



## Multiamperometric-SERS detection of melamine on gold screen-printed electrodes



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### ABSTRACT

A new, simple and fast protocol to generate gold-based SERS substrates is presented in this work. Melamine is a compound widely used in the industry that can be toxic for humans if consumed even in low concentrations. EC-SERS is an excellent alternative to classical methods to detect and quantify this compound because Raman spectroscopy provides a fingerprint of the molecules, providing very good sensitivity. In this work, time-resolved Raman spectroelectrochemistry is employed to generate a SERS substrate and to detect melamine, all in a single experiment. The dynamic character of this technique provides valuable information about the interaction of the molecule with the generated substrate. An optimization of the spectroelectrochemical method based on a multi-pulse amperometric detection has been performed. The new protocol presented in this work shows significant figures of merit in a very short experiment time, just 25 s.

### 1. Introduction

Melamine (Fig. 1) is a nitrogen-rich compound widely used in producing a great variety of products such as plastics, coatings, paints, fertilizers, and pesticides, among others [1–3]. It is also a good precursor to prepare different applications such as medicinal drugs or biologically active compounds [4]. Because of its high nitrogen levels and its low cost, melamine has been illegally added to dairy products to modify the protein concentration in a fictitious way [1]. For example, according to the World Health Organization (WHO), the safety limits are 1 mg/L for milk infant formula and 2.5 mg/L for milk and food products [5,6]. Melamine intakes above these limits can cause serious health problems because the body cannot metabolize this compound, and the kidneys excrete it. Therefore, high concentrations of melamine may cause several kidney diseases and even death [7]. For these reasons, it is essential to determine with high accuracy and in an easy way the concentration of melamine present in dairy food or, for example, in tap water.

There are several techniques to detect melamine, such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), or colorimetric methods [1,7–10], but most of them require expensive instrumentation, long sample preparation time and extended analysis time. Raman spectroscopy could be an easy alternative to these methods because of the high specificity of the Raman spectrum, which can be considered a fingerprint of the molecules. The main drawback of

Raman spectroscopy is its intrinsic low sensitivity, which can be overcome using a SERS substrate because it enhances the Raman signal in several orders of magnitude [11–14]. These SERS substrates consist of nanostructured surfaces with plasmonic properties. SERS enhancement is mainly related to two different effects: electromagnetic (EM) and chemical (CE) mechanisms. The first one is related to the resonance between the electric field of the laser radiation and the located plasmon surface (LPR) of the nanoparticles (NPs) or nanostructures, leading to an enhancement of the Raman signal of  $10^4$ – $10^5$  or even higher. Under specific conditions it can reach enhancement factors up to  $10^8$  [11,15]. The second one, CE, is related to the interaction between the molecule and the SERS substrate, which affects to the polarizability of the molecule [16], obtaining enhancement factors between  $10^2$ – $10^3$ . The total enhancement factor usually reported with a SERS substrate is around  $10^6$ – $10^9$ , which is enough to perform even single molecule detection [15,17]. However, the main challenge for SERS is the preparation of highly reproducible substrates [18]. In literature, different methods to generate arrays of nanoparticles that present good analytical results can be found [19,20], but, in general, the preparation of these substrates are time-consuming. One strategy to develop a SERS substrate is to use an electrochemical procedure [21], which typically consists of roughening a metal electrode to generate a surface with plasmonic properties (gold, silver or copper). This electrochemical procedure can be combined with spectroscopic techniques to obtain the information simultaneously, with the best exam-

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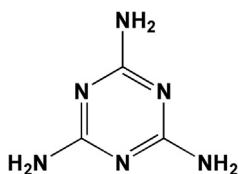


Fig. 1. Chemical structure of melamine.

ple being Raman spectroelectrochemistry (Raman-SEC). This combination improves not only the time required to have a suitable substrate but also the control of the surface potential that can favor the adsorption or interaction of the molecule with the substrate, improving the Raman response [21,22].

It is well-known the strong interaction between melamine and gold NPs [23–25], which induces the aggregation of these NPs. The generation of a EC-SERS substrate in the presence of the target molecule during a Raman-SEC experiment can provide helpful information about the capabilities of this SERS substrate [22,26].

