

Adsorptive Stripping Voltammetric Determination of Morin in Tea Infusions and Chocolate Drinks on a Gold Electrode. Effect of Cetylpyridinium Bromide on the Sensitivity of the Method

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The use of a gold electrode to determine Morin (MO) in the presence of two polyphenolic compounds as Quercetin (Q) and Rutin (RU) by cyclic and adsorptive stripping voltammetry is reported. The effects of various operational parameters such as pH, supporting electrolyte, adsorptive potential and time (E_{ads} , t_{ads}) were optimized. The optimum experimental conditions chosen were: pH 3.0 (phosphate buffer 0,001 mol L⁻¹), E_{ads} : -0.20 V and t_{ads} : 60s. Under these conditions MO was oxidized at +0.49 V and the linear calibration curves ranged from 0.5 and 5.6 $\mu\text{mol L}^{-1}$. When cetylpyridinium bromide (CPB) was added to the electrochemical cell, a Morin/CPB aggregate was formed, increasing the peak current of MO for at least 30 % than in the absence of this cationic surfactant, and the detection limit changed from 0.40 to 0.083 $\mu\text{mol L}^{-1}$ with 15.0 mol L⁻¹ of CPB. The method was validated determining MO in two water samples spiked with MO, Q and RU and finally, was successfully applied to the determination of MO in tea infusions and chocolate drinks samples.

Keywords: Morin; Gold electrode; Adsorptive stripping voltammetry; Tea drink; Chocolate drink.

1. INTRODUCTION

Flavonoids, a naturally occurring group of polyphenolic phytochemicals, are one of the most commonly ingested groups of compounds found in plants such as tea, coffee and fruits based diet. Responsible for a wide range of pharmacological activities, there are ~8,000 flavonoids that can be divided into several classes, including but not limited to: flavanones, flavonols, flavones, and

isoflavones, based on their chemical structure. Overall, flavonoids continue to be extensively studied due to their potential treatment benefits in disorders such as obesity, diabetes, cardiovascular disease, as well as cancer because their antiallergic, antiviral, anti-inflammatory, vasodilatory and antioxidant activity. Among the polyphenols class of plants flavonoids, Morin (3,4',2',5,7-pentahydroxyflavone, Fig. 1) was originally isolated from the members of the *Moraceae* family and is a major component of many fruits, herbs and wine [1-6]. However, the activity of other flavonoids like Q and RU is double [7], which indicates the importance of the 2-OH adjacent in the C-ring structure. The oxidation potential of MO, Q and RU, depends on the properties of the substituents on the ring B and C, these rings are the most active sites [6,8].

Some techniques used to determine MO are UV-VIS spectrophotometry and HPLC with detection limits of 1.0 and 20.0 nmol L⁻¹, respectively [9,10] proving to be a very sensitive technique, but with higher instrumentation cost. On the other hand, electro-analytical techniques have been shown to be sensitive and low operational cost. Therefore, it has been the most used technique to detect MO. Some reports used modified electrodes with vanadium oxide and graphene [2], nano-silver particles [11], carbon nanotubes [12,13] and mercury [14]. On the other hand, the determination of Morin with gold electrode has not been reported yet.

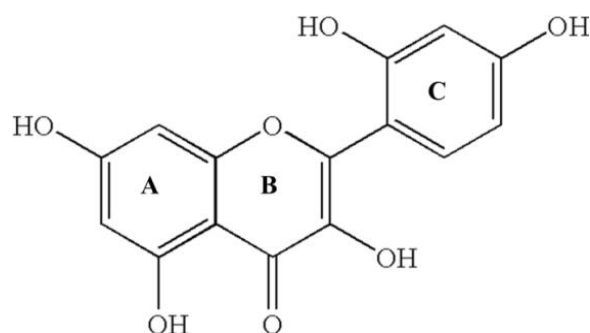


Figure 1. Structure of Morin

In general, surfactants may affect the speed of the electrochemical reactions, increase the reversibility of the system, and increase the oxidation or reduction current [15]. Interaction between flavonoids and surfactants of different charges has been previously reported. This interaction is primarily between the flavonoid and hydrophobic surfactant zone, acting as an extractor agent. However, if the concentration of the surfactant is above the critical micelle concentration the current normally decreases [6,16].

