution for more than 1 h. Then, the substrates were washed several times with ethanol and dried in N_2 condition before Raman test.

3 Results and discussion

3.1 Characterization of the MAGNP-CuF

Figure 1A exhibits the components of the MAGNP-CuF substrates. With the assistance of the graphene film, we could obtain the multilayered plasmonic structure. The “hot spots” exist not only among the in-plane Ag NP but also on the sub-nanometer graphene gap regions, and the density and intensity of “hot spots” is related closely to the Ag NP/graphene layers. The systematic study of the layer-dependent effect of MAGNP-CuF on SERS and plasmon-driven photocatalysis reaction would be well conducted.

The detailed fabrication process of the MAGNP-CuF has been well presented in the experimental section. The 3D structures were prepared through an extremely facile method. We first identify the quality and thickness of the graphene film using the Raman spectroscopy as shown in Figure 1A. The main Raman characteristics of graphene are the D band ($\sim 1350 \text{ cm}^{-1}$), G band ($\sim 1580 \text{ cm}^{-1}$), and 2D band ($\sim 2670 \text{ cm}^{-1}$). The D band associated with defects in graphene originates from the breathing mode of sp^2 hexagonal carbons. The appearance of the D band in the spectrum should be attributed to the damage of graphene film in the evaporation and transfer process. The G band corresponds to in-plane vibration of sp^2 carbon networks and gets activated for the doubly degenerated phonon mode at the Brillouin zone center. The intensity of G band increases almost linearly as the number of graphene layer increases, and the peak intensity ratios (I_{2D}/I_G) have been used to identify the number of graphene layers in many previous reports [24, 25]. The I_{2D}/I_G in this spectrum is ~ 0.97 which indicates a typical spectrum for bilayer graphene. The uniform and dense Ag NP nanostructure with an average size $\sim 22 \text{ nm}$ was directly deposited on the graphene/Cu foil substrate through a thermal evaporation method as shown in Figure 1B. This can be attributed to a kinetic factor: surface diffusion coefficients that relate closely to the number of graphene layers and determine the nucleation, growth, and the density of Ag NP after deposition [26]. Based on the homogeneous bilayer graphene film, the evaporation method provides small enough and uniform gaps among Ag NP without the molecular chains. And it could successfully avoid the aggregation of Ag NP, which is beneficial for the permeation of analyte molecules into “hot spots”. Besides, the diameter and gap size of the Ag NP nanostructure can be well controlled via adjusting the deposition rate and time. To better observe morphology of the MAGNP-CuF, the boundary area of one-, two-, and three-layer structures was exhibited in the inset of Figure 1C and D, and it indicates the successful fabrication of the layered structure. As the increase of Ag NP/graphene layers, we can see that the Ag NP is still well-ordered shown in the SEM images in Figure 1D–G. The Ag NP on the top layers is clear and bright, while the bottom Ag NP is relatively blurry. Bilayer graphene films with an ideal thickness around 0.64 nm separate the vertical Ag NP layers forming “hot spots”, which could generate nearly optimum electromagnetic enhancement [11, 12]. Moreover, the graphene film should be beneficial to protect the bottom Ag nanostructures from oxidative damages under environmental conditions and ensure the stability of SERS signals to some extent. Three-layer Ag NP/graphene was directly fabricated on the copper grid and investigated by TEM and

Figure 1: (A) the schematic illustration of the surface-enhanced Raman spectroscopy (SERS) and surface plasmon-driven reaction on the MAgNP-CuF substrates. (B)–(G) scanning electron microscopy (SEM) images of MAgNP-CuF with different layers. The inset in (B) exhibits the size distribution of Ag NP. The inset in (C) exhibits the boundary region between one- and two-layer structures. The inset in (D) exhibits the boundary region of three-layer structure. (H) and (I) transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images of the three-layer MAgNP substrate.

HRTEM as shown in Figure 1H and I, respectively. The area of the white rectangular frame in Figure 1I clearly show that three Ag NP in different layers are stacked in the vertical direction indicating that the layered structure could be definitely fabricated by the proposed method.

