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Short Communication

In Situ Activation of Thick-Film Disposable Copper Electrodes for Sensitive Detection of Malachite Green Using Electrochemical Surface-Enhanced Raman Scattering (EC-SERS)

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In situ activation of thick-film disposable copper electrodes for sensitive detection of malachite green using electrochemical surface-enhanced Raman scattering (EC-SERS)

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Abstract

In this work, disposable thick-film copper electrodes were used for the detection of malachite green by Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS). In situ real-time SERS detection of malachite green was performed during the activation of copper electrodes by voltammetry. This time-resolved method allowed to find dynamically the optimal potential where the detection sensitivity is increased. Scanning electron microscopy images showed that the generation of new fresh copper nanoparticles are responsible to produce the SERS enhancement. A fast decrease of the SERS signal was observed when the potential was shifted from the optimal potential, which could be due to surface changes on the copper electrode. This fact demonstrates the importance of recording the SERS signal in situ and dynamically to obtain the most sensitive method. The combination of in situ time-resolved EC-SERS with disposable, cost-effective screen-printed electrodes leads to a very appropriate method for the sensitive and fast detection of malachite green.

Keywords: Spectroelectrochemistry, Thick-film electrodes, SERS, EC-SERS, Copper

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Electrochemical surface-enhanced Raman spectroscopy (EC-SERS)^[1] is a technique attracting great interest in recent years because it allows the SERS detection of species involved in electrochemical reactions^[2,3] or the *in situ* activation of electrodes to accelerate the adsorption of molecules. It is essential to differentiate EC-SERS, where the electrochemical processes occur simultaneously with the optical detection to the SERS detection of species using *ex situ* substrates activated by electrochemical techniques. Although many examples have been described of the latter, less studies have been reported using EC-SERS due probably to the difficulty to combine and synchronize electrochemical and Raman instrumentation. SERS^[4] is a technique strongly dependent on the substrate material, being silver^[5] and gold^[6] the most employed materials due to their high enhancement power, although other materials such as copper and platinum^[7,8] have also shown SERS activity. We have recently shown the utility of the EC-SERS technique to get dynamic and quantitative information using silver screen-printed electrodes^[9,10]. Copper has

been less used since the enhancement is usually lower than gold or silver^[11] but it has also been exploited for SERS measurements typically in nanoparticles configuration or electrochemically activated in combination with the adsorption of the sample by drying or contacting for long times^[12–14]. Brandao et al.^[15] reported EC-SERS measurements on ionic liquids using copper electrodes, but the activation was previously performed (*ex situ*) by oxidation-reduction cycling (ORC). Kudelski et al.^[16] studied the adsorption of pyridine at a fixed potential after the *ex situ* activation of the copper surface. However, *ex situ* activation of copper electrodes does not allow to study the time-resolved processes occurring at the electrode surface, it only provides information on the final surface state available for the interaction with the chemical species in the SERS assay, and the influence of time-dependent processes after the activation is not considered. As far as we know, *in situ* dynamic EC-SERS studies during the activation of copper electrodes by electrochemical techniques have not been reported to date.

The goal of this communication is to report the *in situ* time-resolved activation of disposable thick-film copper electrodes to generate a SERS-active substrate by Raman spectroelectrochemistry. We show the usefulness of the EC-SERS method compared to the *ex situ* preparation of substrates as the SERS signal change quickly after the optimum state. Scanning electron microscopy (SEM) is employed to characterize the structure of the electrode surface during the activation process. Malachite green (MG) was used as indicator species because its detection could be interesting since it is a carcinogenic and mutagenic agent^[17].