The aim of this work is to show that adsorptive stripping voltammetry with a gold electrode makes it possible to determine Mo in the presence of Q and RU. The effect of CPB has been studied in the determination of MO using a glassy carbon electrode modified with carbon nanotubes [12]. However, to the best of our knowledge, the effect of CPB on the peak current using a gold electrode has not been reported.

2. EXPERIMENTAL PART

2.1. Chemical and Reagents

Water used for sample preparation, reagent dilution, and rinsing purposes was obtained from Wasselab Purifier System (ASTM D1193). All the chemicals (acetic acid, phosphoric acid, nitric acid, sodium hydroxide, sodium acetate, methanol, etc.) were analytical grade from Merck (Darmstadt, Germany). Morin (MO), Quercetin (Q), Rutin (RU) and cetylpyridinium bromide (CPB) were obtained from Aldrich. Stock solutions containing 5.0 mmol L^{-1} and $5.0 \text{ } \mu\text{mol L}^{-1}$ of MO, Q and RU were prepared in methanol. Phosphate buffer solutions (PBS) were prepared from H_3PO_4 , NaH_2PO_4 or NaHPO_4 (Merck), adjusting the pH required with NaOH or HCl 0.5 mol L^{-1} . Acetate buffers solutions were prepared with acetic acid adjusting the pH required with NaOH.

2.2. Instruments

Cyclic voltammograms (CV) and adsorptive stripping voltammograms (AdSV) were obtained using a potentiostat DropSens 400 μStat (Oviedo, Spain). The electrochemical cell was composed of a gold electrode (1 mm θ), a reference electrode of Ag/AgCl/ KCl 3 mol L^{-1} and a platinum wire used as auxiliary electrode were obtained from CH-Instruments. A magnetic stirring stick was used to homogenize the solution during accumulation time (500 rpm). The pH measurements were made with a Lovibond SD 50 pH meter.

2.3. Procedure for obtaining voltammograms.

In the electrochemical cell were added 9.5 mL of ultra-pure water ($18 \text{ M}\Omega \text{ cm}$), 0.5 mL of 0.02 mol L^{-1} phosphate buffer solution (PBS) and 100 μL of MO 5.0 mmol L^{-1} . Cyclic voltammograms (CV) were recorded from -0.20 to 0.80 V with a scan rate of 50.0 mV s^{-1} , whereas adsorptive stripping voltammograms (AdSV) were recorded from 0.2 to 0.8 V at 100.0 mV s^{-1} with a E_{ads} of -0.20 V during 60 s. Each voltammogram was repeated three times. The calibration curves were obtained and linear regression and detection limits were calculated. With this electrode is not necessary to eliminate oxygen in the cell.

2.4. Sample Preparation

Two kinds of commercial tea infusions and two chocolate drinks were obtained in a supermarket on the Ibagué city. In the electrochemical cell 1.0 and 0.5 mL of the samples without previous treatment were added. To eliminate matrix effects the standard addition method was used. All data were obtained at room temperature ($\sim 25 \text{ }^\circ\text{C}$).