A new, fast and low-cost protocol is presented in this work, following the strategy mentioned above, where the SERS substrate is generated by roughening gold substrates in a very simple way, in presence of melamine. Concurrently, as the substrate is generated, the Raman signal related to melamine, is recorded in a time-resolved (TR) Raman-SEC experiment using disposable gold screen-printed electrodes (SPE).

As melamine is used in pesticides and fertilizers, the development of protocols that allow the detection of this molecule in tap water is interesting. A positive result for melamine in water would mean that some hazardous procedure performed with this product has produced a contamination that could cause health problems. This protocol is low-cost and fast to be applied in routine analysis.

## 2. Experimental section

### 2.1. Reagents and materials

Melamine ( $C_3H_6N_6$ , 99%, Alfa Aesar), potassium chloride (KCl, 99 + %, reagent, ACROS Organics) and hydrochloric acid (HCl, 37%, VWR) were used without further purification. All solutions were prepared using ultrapure water obtained from a Millipore DirectQ purification system (18.2 M $\Omega$  cm resistivity at 25 °C, 2 ppb TOC).

### 2.2. Instrumentation

Time-resolved Raman spectroelectrochemistry (TR-Raman-SEC) experiments were carried out with a customized SPELEC-Raman (Metrohm-DropSens), with an integrated laser of 638 nm, an appropriate spectrophotometer, and a potentiostat. Gold screen-printed electrodes (Au-SPE, DRP-220BT, Metrohm-DropSens) were used in all experiments to ensure good reproducibility. A customized Raman-SEC cell was used to carry out all the experiments with these Au-SPE. These electrodes consist of a 4 mm diameter gold working electrode, a gold counter electrode, and a pseudo-reference silver electrode, all of them printed on a ceramic platform. The laser power was 20 mW (63 mW/cm<sup>2</sup>), and the integration time was kept 1 s for all experiments.

Dropview SPELEC (Metrohm-DropSens) is the software used for the simultaneous acquisition of spectral and electrochemical information, allowing also data treatment.

### 2.3. SEC experiments

Two electrochemical techniques are used in this work:

- (1) Cyclic voltammetry (CV). A CV was carried out to explore the behavior and the SERS response of melamine in 0.1 M KCl medium. The vertex potentials were  $-0.80$  V and  $+1.40$  V, starting at  $+0.70$  V in the anodic direction. All potentials are referred to a silver pseudo-reference electrode. Different scan rates were used ( $0.02$  V·s<sup>-1</sup>,  $0.05$  V·s<sup>-1</sup> and  $0.10$  V·s<sup>-1</sup>).
- (2) Multi-pulse amperometric detection (MAD). This technique shows a better response in a reproducible way after the optimization of the experimental parameters by using the design of experiments (DOE). A protocol with 3 steps is followed: (1) the applied potential is  $+1.15$  V for 5 s, (2)  $0.00$  V for 15 s, and (3)  $-0.70$  V for 5 s. In this way, the whole experiment is performed in just 25 s.

Electrochemical and spectroscopic responses are simultaneously collected, with high temporal resolution (integration time: 1 s).

### 2.4. Tap water sample

A tap water sample was analyzed to demonstrate the good capability of detection of the multi-pulse amperometry detection protocol based on EC-SERS. For this purpose, a  $6.7$   $\mu$ M melamine sample was prepared in 0.1 M KCl using tap water as solvent without any other treatment of the water.

### 2.5. Field Emission-Scanning Electron Microscopy

Characterization of the modified Au SPE surface was performed using Field Emission-Scanning Electron Microscopy (FE-SEM). A Zeiss Gemini 560 microscope operating at 2.0 keV was used to obtain images of the surface at magnification of  $25,000\times$ .