To demonstrate the *in situ* electrochemical activation of thick-film copper electrodes, an oxidation-reduction method was employed, but in contrast to most works in the literature, the Raman signal of MG was followed dynamically. A linear sweep voltammetry from +0.2 V to -0.9 V at a scan rate of 50 mV/s was performed. A solution of 200 $\mu\text{g/L}$ of MG in 0.1 M NaCl was employed. Raman spectra were recorded every 2 s, obtaining one spectra each 100 mV of the voltammogram. **Figure 1A** shows several spectra recorded at different potentials during the potentiodynamic experiment, which illustrates the strong influence of the potential on the Raman signal of MG. The Raman bands observed for MG are in agreement with previous literature^[18,19]. The most noticeable bands could be attributed to stretching of C-C ring (1609 cm^{-1}), N-phenyl stretching (1385, 1355 cm^{-1}), C-H rocking (1206 cm^{-1}), in plane vibrations of C-H ring (1162 cm^{-1}), out of plane bending of C-H ring (783, 901 cm^{-1}). Bands below 300 cm^{-1} could be assigned to copper species (*vide infra*)^[20]. **Figure 1B** shows the voltammogram obtained and the variation of the peak intensity of the Raman band at 1162 cm^{-1} . The voltammogram of the copper electrodes showed an intense initial anodic process assigned to the oxidation of copper. In presence of 0.1 M NaCl, several copper chloride species are likely to be generated at the electrode surface^[21,22], and therefore, the voltammogram could become very complex. Two cathodic processes are observed at peak potentials of -0.16 and -0.28 V, respectively. They could be tentatively assigned to the reduction of different copper species in presence of chloride^[23]. In this case it is difficult to certainly elucidate these processes because unresolved high-current anodic processes were obtained due to the high copper amount involved in the reactions, but the Raman spectra could give some information. **Figure S1** shows the Raman spectra obtained at different potentials during the potentiodynamic experiment. Initially, a band

at 275 cm^{-1} is observed, which disappeared during the reduction. This band could be tentatively assigned to CuCl_2 as previously discussed in the literature^[20]. During the reduction (from 0 to -0.4 V), a new band appeared at 212 cm^{-1} , which could be tentatively assigned to CuCl species^[20]. Interestingly, this band increased during the two reduction processes, suggesting that both processes are generating CuCl species but from a different starting point (different peak potentials). The voltammetric shape of these processes is different: first process is symmetrical and the second process has a long tail, which could indicate adsorption and diffusion rate limiting-control.

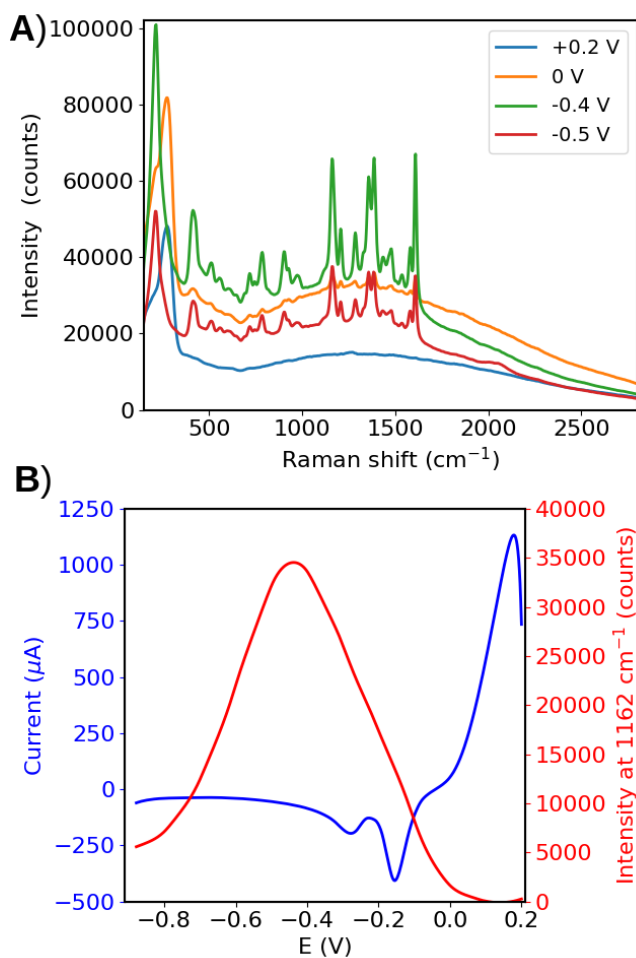


Fig. 1. A) Raman spectra obtained at different potentials during the voltammetric experiment. B) Linear sweep voltammetry (blue line) and evolution of the Raman intensity band at 1162 cm^{-1} (red line) with the potential during the activation of thick-film copper electrodes in presence of 200 $\mu\text{g/L}$ of MG in 0.1 M NaCl.

