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Characterization of SERS Platforms Designed by Electrophoretic Deposition on CVD Graphene and ITO/glass.

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ABSTRACT:

Here we perform electrophoretic deposition (EPD) to control the assembly of citrate-coated Au nanoparticles (NPs) onto ITO/glass and graphene. Monolayer
graphene is obtained by CVD and transferred to ITO/glass free of polymer. A quick surface-enhanced Raman scattering (SERS) test determined that the optimal platform consists in drop-casting MB onto as-deposited Au NPs (600 s at 1.0 V) to achieve 150 x signal enhancement. Therefore, we proceeded the same manner and deposited the Au NPs under identical EPD conditions on graphene/ITO/glass. Then, we tested both platforms upon immersion into 0.3 mM MB solution and found similar SERS intensity on the graphene- and ITO-containing platforms after 10 min and 66 h, respectively which clearly demonstrates the importance of the substrate. To characterize the platforms, linear scan voltammetry (LSV) and SEM were used as fingerprints for assigning the NPs oxidation peaks to their corresponding areas on the image. We find good agreement with respect to the number of NPs per µm² counted by LSV and SEM. We encounter that EPD on graphene leads to six fold larger coverage than on ITO/glass. These Au NPs were mostly assembled in the form of agglomerates located on defects of graphene. If those agglomerates are dissolved by anodic stripping, no SERS signal was found from the MB analyte. This also demonstrates that the platform can be reused for further deposition and SERS analysis. This work provides a simple and controllable method upon the design of advanced SERS platforms.
INTRODUCTION.

The combination of metal nanoparticles (NPs) with graphene has many benefits toward applications in the area of sensing,\textsuperscript{1,2} photocatalysis,\textsuperscript{3} photovoltaics,\textsuperscript{4} electronics,\textsuperscript{5} and detection via Surface-enhanced Raman scattering (SERS).\textsuperscript{2,6} This is possible due to the strong synergy between them.\textsuperscript{2} For example, in light harvesting devices the presence of metallic NPs expands the spectrum to visible while graphene reinforces the electronic transport across the platform.\textsuperscript{7} Detection via molecular sensing and SERS of dye molecules adsorbed on graphene-NPs was possible due to the different stacking configurations of grown graphene on 3D Ni foam.\textsuperscript{8} In SERS applications, it has been demonstrated that while the presence of Au or Ag NPs allowed critical signal enhancement, graphene plays its role by quenching fluorescence and luminescence of the analyte molecules and metallic NPs, respectively.\textsuperscript{2} Previous examples clearly demonstrate that the combination of these two nanomaterials brings about high expectations in identifying some phenomena and discovering new synergies between them. Nowadays new technologies are emerging, as for example, flexible platforms for
point-of-care diagnostics employing SERS as a highly sensitive and non-invasive approach.⁹ Perhaps one of the main challenges is to control deposition of metallic NPs on flexible graphene in order to form heterostructures with enhanced properties. This is a major issue because depending on how plasmonic NPs (active SERS entities) arrange on the vast surface of graphene, some of the aforementioned attributes can be further improved.

Electrophoretic deposition (EPD) becomes the method of choice due to its versatility and control of the nanomaterial to be deposited. It has demonstrated to overrides other deposition methods because it is scalable,¹⁰ film coverage can be easily controlled,¹¹ and formed within seconds.¹² It has gained much attention recently due to the work of Zamborini and co-coworkers who introduced mediate molecules such as peroxide¹² and hydroquinone (HQ)¹³ used to neutralize the negatively-charged nanospheres (NSs) and therefore induce deposition onto the electrode due to charge destabilization. That permitted fine control on the deposition of various sizes metal nanosphere (NS) on ITO/glass electrodes. Size-dependent properties were monitored by linear scan voltammetry (LSV) that exhibited oxidation peaks at different potentials for Ag¹³,¹⁴ and Au¹⁵–¹⁸ NSs during anodic stripping.¹⁹ That comes from the initial work of Ivanova et al.¹³ who experimentally corroborated what Plieth thermodynamically calculated²⁰ about the dependence of clusters size with the oxidation potential. Besides the size of NSs, shifts in peak potential have been demonstrated for same NPs placed on different type of substrates,²¹ different NPs compositions in core-shell configurations,²² NPs agglomeration,²³ functionalized substrates,¹⁴ and different deposition methods.²⁴ For example, Brainina et al. found that oxidation peak potential appeared more positive when
20 nm diameter Au NPs were placed onto Pt or Au electrodes with respect to the same NPs onto glassy carbon (GC). They calculated the Gibbs free energy of the as-deposited NPs on these substrates and demonstrated good agreement with the experimental LSV peaks. In line with that, Masitas et al., electrostatically attached 9 nm diam. Ag NPs on different substrates including ITO-glass, GC-, Pt-, and Au-microelectrodes. They determined that oxidation potentials of Au NSs on Au and Pt microelectrodes also appeared more positive than on GC. Later, Pattadar et al., from the same group, compared different deposition methods including EPD, drop casting, and electrostatically attachment of Au NSs and determined that oxidation potentials for EPD appeared shifted more positive with respect to those from electrostatic method. Allen et al. synthesized citrated-coated 4, 15, and 50 nm diameter Au NSs and varied the pH from 9 to 1.8 in order to follow the different stages of agglomeration by UV-vis and LSV. They determined that as long as pH decreases, the 4 and 15 nm diam. shifted to more positive potentials whereas the 50 nm NSs were unsusceptible to pH changes. These findings are quite relevant to EPD and could be further employed for SERS applications due to the fine control over NPs sizes, NPs distribution and arrangements on the surface, film thickness, etc.

