Deep Eutectic Solvent-Assisted Synthesis of Au Nanostars Supported on Graphene Oxide as an Efficient Substrate for SERS-Based Molecular Sensing

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ABSTRACT: The development of hybrid nanostructures of graphene oxide (GO) and metal nanoparticles (NPs) is of paramount interest for highly flexible surface-enhanced Raman scattering (SERS) substrate-based molecular sensing. In this work, we report a simple and eco-friendly synthesis strategy for the synthesis of a three-dimensional (3D) GO/gold nanostar (3D GO/Au NS) hybrid nanocomposite using deep eutectic solvent (DES) for SERS-based molecular sensing. The 3D GO/Au NS hybrid nanocomposite was obtained by a two-step synthetic process. In the first step, the GO nanosheets of thickness ∼1.25 nm were homogeneously dispersed in choline chloride/urea (molar ratio of 1:2)-derived DES, followed by functionalization of −NH groups using 3-amino-propyltriethoxysilane. Afterward, the presynthesized Au NSs of size ranging between 150−200 nm were then covalently attached on the −NH-functionalized GO nanosheets mediated by DES at 60 °C to obtain 3D GO/Au NS hybrid nanocomposites. Importantly, the SERS substrate fabricated using the 3D GO/Au NS hybrid nanocomposite exhibits highly enhanced SERS activity with an enhancement factor of 1.7 × 10^19 and high sensitivity for the detection of crystal violet with a concentration up to 10^-11 M. The green synthetic approach presented here can be expected to be promising for the large-scale fabrication of GO−metal NP-based hybrid nanostructures for their potential applications in SERS-based sensing.

1. INTRODUCTION

Surface-enhanced Raman scattering (SERS) spectroscopy has been validated as a highly powerful, specific, nondestructive spectroscopy technique for the detection of chemical and biological analyte molecules at a concentration down to a single-molecule level.1−4 After the discovery of Raman enhancement onto rough silver electrodes in 1974,5 it has become a rapidly growing field of research, which stimulated various emerging applications such as biosensors,6 bioimaging,7 biomarkers,8 bioanalyses,2 photothermal therapy,9 and monitoring catalytic reactions.10 The signal enhancement in SERS is largely governed by the intensification of the electromagnetic (EM) field originating from their sharp tips/edges of the Au NSs, which serves as SERS “hotspots” for amplified SERS signal enhancement.17−19

Recently, tremendous research efforts have been focused on the development of flexible and reproducible SERS substrates by combining plasmonic metallic NPs over different support matrices for practical applications.20−22 Among various substrate materials explored, two-dimensional (2D) layered GR or graphene oxide (GO) has attracted a significant attention for application in SERS because of its distinct properties and it is well known to interact easily with various organic analyte molecules via π−π stacking, which facilitates efficient charge transfer from the analyte molecules.23−26 Such excellent properties of GR or GO are of great research interest as an ideal substrate material for various applications in SERS,27 in which the SERS signal arises by the chemical enhancement mechanism (CMEM). To observe CMEM, the target molecules should be close to the substrates for chemical interaction to be effective.28,29 However, the CMEM has a short-range effect, which can contribute to the signal enhancement in the order of 10−100.24 Hence, combination of particular research interest because of their strong and easy tunable LSPR from the visible to near-infrared region and generation of intense localized EM field originating from their sharp tips/edges of the Au NSs, which serves as SERS “hotspots” for amplified SERS signal enhancement.17−19

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of Au NSs with GR/GO is expected to be a promising approach for the SERS substrates because of the synergistic effect of both EM and CMEM. Furthermore, previous studies have demonstrated that the SERS enhancement factors (EFs) and stability of the SERS substrates can be further amplified by forming a 3D GO/NP hybrid nanocomposite in order to diffuse the analyte molecules inside the porous structures.