3. RESULTS AND DISCUSSION

3.1 Effect of the supporting electrolyte.

The study in function of supporting electrolyte was carried out using three different reagents: nitric acid solution, acetate buffer and phosphate buffer (pH: 4.0). The concentration of electrolyte on the solution was always 0.001 mol L^{-1} . It was found that with these electrolytes one signal between 0.49 to 0.56 corresponding to oxidation of the OH groups [8] was observed. Fig.2 shows the cyclic voltammograms of a $0.098 \text{ mmol L}^{-1}$ MO solution (scan rate 50.0 mV s^{-1}). The peak current obtained with nitric acid was $0.37 \mu\text{A}$ (E_{peak} : 0.56 V), with acetate buffer was $0.36 \mu\text{A}$ (E_{peak} : 0.49 V) and with phosphate buffer was $0.49 \mu\text{A}$ (E_{peak} : 0.49 V). In addition phosphate buffer gives the best sensitivity and was selected for further measurements. Using screen printed carbon electrodes modified with carbon nanotubes and ionic liquids, anodic peak current was observed at the same value of potential [17].

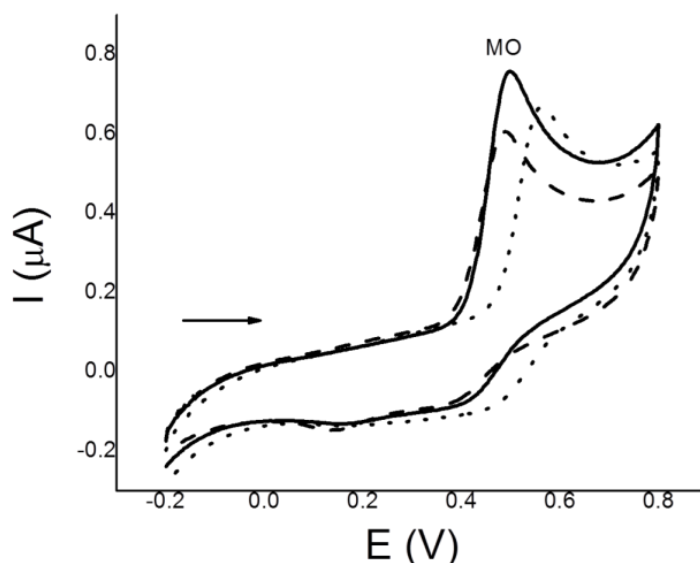


Figure 2. Cyclic voltammograms for Morin $0.098 \text{ mmol L}^{-1}$ using a gold electrode. Experimental conditions: (—) Phosphate buffer, (---) acetate buffer and (····) nitric acid solution. Scan rate 50.0 mV s^{-1}

3.2. Effect of pH

In order to select the optimum pH value at which peak current of MO is maxima, the influence of the pH of the medium was studied in the 2.0–7.0 range using phosphate buffer solutions (0.001 mol L^{-1}). Fig.3A shows the cyclic voltammograms of a $0.098 \text{ mmol L}^{-1}$ MO solution obtained to different pH (scan rate 50.0 mV s^{-1}) and Fig.3B shows their effect on the peak current.

The peak potential of oxidation of MO shifted towards less positive values with increasing pH, (between 0.53 to 0.35 V) and maximum peak current was obtained at pH 3.0, indicating that there are protons involved in the oxidation process. The slope of the curve was 0.046 which is close to 0.059, indicating that the number of H^+ is equal to the number of electrons ($2\text{H}^+ = 2\text{e}^-$) [8]. A slope of 0.048

was reported using a glassy carbon electrode modified with carbon nanotubes [13]. Further measurements were carried out at pH 3.0.