3.2 Layer-dependent properties of the MAgNP-CuF

It is crucial to increase the “hot spots” density and intensity for improving the SERS sensitivity and plasmon-driven, surface-catalyzed reaction. For the multilayered structures, it is seemed that the number of plasmonic layers is the more the better. The diameter of the incident light spot is limited and the more stacked plasmonic layers would stimulate more plasmonic couplings on the vertical plane, where the multiple plasmonic couplings could further enhance the intensity of the “hot spots” [27, 28]. However, the finite penetration depth of the incident laser into the multilayered structures should be considered. The Raman detector could not easily collect the scattered light that emerges from the multilayered structures with low transmittance. Thus, the number of plasmonic layers is limited and that should be a key point for the SERS performance in multilayered structures. In this work, combining highly transparent graphene film with the only 2.5 nm thickness Ag NP as plasmonic layer is vitally important for the increase of the stacked layers that incident light could penetrate. Figure 2A clearly shows the SERS performance of MAgNP-CuF from one to six layers using R6G (10^{-6} M) as the probe molecules. Note that the SERS signal increases obviously as the plasmonic layers stacking, and this could be attributed to the enhancing multiple plasmonic couplings on the vertical plane. However, when it increases to more than four-layer stacking, the SERS signal exhibited even a slight decreases. To verify this experimental result, we choose CV (10^{-6} M) as another probe molecules and the SERS test is conducted as shown in Figure 2B. The variation trend of the SERS signals is same with that of R6G. Although the intensity of the incident light would decrease more as it penetrates into the “thicker” MAgNP-CuF, and the scattered light are more difficult to emerge from the “thicker” structures and be collected by the Raman detector. However, in this case, we think this phenomenon should be mainly attributed to the molecular accumulation on the top layer of MAgNP-CuF. As shown schematically in Figure 2C, the molecules should mainly concentrate on the top layer of MAgNP-CuF for the graphene barrier after dropping the analytes on the substrate. The amount of molecules continuously decrease as the increase in depth

of MAgNP-CuF. And for the six-layer MAgNP-CuF, there are few molecules distribution in the bottom layer. These factors can well explain the slight drop of the SERS signal intensity above four layers.

The reflectance spectra of MAgNP-CuF was observed from the one to six layer substrate in the visible wavelength range, as presented in Figure 2D. In the whole spectral range of 200–900 nm, samples show a great decrease in reflectivity and correspondingly greater absorption for light with the plasmonic layers stacking, which suggests the enhancement of electromagnetic field at these “thicker” MAgNP-CuF. It has been reported that the SERS effect increases almost linearly with the decreasing reflectance in a semilog plot under the excitation wavelength [29, 30]. And thus the six-layer MAgNP-CuF should perform the best SERS response. To prove this, the following SERS test experiment was conducted. The bilayer graphene film create the high strength “hot spots” and can be also regarded as a kind of special molecules distributing in the entire MAgNP-CuF for SERS assessment. The SERS spectra of graphene collected from MAgNP-CuF substrates with graphene/Cu film as contrast are shown in Figure 2E. It is observed that the SERS signals of graphene still keep a typical spectrum for bilayer graphene. The intensity of the SERS continuously increase as the Ag NP/graphene layers from one to six, which is identified with the above analysis. Furthermore, we can see that the SERS signals of graphene from MAgNP-CuF is much stronger than that from the graphene/Cu film indicating the huge electromagnetic enhancement in nanogaps area.

The role of the Cu film on the SERS performance was also evaluated. Figure 2F shows the comparison of SERS signals at 612 cm^{-1} for the MAgNP with or without Cu film. The variation trend of the SERS signals is the same for both kinds of substrates. However, the signal intensity from MAgNP-CuF is much stronger than that of the MAgNP-SiO₂, even when the number of plasmonic layers exceeds four. It indicates the reflected excitation and scattered photons contain a component coming from the Cu film and further conduce to the Raman signal enhancement.