Over the past few years, numerous synthetic approaches have been explored for the controlled synthesis of metal NPs supported over GR/GO nanosheets to obtain GO/NP hybrid nanocomposites. The ionic liquids have shown to be promising as an efficient medium for the controlled synthesis and functionalization of a variety of nanostructures because of their unique physicochemical properties. Recently, deep eutectic solvents (DESs) emerged as a new and advanced type of IL analogues, which are considered to be a potential alternative to standard ILs, have attracted great attention in the past few years for the synthesis of different nanostructured materials because of their excellent properties and potential technological applications. DESs are a class of liquids formed by the mixing proper ratios of two compounds having high melting points (i.e., hydrogen bond acceptor and hydrogen bond donor), which lead to the formation of eutectic mixtures with low melting points that are close to room temperature.

The main advantages of DES are as follows: it is derived from natural origin, has low toxicity, high biocompatibility, biodegradability, and low cost, and plays a dual role as a solvent and template for the nanostructure synthesis. Such fascinating properties of DES provide a promising platform for designing functional nanostructured materials that include controlled surface functionalization of NPs and syntheses of a variety of metallic, semiconducting, and porous carbon-based nanostructures. For example, Pethsangave et al. described a novel route using DES-assisted functionalization of GR and their potential application as an effective flame-retardant material. Recently, Guan et al. fabricated a porous carbon–GO composite using eutectic mixture composed of phosphoric acid (85% in water, PA), ethylamine hydrochloride (EA), and glycerol (Gly) in a 1:2:1 molar ratio. However, DES-assisted surface functionalization and synthesis of a metal NP-supported GO-based hybrid nanocomposite have not been explored enough.

In this work, we designed a facile and green synthetic approach for the controlled synthesis of a 3D GO/Au NS hybrid nanocomposite using ChCl/urea (molar ratio of 1:2)-based DES as a large-area SERS substrate. DES can be used as an effective solvent and template for surface functionalization of NH2 groups on GO and also to support Au NSs on the GO surface to form 3D hybrid nanostructures. Owing to the unique 3D structure, the resultant GO/Au NS hybrid nanocomposite manifests greatly enhanced SERS performance for detection of crystal violet (CV) probe molecules with concentration as low as 10−11 M and reproducible SERS signals. This DES-assisted synthetic approach to controlled synthesis, and assembly of Au NSs on GO nanosheets has several advantages such as eco-friendly, large-scale synthesis, high control over uniformity, and economic feasibility in comparison with other standard ionic liquid-assisted synthesis and wet-chemical synthetic routes.

2. RESULTS AND DISCUSSION

2.1. Synthesis and Structural Studies of the 3D GO/Au NS Hybrid Nanocomposite. As schematically shown in Figure 1, the hybrid 3D GO/Au NS nanocomposite was prepared by two-step synthetic steps using ChCl/urea (ratio of 1:2)-based DES as the solvent. In the first step, the GO nanosheets were uniformly dispersed in DES, followed by controlled functionalization of −NH groups on GO. For this purpose, the GO nanosheets were first treated with 3-aminopropyltriethoxysilane (APTES) in order to facilitate the amine (NH3) group-terminated surface through reacting NH2 groups of APTES with carboxylic groups of GO surfaces using DES as the solvent at 60 °C. In the second step, uniform Au NSs (size of 150–200 nm) were synthesized separately in DES using the HAuCl4 precursor and l-AA as reducing agents at 60 °C. The as-synthesized Au NSs are surface-passivated by Cl− because of the presence of a large amount of Cl− in the ChCl/urea-based DES. The pre.synthesized Au NSs were functionalized onto NH2-terminated surfaces through covalent coupling. Notably, in the presence of the dense NH2-functionalized surface of GO, the Au NSs tend to assemble into a one-dimensional (1D) chain-like structure. Previous studies demonstrated that the passivation of Cl− onto Au NSs shows the strong affinity between two adjacent particles, which also trigger assembly into a chain-like structure. In the present synthesis, both surface-functionalized NH2 groups and surface-passivated Cl− ions onto Au NSs favor the formation of a 1D assembled structure on the GO surface. To understand the synthesis better, we performed an additional one-step experiment by surface functionalization of APTES and subsequent in situ reduction of Au3+ into Au on GO nanosheets by keeping all other conditions the same. The resultant composite showed that flower-shaped Au nanostructures are effectively supported over the GO nanosheets (Figure S1, Supporting Information), in which no ID assembly was observed, suggesting that the Cl−-passivated Au NSs can play an important role in 1D assembly over GO.
The morphology of the as-synthesized 3D GO/Au NS samples was characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis. Figure 2a,b shows the SEM image of the 3D GO/Au NSs, which revealed the formation of a porous 3D-like structure, in which the Au NSs were supported over GO nanosheets. In addition, the energy-dispersive X-ray spectroscopy (EDS) mapping analysis demonstrated that the C, O, and Au elements are uniformly distributed as shown in Figure 2c−f, further suggesting the uniform dispersion of Au NSs on GO supports. Furthermore, EDS elemental analysis presented in Figure S2 also confirms the existence of only C, O, Si, and Au peaks, indicating that no additional elements are present in the hybrid nanocomposite. It should be noted that the existence of Si peaks in the sample indicates of successful APTES-functionalized GO nanosheets.