## 3. Results and discussion

### 3.1. Detection of melamine

The first objective of this work was the development of a Raman-SEC protocol to detect and identify melamine at low concentrations. The EC-SERS spectrum obtained in a  $50$   $\mu$ M solution was compared with the Raman spectrum of solid melamine and the Raman spectrum of 0.01 M melamine in water. These spectra are highly correlated, as can be seen in Fig. S1 (Supplementary material). Differences between them are due to the influence of water and the interaction between the molecule and the EC-SERS substrate. It should be noted that the spectrum for melamine without an EC-SERS substrate was obtained with a higher integration time (10 s) and a higher concentration to obtain a suitable Raman response. The main band at around  $677$  cm<sup>-1</sup> is related to the ring vibration, as shown in Table 1. EC-SERS spectrum was obtained during a TR-Raman-SEC experiment, at  $-0.70$  V, where the maximum Raman signal was recorded.

Cyclic voltammetry (CV) is the most employed technique to generate an EC-SERS substrate [22,28,29]. This electrochemical technique supplies a roughened and nanostructured surface by applying of subse-

**Table 1**  
Vibrational assignment of SERS spectrum of melamine.

Raman shift/cm <sup>-1</sup>	Assignment [27]
383	$\delta$ (CN)
677–707	ring breathing
992	$\delta$ (CNC) + $\delta$ (NCN)
1448	$\nu$ (CN) + $\delta$ (NH <sub>2</sub> )
1587	$\delta$ (NH <sub>2</sub> )

$\delta$ : scissoring;  $\nu$ : stretching.

quent oxidation and reduction cycle (ORC) to a metal electrode. In this case, Au-SPEs were used as source of gold in solution by oxidation of the surface in the presence of KCl, which favors the formation of the  $[\text{AuCl}_4]^-$  complex. The reduction of this gold complex generates the nanostructures/NPs responsible for the Raman enhancement. This procedure, and the dynamic character of Raman-SEC, allows us to explore the behavior of melamine Raman response during the CV. The evolution of the full Raman spectra can be followed during the ORC. During the oxidation stage, only Raman bands related to gold oxides can be detected. After the gold complex reduction, intensity of melamine Raman spectra starts to grow up. To show these changes, the main band of melamine can be plotted versus the applied potential. This representation is called voltaRamagram [22] (Fig. 2, blue line).

Fig. 2 shows the CV response (right axis, garnet line) compared to the voltaRamagram at  $677\text{ cm}^{-1}$  (left axis, blue line). CV shows an anodic peak related to the oxidation of the gold electrode to form the complex  $[\text{AuCl}_4]^-$  and gold oxides [30] and a cathodic peak associated with the reduction of this complex and oxides to form AuNPs. When the voltaRamagram is analyzed, the increase of the Raman signal related to melamine is observed from  $-0.50\text{ V}$  onwards, with the maximum value being reached at  $-0.70\text{ V}$ . Then, the Raman intensity decreases until the vertex potential is reached ( $-0.80\text{ V}$ ). In the forward scan it can be observed a small growth of the Raman signal that decreases from  $-0.55\text{ V}$  onwards. This behavior can be explained in terms of adsorption of melamine due to the applied potential. Melamine molecules could be adsorbed onto the electrogenerated SERS substrate, promoting the enhancement of the Raman signal. At potentials lower than  $-0.70\text{ V}$ , other phenomena related to the electrolytic media, such as adsorption of ions or oxygen reduction, imply a displacement of melamine molecules from the EC-SERS substrate.

The analysis of all the Raman spectra registered during this CV experiment shows significant differences depending on the applied potential (Fig. 3), which supports our hypothesis of melamine adsorption on the EC-SERS substrate. The orientation of the adsorbed molecule on a SERS-active surface seems to be a key factor and responsible for the differences in the Raman spectra at different points in the CV.

It should be noted that the Raman response starts showing a band around  $707\text{ cm}^{-1}$ , accompanied by the band at  $1442\text{ cm}^{-1}$ . When the potential is changed to more negative potentials, a second band appears at lower Raman shifts,  $677\text{ cm}^{-1}$  (Fig. 3A); even for a few spectra, in a narrow potential window, the two bands ( $677\text{ cm}^{-1}$  and  $707\text{ cm}^{-1}$ ) can be observed; the band at  $1442\text{ cm}^{-1}$  is less notable at these potentials, and a band at  $1000\text{ cm}^{-1}$  is also noticeable.