In order to understand the origin of electric enhancement, the simulation was conducted by means of the (finite-difference time-domain) FDTD method. The simulation model consists of different layers of graphene/Ag NP as shown in Figure 3A. In the layer, the Ag NP wrapped with hat-shaped graphene film (0.64 nm) form nanoarrays. The diameter of the Ag NP set as 20 nm and the inter-particle distance is 10 nm according to the SEM and TEM images. After the incident of 532 nm wave with polarization along the y-direction, we found that the primary EM mainly distributes in the gaps area produced by the graphene films

Figure 2: (A) The surface-enhanced Raman spectroscopy (SERS) spectra of R6G (10⁻⁶ M) collected from one- to six-layer substrates. (B) The SERS spectra of CV (10⁻⁶ M) collected from one- to six-layer substrates. (C) The schematic illustration of the analytes distribution using the dropping method. (D) The reflectance spectra of the MAGNP-CuF substrate. (E) The SERS spectra of graphene collected from MAGNP-CuF substrates with different layers, with graphene/Cu film as contrast. (F) Comparison of SERS signals at 612 cm⁻¹ for the MAGNP with or without Cu film. Each value was the mean of 20 random spots collected from each MAGNP-CuF substrates.

Figure 3: (A) Simulation set-up of the MAGNP-CuF substrate. (B) The y-z views of electric field distribution for MAGNP-CuF with different Ag NP/graphene layers at 532 nm wavelength. (C) Electric field enhancement (E/E_0) for the MAGNP-CuF.

between the upper and lower Ag NP layers as presented in Figure 3B, which indicates the significant role of bilayer graphene in the SERS effect. In these models, it is clearly observed that the density and the strength of the “hot spots” gradually increase as the plasmonic layers stacking as exhibited in Figure 3C, which should be beneficial for

the SERS and plasmon-induced reaction efficiency. The variation tendency in the simulation results is in accordance with the experimental results of graphene SERS spectra. Interestingly, the maximum EM appear on the nanogap region between the topmost and the second-layer Ag NP, which would effectively facilitate the use of simple

dropping method for MAgNP-CuF in SERS detection. Nevertheless, the intensity of the “hot spots” is reduced from top to bottom in one model for the finite penetration depth of the incident laser.

3.3 Plasmon-induced reaction efficiency of the MAgNP-CuF

After verified by the experimental and theoretical results, it is concluded that the intensity and density of “hot spots” in MAgNP-CuF could be tuned by the plasmonic layers. Under this circumstances, more hot electrons would be generated from plasmonic decay by increasing the plasmonic layers, and hot electrons could transfer from the surface Ag NP to graphene, which should be beneficial to the surface-catalyzed reaction. The plasmon-driven catalytic reactions have been extremely critical in environmental protection solar energy, and chemical industry for low-energy requirements and high efficiency [4]. Thus, the MAgNP-CuF SERS substrates were used to in-situ monitor the reduction reaction of PNTP dimerizing into DMAB and verify the catalytic ability. To guarantee the better distribution of PNTP molecules in the entire structure, the MAgNP-CuF substrates were immersed in PNTP ethanol solution for more than 1 h and washed several times with ethanol and dried in N_2 condition before Raman test. As shown in Figure 4A–F, the time-dependent reaction processes of PNTP dimerizing into DMAB for MAgNP-CuF were clearly proved for the decreasing intensity of the bands at 1340 cm^{-1} , representing the $\nu(\text{NO}_2)$ of PNTP, and the emerging peaks at 1397 and 1445 cm^{-1} for the $\nu(\text{N}=\text{N})$ represent the formation of DMAB. As the reaction progress, the intensities of the $\nu(\text{NO}_2)$ become weak gradually, while the $\nu(\text{N}=\text{N})$ peaks are getting stronger, demonstrating that the increased production of DMAB during the reaction. For the one-layer MAgNP-CuF, the intensity of the nitro group is nearly equal to that of the $\nu(\text{N}=\text{N})$ bands of DMAB after 10 s reaction, which indicates a relatively low conversion efficiency. When the number of plasmonic layers increased to two or more, we note that the intensity of $\nu(\text{N}=\text{N})$ bands are much stronger than that of the nitro peak at the beginning of the reaction, suggesting that increasing the catalytic efficiency is closely related to the intensity and density of “hot spots”. Especially, for the six-layer MAgNP-CuF, the reaction is totally complete after 90 s for the disappearance the nitro peak as shown in Figure 4F.

