Research Article

Economically reproducible surface-enhanced Raman spectroscopy of different compounds in thin film

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ABSTRACT

We report herein an economically cheap and functionally stable surface-enhanced Raman scattering (SERS) protocol of two photoactive pigments Rhodamine 6G (R6G) and Kiton Red (KR), implemented in thin films of silver (Ag) and gold (Au) nanoparticles (AgNPs and AuNPs). Both commercially available and chemically synthesized nanoparticles were used. The suitability of the nanoparticles toward SERS activity was tested through UV-visible absorption spectroscopy and scanning electron microscopy (SEM). The AgNPs and AuNPs based SERS substrates in the form of films were fabricated onto square-sized aluminum (Al) plates by simple drop deposition of colloidal nanoparticles solution onto their polished surfaces. The prepared nanoparticle films were sufficiently dried and coated further with the probe (R6G and KR) molecules by employing the identical deposition technique. The enhanced Raman signals of R6G and KR in such composite film structures were then recorded through a custom-built dispersive Raman spectrometer with He-Ne laser excitation at 632.8 nm. Our AgNPsfilm-based SERS protocol could yield the magnitude of the Raman signal enhancement up to 10^4 times for both R6G and KR. Moreover, AuNPs-based film was found to be less efficient toward the Raman enhancement of both compounds. Our SERS substrates can be easily fabricated, and SERS spectra are reproducible and stable, allowing one to consistently get a reproducible result even after 6 months.

Introduction

Raman signal arises mainly from a magnetic dipole active vibrational transition of a molecule; therefore, it is intrinsically very weak in nature (Campion and Kambhampati, 1998). To increase the Raman signal, a versatile technique, surface-enhanced Raman spectroscopy (SERS), is often introduced (Otto, 1982). SERS is better in terms of the capability of identifying chemical species and obtaining structural information in a wide variety of fields, including polymer and materials science, biochemistry and bio-sensing, catalysis and electrochemistry, and so on (Sharma et al., 2012; Cardinal et al., 2017). SERS enhancement is hypothesized to originate from two separate phenomena: (i) enhancement of the local electromagnetic field surrounding the small object of nanoscale dimension optically excited near an intense and sharp plasmon resonance. The enhanced fields excite the adsorbate (probe), and the SERS signal is produced (Lee et al., 2007), (ii)

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chemisorption of specific functional groups like an amino group to the noble metallic nanoparticle such as Ag/Au, whereby the transfer of lone pair electrons takes place from the probe molecule to the metallic nanoparticle (Campion et al., 1995) and hence producing charge-transfer species. The first phenomenon is known as electromagnetic enhancement, while the second one is referred to as chemical enhancement.

SERS obtained through colloidal nanoparticle solution requires minimal sample preparation and demonstrates high enhancement; however, lack of reproducibility is a major drawback. On the other hand, thin films of nanoparticles made from dried colloids on a base substrate offer more reproducible enhanced Raman signal intensities, which is considered as a more accessible alternative for obtaining SERS. To get a significantly improved signal from metallic nanoparticles/nanoclusters-based film, selecting a suitable base that supports strong plasmon resonances is of fundamental importance. Different types of film-based SERS substrates are traditionally fabricated onto Au (Michota and Bukowska, 2003; Abdelsalam et al., 2005), Ag(Shanmukh et al., 2006), paper (Zhu et al., 2015), glass (Liu et al., 2012), or silicon (Chan et al., 2005) bases via deposition of the nanoparticles/clusters (Markelonis et al., 2015). Structures made of Au or Ag are mainly used for SERS (Farcau and Astilean, 2010) as they have the suitable optical properties to sustain good plasmon resonances in the visible/near-infrared range (~400-1000 nm) (Liu et al., 2011; Fan et al., 2011). Although Au, Ag, or silicon substrate is suitable for SERS but has the disadvantage of a high cost. On the other hand, common glass is a cheap material but presents a high fluorescence signal overlapping the SERS signal. Aluminum and copper foil (Gudun et al., 2017; Markelonis et al., 2015; Jiang et al., 2013) have the disadvantage of being too flexible, making the surface nonuniform. SERS on paper are not adequately stable, and the signal intensity decreases significantly just after a few days (Chamuah et al., 2017). Low-cost SERS substrate yielding enhanced and reproducible Raman signal even after months of storage has been a long-standing goal for researchers for years. Herein, we employed Al plate as the base for AgNPs and AuNPs associated SERS substrate for inducing enhancement on the Raman signals of two selective Raman active chromophores R6G and KR. For doing so, we have fabricated a thin layer of AgNPs or AuNPs onto polished Al plates to adsorb the R6G or KR and concomitantly observe their Raman signals enhancement via a custom-built dispersive Raman spectrometer. Our AgNPs based SERS film protocol could yield up to 10^4 times larger Raman signal for both the two chromophores with economical reproducibility and high stability (stable SERS spectrum even after 6 months).