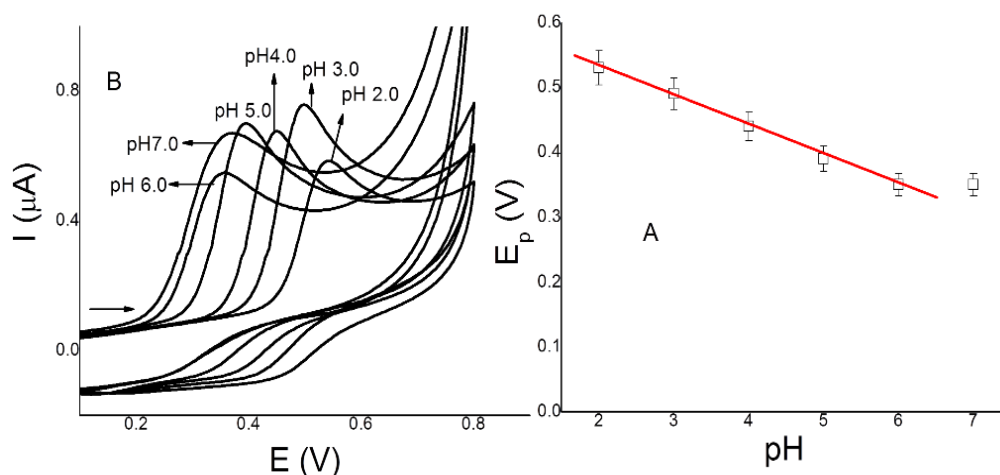


Figure 3. A. Cyclic voltammograms for Morin $0.098 \text{ mmol L}^{-1}$ in function of pH (phosphate buffer solutions). B. Effect of pH on the peak current of MO. Experimental Conditions: scan rate 50.0 mV s^{-1}

3.3. Effect of scan rate of the cyclic voltammograms

In order to know the mass transport process, the influence of the scan rate (v) (between 10.0 to 100.0 mV s^{-1}) on anodic peak current for MO was studied. Fig.4A shows plots of cyclic voltammograms and Fig.4B the respective plot peak current in function of scan rate. Peak current of oxidation of MO increased linearly with stirring speed until 100.0 mV s^{-1} . Further measurements were carried out at scan rate of 100.0 mV s^{-1} . The curve equation was $i_{\text{peak}} = -0.0019 + 0.0048v$ ($R: 0,994$). These results indicate that the process is controlled by adsorption. Similar results were observed with a glassy carbon electrode modified with carbon nanotubes [13].

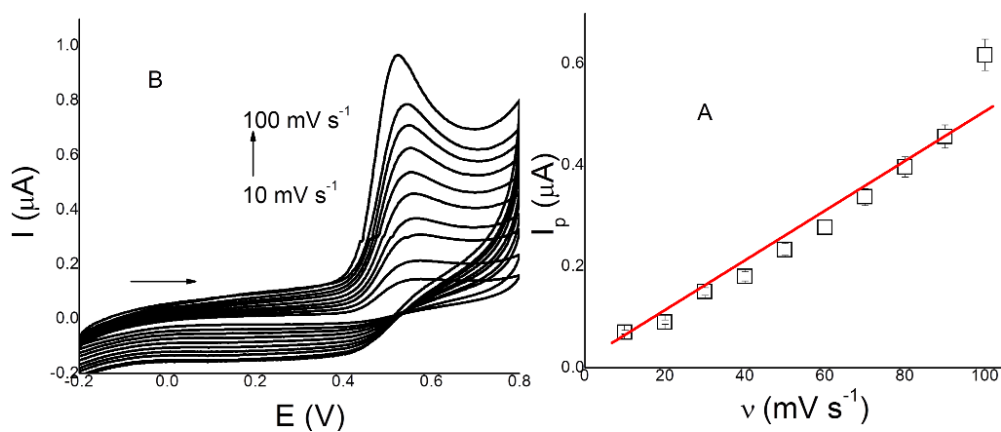


Figure 4. (A) Cyclic voltammograms of MO ($0.098 \text{ mmol L}^{-1}$, pH: 3.0) as a function of scan rate (10.0 - 100 mV s^{-1}). (B). Influence of scan rate on the peak current of MO.