The generation of a fresh and nanostructured metallic surface is normally required to obtain a significant SERS effect and, in this case, the SERS signal increased starting from 0 V, which coincides with the onset of the first cathodic process. Raman bands continue to grow until reaching a maximum at about -0.40 V, just after the peak potential of the second cathodic process. Therefore, it is not excluded that this second reduction process can also influence the SERS effect in the thick-film copper electrodes. It is remarkable that after the maximum of the SERS signal at a very specific potential (about -0.4 V), the signal decreased quickly. This fact could be due to the fast passivation of copper in presence of oxygen, and as described in the literature previously, the SERS enhancement with copper oxide materials is low [14]. Another factor could be the different adsorption properties of malachite green with the potential variation (which changes the charge of the surface) or a change in the orientation of the molecule. Therefore, this demonstrates that the *in situ* dynamic EC-SERS may provide results avoiding time-dependent or potential-dependent surface changes such as passivation, which could decrease the analytical signal. This is one of the advantages of *in situ* dynamic EC-SERS in comparison to the typically employed oxidation-reduction cycling (ORC) *ex situ* activation because the surface between the electrochemical activation and the SERS assay could lose some SERS activity. This is especially important with metals suffering fast surface changes such as copper.

The changes of the microscopic surface of the copper electrodes during the electrochemical activation was monitored by recording SEM images at different potentials. Initially, the copper surface shows a surface composed by large micrometric copper particles (**Figure 2A**). The copper surface shows some crevices when the voltammetry was performed between +0.2 V and +0.05 V (only the initial anodic process occurs in this range) (**Figure 2B**). These crevices could be attributed to the oxidation of the copper, which is removed from the electrode surface leaving these features. When the activation of the surface is performed between +0.2 and -0.4 V, numerous small brighter particles are generated on the electrode surface (**Figure 3B**). The emergence of these particles coincides with the increment of the SERS effect observed for MG at these potentials. Therefore, it is reasonable to assume that these particles are responsible to enhance the Raman signal, which also agrees with the theoretical foundations of the SERS effect. However, we cannot assure this completely, and other hidden surface effects could also influence the strong SERS enhancement. **Figure S1** shows the EDS

analysis of the electrode surface, confirming the copper presence in both non-activated and activated surfaces. Interestingly, no chloride presence was observed in any surface. This fact could be due to have a low amount of chloride below the limit of detection of the SEM, or to the possible generation of the copper oxide species on the surface by the ambient oxygen, which inhibits the SERS effect.