Figure 3a,b shows the TEM images of the obtained pristine Au NSs using DES as the solvent, which displays highly uniform Au NSs with an average size ranging between 150 and 200 nm. Figure 3c,d presents the TEM image of pristine GO nanosheets utilized for supporting Au NSs, which showed that the highly smooth surface is estimated to be 2−5 μM in length and ca. 200 nm in thickness. The TEM images of the 3D GO/Au NS hybrid nanocomposite are displayed in Figure 3e,f. As can be seen from Figure 3e,f, the uniform Au NSs are effectively supported over the GO nanosheets, in which Au NSs are assembled as 1D chain-like structures. In addition, a close observation of Figure 3f shows that the GO nanosheets are transformed into 3D porous structures after surface functionalization and decoration of Au NSs in DES. The TEM results are in good agreement with the SEM images in Figure 2.

To further demonstrate the structural details, the atomic force microscopy (AFM) analysis was also performed. Figure 4a−d displays the AFM images of the single GO nanosheet and 3D GO/Au NS hybrid nanocomposite and corresponding line profiles. As can be seen from the AFM topographical image of the pristine GO sheet in Figure 4a, a thin layered GO structure with a smooth surface was observed. The surface height profile was scanned across the single GO nanosheet (Figure 4c), which revealed that the thickness is about ∼1.25 nm and the size is about 1 μM. Figure 4b shows the typical AFM image of the GO/Au NS hybrid nanocomposite, which showed that some Au NSs are attached onto the surface of GO as can be visualized as bright spots. The structure of the Au NSs is not clearly visible, which may be due to the presence of the sharp edges in the Au NSs and porous structure of the support. The corresponding line-profile analyses scanned across the Au NSs in the resultant nanocomposite indicate that the height of Au NSs is about 200 nm (Figure 4d). Interestingly, a close observation of Figure 4b shows the formation of a 3D structure, which is in good agreement with the SEM and TEM results. The AFM results further support the successful incorporation of Au NSs over GO and formation of a 3D hybrid structure in the resultant sample.

To understand the structure and interaction in the GO/Au NS nanocomposite, we applied various spectroscopic characterization techniques. Figure 5a shows the UV−vis absorption spectra of the GO nanosheets, pristine Au NSs, and 3D GO/Au NS hybrid nanocomposite samples. The Au NSs exhibit two absorption peaks at 547 nm and a less intense peak at around 300 nm, indicating the LSPR peaks of an anisotropic

Figure 2. (a) Low- and (b) high-magnification SEM images of the 3D GO/Au NS nanocomposite. (c−f) Scanning TEM and corresponding EDS elemental mapping of the GO/Au NS nanocomposite.

Figure 3. (a) TEM image of the as-synthesized Au NSs with an average size of 150−200 nm and (b) self-assembled Au NSs. (c,d) TEM images of pristine GO nanosheets and (e,f) 3D GO/Au NS nanocomposite, respectively.
After supporting Au NSs over GO nanosheets, the appearance of both peaks of Au NSs and GO nanosheets suggests the effective formation of GO/Au NS hybrid nanocomposites. The powder X-ray diffraction (p-XRD) of GO and the GO/Au NS composite is shown in Figure 5b. The GO nanosheets exhibit a sharp peak at 2θ = 9.85°, which is ascribed to the d-spacing of 8.97 Å for the (001) plane. In comparison with GO, the obtained GO/Au NS nanocomposite samples display four additional peaks at 2θ = 38, 44.3, 64.45, and 77.3°, which are assigned to the (111), (200), (220), and (311) planes of the face-centered cubic (fcc) structure of Au.