**Experimental**

**Chemicals:** Silver nitrate (99.8%), tri-sodium citrate (99%), R6G (99%), KR (99.5%), and the commercially available colloidal solutions of AgNPs/AuNPs (will be termed as commercially available AgNPs/AuNPs) were purchased from Sigma-Aldrich. All chemicals and commercially available AgNPs/AuNPs were used as received without further purification.

**Preparation of Silver Colloidal Suspension:**

AgNPs were prepared by reducing silver nitrate (AgNO₃) with sodium citrate based on the method reported by Lee and Meisel, (1982). Briefly, 90 mg of AgNO₃ was dissolved in 500 mL distilled water. The
solution was heated until boiling. Then, a 10 mL aliquot of 1% sodium citrate was added dropwise into the solution, and boiling was maintained until the volume was reduced to half the initial volume. The drop-wise addition of the sodium citrate was accompanied by vigorous stirring. The formation of the AgNPs is primarily confirmed by monitoring the gradual change of the color of the solution to pale yellow. The AgNPs colloid prepared through this protocol typically has a size of less than 50 nm (Lee and Meisel, 1982).

The absorption maximum of the synthesized AgNPs colloidal solution was observed at around 438 nm. The colloidal AgNPs prepared with this method were further characterized using the SEM technique. The average diameter of the AgNPs was found to be 60 nm.

**Preparation of Samples for Raman Measurements:** The thin films of probe molecule (R6G or KR \(\sim 10^{-4} \text{ M}\)) for Raman measurement were prepared onto the polished surface of a square-sized Al plate by drop-casting of dye solution followed by spontaneous evaporation of the solvent and dye thin film on Al plate was obtained for Raman measurement.

**Preparation of Samples for SERS Measurements:** To prepare the SERS substrate, a thin film of AgNPs/AuNPs was deposited onto the polished surface of a square-sized Al plate by drop-casting (Martínez-Torres et al., 2015) of the aqueous solution of NPs followed by spontaneous evaporation of the solvent (Fig. 1). Finally, the probe molecule (R6G or KR \(\sim 10^{-4} \text{ M}\)) was adsorbed onto the prepared thin film of NPs on Al plate by following the same deposition technique, i.e., drop-casting of dye solution followed by spontaneous evaporation.

**Surface-Enhanced Raman Scattering Measurements:** All SERS measurements were carried out using a custom-built dispersive Raman spectrometer equipped with a He-Ne laser excitation (Fig. 2). The 632.8 nm He-Ne laser at a power of 10 mW was used for all the (Raman and SERS) measurements.

The laser beam was focused on the sample, and the subsequent Raman-scattered radiation was collected at an oblique collection geometry at (70 to 90)\(^\circ\) a large-aperture convex lens (f/0.98), which was ultimately focused to the entrance window of the optical fiber. A Weuseda 632.8 nm notch filter (COHERENT) was used just in the front of the optical fiber entrance, which in turn allows the desired Raman signals to pass through it and thus attenuating the unwanted Rayleigh scattering. The outlet of the optical fiber was connected to the entrance slit of Acton Spectra Pro-2758, the spectrograph with a Czerny Turner configuration. We used the grating 600 grooves/mm blazed at 500 nm for recording all SERS spectrums in this study. The output port of the spectrometer is equipped with a 1024×1024 pixels imaging array CCD camera (Princeton PI-MAX with unigene II coating) cooled to \(-20^\circ\text{C}\) by a Peltier cooler which records the spectrum. Ten SERS spectra were acquired from each sample using the Winspec/32 software and were averaged to obtain the SERS spectra presented in this study. The exposure time and accumulation were 60 s and 1, respectively. No additional data processing was used for any of the SERS spectra presented in this study.

**Stability performance.** The stability performance of the proposed SERS substrate (the thin film of synthesized AgNPs/AuNPs on a square-sized Al plate) has also been evaluated for both chromophores R6G and KR. To do that
R6 and KR were adsorbed on SERS substrate, which was then stored inside a desiccator at ambient temperature at dark for six months. The reproducibility of the SERS protocol was verified by comparing the Raman signals captured under the same experimental conditions before and after the six months storage period.

**Spectrophotometer:** The absorption spectra of the prepared colloidal solution of AgNPs and AuNPs have been recorded in the wavelength range 200 nm to 800 nm using a spectrophotometer (UV 1800, Made in Japan) with a 1-cm optimal length cuvette.

**Scanning Electron Microscope:** A Hitachi-S3400N model SEM instrument was used to determine the microstructure and particle size of nanoparticles onto the Al substrate. The samples were imaged after spotting on the substrate with an accelerating voltage of 10 kV.