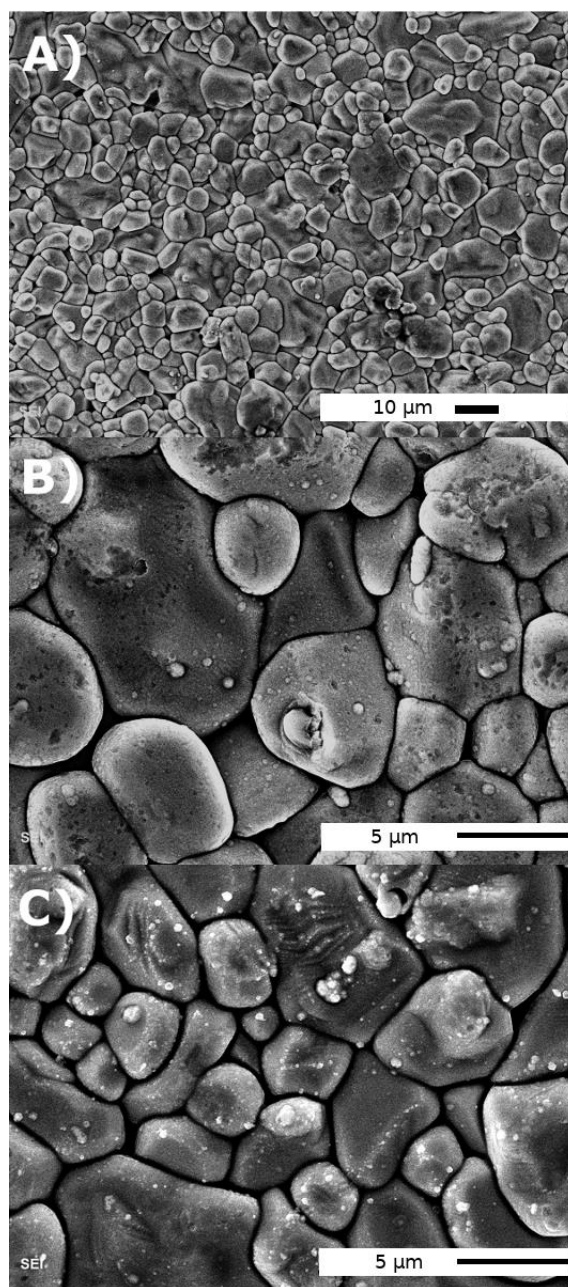


Fig. 2. SEM images of the surface of thick-film copper electrodes at A) the initial stage (pristine electrode), B) after performing the electrochemical pretreatment from +0.20 V to +0.05 and C) from +0.20 V to -0.4 V.

Figure 3A shows the Raman spectra obtained at different concentrations of MG (1-200 $\mu\text{g/L}$) (2.7×10^{-9} M to 5.5×10^{-7} M). Peak intensity of the 1162 cm^{-1} band increased linearly with MG concentration as shown in **Figure 3B**. The linear equation obtained was: $I_p \text{ (counts)} = 6.6 + 442 \cdot [\text{MG}] \text{ (}\mu\text{g/L)}$ ($R^2 = 0.991$) and the limit of detection estimated was $0.15 \mu\text{g/L}$ (4×10^{-10} M). The enhancement factor (EF) from the SERS effect was calculated using the following known equation:

$$\text{EF} = I_{\text{SERS}} C_{\text{Normal}} / I_{\text{Normal}} C_{\text{SERS}}$$

where I_{SERS} and I_{Normal} are the intensities of the MG band at 1162 cm^{-1} for SERS and normal Raman spectra, respectively, and C_{SERS} and C_{Normal} are the concentrations of MG used in the experiments. The calculated EF was 4.5×10^6 , which demonstrates the strong enhancement obtained with the *in situ* EC-SERS method using thick-film copper electrodes. Precision was good considering the typical issues of the SERS technique on this matter: the reproducibility of the calibration slopes was 8.2% (RSD, $n=3$) and the reproducibility for the same solution (100 $\mu\text{g/L}$) with different electrodes was 12.4% (RSD, $n=5$). The use of a specific cell for screen-printed electrodes (and also the mass-fabrication of devices) allows to minimize factors that can influence the precision such as the placement of the probe and the substrate surface between measurements. Furthermore, the utilization of a probe with a $200 \mu\text{m}$ diameter laser spot, which allows to obtain an average SERS signal from a large surface, seems also important to obtain precise SERS experiments because the influence of hot spots is minimized. These analytical results demonstrate that thick-film copper electrodes can be used for the detection of trace concentrations of MG in a simple and precise way using EC-SERS and opens the door for the development of new analytical methods based on these tools for the detection of other species of interest. Other works describing the detection of MG by SERS only seem to reach or improve these analytical characteristics when the sample is dried on the substrate^[19] and the acquisition time is considerably increased^[24] leading to longer analysis times. If the measurement is performed in solution (although also with some incubation time)^[25,26], similar detection limits are obtained but with shorter linear ranges and complex procedures or the synthesis of nanosubstrates are required. In this work, we demonstrate the use of readily available electrodes activated in a simple way for the *in situ* detection of MG with excellent detection limits in a few seconds analysis and without the need to perform complex procedures, dry the sample on the substrate or previously synthesize functional nanomaterials with high SERS activity. This *in situ* method works with different species and screen-printed

electrodes as demonstrated previously for ferricyanide, $[\text{Ru}(\text{bpy})_3]^{2+}$ or Naratriptan using silver screen-printed electrodes^[9,10].