Surprisingly, EPD on graphene has been scarcely explored. For instance, Semaltianos et al. obtained Au NPs by laser ablation and performed EPD at 30 V during 20 and 60 min. and found relatively well-distributed NPs on graphene. However, at such high potentials and longer times graphene became oxidized. Most of the studies related to deposition of nanoclusters on graphitic structures (i.e., HOPG) started with Penner’s group who selectively deposited metal and metal-oxides nanoclusters via electrochemical...
Selective deposition sites were previously studied by McCreery and co-workers who demonstrated higher electron transfer on generated defects on graphite (i.e., step-edges) caused by laser or electrochemical pre-treatments. For instance, anodization of HOPG led to the formation of oxygen-containing groups preferentially at step edges. Interestingly, other groups have shown electroless deposition of metal NPs on HOPG and on graphene. For instance, Lomax and Dryfe performed in-situ AFM and electrochemistry at an open cell and observed in-situ growth of Au NPs on HOPG. Kong and co-workers demonstrated that less than 20 s exposure of CVD graphene to Au caused spontaneous nucleation and growth of Au NPs preferentially formed on wrinkles of graphene. This particular phenomena has found practical uses such as healing graphene at defect areas for further improving conductivity by the presence of conducting NPs. Dalfovo et al. demonstrated other forms of spontaneous deposition of metal NPs on graphene via van der Waals interactions between the organic alkane chains surrounding the Au core and the hydrophobic surface of graphene. This strong interaction was used later for forming heterostructures (graphene-NPs) and transferring graphene to a desired substrate. Since the EPD process requires positive potentials (1.0 V) to attract the negatively-charge Au NPs, it is reasonable to expect oxygen-functional groups already formed on the graphene surface. Those specific areas can be wrinkles, grain boundaries, and step edges on graphene that may act as active sites for deposition of negatively-charged citrate-coated Au NPs. In this context, Ananthoju et al. systematically engineered structural defect on basal planes of CVD graphene in order to preferentially electrodeposit Au NPs for improving SERS. They were able to detect
concentrations as low as $10^{-16}$ M of Rhodamine 6G which is $10^8$ orders of magnitude higher Raman signal.

Our goal in this work is to characterize the already constructed SERS platforms consisting in EPD of Au NPs on ITO/glass and graphene/ITO/glass followed by drop-casting or immersion of the platforms in 0.3 mM methylene blue (MB). In both platforms we encountered agglomerated Au NPs likely induced by ethanol-containing MB dye however; those in graphene were more frequent and prominent. OES, SEM and LSV techniques were crucial for determining the NPs size in solution and later assigning each corresponding area to a specific oxidation potential. Characterization of the SERS platform is very important because, for example the literature has shown that EPD of Au NSs on ITO (prior to MB) exhibited well-distributed NPs along the entire surface\textsuperscript{17} with no agglomerates whatsoever whereas here, there are some agglomerated NPs as determined by SEM and LSV peaks. Au NPs on graphene are much more and way more aggregated than on ITO which is likely due to effect of the substrate since the EPD geometrical area was kept constant for ITO and graphene. Interestingly, those agglomerated Au NPs on graphene oxidized at lower potentials compared to the same NPs on ITO/glass. A quick SERS test by drop casting MB on EPD samples determined that just 600 s deposition of Au NPs on ITO/glass is enough for 1.5 orders of magnitude greater Raman signal. On the other hand, immersion of both platforms (600 s EPD) into 0.3 MB dye demonstrated that for achieving a similar SERS, graphene required just 10 min immersion while ITO/glass needed 66 h. In this work we explore and characterize optimal platforms for SERS applications. The construction of these platforms is achieved
by well controlled techniques such as EPD along with an exhaustive characterization carried out with SEM and LSV.