**Results & Discussion**

All the Raman spectroscopic investigations reported in this study were performed on a modified dispersive Laser Raman system, and unlike the one we reported earlier (Wahadoszamen et al., 2015), this Laser Raman system was constructed with a
spectrograph coupled to an intensified CCD (ICCD) camera. Therefore, to optimize and ensure the newly configured and modified Raman system's reliability, we first carried out the Raman protocols on different substances that have well-recognized Raman signals (like Benzene, Aniline, and Chlorobenzene). Upon having the reliable Raman setup, we approached to develop a reliable, stable, and reproducible, cost-effective SERS protocol in a thin film of metallic nanoparticles. A successful SERS protocol has several simultaneous requirements, such as selecting suitable Raman active compounds and suitable metallic nanoparticles (with typical size within 100 nm) and proper excitation wavelength. In addition, a suitable metallic surface of nm order surface roughness can be employed for observing SERS activity (Lee et al., 2015). For carrying the SERS experiment, the thin film of AgNPs or AuNPs (30 nm-70 nm) on the polished surface of a square-sized Al plate was used in this study as SERS substrate, whereas R6G and KR served as the SERS probes. The UV-VIS absorption spectra (200 nm-700 nm) of the colloidal solutions of AgNPs and AuNPs are shown in Fig. 3. The colloidal solutions of chemically synthesized and commercially available AgNPs exhibit well-defined absorption peaks at around 438 nm and 426 nm, respectively, whereas AuNPs give defined and characteristic absorption peaks at 512 nm. On the other hand, from the SEM images (Fig. 4), we can observe that the colloidal solutions of AgNPs and AuNPs are nearly spherical with a narrow size distribution within 30-70 nm, hence pointing the good utility of these metallic nanoparticles towards SERS activity.

Fig. 3. UV-VIS absorption spectrum of chemically synthesized AgNPs (Red line), commercially available AgNPs (Black line) & AuNPs (Green line).

For Raman measurement, the 632.8 nm He-Ne excitation source was used to overcome the severe fluorescence of some samples while using the 514 nm excitation of the Argon-ion laser. Before implementing the SERS protocol, we attempted to measure the intrinsic Raman signatures of R6G and KR (of concentration $10^{-4}$ M) by coating their thin film onto the Al plates. The measured Raman signals are shown in Fig. 5. One can easily notice that both the dyes at this concentration exhibit weak and barely resolved Raman signals.

Then we focused on detecting enhanced Raman signals for both the compounds by using the thin films of AuNPs (commercially available) and two batches of AgNPs (both commercially available and chemically synthesized). As discussed in the material method section, the thin films of the nanoparticles were prepared by drop-casting their colloidal solutions onto the polished surface of Al plates followed by spontaneous evaporation of the solvent at ambient temperature without any further special treatment on the Al surface.
A key motivation for such preparation was laid on the fact that an inhomogeneous deposition of the nanoparticle thin film produces inhomogeneous roughness of nanometer-scale onto the slide surface, generating super-active hotspots for SERS activity (Kleinman et al., 2013). Therefore, after forming a thin layer of metallic nanoparticles, dye solution (R6G and KR of concentration $10^{-4}$ M) is poured drop-wise onto the thin NP layer and subsequently dried to produce a composite film of probe molecules and NPs onto the Al base plate. In such a preparation, the probe molecules are supposedly in good contact with metallic nanoparticles and are suitable for obtaining SERS signals.

Fig. 6 represents the Raman signal of R6G (a) and KR (b) taken on their composite films with chemically synthesized AgNPs. It is evident that, while measured through a composite thin film with AgNPs, the barely

Fig. 4. SEM micrograph of (a) chemically synthesized AgNPs, (b) commercially available AgNPs, and c) commercially available AuNPs.

Fig. 5. Raman signal of (a) R6G, (b) KR (of concentration $10^{-4}$ M). All Raman spectra were recorded with He-Ne laser with an acquisition time of 60s. For the Raman measurement, a thin film of dye solution (R6G or KR $\sim 10^{-4}$ M) is coated on square-sized Al plates by simple drop deposition of dye solution onto their polished surfaces followed by spontaneous evaporation of the solvent.
distinguished and weak Raman signals of both compounds become spectrally well resolved and distinct and characterized by remarkable intensities. In addition, some of the bands which could not even be detected in the flat Raman background signal of both the compounds, exhibit spectacular appearance in the enhanced Raman spectrum (Fig. 6). These findings demonstrate the superb sensitivity of the chemically synthesized AgNPs thin film towards inducing considerable enhancement of Raman signals of both compounds.

A similar protocol was implemented for both compounds using commercially available AgNPs and AuNPs. We observed that a thin layer of commercially available AgNPs also enhances the Raman signal of both the compounds, however, the magnitude of the enhancement was found to be not as large as the one obtained by the chemically synthesized AgNPs thin film. The less enhancement of the Raman signal of commercially available AgNPs thin film may be associated with some stabilizing agents often used for capping the nanoparticles to prevent possible coagulation.