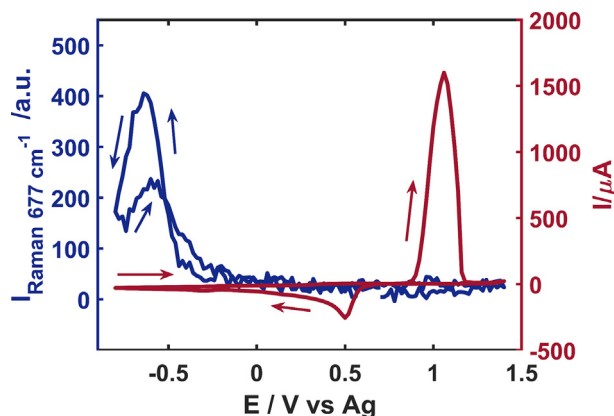


Fig. 2. CV (garnet line, right axis) and voltaRamagram (blue line, left axis) of  $10\text{ }\mu\text{M}$  melamine in  $0.1\text{ M KCl}$ . Au-SPE is used as substrate to carry out the experiment. VoltaRamagram shows the evolution of the main Raman band of melamine ( $677\text{ cm}^{-1}$ ) with the applied potential. Experimental conditions described in experimental section.

Melamine is orientated differently on the surface depending on the applied potential. Both bands at  $677\text{ cm}^{-1}$  and  $707\text{ cm}^{-1}$  are related to the breathing of the ring, so the main change observed in the onward scan can be associated with the orientation of melamine on the EC-SERS substrate [31,32]. In this case, as these bands are significantly more intense around  $-0.80\text{ V}$ , it means that the molecule is placed parallel to the electrode surface at cathodic potentials. Moreover, the band at  $383\text{ cm}^{-1}$ , which is related to  $\delta(\text{CN})$ , disappears at potentials more negative than  $-0.40\text{ V}$ , and the band at  $1442\text{ cm}^{-1}$  increased up to about  $-0.50\text{ V}$ , losing intensity from  $-0.50\text{ V}$  onward. The evolution of the Raman bands confirms changes in the orientation of the molecule on the EC-SERS substrate. The comparison of the voltaRamagram at different Raman shifts gives significant information about these changes, as shown in Fig. S2 for the bands peaking at  $677$  and  $707\text{ cm}^{-1}$ .

When the potential is scanned in the opposite direction, towards potentials more positive than the vertex potential ( $-0.80\text{ V}$ ), all these changes can also be observed (Fig. 3B), but in the opposite direction. This fact evidences the importance of the applied potential and the advantages of using TR-Raman-SEC.

As demonstrated, TR-Raman-SEC is a helpful technique to detect melamine and it can be used to study their interaction with nanostructured gold surfaces. But, in order to develop a new analytical method, an improvement in both sensitivity and reproducibility of the SERS signal is mandatory.

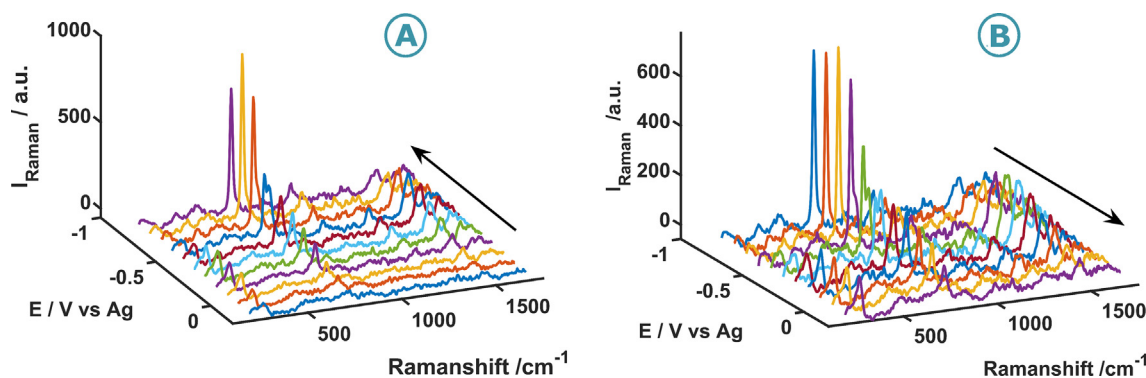
### 3.2. Optimization of the measurement methodology to generate the EC-SERS substrate