Raman spectroscopy was used to examine the crystalline structure of the obtained pristine GO and GO/Au NS nanocomposite (Figure S3, Supporting Information). It is noticed from Figure S3a that the GO nanosheets exhibit a G-band peak at 1591 cm−1, which is higher relative to the original G-band peak of graphite (1685 cm−1). In addition, after decoration of Au NSs, the intensity of the Raman peaks increased, and a slight negative shift in the G-band peak from 1691 to 1578 cm−1 was observed. These changes are attributed to the strong enhancement of plasmonic effect caused by the supported Au NSs in the GO/Au NS nanocomposite sample. Furthermore, Fourier transform infrared (FTIR) spectra of the GO/Au NS nanocomposite were compared with those of the pristine GO nanosheets, as shown in Figure S3b (Supporting Information). The FTIR spectra of functionalized GO nanosheets showed several peaks such as hydroxyl bending vibrations (C−OH, 3326 cm−1), carbon−ketone bonds (1724 cm−1), C=O stretching vibrations of aromatic carbon atoms (C=O, 1656 cm−1), stretching vibrations of epoxide bonds (C−O−C, 1370 cm−1), and C=H bending and C−O stretching vibrations (1194 and 1039 cm−1), respectively. The peak positions are well consistent with the previous report of GO nanosheets. After supporting Au NSs onto the GO sheets, significant reduction in the intensity of the peak at 1724 cm−1 and slight decrement in the peaks at 1587 and 3326 cm−1 are associated with the C=O and O−H stretching vibrations, indicating the reduction of oxygen groups, which confirm the effective interaction of Au NSs with the GO nanosheets in the resultant composite.

Furthermore, the oxidation states and chemical interaction between Au NSs and GO were further investigated using X-ray photoelectron spectroscopy (XPS) analysis. Figure 6a shows that the high-resolution XPS spectrum for the C 1s region is fitted to three peaks. The prominent peak at 284.5 eV, which can be assigned to the C−N bonds, arose from the amine functional groups.
functionalization in the GO structure, while other two weak peaks at 286.80 and 289.60 eV, corresponding to the carbon–oxygen bonds (C–O or C–OH) and carboxylic acid groups (C=O), indicate the oxidized form of the sp² atomic structure of the graphene.53,55,56 Figure 6b shows that the O 1s XPS spectrum is fitted with two peaks at 532.7 and 530.6 eV, which are assigned to the C–O and C=O bonds, respectively. The N 1s XPS spectrum of the GO/Au NS hybrid nanocomposite in Figure 6c showed two peaks at 400.54 and 404.40 eV, corresponding to the carbon–amine bonds (C–N) and quaternary nitrogen atoms.55 The Au 4f XPS spectrum of the GO/Au NS nanocomposite sample is presented in Figure 6d. It exhibits peaks at 83.87 and 87.76 eV, which are assigned to the Au 4f⁷/₂ and 4f⁵/₂ core levels of metallic Au (Au⁰), whereas other less intense peaks at 84.86 and 89.88 eV correspond to the Au 4f⁷/₂ and 4f⁵/₂ core levels of the unreduced form of Au (Au³⁺), respectively. Notably, the presence of less intense peaks at 84.86 and 89.88 eV,

Figure 6. XPS spectra of the as-synthesized 3D GO/Au NS nanocomposite, (a) C 1s XPS spectrum, (b) N 1s XPS spectrum, (c) O 1s XPS spectrum, and (d) Au 4f spectrum, respectively.

Figure 7. SERS performance and sensitivity of the SERS substrates. (a) Comparison of the SERS signal of the GO nanosheet, GO/Au NPs, Au NSs, GO/Au NSs, and 3D GO/Au NSs and (b) corresponding SERS EFs. (c) Raman spectra of CV probe molecules with varying concentrations from 10⁻⁶ to 10⁻¹¹ M on the 3D GO/Au NS hybrid nanocomposite and (d) corresponding linear calibration plot at the SERS peak at 1620 cm⁻¹.
suggesting that the majority of Au are present in the metallic state (Au0).