On the other hand, we could observe the least amount of Raman enhancement for both compounds through the composite film of commercially available AuNPs. The lower magnitude of the Raman enhancement may be associated with the larger surface Zeta potential of AuNPs, which may inhibit the probe molecule from making better noncovalent contact for Raman enhancement (Alvarez-Puebla et al., 2005). To better compare the magnitude of the Raman intensity, we have overlaid all the compounds' spectra in two panels, as shown in Fig. 7. We then finally estimated the magnitude of the Raman enhancement of different Raman modes resolved in the intensity enhanced spectra. As we observed maximum intensity enhancement for both compounds using the film of synthesized AgNPs, we only estimated the magnitude of the intensity enhancement for this film.
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Fig. 7. (a) The comparison among Raman signals (Black line) and enhanced Raman signals of R6G for AuNPs thin film (Magenta line), commercially available AgNPs thin film (Blue line), and synthesize AgNPs thin film (Red line). (b) The comparison among Raman signal (Black line) and enhanced Raman signals of KR for commercially available AuNPs thin film (Magenta line), commercially available AgNPs thin film (Blue line), and synthesize AgNPs thin film (Red line). All Raman spectra are captured with an He-Ne laser with an acquisition time of 60s.

Fig. 8. Comparison of enhanced Raman signal of R6G adsorbed on AgNPs thin-film coated Al plates, recorded under identical experimental conditions.
The maximum magnitude of Raman intensity enhancement for R6G and KR (concentration \( \sim 10^{-4}\text{ M} \)) adsorbed on chemically synthesized AgNPs coated Al substrate was estimated to be of the order of \( 10^4 \). In our earlier work, we note that we reported a lower magnitude of Raman signal enhancement (\( \sim 10^3 \)) of R6G by implementing SERS protocol using the colloidal solution of synthesized AgNPs (Wahadoszamen et al., 2015). Therefore, the thin film of AgNPs seems to be more efficient in producing the Raman enhancement of R6G in agreement with the hypothesis of super-active hotspots formation (Kleinman et al., 2013). Martínez-Torres et al. (Martínez-Torres et al., 2015) reported enhancement factor as \( \sim 10^6 \) using a solution of R6G on Al-foil where Au nanocuboids have been drop cast and allowed to dry spontaneously. Martinez-Garcia et al. (2016) measured EF in the order of \( \sim 10^5 \) using the drop-dry method to obtain dried concave gold nanocubes solution on Al-6063 slides on which the drop of the sample (R6G) had subsequently dried. Although our study shows less magnitude of Raman enhancement, however, the study's novelty lies in the fact that the prepared composite film can retain the enhancement even after six months (Fig. 8).

Glass, silicon wafer, and quartz surfaces are generally used as solid SERS substrates. Physical and chemical procedures need to be performed to prepare the surface of solid substrates before depositing colloidal metal nanoparticles (Saleh et al., 2016). However, in our protocol, the Al substrate is proposed as solid substrates, which can be used without special treatments on its surface. Unlike glass, it does not exhibit fluorescence. Although the uses of Al-foil (Martínez-Torres et al., 2015), paper (Chamuah et al., 2017), or sandpaper (Fan et al., 2014) are cost-effective as SERS substrate, they are suffering from poor stability and are too flexible to store for a long time. Periodic nanoholes on Al substrate (Lee et al., 2015) have also been employed as SERS substrate; however, it involves high cost due to the use of advanced techniques such as electron beam lithography, focused ion beam, and soft lithography (Kahraman et al., 2017). Proposed Al-alloy as solid SERS substrate is advantageous over traditional SERS substrate because of the requirement of minimum processing, low cost, and most importantly, high stability, as the observed intensity of SERS signal is well-preserved (with 5-10% drop of average intensity) even after 6 months(Fig 8).

**Conclusion**

We have demonstrated the enhancement of the Raman signal of R6G and KR on a thin film of AgNPs/AuNPs on an Al substrate where the thin film was obtained by simple drop-casting of NPs followed by drying. Based on the developed SERS substrate, we could measure an enhancement of the order of \( 10^4 \) by detecting R6G and KR with a concentration as low as \( 10^{-4} \text{ M} \). The spectral intensity for the developed NP thin film on Al SERS substrate is maintained even after six months unlike conventional SERS substrates and hence can be considered as remarkably stable. In conclusion, it is plausible to state that the proposed SERS substrate could be used as an ultra-low-cost, reliable, and storable SERS substrate for detection, quantification of Raman signals of various chemicals and biological samples.
Data Availability
The data used to support the findings of this study are included in the article.

Conflicts of Interest
The authors declare that they have no conflicts of interest regarding the publication of this article.

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