Time-dependent SERS intensity ratios $I_{\nu(\text{N}=\text{N})}/I_{\nu(\text{NO}_2)}$ are used to evaluate the reaction efficiency, where the $I_{\nu(\text{N}=\text{N})}$ represents the DMAB band at 1445 cm^{-1} and $I_{\nu(\text{NO}_2)}$ represents the DMAB band at 1340 cm^{-1} as shown in Figure 4G. It

is demonstrated that the time to achieve equilibrium in the reaction gets shorter as the Ag NP/graphene layers increase. It has been reported that the graphene film would facilitate the transfer of hot electrons from the surface of Ag and could effectively extend the lifetime of the hot electrons [31]. Considering the fact that the reduction process of PNTP dimerizing into DMAB needs the participation of the hot electrons, the “thicker” MAgNP-CuF processing the high intensity and density of “hot spots” is supposed to produce more “hot electrons” to accelerate this reaction. Moreover, the layer-dependent SERS intensities of the $\nu(\text{N}=\text{N})$ band at 1445 cm^{-1} after 90 s reaction collected from MAgNP-CuF with different layers are exhibited in Figure 4H. The SERS signals continuously increase as the Ag NP/graphene layers stacking, which is in keeping with the trend of graphene SERS spectra and the simulated results. This discovery indicates that the six-layer MAgNP-CuF still do not reach the penetration depth of the incident laser. And the more stacked Ag NP/graphene layers could be stimulated and would obtain the higher density and intensity of the “hot spots”.

3.4 SERS performance of the MAgNP-CuF SERS substrates

MAgNP-CuF substrates combined multiple plasmonic couplings with chemical enhancement produced by graphene are expected to perform excellent SERS effect. To assess the potential of the MAgNP-CuF as SERS substrate, a series of experiments were conducted. Figure 5A shows the SERS signals of the R6G with the concentration range from 10^{-6} M to 10^{-12} M . The characteristic peaks of R6G ($612, 773, 1185, 1361, 1508$ and 1650 cm^{-1} etc.) can be clearly observed and distinguished. The limit of detection (LOD) for R6G is determined to be 10^{-12} M . Moreover, it is worth noting that the logarithmic concentration of the R6G shows a good linear relationship with a correlation coefficient (R^2) of 0.933 at 612 cm^{-1} from 10^{-12} to 10^{-6} M , as shown in Figure 5C, which indicates that quantitative detection of unknown concentration of R6G molecules can be achieved in this range of concentration. The intensity change is represented by empirical equations: $\text{Log } I = 0.403\text{Log } C + 6.735$ for 612 cm^{-1} , where I represents the intensity of the probe molecules and C express the concentrations.

The above results clearly show that the MAgNP-CuF structure exhibit an outstanding SERS sensitivity. To test the compatibility, the CV molecules, a toxic chemicals, widely used in aquaculture and fisheries were selected. The SERS signals of CV with the characteristic peaks at $528, 916, 1179, 1368, 1588$ and 1622 cm^{-1} are shown in Figure 5B.

Figure 4: (A)–(F) The dual function of the catalytic activity and in situ surface-enhanced Raman spectroscopy (SERS) monitoring the reaction process of p-nitrothiophenol (PNTp) dimerizing into p,p'-dimercaptobenzene (DMAB) for MAGNP-CuF. (G) Time-dependent SERS intensity ratios of the $\nu(\text{N}=\text{N})$ and $\nu(\text{NO}_2)$ bands for MAGNP-CuF with different Ag NP/graphene layers under 532 nm laser excitation (0.48 mW). (H) Layer-dependent SERS intensities of the $\nu(\text{N}=\text{N})$ band.