Disposable thick-film copper electrodes can be activated electrochemically to *in situ* generate a SERS-active surface for the detection of malachite green at trace concentrations. The potential applied to the electrode showed a clear influence on the SERS effect with a maximum that happens at potentials where fresh copper nanoparticles are generated at the electrode surface. The SERS detection is carried out simultaneously to the generation of the nanostructured surface ensuring that the copper nanoparticles does not suffer time-dependent surface changes such as passivation, which could decrease the SERS effect. Malachite green was easily detected with a limit of detection below ppb following the *in situ* electrochemical activation to generate the SERS effect.

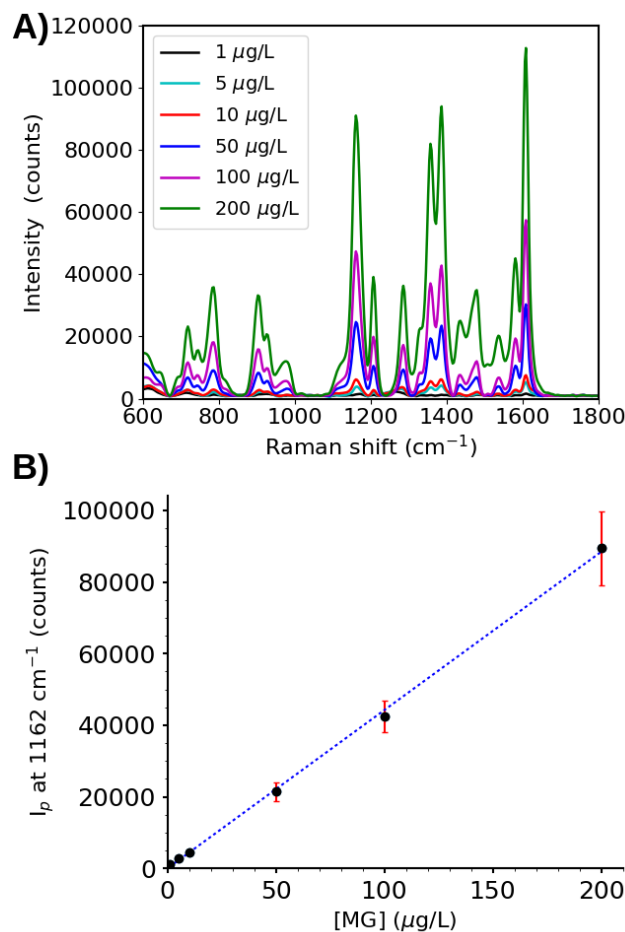


Fig. 3. A) Variation of the optimum Raman spectra (baseline subtracted) with increasing MG concentrations (1-200 $\mu\text{g/L}$). B) Calibration plot that illustrates the relationship between the peak intensity of the band at 1162 cm^{-1} and the MG concentration.

Experimental

Instrumentation

In situ dynamic Raman spectroelectrochemistry was performed by using a compact and integrated instrument, SPELEC RAMAN (DropSens), which contains a laser source of 785 nm. This instrument was connected to a bifurcated reflection probe (DRP-RAMANPROBE) and a specific cell for screen-printed electrodes (DRP-RAMANCELL). **Figure S3** shows the instrumental setup employed to perform the electrochemical experiments and **Figure S4** shows a scheme of the spectroelectrochemical cell. The instrument was controlled by DropView SPELEC software, which allows to perform simultaneous and real-time spectroelectrochemical experiments, with totally synchronized data acquisition.

Thick-film copper electrodes (DRP-CU10, DropSens) were used throughout the work. These devices consisted of a ceramic card where the working electrode (circular, 4 mm diameter) was made by copper sputtering and the auxiliary and pseudoreference electrodes were made by screen-printing using carbon and silver inks, respectively. The thickness of the copper film was about 1 μm . Reported potentials are related to the silver pseudoreference electrode. A JEOL 6610LV SEM was used to characterize the working silver electrodes at different stages of the electrochemical activation.

Reagents and solutions

Malachite green and sodium chloride were purchased from Sigma. Ultrapure water obtained with a Millipore DirectQ purification system from Millipore was used throughout this work.

