REMOVABLE SUBSTRATE FOR SURFACE ENHANCED RAMAN SPECTROSCOPY FOR THE STUDY OF PIGMENTS FROM THE COLONIAL ERA

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Introduction. Raman spectroscopy detects the vibrations of a molecule that scatters light inelastically (Raman scattering, with an occurrence of 1 in 10⁸ scattered photons for this phenomenon to be generated). Its characteristics of high molecular specificity, semi-destructive nature, and ability to perform in situ analysis provide it with great versatility for the analysis of works of historical value [1]. It has been used successfully in the analysis of historical paints to identify pigments, dyes, and organometallic complexes [2, 3]. However, organic molecules often produce the fluorescence phenomenon that analytically overlaps the analyte signal due to the longer extinction time compared to Raman scattering [4, 5]. A technique developed to avoid the generation of the fluorescence phenomenon and the risk of carbonization or alteration of the sample during the analysis, is Surface-Enhanced Raman Spectroscopy (SERS) [1].

In the SERS technique, electrons in the conduction band of a noble metal nanostructure (also called a SERS substrate) oscillate in resonance with incident light from a laser upon contact with the surface of the substrate, known as Localized Surface Plasmon Resonance (LSPR) [1]. This resonance condition is fulfilled in the visible region of the silver (Ag) and gold (Au) nanoparticles (NPs) [1, 6]. If a Raman-active molecule is in the vicinity of the nanostructured surface, the occurrence of LSPR causes the Raman signal of the analyte to become stronger than in the Raman technique [7]. In this way, low laser intensities can be used to avoid damaging the analysis surface and reduce the generation of fluorescence that many complex samples produce.

The main problem of the SERS technique is related to the form of application of the substrates on the surface of the sample, making it impossible to use them in the form of colloids for the analysis of cultural heritage works, because it leaves residues that are difficult to remove, without invade the surface of the sample resulting in its contamination by the metallic NPs [8].

In this type of analysis, applying the SERS technique requires developing a substrate that presents the main characteristic that, after the measurements, it
can be removed and limits residues on the surface under study. It is proposed to prepare and characterize a viscous active SERS gel that is constituted of Ag or Au NPs, where SERS measurements can be performed [8–10]. Methylcellulose can be chosen as a suitable substrate to produce the matrix that adds the metal NPs, since it is one of the most stable cellulose ethers that tends to be resistant to thermally induced discoloration and is inert to the electromagnetic radiation used to produce the Raman effect [10].

**Methodology.** The reagents used for the production of removable SERS substrates (gels) were: silver Nitrate (AgNO$_3$, 99% of metal traces), gold chloride hydrate (III) (HAuCl$_4$·xH$_2$O, 99% of metal traces), dihydrated tribasic sodium citrate (C$_6$H$_5$Na$_3$O$_7$·2H$_2$O), sodium hydroxide (NaOH, ≥98%), methylcellulose 4000 CP, all acquired from Sigma Aldrich; hydroxylamine hydrochloride (NH$_2$OH·HCl, ACS ≥99%) of Fermont, and ultra-pure water (type 2) of Milli-Q.

**Silver nanoparticle colloid**

C$_6$H$_5$Na$_3$O$_7$·2H$_2$O (Lee-Meisel Modification: C-Ag@Cit). 10 mL of a fresh 0.01 M C$_6$H$_5$Na$_3$O$_7$·2H$_2$O solution were prepared, adjusting the pH of the solution to a value of 9. Subsequently, 5 mL of the 0.01 M C$_6$H$_5$Na$_3$O$_7$·2H$_2$O solution were added to a reflux system at temperature and constant stirring. After five minutes, 2 mL of 0.1 M AgNO$_3$ were added. Finally, one hour was waited to stop the reaction. The result is a colloid with a thick green color.