2.2. SERS Performance of the GO/Au NS Hybrid Nanocomposite. It is well-known that, in comparison with the bare Au NSs, the GO/Au NS nanocomposite can produce much stronger SERS signal because of the synergetic effect of both EM and CMEM.34,57,58 Furthermore, controlled assembly of Au NSs over GO nanosheets can provide a large amount of inherent SERS “hotspots” because of the existence of gaps between the adjacent Au NSs, thereby generating extremely intense EM field at the conjunctions, resulting in remarkable enhancement in the overall SERS signal response.59 Thus, the SERS substrate fabricated using the as-synthesized 3D GO/Au NS hybrid nanocomposite is expected to exhibit highly improved SERS performance. The SERS measurements were carried out on the 3D GO/Au NS hybrid structure using a 633 nm excitation laser source and most commonly employed probe molecules (CV). Owing to its 3D structure, the GO/Au NS nanocomposite can enable to adsorb the analyte probe molecules (CV) from aqueous solution, making it possible to detect the specific analyte molecules from solution. Therefore, first we performed the SERS analysis in an aqueous dispersed sample with the same concentration, which was mixed with the aqueous solution of CV and incubated for 2 h before the SERS measurements. The SERS substrate of 3D GO/Au NSs showed higher SERS signals relative to the pristine GO and pristine Au NSs, respectively (Figure S4, Supporting Information).

Furthermore, it was well accepted that the SERS signal dramatically improved when the Au NP deposited over any substrates by forming multiple inherent “hotspots” over the substrates.20 Thus, substrate-based SERS measurements were carried out, and the SERS performance was evaluated. For preparation of the SERS substrate, a known amount of samples was deposited over the silicon (Si) substrates, followed by depositing the analyte molecule, CV (10−6 M), by a simple drop-casting technique and dried well prior to SERS analysis. To understand the SERS enhancement of the 3D GO/Au NS hybrid nanocomposite-based SERS substrate, we have prepared two different SERS substrates, and their SERS performance was compared with the 3D GO/Au NS nanocomposite. First, the spherical Au NPs (average size of 9 nm) were uniformly decorated onto GO nanosheets (Figure S5a,b). Second, the Au NSs were simply supported on GO nanosheets, without introduction of APTES functionalization steps that contain the same concentration of Au NSs (Figure S5c,d). As shown in Figure 7a, the substrate fabricated with the 3D GO/Au NS nanocomposite exhibited higher SERS signal intensity compared with the other tested SERS substrates. The higher SERS signal for 3D GO/Au NS nanocomposites can be due to the availability of a large amount of SERS “hotspots” between two adjacent Au NSs and efficient diffusion and adsorption of CV molecules in the 3D structure.

To evaluate the SERS performance of the 3D GO/Au NS hybrid nanocomposite, SERS EF was quantitatively estimated according to the previous report of Meng et al.60 by using the following equation

$$\text{EF} = \frac{I_{\text{SERS}}}{N_{\text{SERS}}} / \frac{I_{\text{Nor}}}{N_{\text{Nor}}} \quad (1)$$