In a first stage, the effect of the KCl concentration was studied. As  $\text{Cl}^-$  ions are mandatory to form the complex  $[\text{AuCl}_4]^-$ , the concentration of  $\text{Cl}^-$  seems to be a key factor. As shown in Fig. S3, three different KCl concentrations were evaluated, obtaining the best Raman response for melamine with  $0.1\text{ M KCl}$ . A higher KCl concentration does not yield a good EC-SERS substrate because too much  $[\text{AuCl}_4]^-$  was generated, giving a high amount of gold deposited on the electrode but also a high amount of gold oxide, as can be deduced from the Raman spectra obtained at the end of the experiments, Fig. S3C. On the contrary, a concentration lower than  $0.1\text{ M}$  did not generate enough  $[\text{AuCl}_4]^-$  complex to be reduced in the forward scan, generating an EC-SERS substrate less sensitive to melamine than the one obtained at  $0.1\text{ M KCl}$ .

A pH optimization was also carried out to study the effect of the pH in the EC-SERS substrate generation. An alkaline medium was discarded due to the easy formation of oxides which difficult both melamine adsorption and AuNPs generation. Therefore, a comparison between results obtained at  $\text{pH} = 1$  ( $0.1\text{ M HCl}$ ) and at  $\text{pH} = 6.1$  ( $0.1\text{ M KCl}$ ) was made, detecting significant differences between them (Fig. S4). Experiments carried out in  $0.1\text{ M KCl}$  ( $\text{pH} = 6.1$ ) provided more intense Raman spectra, selecting this electrolytic medium as the most appropriate to obtain EC-SERS substrates sensitive enough to melamine.

Different potential scan rates were also evaluated to minimize the experiment length. The scan rate selected should greatly affect the formation of the nanostructures, as is a key factor in the nucleation and growth steps of the nanostructures. Three different scan rates were evaluated:  $20\text{ mV}\cdot\text{s}^{-1}$ ,  $50\text{ mV}\cdot\text{s}^{-1}$  and  $100\text{ mV}\cdot\text{s}^{-1}$  (involving experiment lengths of  $220\text{ s}$ ,  $88\text{ s}$  and  $44\text{ s}$ , respectively). As can be seen in Fig. S5, the highest Raman response is obtained with a scan rate of  $50\text{ mV}\cdot\text{s}^{-1}$  although the lack of reproducibility is notorious. A scan rate of  $20\text{ mV}\cdot\text{s}^{-1}$  achieves a higher reproducibility without losing much sensitivity but implies a longer experimental time.

Therefore, with these results, other experimental strategy was explored to improve both the length, the reproducibility and the sensitivity of the experiment. Since the growth rate is a key factor in the properties of the electrogenerated EC-SERS substrates, and a low



**Fig. 3.** Raman spectra of 50  $\mu\text{M}$  melamine vs. potential scan in 0.1 M KCl medium. In part A, the potential evolves from +0.20 V to  $-0.80$  V in the cathodic direction. Part B shows the opposite direction (anodic direction), from  $-0.80$  V to +0.20 V. All spectra are registered with an integration time of 1 s and a scan rate of  $20 \text{ mV}\cdot\text{s}^{-1}$ .

scan rate favors the growth of more reproducible nanostructures, it is possible to control it with the applied potential.