NH$_2$OH·HCl (Peng-Zhong Modification: C-Ag@HX). 10 mL of 0.01 M NH$_2$OH·HCl were prepared, adjusting the pH of the solution to a value of 9. Subsequently, with constant stirring, 1 mL of 0.1 M AgNO$_3$ was added. Finally, the reaction was stopped for one minute. As a result, a colloid with a thick brown color appearance was obtained.

**Gold nanoparticle colloid**

C$_6$H$_5$Na$_3$O$_7$·2H$_2$O (Turkevich Modification: C-Au@Cit). 10 mL of a fresh solution of 0.034 M C$_6$H$_5$Na$_3$O$_7$·2H$_2$O were prepared. Subsequently, 2 mL of 0.01 M HAuCl$_4$·xH$_2$O were diluted to obtain a 0.002 M concentration. Then the 0.002 M HAuCl$_4$·xH$_2$O solution was added to a reflux system with temperature and constant stirring. After five minutes, 2.4 mL of 0.034 M C$_6$H$_5$Na$_3$O$_7$·2H$_2$O were added. Finally, one hour was waited to stop the reaction. A violet-colored colloid was obtained as a result.

NH$_2$OH·HCl (Luca-Filippo Modification: C-Au@HX). 10 mL of a 0.1 M NH$_2$OH·HCl solution were prepared, adjusting the pH of the solution to a value of 12. Subsequently, with constant stirring, 1 mL of 0.01 M HAuCl$_4$·xH$_2$O was added. Finally, 1 minute was waited to stop the reaction. A blue colloid was obtained.

**Removable SERS substrate (G-(Ag or Au)@(Cit or HX)).** For the preparation of the substrate, 1%, by mass of methylcellulose, of the total volume obtained from each synthesis, was added to each newly synthesized colloid.
Finally, it was constantly stirred cold, until the complete dissolution of methylcellulose in the colloid.  

**Characterization of metallic NPs.** For the characterization of the metallic NPs, the UV-Vis spectroscopy technique was used, by means of an FS5 spectrofluorometer (Edinburgh Instruments) to identify the maximum absorbance wavelength ($\lambda_{\text{max}}$) of the LSPR by means of a spectrophotometric scan (300-700 nm). A Gamma Zetasizer Advance (Malvern Panalytical) was also used for the theoretical determination of the particle sizes obtained in the colloids and gels, as can be seen in the Fig. 1.

![UV-Vis and DLS spectra](image)

**Fig. 1.** UV-Vis (left) and DLS spectra (right) of non LSPR gels (up) and Au@Cit (down).

**Evaluation of the efficacy of the SERS substrate.** To observe the efficacy of the removable SERS substrate, 2.5 uL of gold gel were added to the surface of 25 pigments used during the colonial period, provided by the National Laboratory of Sciences for Research and Conservation of Cultural Heritage (LANCIC) of the Institute of Physics from the Autonomous University of Mexico (IF-UNAM). Subsequently, Raman scattering was measured using Raman i-Raman Plus (B&W Tek) spectrophotometers with 532 and 785 nm wavelength lasers. Finally, the spectra obtained were contrasted with spectra from the Pigments Checker and RUFF databases, and with the 2016 article by García-Bucio et al, mainly, as can be seen in the Figure 2.
Conclusions. It is possible to synthesize small volumes of metallic NPs based on gold reduction using C₆H₅Na₃O₇·2H₂O. Otherwise, it is not possible to stabilize silver NPs reduced with C₆H₅Na₃O₇·2H₂O and Ag and Au NPs reduced with NH₂OH·HCl, in small volumes, with methylcellulose. The production of a removable SERS substrate, constituted by a matrix of methylcellulose and Au NPs, allows the identification of yellow ochre, orpiment, hematite, and indigo from the colonial period. Employ XRF allows us to observe that the ability to remove the removable SERS substrate from the surface of replicas of paintings from the colonial period is effective. Use methylcellulose allows designing SERS substrates that can be removed from the surface to avoid damaging the sample.

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