Raman spectroelectrochemical measurements

Raman spectroelectrochemical experiments were performed by applying a linear sweep voltammetry from +0.2 V to -0.9 V at a scan rate of 50 mV/s, a step potential of 2 mV and equilibration time of 1 s. Raman spectra were acquired with an integration time of 2 s and a laser power of 130 mW (laser spot diameter is about 200 μm). For the analytical detection, the scan rate was 25 mV/s, the integration time was 8 s and the laser power was 45 mW. All spectroelectrochemical measurements were performed at room temperature and using a solution of 60 μL .

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Supporting Information

In situ activation of thick-film disposable copper electrodes for sensitive detection of malachite green using electrochemical surface-enhanced Raman scattering (EC-SERS)

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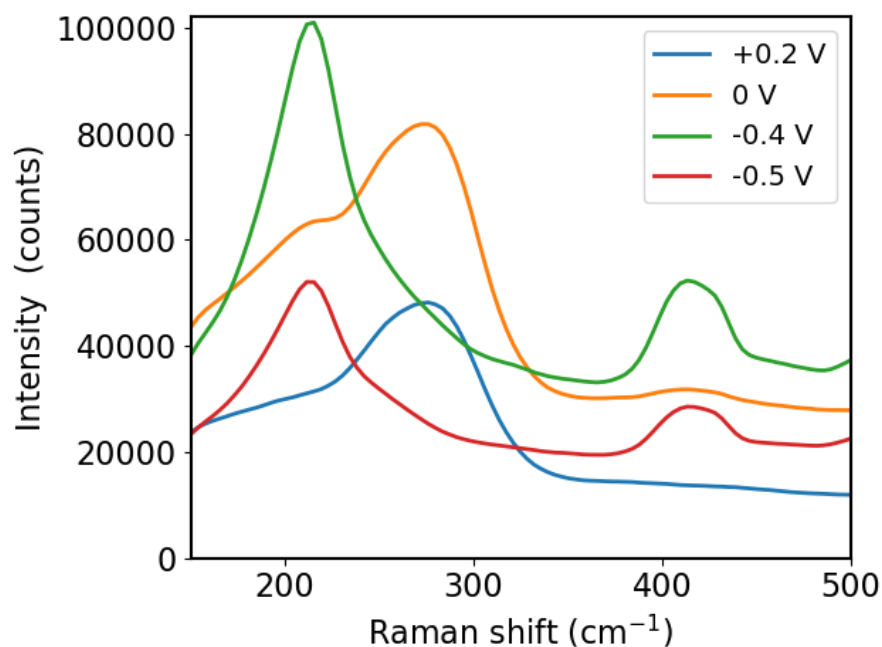


Figure S1. Low-shift range of the Raman spectra obtained at different potentials during the *in situ* voltammetric experiment.