EXPERIMENTAL SECTION.

Chemicals. Sodium borohydride (99%) and hydroquinone (99%), trisodium citrate salt, potassium perchlorate, nitric acid, ammonium persulfate, methylene blue and potassium bromide were purchased from Sigma Aldrich Chemical. HAuCl₄·3H₂O was prepared from a high purity Au coin. Milli-Q grade water was used for all the solutions.

Chemical Synthesis of Citrate-Stabilized Au NPs. Aqueous Au NPs dispersion was synthesized following the seed method by Murphy.₃₈ Briefly, 0.5 mL of 0.01 M of the metal salt and 0.5 mL of 0.01 M trisodium citrate were added to 18.5 mL nanopure water. Then, 0.6 mL ice-cold 0.1 M NaBH₄ was added to the solution and stirred moderately for 2 h. The color of the solution changed to orange immediately after the addition of NaBH₄ and then turning reddish indicating the formation of Au NPs.

CVD graphene and transfer. Graphene (G) was grown by chemical vapor deposition (CVD) on a 25-μm thick Cu foil (MTI Corp.) following the same procedure as reported recently.₃₉ The transfer protocol follows the biphasic transferring method by Zhang et al. with some modifications.₄₀ Briefly, once graphene is etched away from the bottom side of Cu with 4.0 M HNO₃, the Cu piece (1 x 1 cm) with just graphene on top is brought into the biphasic solution consisting of 0.1 M ammonium persulfate and hexane. The bottom aqueous solution is extracted gently several time and refilled with nanopure water to complete volume. When all Cu is completely dissolved, the graphene film is transferred to a clean ITO/glass electrode.
UV-vis spectra and OES calculations. UV-vis spectroscopy of as-synthesized Au NPs dispersions was performed on a UV–vis Perkin-Elmer Lambda 35 spectrophotometer in a wavelength range between 300 and 900 nm. Optical extinction spectroscopy (OES) was used to determine the size distribution of Au NPs in the suspensions obtained from the fit of the experimental extinction spectra and calculated by Mie-Gans theory with size corrections.41

EPD conditions. Electrochemical experiments were carried out using an Autolab (PGSTAT30 ECOCHEMIE) and the 600 E (CH Instruments Inc.) potentiostat for EPD and LSVs experiments consisting in a 3-electrode setup with ITO/glass (Rs = 8–12 Ω, purchased from Delta Technologies Ltd.) as a working electrode, platinum foil was used as counter, and saturated calomel electrode (SCE) as reference electrode (0.243 V vs. RHE). The three electrodes were facing each other and spaced apart approximately 1.0 cm with the conductive side of the ITO/glass facing toward the reference and counter electrodes. EPD experiments were performed keeping the same parameters as follows: the same geometric area (0.75 cm²) of the working electrodes (ITO/glass and transferred G on ITO/glass) was immersed in a solution containing 13.4 mL nanopure water, 3.3 mL of NPs solution and 3.3 ml of 0.1 M HQ added few seconds before the experiment. Finally, 1.0 V was applied to the ITO/glass working electrode for different times including 120, 600, and 1800 s. Transients (current vs. time) for each time were recorded.

Characterization of SERS platforms. Linear Scan Voltammetry (LSV). LSVs curves were obtained by scanning the potential of electrodes (with the as-deposited Au NPs) from -0.2 to 1.2 V in 0.01 M KBr plus 0.1 M KClO₄ electrolyte solution at 0.01 V/s. The as-deposited Au NPs on ITO/glass and G/ITO/glass, a platinum foil, and saturated
calomel electrode (SCE) were used as working, counter, and reference electrodes, respectively. **SEM.** SEM images were recorded with a FE-SEM (Zeiss Sigma, Model JEOL JSM-6700F FEG) microscope operating at 10.0 kV and 5.0 kV acceleration voltages used for ITO/glass and graphene/ITO/glass samples, respectively. Samples were grounded using a copper tape in order to ensure a good electrical contact to the holder.