Thus, in this work, a new strategy based on a multi-pulse amperometric detection (MAD) protocol is proposed to obtain a better response in a fast way by applying different potential steps. A careful selection of the number of steps required to obtain a suitable EC-SERS substrate is needed to optimize the MAD protocol. In a first stage, a set of experiments with 9 potential pulses related to the oxidation of gold, the reduction of the gold compounds generated during the oxidation and the adsorption of the target molecule observed in the CV (Fig. 2) was made. The studied potential pulses were: +0.70 V (5 s), +0.97 V (5 s), +1.05 V (5 s), +1.15 V (30 s), +1.25 V (5 s), +0.55 V (20 s), +0.25 V (20 s),  $-0.30$  V (20 s) and  $-0.70$  V (20 s). In this multi-pulse experiment, a similar Raman response than the one observed in the CV experiment was obtained. This result could be expected because we are reproducing the potentials applied in the CV, oxidizing the gold electrode in a step-by-step way, and reducing the oxidized gold in a similar way than in the CV. However, not all potential pulses should be necessary to obtain a good EC-SERS substrate. An experimental strategy based on the removal of pulses one by one was used to select the most important potential pulses. In this regard, in a series of 9 experiments, a different potential pulse was removed each time to determine and understand the effect that each potential pulse has on the EC-SERS response (data not shown).

From the results obtained from this set of experiments it was concluded that only 4 steps were essential to generate a suitable EC-SERS substrate (+1.15 V, +1.25 V, +0.25 V, and  $-0.70$  V) because the other potentials did not enhance significantly the Raman response. Of course, another significant factor to consider is the length of these potential pulses. Design of experiment (DOE) is a useful statistics tool to optimize the experimental parameters [33,34]. Thus, the potential pulse duration effect was evaluated exploring 2 different times (long time, 30 s, and short time, 5 s). To clarify this point, a DOE with 4 factors (being each factor one potential step) and 2 levels (long and short time) was made using 19 experiments (see Fig. S6 and Table S1 to get more information). This study reflects that the reduction stage (at +0.25 V) is the most influential process and needs the most extended time, as can be seen in the Pareto chart (Fig. S6). From these results can be deduced that there is a small influence of the oxidation process. Besides, a short oxidation time is enough to obtain a good Raman response.

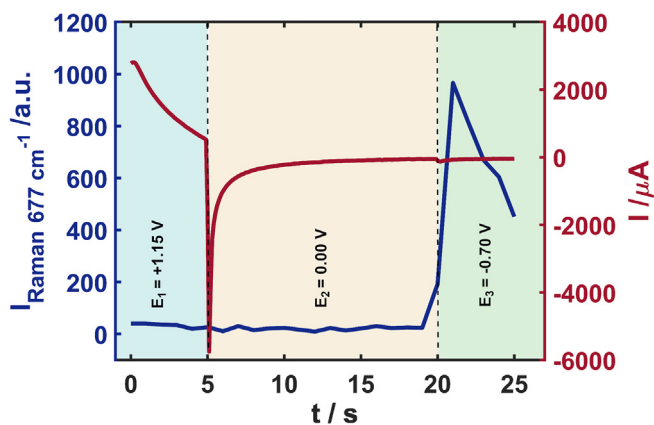
Based on all the information gathered, it was necessary to perform and additional series of experiments to find the best potentials to apply and the most suitable length of the potential pulses to obtain the most melamine-sensitive EC-SERS substrate and the shortest analysis procedure. These experiments consist of applying only one oxidation step during 5 s (+1.15 V or +1.25 V), and different reduction potentials

during 15 s (+0.40 V, +0.30 V, +0.20 V and 0.00 V). Finally, after this exhaustive study, it was demonstrated that a protocol with only three steps is suitable for obtaining a good EC-SERS response in a reproducible way. The optimized experimental conditions consisted of a three-step protocol: (1) +1.15 V for 5 s in order to generate  $[\text{AuCl}_4]^-$  in solution, avoiding the generation of gold oxides which were not observed in the Raman spectrum; (2) 0.00 V for 15 s to reduce the  $[\text{AuCl}_4]^-$  in solution, generating the SERS substrate; and (3)  $-0.70$  V for 5 s in order to adsorb melamine molecules on the SERS substrate. The whole experiment lasts only 25 s.

Fig. 4 shows a comparison between the evolution of the current (chronoamperogram) and the evolution of the main Raman band of melamine at  $677 \text{ cm}^{-1}$  with time (chronoRamangram) during the three-step protocol.