where $I_{\text{SERS}}$ and $I_{\text{Nor}}$ are the SERS signal intensities of the CV-adsorbed GO/Au NS substrate and normal Raman spectra of CV at the prominent peak at 1620 cm−1 and $N_{\text{SERS}}$ and $N_{\text{Nor}}$ represent the corresponding number of CV molecules in the focused incident laser spot. It should be mentioned that it is extremely difficult to calculate the number of analyte molecules adsorbed onto the substrate and the number of CV molecules present at the laser irradiation site. Thus, by assuming uniform distribution of CV molecules over the GO/Au NS hybrid substrates, the substituted values of $N_{\text{SERS}}$ and $N_{\text{Nor}}$ by the concentration of CV subjected to obtain SERS spectra and the normal Raman spectra of CV (without substrate) are 1.0 × 10−6 and 1.0 × 10−3 M, respectively (Figure S6, Supporting Information).
Figure 7b compares the calculated SERS EFs for different obtained SERS substrates. The EF for 3D GO/Au NSs is about $1.7 \times 10^6$, which is higher relative to the GO/Au NSs ($1.6 \times 10^5$), Au NSs ($1.1 \times 10^5$), and GO/Au NPs ($0.3 \times 10^5$), respectively. The greater SERS EFs of the fabricated 3D GO/Au NS hybrid nanocomposite are due to the following parameters: (i) the assembly of uniform Au NSs over GO can provide a large number of gaps between two adjacent Au NSs, which serves as “hotspots” for SERS signal enhancement, and (ii) the 3D structure of the GO/Au NS nanocomposite permits the analyte molecule (CV) to diffuse inside the pores or cavities, which could produce stable SERS signals. The sensitivity of the substrates was estimated using the CV molecule that was adsorbed onto the GO/Au NS hybrid nanocomposite by varying the CV concentration. Figure 7c displays the Raman spectra obtained by varying the CV concentration from $10^{-6}$ to $10^{-11}$ M. As can be seen from Figure 7c, the Raman intensity increases gradually with increasing concentration from $10^{-11}$ to $10^{-6}$ M, and even at $10^{-11}$ M of CV concentration, the Raman peak was observed. In addition, the changes of the Raman intensity at a prominent peak at 1620 cm$^{-1}$ were plotted as a function of CV concentration (Figure 7d), revealing the linear dependence. Importantly, the observed sensitivity is greater or comparable with the previous report of the GO/metal NP hybrid nanocomposite as summarized in Table S1, suggesting the excellent sensitivity of the fabricated 3D GO/Au NS hybrid nanocomposite-based SERS substrate.

The reproducibility of the substrate is another important parameter for SERS-active substrates for their applications in various platforms. Thus, two different reproducibility tests were performed, and their reproducibility was evaluated. First, spot-to-spot Raman scanning in the same substrate was examined to evaluate the SERS reproducibility. Figure 8a depicts the Raman spectra of 15 random spots in the same substrates. The SERS intensities for 15 obtained SERS spectra are highly uniform, indicating that the SERS substrate is highly reproducible and stable. To quantitatively evaluate the SERS reproducibility, we estimated the relative standard deviation (RSD) of a prominent peak of the CV peak at 1620 cm$^{-1}$ for 15 SERS spectra (Figure 8b). The RSD value is estimated to be $\pm 13.2\%$, which suggests the excellent SERS reproducibility of the as-fabricated SERS substrate. Second, the substrate-to-substrate reproducibility of the 3D GO/Au NS substrate is carried out by collecting a SERS signal from eight different substrates with the same condition. Figure 8c shows the SERS spectra of eight different substrates with the same CV concentration ($10^{-6}$ M). The RSD of the Raman intensity of the most prominent peak for CV at 1620 cm$^{-1}$ is calculated to be $\pm 11.4\%$. This value is significantly lower than the maximum threshold RSD for any SERS-active substrate, which is determined to be about 20% which indicates the outstanding reproducibility of the designed substrates. Furthermore, the stability of the fabricated GO/Au NS hybrid nanocomposite substrate was evaluated by collecting the SERS spectra under continuous laser irradiation at the same spot (Figure S7, Supporting Information). As can be observed in Figure S7, the prominent Raman peak intensity at 1620 cm$^{-1}$ of the probe molecule (CV) gradually decreases with the repeated irradiation by the laser beam, and the SERS peaks are visualized after six irradiation cycles at the same site of the substrate, suggesting reasonable stability of the substrate. The decrease in the SERS signal upon continuous irradiation can be understood by the fact that damage of the laser irradiation site on the substrate as well as due to partial degradation of CV molecules.