**Raman.** Raman spectra were recorded by using a LABRAM-HR 800 Horiba Jobin Yvon confocal microscope, with spectral resolution of 1.5 cm$^{-1}$ for wavelength (laser energy) 514.5 nm (2.41 eV). A 100 × objective lens (0.9 NA) for focusing the laser beam and collecting the backscattered Raman signal was used and 10 s acquisition time with average of 10 s. The laser spot size was $\sim$ 1 µm diameter at the sample surface and the grating grid was 1800 lines/mm.

**SERS.** We initially performed a quick SERS test on three different platforms constructed at various EPD times as follows: 120, 600, and 1,800 s EPD of Au NPs on ITO/glass. Then, 0.3 mM MB was drop cast deposited on the three platforms, let them dry for couple of minutes, and subjected to SERS analysis. Once found the optimal time for SERS, we proceeded the same manner with graphene. ITO and graphene platforms were also immersed into 0.3 mM MB for 10 min and 66 h for SERS analysis. MB signal enhancement for each trial was calculated based on the intensity ratio between the MB stretching mode at 1617 cm$^{-1}$ recorded after immersion of the platform in 0.3 mM MB solution and that of Raman spectrum of 0.3 MB bulk solution.

**RESULTS AND DISCUSSION**
Characterization of EPD of Au Nanoparticles on ITO/glass. Fig. 1A exhibits UV-vis spectrum of as-synthesized citrate-coated Au NPs along with red dashed curve which represents the theoretical fit obtained by optical extinction spectroscopy OES. Figure 1B shows linear scan voltammetry (LSV) of as-deposited Au NPs which were dissolved after anodic stripping in 10 mM KBr plus 0.1 M KClO₄ solution at scan rate of 0.01 V.s⁻¹. Au NPs were deposited by electrophoretic deposition (EPD) method run for 600 s (further details in Experimental Section). The UV-vis spectrum taken from Au NPs dispersion shows a single plasmon band centered around 515 nm, whereas the anodic stripping of as-deposited Au NPs exhibits two different oxidation peaks potentials (Eₚ,ox) located at ~0.95 and ~1.21 V. These peaks correspond to the bromide-induce oxidation and dissolution of the Au NPs deposited on the electrode whose peak potential (Eₚ,ox) and area under these peaks (yellow shaded) provide information about the NPs size and the amount of oxidized metal after anodic stripping. Therefore, the appearance of two peaks from the same synthesis suggests the coexistence of at least two size populations. Accordingly, from the UV-vis spectrum we performed optical extinction spectroscopy (OES) calculations to more precisely determine the size of Au NPs in solution as shown in Fig. 1C. Fig. 1C shows NPs distribution of 2.4 and 5.0 nm diam. Au NPs corresponding to the modal and average sizes, respectively. At this point, the two size populations of NPs calculated by OES are consistent with two peaks in LSV. OES and LSV are extraordinary tools because the former is capable of revealing two or more sizes within the same batch of Au NPs otherwise quite difficult to achieve with just one UV-vis plasmon band and the latter, provides information of the already deposited Au NPs. Figure 1D exhibits a selected SEM image of as-deposited and as-treated Au NPs. It
shows small and large individual NPs which are relatively well-distributed along the surface. A closer look, though, exhibits small agglomerates comprised of just a few NPs pointed out with red arrows in the zoom-in image below Fig. 1D. Figure 1E shows a histogram taken from the zoom-in image. The histogram shows a size range between 5 and 20 nm with a predominant size population around 20 nm diam. It should be pointed out that even though Au NPs smaller than 5 nm diam. were seen on the substrate they were difficult to sizing due to the rough ITO background. The 20 nm diam. Au NPs is consistent with agglomerates of 3 to 4 original NPs which were counted as one large NP.

In conclusion, OES provides information about small size Au NPs in solution (i.e.; 2.5 nm diam.) while SEM tells about larger NPs sizes and their arrangement on the substrate.