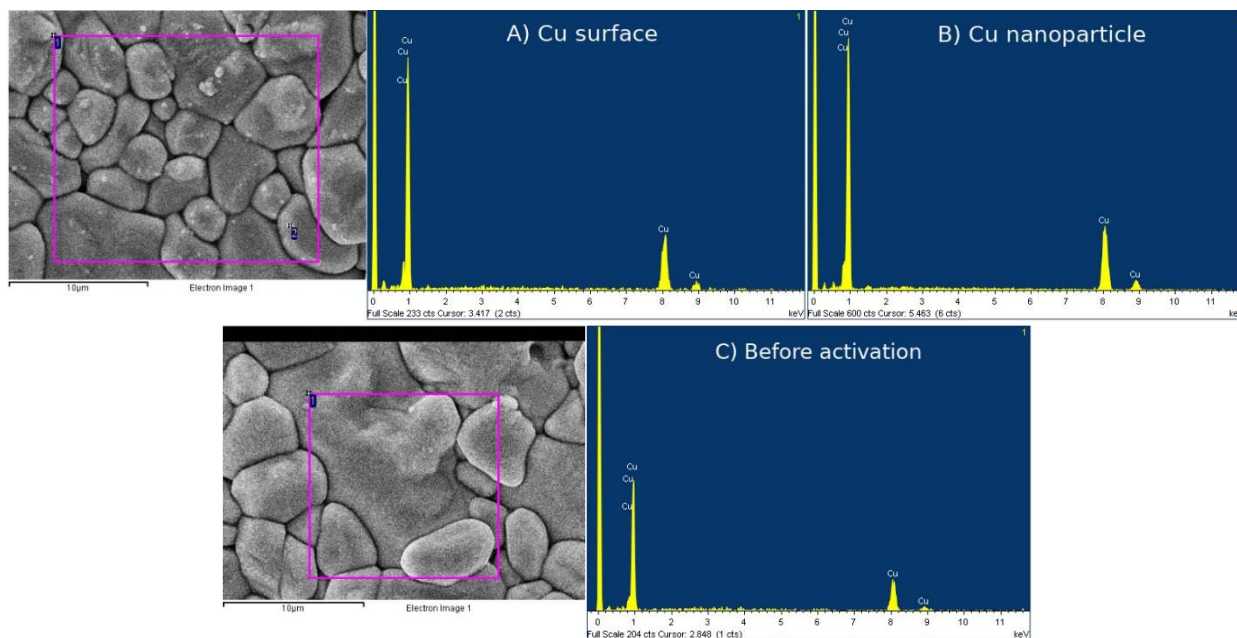


Figure S2. Energy Dispersive X-ray spectra of two different parts of the copper electrode after electrochemical activation: A) 20x20 μm surface, B) Cu nanoparticle generated after electrochemical activation. C) Spectrum obtained before the electrochemical activation.

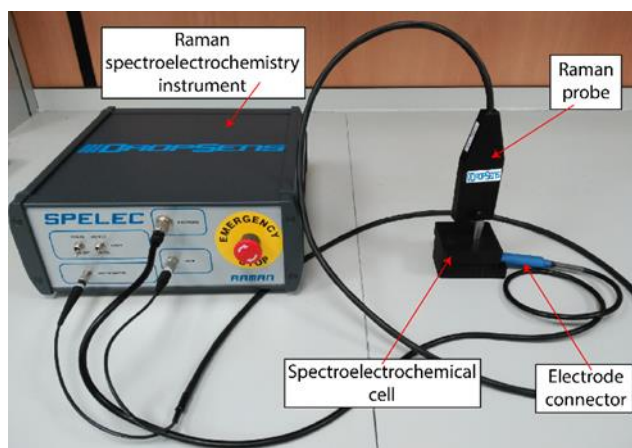


Figure S3. Experimental setup used to perform the Raman spectroelectrochemical experiments composed by the SPELEC RAMAN instrument, a bifurcated Raman probe and the spectroelectrochemical cell for screen-printed electrodes

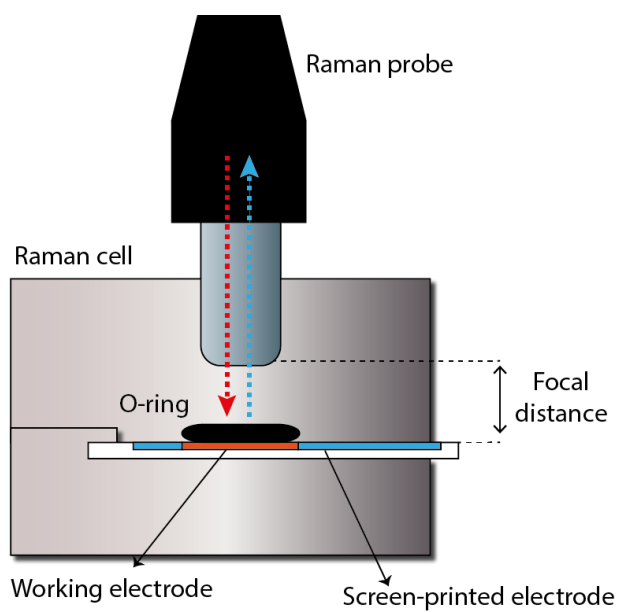


Figure S4. Illustrative drawing of the Raman spectroelectrochemical cell.