3. CONCLUSIONS

In summary, an eco-friendly route to fabricate a 3D GO/Au NS hybrid nanocomposite using choline chloride/urea (1:2 molar ratio)-based DES as an efficient substrate for SERS-based molecular sensing is reported. Specifically, the NH-functionalized GO was decorated with Au NSs of size ranging between 150–200 nm by an ex situ synthesis route using nonaqueous DES. DES was found to play an important role as both a solvent and soft template for controlled surface functionalization of GO nanosheets with $\text{NH}_2$ groups and subsequent formation of a 3D GO/Au NS hybrid nanocomposite. Importantly, the SERS substrate fabricated using the 3D GO/Au NS hybrid nanocomposite exhibited enhanced SERS activity and stability for the detection of a probe molecule (CV) with high sensitivity of CV concentration as low as $10^{-11}$ M. Furthermore, the SERS substrate showed excellent stability and reproducibility with a small RSD of 13.2%. The excellent SERS performance of the 3D GO/Au NS nanocomposite is mainly related to the presence of abundant SERS “hotspots” in the composite as well as 3D morphology. Furthermore, the 3D morphological features allowed for the CV molecule to diffuse into the porous structure, resulting in a stable and reproducible SERS signal. This green, DES-assisted synthetic approach to design 3D hybrid nanocomposites is expected to be a promising platform for controlled fabrication of GO-based hybrid nanocomposites in a nonaqueous environment for their potential applications in SERS-based sensing.

4. EXPERIMENTAL SECTION

4.1. Chemicals. Graphite powder, potassium permanganate ($\text{KMnO}_4$), sulfuric acid ($\text{H}_2\text{SO}_4$), hydrogen peroxide ($\text{H}_2\text{O}_2$), choline chloride (ChCl), urea, chloroauric acid ($\text{HAuCl}_4$·$4\text{H}_2\text{O}$, 99.9%), l-ascorbic acid (AA, 99%), sodium borohydride ($\text{NaBH}_4$, >98%), APTES (99%), and CV (90%) were purchased from Sigma-Aldrich, Mexico. All the chemicals were utilized as received, and all the glasswares were washed thoroughly using deionized (DI) water prior to their use in experiments.

4.2. Synthesis of GO Nanosheets. GO was synthesized from graphite powder by following a modified Hummer’s method. Briefly, 2 g of graphite powder was dispersed in 46 mL of $\text{H}_2\text{SO}_4$ at 0 °C, followed by the addition of 6 g of $\text{KMnO}_4$. The mixture was heated to 35 ± 2 °C under vigorous stirring and reacted for 2 h. After that, 92 mL of distilled water ($R = 15 \text{ MΩ·cm}$) was slowly added to the reaction mixture and kept under magnetic stirring for 15 min. The reaction was terminated by transferring the mixture to a beaker containing $\text{H}_2\text{O}_2$ at 1% to remove the remaining $\text{MnO}_2^-$, For further purification, the mixture was washed using centrifugation first with 1% HCl and then with distilled water several times at 3000 rpm for 5 min. The resulting GO was dried for 12 h at 65 °C, and then GO powder was exfoliated in a sonication bath (Bransonic 1510, 40 KHz) in 10 mg/mL of water for 3 h to obtain a brown-colored GO nanosheet dispersion.

4.3. Preparation of ChCl/Urea-Based DES. Choline chloride (ChCl)/urea-derived DES (molar ratio of 1:2) was prepared using the previously reported protocol. Prior to the
preparation, ChCl was heated to 90 °C for 2 h in a vacuum oven in order to dry the moisture content if any. DES was obtained by mixing ChCl/urea with a molar ratio of 1:2, followed by heating the mixture at 80 °C for 2 h, and then a colorless liquid is obtained. After that, the formed DES was cooled down to room temperature, closed tightly, and then saved in an ambient condition for further synthesis.

4.4. DES-Assisted Synthesis of the 3D GO/Au NS Nanocomposite. The Au NSs were first synthesized following our previously reported protocol. Briefly, 10 mg of gold precursor (HAuCl₄·4H₂O) was added to 20 mL of freshly prepared DES and magnetically stirred at 60 °C until a homogeneous dispersion was obtained. Separately, 50 mg of 1-AA was mixed with 10 mL of DES solution and then magnetically stirred at 60 °C until 1-AA was completely dissolved. Then, the DES-containing 1-AA solution was quickly added to the reaction mixture and magnetically stirred for another 30 min in a closed environment. The yellow-colored solution mixtures immediately turned transparent and then slowly changed into a dark brown color, suggesting the formation of Au NSs. The resultant Au NS was separated by adding an equal amount of DI water and centrifuged at 7000 rpm for 15 min 4 times.