We recently performed the same synthesis of citrate-coated Au NPs and observed two similar size populations and two distinct oxidation peaks obtained by OES and LSV however, those $E_{p,ox}$ appeared more negative. In addition, the LSV curve in Fig. 1C shows an important increase in current starting at 0.7-0.8 V and two peaks that seem to overlap. Such positive shifts in potential and some the aforementioned LSV profiles can be associated with NPs agglomeration caused by evaporation of ethanol used as solvent for the MB dye. Allen et al. studied NSs agglomerations by monitoring changes in UV-vis spectroscopy and LSV. They synthesized citrated-coated 4, 15, and 50 nm diameter Au NPs and varied the pH from 9 to 1.8 in order to follow the different stages of agglomeration to finally conclude that oxidation peaks shifted more positive consistent with the formation of agglomerates. For small size NSs (4 and 15 nm) there were always two overlapped LSV peaks that appeared between ~ 0.7 and ~ 1.0 V attributed to isolated and aggregated Au NPs, respectively. They also determined that the maximum shift in
potential for 15 nm Au NPs at the lowest pH was limited by potential oxidation of bulk Au at ~ 0.9 V. Surprisingly, our LSV peak $E_{p2,ox}$ compared to theirs appeared even more positive (at ~ 1.2 V) than that of dissolution of bulk Au while $E_{p1,ox}$ appears at the potential at which high agglomerates were observed by Allen.\textsuperscript{23} LSV profiles are similar because both peaks are somehow overlapped. Positive shift in potential were also studied later by Pattadar et al. who demonstrated that EPD of Au NSs on bare ITO electrodes required higher overpotential to be oxidized as compared to the same NPs deposited on electrostatically-modified electrodes (i.e., APTES).\textsuperscript{24} In conclusion, our results indicate that more positive potentials are needed for oxidation of Au NP which can be attributed to a combination of elements associated with the formation of agglomerates in the first place, the deposition method (EPD), and adsorption of methylene blue (MB) onto Au NPs.\textsuperscript{44}

**EPD of Au NPs on graphene/ITO/glass.** Figure 2A shows a selected LSV obtained from as-deposited and as-treated Au NPs on G/ITO/glass. It should be pointed out that CVD graphene (G) was transferred to the ITO/glass without the use of polymers\textsuperscript{40} (see Experimental Section for more details). SEM images in Figure 2B, C and D correspond to different samples showing as-deposited citrate-coated Au NPs on as-transferred G. It should be recalled that EPD and LSV parameters were kept constant during the construction of the SERS platforms. As in Fig. 1B, the shaded areas under the LSV peaks represent the amount of dissolved metal after anodic stripping. The LSV curve shows, from negative to positive potentials, two minors and one prominent oxidation peaks labeled as $E_{ip,ox}$, $E_{2p,ox}$, and $E_{3p,ox}$ located about 0.04, 0.70, and 1.10 V, respectively. The shaded areas for the two minor peaks, shown as zoom-in on top of the
Figure, represent almost two orders of magnitude less charge (in coulombs, C) with respect to the $E_{3p,ox}$ (at 1.10 V). Table 1 shows the oxidation potential for each peak ($E_{p,ox}$), the calculated charge (C) under those peaks, the estimated area of metal NPs being oxidized, the total charge, and the moles of oxidized Au NPs. We assigned each $E_{p,ox}$ to one particular SEM area on graphene based on the potential at which those peaks appeared and the quantity of oxidized metal. There are striking differences between as-deposited Au NPs on ITO/glass compared to G/ITO/glass seen in Fig. 2 B. First, NPs on graphene exhibit some empty spots, isolated NPs, and majority of agglomerated areas. For instance, those NPs circumscribed with blue circles exhibit sort of isolated NPs aligned along edges or defects of graphene. Defects can be distinguished by dark spots on the SEM image which are characteristic of vacancies. This type of NPs arrangement is the least frequent. NPs inside the dotted red and complete red circles exhibit the so called “low agglomeration” and “high agglomeration” areas which are predominant along the surface of graphene. Based on the SEM images, the peak potential, and the area under those peaks we assigned $E_{2p,ox}$ and $E_{3p,ox}$ to “isolated NPs” and “low” and “high” agglomerates as marked with blue, dotted red, and red circles as indicated in the SEM image; respectively. The peak named $E_{1p,ox}$ shown in Fig. 2A (green square) will be discussed later.