The LOD for CV is low relative to 10^{-10} M, which could satisfy the measurement requirements of the banned substance [32]. The linear fitting calibration curve for the average intensity of SERS signals at 612 cm^{-1} versus the concentration of R6G is shown in Figure 5D. The coefficients of determination (R^2) is 0.998 representing an excellent linear relation. Thus, the quantitative detection for CV molecules can be achieved in this concentration range. The intensity change is also represented by empirical equations: $\log I = 0.533 \log C + 7.139$ for 916 cm^{-1} .

The above results indicate that the sensitivity and quantitative detection ability of MAGNP-CuF have great

potential in trace molecule recognition, which can be attributed to the following factors: Firstly, sub-nanometer gap among stacked Ag NP/graphene layers produce huge electromagnetic field enhancement; Secondly, graphene film could effectively promote the adsorption ability especially for the aromatic organic molecules and possess superior chemical enhancement, which would greatly attribute to the SERS detection. Most of all, the incident light could penetrate the numerous stacking layers for the highly transparent graphene film and the only 2.5 nm thickness Ag NP. Apart from the sensitivity, the uniformity of SERS signals are also crucial for practical applications.

Figure 5: (A) Surface-enhanced Raman spectroscopy (SERS) spectra of R6G with varying concentrations ranging from 10^{-6} to 10^{-12} M. (B) SERS spectra of CV with varying concentrations ranging from 10^{-5} to 10^{-10} M. (C) Log–log plot of average intensity of SERS signals at 612 cm^{-1} versus the concentration of R6G. (D) Log–log plot of average intensity of SERS signals at 916 cm^{-1} versus the concentration of CV. (E) Corresponding Raman mapping of the 612 cm^{-1} characteristic peak of R6G (10^{-6} M).

The Raman mapping of R6G at 612 cm^{-1} was measured with 1 μm step-size as shown in Figure 5E. It could be seen that most of the area are red colored and the color variations are very small indicating the highly reproducible SERS signals collected from the MAgNP-CuF.

The SERS performance of MAgNP-CuF substrates can be evaluated by calculating the EF. According to the formula, the EF of R6G molecule was calculated according to the previous reports [13, 28, 33]:

$$\text{EF} = \frac{I_{\text{SERS}} \times N_{\text{Cu}}}{I_{\text{Cu}} \times N_{\text{SERS}}}$$

where I_{SERS} and I_{Cu} represent the SERS spectra and normal Raman intensity of the same bands obtained from SERS substrate and Cu foil, respectively. N_{SERS} and N_{Cu} represent the number of R6G molecules in the laser spot on the SERS substrate and Cu substrate, respectively. The 10^{-12} M R6G was selected as the LOD for the EF calculation, while the Raman signal of 10^{-2} M R6G collected from the Cu foil was used as comparison as shown in Figure 6A. The calculated EF of R6G for the four-layer MAgNP-CuF is about 7.8×10^9 , which is much higher than other multilayered SERS substrates as shown in Table 1. For the multilayered structures, the more stacked plasmonic layers would

stimulate more plasmonic couplings on the vertical plane, which is related closely to the density and intensity of the “hot spots”. However, the finite penetration depth of the incident laser affects the stacked plasmonic layers that could be stimulated. In the work, six or more layers could be penetrated, which is much more than other multilayered structures and that should be a key point for the best SERS performance. Take the same method, the EF of CV molecules was also calculated to be 3.24×10^8 as shown in Figure 6B.

The stability of the SERS substrate is a crucial parameter for practical application. Ag Nanostructures are known to possess strong SPR, but their poor chemical stability in general environments has limited applications in many fields. The stability of MAgNP-CuF was assessed through monitoring the variations of the SERS signal over time. As shown in Figure 6C and D, the attenuation of measured SERS signals from the MAgNP-CuF is very small at time passed. One month later, the average intensity of the SERS signals at 612 cm^{-1} is only decreased 19.3 %. While for the sole Ag NP substrate, the measured SERS signals decrease 66.7 % at time passed as shown in inset in Figure 6C and D. This maybe because that the graphene film could isolate the lower Ag NP layers from the outside environment and