For the preparation of GO/Au NSs, 5 mg of presynthesized clean GO nanosheet powder was dispersed in 20 mL of DES and magnetically stirred at 60 °C for 15 min to obtain a well-dispersed GO/DES mixture. Then, 0.1 mL of APTES solution was slowly added into the reaction and magnetically stirred at 60 °C for 6 h to obtain NH₂-terminated GO nanosheets. After that, 10 mg of presynthesized Au NSs was added to the reaction under magnetic stirring for another 30 min to obtain 3D GO/Au NS nanocomposites. The resultant product was cleaned and separated using the similar steps as that used for Au NSs. In a typical in situ synthesis of GO/Au NSs, 5 mg of GO nanosheets was dispersed in 20 mL of DES, and 0.1 mL of APTES was added into the solution mixture and magnetically stirred at 60 °C for 6 h. After that, 10 mg of the HAuCl₄ precursor was added to the solution mixture and magnetically stirred for another 30 min. Subsequently, 50 mg of AA was dissolved separately in 10 mL of DES, and the 1-AA-containing DES was added to the solution mixture and magnetically stirred for 30 min. The final product was separated by the same protocol used for the ex situ synthesis.

4.5. Characterization Techniques. The morphology of the Au NSs and GO/Au NS samples was analyzed by TEM using a JEOL JEM-1010 microscope operating at 80 kV. The samples for TEM measurement were prepared by dispersing the colloidal nanostructures over carbon-coated Cu grids and subsequent drying at room temperature overnight. The AFM of the samples was carried out using a Bruker (scanAsyst-Air model) AFM microscope operating in tapping mode. For AFM analysis, the dispersed samples were deposited onto the Si substrate and dried at ambient conditions. UV−vis spectra of the samples were recorded with an Agilent 8453 UV−vis spectrophotometer. The XRD patterns were obtained with an X-ray diffractometer (Rigaku Ultima IV, using Cu Kα radiation) using parallel-beam geometry with 2θ scan between 20 and 80°. The XPS spectra of the samples were obtained using an XPS spectrometer (Intercovamex-XPSS110) using nonmonochromatic Al Kα (1486.7 eV) X-ray source. All the recorded XPS spectra were corrected utilizing the C 1s line at 284.8 eV. Infrared transmittance spectra (FTIR) of the samples were collected from a Thermo Scientific (NICOLET 6700) spectrometer, equipped with a universal ATR accessory in the spectral range of 4000−650 cm⁻¹. Raman and SERS spectra of the samples were collected utilizing a Bruker (SENTERRA) Raman spectrometer equipped with a He−Ne laser of 633 nm excitation wavelength (λex).

4.6. SERS Measurements. Solution-based SERS performance was carried out by the same amount of as-synthesized GO/Au NS hybrid nanocomposite samples (1 mg/mL in DI water) that were mixed with 25 μL of an aqueous solution of CV with a concentration of 10⁻⁶ M by magnetic stirring for 2 h. After that, the solution containing CV-adsorbed samples was transferred to a glass cuvette to collect the SERS spectra. For the substrate-based SERS measurements, the samples of the same concentrations (1 mg/mL) were first dispersed in a DI water using sonication for 30 min, and then 0.1 mL of well-dispersed samples was drop-casted over silicon (Si) wafer (8 mm × 8 mm). After that, 25 μL of an aqueous CV solution (10⁻⁶ M) was dropped over the sample and dried under the ambient condition. The CV deposited substrates were then washed with DI water and dried prior to the SERS analysis. Raman spectra were collected using an excitation wavelength of 633 nm He−Ne laser with a laser power of 4.7 mW under 50× lens, and the signal acquisition time was fixed at 2 s for all samples.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02759.

■ TEM and SEM images of GO/Au NSs, FTIR, Raman spectra of the GO/Au NSs, SERS spectra of reproducibility, and stability analysis (PDF)

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Notes

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