The peaks potential for Au NPs on graphene at 1.10 and 0.70 V appeared more negative (~ 100- 250 mV range) with respect to the peaks of agglomerated and individual NPs, respectively seen on ITO/glass (Fig. 1B). This indicates that despite the large agglomerates, Au NPs dissolved at lower potentials on graphene than on ITO/glass. Brainina et al. studied the influence of different substrates on the oxidation of Au NPs by...
correlating the potential shifts with the Gibbs free energy. They compared NPs deposited on glassy carbon (GC) with macroscopic electrodes and demonstrated that electrooxidation of Au NPs deposited on bulky Au and Pt required higher potentials due to the stronger NPs-electrode interaction. Similarly, Ag NSs deposited electrostatically on amino-terminated ITO/glass, GC-, Au- and Pt-µdiscs demonstrated lower overpotentials in GC with respect to Au and Pt electrodes.\textsuperscript{14} Our results are consistent with the literature since GC electrode can be considered similar to our CVD graphene. With regards to the small peak seen at \textasciitilde 40 mV, it is shifted way more negative with respect to the other peaks at 1.10 and 0.7 V indicating that the energy required to oxidize those NPs is quite insignificant. Dramatic negative shift potentials have been reported by Masitas and Zamborini who observed few small peaks between 0 to 400 mV for 2.5 nm diameter Au monolayer-protected clusters.\textsuperscript{15} Another possibility could be the presence of metallic impurities remaining from etching the Cu substrate with (NH\textsubscript{4})\textsubscript{2}S\textsubscript{2}O\textsubscript{8} during the biphasic transferring procedure. Most related to our work is the study of Iost \textit{et al.} who performed CV experiments of a graphene film after being transferred from Cu foil with ferric chloride.\textsuperscript{45} They observed a peak around 40 mV which was assigned to anodic stripping of Cu. Our $E_{Ip,ox}$ exactly coincides with theirs strongly suggesting that this peak can be associated with Cu impurities. It is clear that more research is needed to conclusively determine this oxidation peak.

Table 2 compares the two platforms constructed by 600 s EPD of Au NPs on ITO/glass and G/ITO/glass. The Table indicates the oxidation peaks, the current-time transient charge achieved during EPD (Fig. S1 in Supplementary Information), total charge under the peaks (sum of all the areas under the peaks), and the total number of Au
NPs per µm² accounted with LSV and SEM. For the LSV technique we considered the area under the curve (C) and the average size of the Au NPs obtained by OES (between 2.4 and 5.0 nm) to estimate the total number of NPs per area. We choose 1 µm² because it approximates to the size of the Raman spot. There are three remarkable differences in the Table. First, to the best of the LSV sensitivity, there are at least three oxidation peaks in graphene. Second, the coverage of NPs on graphene is six fold larger than EPD on ITO/glass as measured by LSV and SEM, respectively. Last, the total number of Au NPs per µm² is different between LSV and SEM and between ITO and G/ITO. With regards to the latter, the discrepancy about NPs counted by SEM (56 Au NPs/µm²) with respect to LSV (85 Au NPs/µm²) for Au NPs/ITO/glass is reasonable since some of those agglomerated NPs were counted as a large 20 nm diam. NP. The difference by a half in NPs/µm² for graphene is also reasonable because Au NPs were counted in low agglomerated areas as shown in Fig. 2C. High agglomerated areas are impossible to count while individual Au NPs are not representative of the sample at all. SEM and histogram taken form “low agglomerates” are shown in Figure S2 (Supplementary Information).

Deposition of metal NPs on graphitic structures has been initially investigated by Penner’s group who performed electrochemical deposition experiments on HOPG substrates. By controlling the time pulses and potential applied, they demonstrated that metal NPs can nucleate and growth preferentially at defects on HOPG which ultimately led to the growth into mesowires as long as the length of the step edges. The propensity of nucleation at defect sites was explained on the basis of anodization of HOPG which leads to oxygen-containing (surface functionalization) groups preferentially
located at those defects. However, other groups showed that there is no need of an applied potential for nucleation and growth of metal NPs on HOPG and graphene. Lomax et al. observed in-situ nucleation and growth of Au NPs on HOPG by AFM performed by electrochemistry at an open cell. Since the EPD process requires positive potentials, it possible that specific areas on graphene were already oxidized prior deposition. Therefore it is reasonable that wrinkles, grain boundaries, and step edges on graphene may act as active sites for deposition of negatively-charge citrate-coated Au NPs as well as more thermodynamically available for anodic dissolution. Although more research is needed to conclusively assign each oxidation peak, at this point the formation of large agglomerates and large coverage of Au NPs on graphene seem promising upon SERS studies.