Figure 6: (A) and (B) The surface-enhanced Raman spectroscopy (SERS) spectra of R6G (10^{-12} M) and CV (10^{-10} M) collected from the four-layer MAGNP-CuF and the Raman spectra of R6G (10^{-2} M) and CV (10^{-2} M) collected from the Cu foil as comparison. (C) SERS spectra of R6G from MAGNP-CuF with different aging times. The inset in (C) exhibits the SERS spectra of R6G from Ag NP with different aging times. (D) SERS intensity ratios of the I_{time} and I_{origin} for MAGNP-CuF and Ag NP. (E) SERS spectra of thiram at varying concentrations ranging from 10^{-2} to 10^{-6} M. (F) Log-log plot of average intensity of SERS signals at 1385 cm^{-1} versus the concentration of thiram.

effectively protect Ag NP from oxidation and endow the substrate long-term stability [35].

Tetramethylthiuram disulfides, commonly known as thiram, are widely used as plant fungicides to prevent bacterial infections, or used as a preservative to make ripe fruits or vegetables easy to store [36, 37]. However, improper or excessive use of thiram, would pose a health threat to human body. Therefore, it is necessary to realize the detection for safe levels of thiram, the general default maximum residue limit (MRL) set by China's National Food

Safety Standard (GB2763-2019) [36]. The absorption band of thiram locates at around 254 nm, and there is no resonance Raman under the 532 nm excitation [38]. The SERS spectra of thiram solutions ranging from 10^{-2} to 10^{-6} M are detected and exhibited in Figure 6E. The characteristic peaks of thiram at 567, 936, 1143, 1385, and 1512 cm^{-1} can be distinctively observed in the spectra. The LOD reached 10^{-6} M combined with the excellent linear relationship between intensity of SERS signal and concentration ($R^2 = 0.885$). This indicates that the quantitative detection

Table 1: A comparison between the reported multilayered nanostructures and the MAgNP-CuF substrate.

Structure	The layer number for maximum Raman enhancement	EF	Reference
Multilayered gold nanorods	One layer	1.23×10^7	[18]
Multilayered gold nanoparticles onto colloidal spheres	Two layers at 633 nm and three layers at 785 nm	9×10^5 at 633 nm	[19]
Multilayer Ag nanocubes	Two layers	3.76×10^7	[20]
Multilayer Ag NP using GO as spacer	Four layers	7×10^8	[28]
3D cross-point Au nanowires nanostructures	Five layers	4.1×10^7	[33]
Multiple Ag NP	Four layers	1.5×10^7	[34]
MAgNP-CuF	Six layers or more	7.8×10^9	This work

of thiram can be achieved in the concentration range of 10^{-2} – 10^{-6} M shown in Figure 6F, which is one order of magnitude lower than the MRL. The relationship was represented using the empirical equation: $\log I = 0.284 \log C + 3.617$. Hence, the MAgNP-CuF substrate is totally suitable for the actual detection of the pesticide thiram.

4 Conclusion

In summary, we have systemically studied a layer-dependent effect of MAgNP-CuF on SERS and surface-catalyzed reaction, combined with theoretical simulations. For this multilayered structures, the finite penetration depth of the incident laser affects the stacked plasmonic layers that could be stimulated. In the work, six or more layers could be penetrated for the highly transparent graphene film and the only 2.5 nm thickness Ag NP as plasmonic layer, which is much more than other multilayered structures and that should be a key point for the SERS performance. The role of the Cu film on the SERS performance was also evaluated, and it indicates Cu film reflect the excitation and scattered photons and conduce to the Raman signal enhancement. By way of contrast, an improved EF (7.8×10^9) is obtained, and the MAgNP-CuF realized ultra-low detection limit for toxic molecules such as the R6G, CV, thiram, etc. Moreover, the PNTp to DMAB conversion are also carefully studied on MAgNP-CuF. Interestingly, the plasmon-driven, surface-catalyzed reaction is also related closely to the number of Ag NP/graphene layers. We anticipate that the bifunctional MAgNP-CuF substrate would be widely used for both SERS sensors and plasmon-driven photocatalysis applications.

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