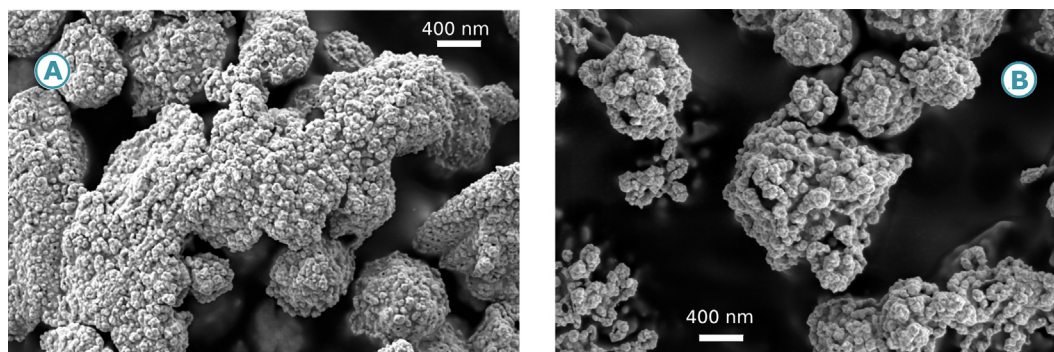
Paying attention to the chronoRamangram, it can be observed that Raman signal of melamine is not detected until the third step, in which a potential that implies the adsorption of melamine is applied, although the formation of the gold nanostructures takes place in the second step. This experiment reveals the importance of the electrochemical induced adsorption of the molecules in the SERS phenomenon.

The morphology of the nanostructures generated during the first reduction step at 0.00 V for 15 s are shown in Fig. 5A. FE-SEM images show the generation of nanostructures on the gold microparticles that form the pristine Au SPE. After the adsorption of melamine at  $-0.70$  V for 5 s, no important morphological changes are observed in the SERS



**Fig. 4.** Chronoamperogram (red line, right axis) and ChronoRamangram at  $677 \text{ cm}^{-1}$  (blue line, left axis) of  $10 \mu\text{M}$  melamine in 0.1 M KCl, applying the optimized protocol with 3 step potentials.





**Fig. 5.** FE-SEM images of the Au SPE used in the experiment shown in Fig. 4. (A) image obtained after step 2, at 0.00 V for 15 s to reduce the  $[\text{AuCl}_4]^-$  in solution, generating the SERS substrate; and (B) after step 3 at  $-0.70$  V for 5 s in order to adsorb melamine molecules on the SERS substrate.

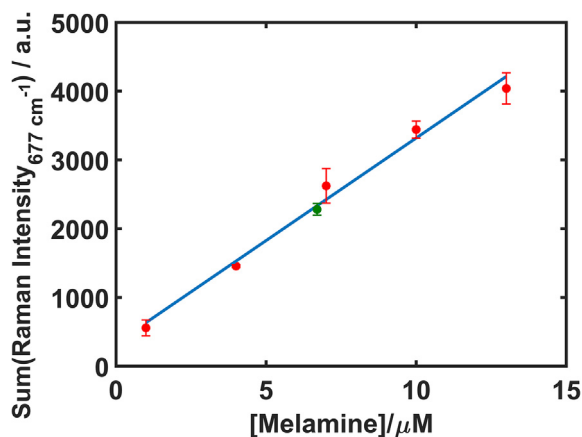
substrate formed in the second step, indicating that the enhancement of the Raman signal is related to the adsorption of the molecule on the plasmonic gold nanostructures.

It should be highlighted that this protocol provides a better response than the one obtained for the CV protocol, and in a faster way. As illustrated in Fig. 4, a good SERS substrate is electrogenerated, and melamine is detected, in only 25 s. What is more important, MAD protocol has improved the analytical signal, becoming twice the one provided by the CV protocol (see Fig. 2), which allows us to achieve better sensitivity and lower detection limits. If a higher sensitivity were needed, the step of melamine adsorption could be longer. This new protocol was used to perform the determination of melamine.

### 3.3. Melamine determination and figures of merit

Once the best protocol was found, a linear regression model was performed in the melamine concentration interval of  $1 \mu\text{M}$  and  $13 \mu\text{M}$ . This range is adequate to detect and quantify melamine in the low levels required for WHO [5], establishing the safety limit in  $8 \mu\text{M}$ . It was also included a test sample of tap water in which  $6.7 \mu\text{M}$  melamine was spiked. The Raman response for the calibration curve (Fig. 6) is obtained by the cumulative sum of the Raman spectra obtained during the 3<sup>rd</sup> step, in this way the sensitivity is improved. Three samples were measured for each concentration in the calibration curve shown in Fig. 6 to demonstrate the good reproducibility of the EC-Raman measurements.