3.4. Effect of CPB concentration

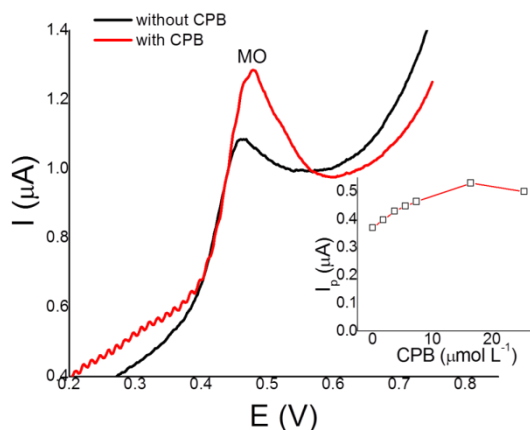


Figure 5. Adsorptive stripping voltammograms for MO ($10.0 \mu\text{mol L}^{-1}$) in function of CPB (0.0 and $15.0 \mu\text{mol L}^{-1}$). Insert: Effect of CPB concentration on the peak current of MO. Conditions: pH 3.0 (PBS), E_{ads} : -0.2 V , t_{ads} : 60 s

One possible way of enhancing the adsorptive process is the use of surfactants, whose beneficial effects are unpredictable, as they tend to interfere by competitive adsorption. The surfactant may interact with MO, adsorbing on the electrode surface increasing their concentration in the accumulation step and therefore increasing the sensitivity of the method. The influence of cationic surfactant as cetylpyridinium bromide (CPB) on the oxidation peak current of MO was studied in the concentration range of 2.5 to $25.0 \mu\text{mol L}^{-1}$ using adsorptive stripping voltammetry technique. Previous measurements were carried out as a function of the adsorption potential and time finding that the adsorption of MO on the gold electrode is maximum at -0.20 V (t_{ads} : 60 s). As illustrated in Fig.5, the peak current of MO increased about 30 % in the presence of $15.0 \mu\text{mol L}^{-1}$ of CPB, and the signal was displaced only 20 mV towards more positive potentials. On the other hand, the peak current of MO decreased slightly for concentrations greater than $15.0 \mu\text{mol L}^{-1}$. This concentration of CPB gives the best sensitivity and was selected for further measurements. CPB interacts with the MO through its hydrophobic or chain aliphatic [6] increasing of MO on the electrode surface. On the other hand, in the presence of sodium dodecyl sulphate (anionic surfactant) peak current of MO decreased.

3.5. Linear range, detection limit, and repeatability of the method.

The calibration graph for the determination of MO was obtained under the optimized conditions: pH 3.0 (0.001 mol L^{-1} , phosphate buffer solution), E_{ads} -0.2 V , t_{ads} 60 s and scan rate 100 mV s^{-1} . Under these conditions the peak current was proportional to the concentration of MO over the 0.49 – $5.60 \mu\text{mol L}^{-1}$ range, with a 3σ detection limit of $0.40 \mu\text{mol L}^{-1}$ without CPB and $0.083 \mu\text{mol L}^{-1}$ in the presence of CPB ($15.0 \mu\text{mol L}^{-1}$). Fig.6A shows adsorptive stripping voltammograms in the presence of CPB and Fig.6B shows the calibration curves for MO in the presence and absence of CPB. These results show that the slope of the MO calibration curve increases in the presence of CPB

indicating higher sensitivity. Therefore the current was greater, and the detection limit calculated is less than $0.10 \mu\text{mol L}^{-1}$. Repeatability for a $50.0 \mu\text{mol L}^{-1}$ MO solution was 2.0 % ($n = 7$).

By comparing the sensitivity with other techniques and modified electrodes, the result of this work showed to be sensitive with others electrodes presented in Table 1 In others reports were necessary to modify the electrodes, The greater sensitivity was presented with modified electrodes with metal complexes. Whereas in our work we used an unmodified gold electrode.