**SERS of MB.** SERS experiments were conducted by drop-casting (quick test) and immersing the platforms in 0.3 mM MB dissolved in ethanol (see Exp. Sect. for more details). We first run a quick SERS test by drop-casting MB on 3 different platforms constructed by 120, 600, and 1800 s EPD on ITO/glass in order to achieve the optimal coverage (see Experimental Section for more details). Figure 3A shows selected Raman spectra of the four trials which included MB on ITO/glass used as blank. Figure 3B is a three-entry plot that shows Raman intensity vs. MB signal enhancement vs. all the trials. It is clear that 600 s EPD outperforms SERS with respect to the other evaluated samples however we realized that drop casting the analyte, although is a quick method, it is not fully reliable and reproducible since the concentration of MB may be uneven distributed along the platform. Therefore, we proceeded by immersing the platforms into MB solution in order to let the adsorption of the dye be self-regulated. Figure 4A shows
Raman spectra of pristine MB (used as a blank) and as-deposited Au NPs on bare ITO/glass and G/ITO/glass immersed in MB for 10 min as indicated. Figure 4B shows LSV run twice on the Au NPs/G/ITO/glass sample to make sure that all Au NPs were dissolved. Figure 4C shows Raman of the G/ITO/glass sample after dissolving the Au NPs electrodeposited on graphene. Table 3 exhibits the MB band position, intensity, SERS of MB, and SERS after dissolving the Au NPs from the platform.

Figure 4A and Table 3 demonstrate that 10 min immersion of Au NPs/G/ITO is enough to achieve ~ 3 fold Raman enhancement of MB with respect to Au NPs deposited on ITO/glass. A control experiment determined that MB on Au NPs/ITO/glass needs 66 h of immersion time to achieve a similar SERS performance (See Fig. S3 and Table S1). Another control experiment demonstrates that when Au NPs are dissolved from the platform there is no SERS at all. This clearly indicates that Au NPs are the main responsible of SERS. Interestingly, the dissolution of Au NPs from graphene allows reusing the platform for further EPD and SERS experiments. These experiments strongly highlight the synergy between Au NPs and graphene because the former allows SERS while the latter causes Au NPs to agglomerate along wrinkles and defect areas. It is possible that the great affinity between MB and graphene is the responsible for improved SERS signals in such a short time.

Conclusions.

In conclusion, EPD has demonstrated to be versatile, fast, and controllable method to construct optimal SERS platforms. We found that the substrate plays a very important role during the EPD of citrate-coated Au NPs comprised of ~ 5 and ~ 2.4 nm
diameters as calculated by optical extinction spectroscopy (OES). For instance, as-deposited Au NPs on ITO/glass appear well-distributed along the surface, whereas the same NPs under identical EPD parameters on graphene exhibited mostly agglomerated areas. OES, LSV, and SEM techniques were used as fingerprints because the former revealed two size populations from a same batch of Au NPs, whereas LSV and SEM permitted correlation between the oxidation peaks and the different NPs arrangements. Calculating the area under the oxidation peaks and the diameter of Au NPs we were able to count how many NPs per \( \mu \text{m}^2 \) (diameter of the laser spot) were available on each platform for SERS studies. It was determined that NPs coverage on graphene was about 6 times greater than on ITO/glass and the majority of those NPs appeared in the form of large agglomerates. Based on the oxidation peaks, the area under the curve, and the type of Au NPs arrangement we were able to assign each LSV peak to a particular SEM area. A quick SERS test determined that drop-casting MB on the optimal EPD platform (600 s EPD of Au NPs on ITO/glass) led to more than 1.5 orders of magnitude signal enhancement. We also tried immersion of both platforms in MB and encountered that G/ITO/glass immersed for 10 min. was enough for an improved Raman signal similar to ITO/glass after 66 h. Dissolving the Au NPs from graphene indicated that Au NPs are the main responsible for SERS of MB. It also indicated that the platform can be reused for further EPD and SERS analysis. The synergy between Au NPs on graphene will be further explored in the future upon the design of advance plasmonic structures.