Table 2 shows the main figures of merit of the calibration model ( $I_{\text{Raman}, 677 \text{ cm}^{-1}} = 298.31 \cdot C_{\text{melamine}} + 335.5$ ). The excellent figures



**Fig. 6.** Regression curve of  $1\text{--}13 \mu\text{M}$  melamine with the electrochemical protocol shown in Fig. 4. The Raman response corresponds to the sum of the height of Raman band at  $677 \text{ cm}^{-1}$  from the 5 spectra obtained during the 3<sup>rd</sup> step potential, each sample was measured three times.

**Table 2**

Regression parameters of the linear calibration model for the determination of melamine in  $0.1 \text{ M KCl}$  by TR-Raman-SEC and EC-SERS gold substrates.

$R^2$	Syx	RSD slopes (%) (n = 3)	LOD (M)	LOQ (M)
0.99	178.9	5.3	$2.6 \cdot 10^{-7}$	$8.6 \cdot 10^{-7}$

of merit become this method as a powerful analytical tool. The low value of the standard deviation of residuals ( $S_{yx}$ ) indicate the low dispersion of data. The methodology presented in this work also shows a high reproducibility with a %RSD between slopes of 5.3 %, for 3 replicated calibrations, much lower than those usually reported in SERS measurements [11].

The prediction of the test sample (tap water) with the calibration model is  $6.5 \pm 0.7 \mu\text{M}$ , with a recovery of 97.4 % and a low relative standard deviation (RSD = 4.4 %, n = 3).

This protocol provides a high sensitivity. With the objective to calculate the limits of detection (LOD) and quantification (LOQ) [35], a new calibration curve model was constructed in a lower concentration range than the previous regression curve, including ( $0\text{--}7 \mu\text{M}$ ), achieving limits of detection and quantification of  $260 \text{ nM}$  and  $860 \text{ nM}$ , respectively. It was demonstrated that using 5 s in the detection step (3<sup>rd</sup> MAD step) the Raman response is high enough to detect melamine under the safety limit, but the LOD could be improved by increasing the time of this step. The new MAD-based EC-SERS detection method provides similar results to other analytical methods (Table S2) but using a very fast and simple analytical approach. This protocol should be useful for the analysis of more complex samples, but always after a good preparation of the test sample. As is described in literature [36–40], removing of proteins, for example in milk or food products, precipitation of interferents, filtration and/or centrifugation should be needed to obtain a suitable sample without potential interferents of the Raman response.

## 4. Conclusions

A new TR-Raman-SEC method using Au-SPEs has been proposed for the quantitative analysis of melamine, a widely used compound that can cause several health problems if ingested, even death.

This method is based on multi-pulse amperometric detection, which allows us to generate an EC-SERS substrate in a highly reproducible, fast and simple way. The optimization of several variables ( $\text{Cl}^-$  concentration, pH, scan rate, potential applied, potential pulse length) has been performed, proving the importance of adsorption in EC-SERS measurements and allowing us to detect and quantify melamine at levels below the limit of safety fixed by WHO. The limits of detection and quantification were  $260 \text{ nM}$  and  $860 \text{ nM}$ , respectively.

This new protocol for preparing EC-SERS substrates can be similarly extrapolated to other molecules, optimizing the accurate potentials, and promoting a new easy protocol integrated into water routine analysis.

### Author contributions

S.H., L.G, M.P.E and W.Ch. contributed to the acquisition of data. S.H, W.Ch, M.P-E, A.H and A.C. contributed to the conception, design and implementation of the research and to the analysis and interpretation of the results. S.H, W.Ch, M.P-E, A.H and A.C. contributed to the writing of the manuscript and to the revision of the manuscript critically for important intellectual content. This work has been headed by A.C. and S.H.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jelechem.2022.116478>.

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