3.6 Validation of the method and interferences study.

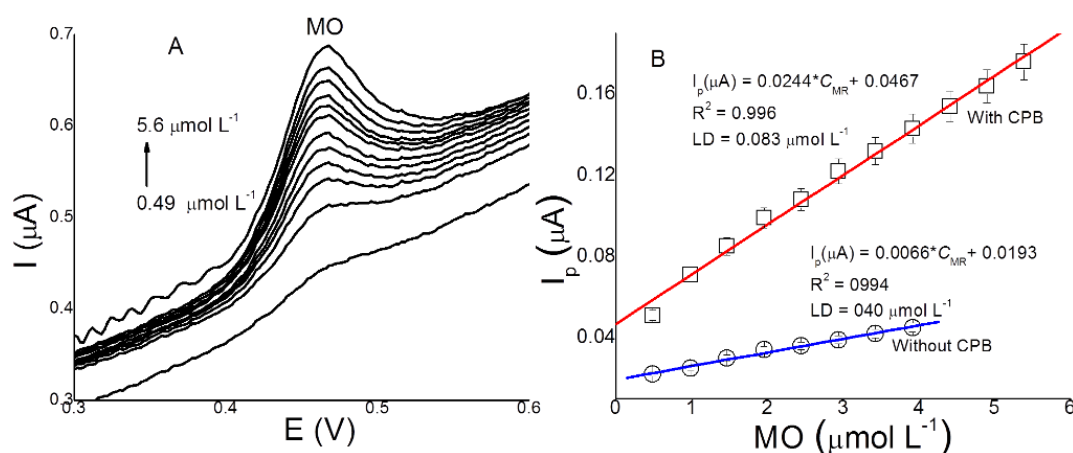


Figure 6. (A) Adsorptive stripping voltammograms in function of MO concentration. (B) Plot i_{peak} vs. MO concentration without CPB (blue) and with CPB (red). Conditions: pH 3.0, $C_{\text{CPB}} 15.0 \mu\text{mol L}^{-1}$; $E_{\text{ads}}: -0.20 \text{ V}$, $t_{\text{ads}}: 60 \text{ s}$.

Table 1. Determination of Morin using different electrodes

Technique	Electrode	Matrix	Detection limit ($\mu\text{mol L}^{-1}$)	Reference
DPV	SWNT-COOH/GCE	mulberry	0.028	[12]
SWV	MWCNT-IL-GCE	plants	0.054	[18]
CV	CNTPE	glycyrrhiza	0.001	[11]
SWV	HMDE	urine	0.0071	[12]
DPV	$\text{V}_2\text{O}_5 \text{ NF/GCE}$	fruits	0.050	[2]
SWV	AgNPsAETGO/GCE	grapes	0.003	[9]
AdSLV	GE	tea, chocolate	0.083	This work

DL: detection limit; GCE: Glassy carbon electrode; CPE: carbon paste electrode; GE: gold electrode; MWCNT: multi walled carbon nanotube; NF:nafion; IL: ionic liquids.

The usefulness of the present method was evaluated by determining MO in two water samples spiked with MO 18.0 and $10.0 \mu\text{mol L}^{-1}$. The results were $14.1 \mu\text{mol L}^{-1} \pm 0.10$ (RE 20.0 %) and $11.2 \mu\text{mol L}^{-1} \pm 0.12$ (RE 12.0 %) respectively. On the other hand, the potential interfering in the determination of MO are other flavonoids such as Quercetin and Rutin, which can be oxidized to a similar potentials of MO. **Fig.7** shows the cyclic voltammograms for MO, Q and RU (0.10 mmol L^{-1})

on a gold electrode at pH 3.0 and scan rate of 100 mV s^{-1} . Peak oxidation of the MO, Q and RU complex occurs at 0.41; 0.44 and 0.59 V respectively. This difference indicates that the determination of MO in the presence of RU is possible, but this method (with optimum parameters for MO determination) is not possible in the presence of Q because although the peak current for Q is lower, the signals overlap. Dyes like tartrazine, amaranth, and allura red not showed anodic peak in this zone of potential on the gold electrode.