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Figure 1. UV-vis Extinction spectrum of as-synthesized citrate-coated Au NPs along with red dashed curve which represents the theoretical fit obtained by optical extinction spectroscopy OES (A). Linear scan voltammetry (LSV) of as-deposited (EPD 600 s) citrate-coated Au NPs on ITO/glass electrode in 0.1 M KClO₄ plus 0.01 M KBr at a scan rate of 0.01 V/s (B). OES calculation indicating 2.4 and 5.0 nm modal and average diameter Au NPs in solution, respectively (C). Inset shows a zoom-in of the LSV curve with color shaded under the oxidation peak. SEM image of as-deposited Au NPs on ITO/glass along with a zoom in area marked with a red dotted line (D). Histogram constructed from the zoom-in SEM image (E). Red arrows point out islands of dimmers and trimmers.
Figure 2. Linear scan voltammetry (LSV) of as-deposited Au NPs on graphene previously transferred to ITO/glass (A) and SEM images taken from different areas and magnifications of graphene (B, C and D). Blue, dotted red, and red circles indicate isolated Au NPs and “low” and “high” agglomerates, respectively.
Table 1. Table shows oxidation peaks potential ($E_{p,ox}$), charge (in coulombs) for each peak, the SEM correlated area of NPs (and potential impurities on graphene), total charge (C), and total moles of Au NPs stripped off from the G/ITO/glass electrode. $^a$assigned areas based on SEM, $E_{p,ox}$, and the area under the peaks.

| Peaks potential | Charge (C) | Possible SEM area $^a$ | Total charge (C) | Total moles Au (mol) |
|-----------------|------------|------------------------|-----------------|---------------------|
| $E_{p1,ox}$     | $8.3 \times 10^{-7}$ | Cu impurities?         | $6.5 \times 10^{-5}$ | $2.2 \times 10^{-10}$ |
| $E_{p2,ox}$     | $3.3 \times 10^{-7}$ | Isolated NPs           |                  |                     |
| $E_{p3,ox}$     | $6.4 \times 10^{-5}$ | Low and high agglomerates |            |                     |

Table 2. Table compares EPD of Au NPs on ITO/glass vs. G/ITO/glass exhibiting the oxidation peaks potential ($E_{p,ox}$), transient charge (C) reached during EPD, total charge under the peaks (C), the # of NPs counted per $\mu$m$^2$ based on LSV and SEM. $^a$ # of NPs counted from SEM considering a low agglomerate (See histogram in Fig. S2).

| Platform           | $E_{p1,ox}$ (V) | $E_{p2,ox}$ (V) | $E_{p3,ox}$ (V) | Transient charge during EPD (C) | Total Charge under the peaks (C) | # NPs/$\mu$m$^2$ (LSV) | # NPs/$\mu$m$^2$ (SEM) |
|--------------------|----------------|----------------|----------------|--------------------------------|---------------------------------|------------------------|------------------------|
| Au NPs/ITO/glass   | 1.21           | $\sim 0.96$   | /              | 0.12                           | $1.1 \times 10^{-5}$           | 85                     | 56                     |
| Au NPs/G/ITO/glass | 1.10           | 0.70           | 0.04           | 0.12                           | $6.5 \times 10^{-5}$           | 500                    | $\sim 226^a$          |
Figure 3. Selected Raman spectra of 0.3 mM MB blank sample and the three different quick SERS tests consisting in 120, 600, and 1800 s EPD of Au NPs on ITO/glass (A). Plot show three-entry plot of Intensity vs. MB signal Enhancement vs. all the trials performed in this work including the blank (B). Spectra in A are off-set for better comparison.
Table 3.

| Sample                  | MB Band position (cm⁻¹) | Intensity | SERS | SERS after anodic stripping |
|-------------------------|-------------------------|-----------|------|-----------------------------|
| Blank (0.3 mM MB)       | 1624                    | 8         | /    | /                           |
| MB + Au NPs/ITO/glass  | 1617                    | 20        | 2.5  | Not observed                |
| MB + Au NPs/G/ITO/glass| 1617                    | 68        | 8.5  | Not observed                |

Figure 4. Selected Raman spectra of a blank (0.3 mM MB solution) and 600 s EPD of Au NPs on ITO/glass and G/ITO/glass after being immersed in 0.3 mM MB for 10 min (A). LSV curves showing 1st and 2nd anodic scan from -0.2 to 1.2 V (B). Second scan confirms the disappearance of the oxidation peaks due to dissolution of Au. Raman spectra taken after dissolution of Au NPs from graphene and compared to a blank sample (just MB) (C). Raman spectra and LSV curves are off-set for better comparison. Table 3 indicates MB immersion time, MB band position, Intensity (a.u.), SERS, and SERS after anodic stripping.