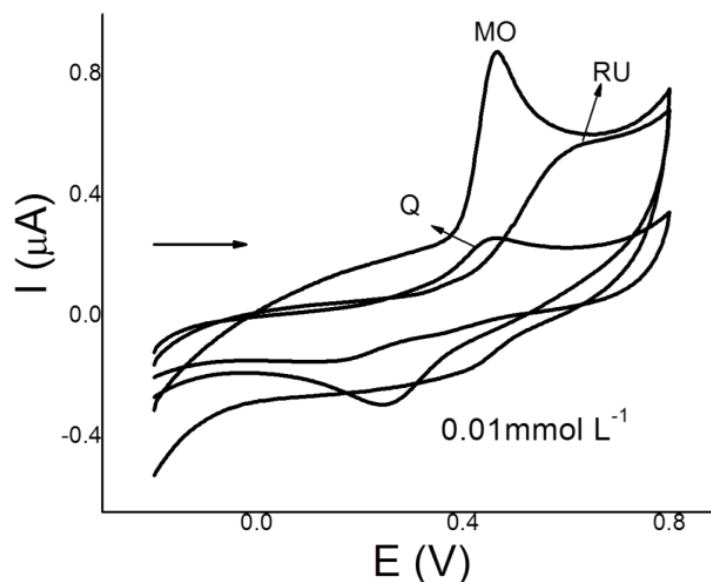


Figure 7. Cyclic voltammograms of MO, Q and RU solutions (0.10 mmol L^{-1}) using a gold electrode. Scan rate 100 mV s^{-1} . Others conditions as in Fig. 3.

3.6. Real sample analysis

The proposed method was successfully applied to the determination of MO in two commercial tea infusions and two commercial chocolate drinks obtained in a supermarket of Ibagué, Colombia. The results are presented in Table 2. Fig. 8 shows adsorptive voltammograms and calibration curve of the tea infusion 1 sample. The values obtained for MO in tea infusions and chocolate drinks samples analyzed were lower compared with mulberry (5.26 mmol L^{-1}) [12]. On the other hand, the values founded were similar to those reported in medicinal plants [17].

Table 2. Analysis of MO in tea infusions and chocolate drinks samples

Samples	Found ($\mu\text{mol L}^{-1}$)	%RSD
*Tea infusion 1	9.0	2.5
Tea infusion 2	7.6.0	1.1
Chocolate drink 1	1.97	1.0
Chocolate drink 2	2.8	1.5

*AdSV and calibration curve showed in Fig.8.

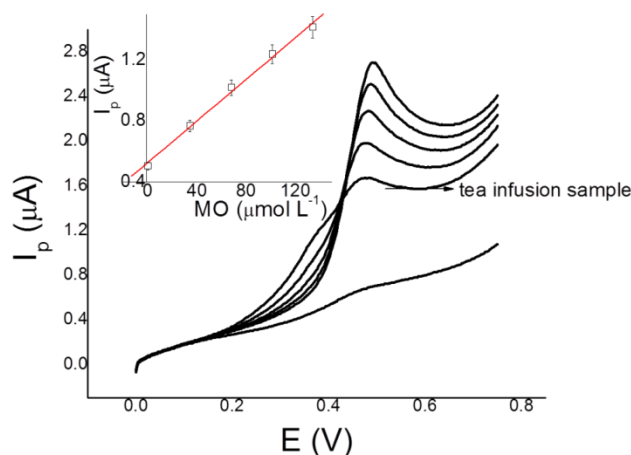


Figure 8. Adsorptive stripping voltammograms and calibration curve (insert) for sample 1* (Table 2) using a gold electrode. Others conditions as in Fig. 5

4. CONCLUSIONS

The herein proposed method for determination of Morin using a gold electrode shows clear advantages in terms sensitivity, selectivity and cost with respect to several reported methods and may be used for quick analysis of tea infusions and chocolate drinks. The presence of CPB enhanced the sensitivity of the method.

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