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the development of screen-printed electrodes [26] presenting a linear response range between 2×10^{-3} and 2 mol L^{-1} .

The present work takes advantage of the electrocatalytic activity of a cobalt(II)–phthalocyanine modified carbon paste electrode for citric acid oxidation towards developing a very straightforward amperometric flow injection analysis method well suited for the analysis of commercial citric juices with minimum sample pretreatment. Ascorbic acid interference is circumvented by sample pretreatment with ascorbate oxidase. The proposed method is more sensitive than the other voltammetric methods already developed [25–27] with additional advantages such as some lowering of the working potential and the ease of renewing the electrode surface. Furthermore, a higher sensitive and selective square-wave voltammetric determination procedure is also presented.

2. Experimental

2.1. Reagents and solutions

Cobalt(II)–phthalocyanine (CoPC) 97% (catalog number 307696), mineral oil and graphite powder ($\leq 20 \mu\text{m}$) were acquired from Sigma-Aldrich, while citric acid (CA) was from VETEC. All other chemicals were of analytical-reagent grade and used as received. All solutions and subsequent dilutions were prepared in ultra pure water taken from a Millipore Milli-Q system (resistivity $\geq 18 \text{ M}\Omega \text{ cm}$). Unless specified elsewhere, an acetic acid/sodium acetate buffer pH=4.5 (0.1 mol L^{-1}) was employed as the supporting electrolyte in the experiments. The supporting electrolytes used for pH studies were H_2SO_4 pH=1.2, biphthalate/hydrochloric acid pH=3.0, biphthalate/sodium hydroxide pH=5.9, hydrogen phosphate/dihydrogen phosphate pH=7.0 and ammonia/ammonium chloride pH=9.0.

2.2. Samples and standard solutions

Samples of commercial citric juices were acquired from local grocery stores. Aliquots of 20 mL were stirred for 30 min in a glass vessel containing about 8 g of thin slices of the mesocarp of cucumber (*Cucumis sativus*) and, afterward, cleaned using a syringe filter with $0.45 \mu\text{m}$ porous membranes (Whatman®). Next, the samples were properly diluted in the supporting electrolyte before analysis. A standard stock solution of 4.0 mmol L^{-1} citric acid, standardized by acid-base titration, was also properly diluted in the same supporting electrolyte.

2.3. Apparatus and measurements

All voltammetric experiments were performed using an Eco Chemie, potentiostat/galvanostat Autolab (Model PGSTAT302N) three-electrode system. Cyclic and square wave (SW) voltammetric studies were carried out in a conventional 10 mL cell. Amperometric flow injection analysis (FIA) experiments were performed using a carbon paste working electrode (about 1 mm apart from the tip of flow stream nozzle) in a wall-jet flow cell configuration which had already been presented in the literature [28] coupled to a Ag/AgCl (KCl, 3 mol L^{-1}) and a platinum wire as reference and auxiliary electrodes, respectively. An Ismatec peristaltic pump (Model IPC-8) and a manually operated proportional injector were employed in the FIA system assembly. All the experiments were performed at room temperature. The FIA assembly is presented in Fig. 1.

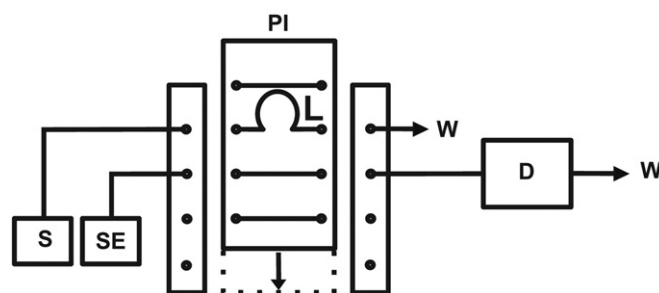


Fig. 1. FIA system diagram. S, sample or standard solutions; SE, supporting electrolyte; L, sampling loop of $140 \mu\text{L}$ (28 cm); PI, proportional injector; D, detector; W, waste. Flow rate of 2.5 mL min^{-1} .

2.4. Preparation of the carbon paste electrodes

Bare and modified carbon pastes were prepared by direct mixing (w/w), for 10 min in a mortar, $x\%$ of CoPC ($x=0, 1, 3, 6$ and 9), $(60-x)\%$ of carbon powder and 40% of mineral oil. The electrodes were built by packing the paste inside a glass tube with a 3 mm inner diameter containing a copper wire at the opposite side for electrical contact. The electrode surface was then smoothed with a weighing paper and, before being used, each electrode was electrochemically pretreated by cyclic voltammetry at 100 mV s^{-1} (10 cycles from $+0.6$ to $+1.2 \text{ V}$) in an acetate buffer, pH=4.5.

2.5. FIA procedure

A simple FIA system protocol was required. The working electrode was polarized at a fixed potential of 0.95 V and the flow rate set at 2.5 mL min^{-1} . Afterwards, aliquots of $140 \mu\text{L}$ of the sample or standard solutions were injected in the carrier stream and their signals registered.

3. Results and discussion

3.1. Redox behavior of citric acid at CoPC-CPE

To ensure a satisfactory electrochemical performance, the CoPC-CPE was mechanically polished with a weighing paper and, then, submitted to 10 consecutive cyclic voltammetric scans between $+0.6$ and $+1.2 \text{ V}$ before use. Such a conditioning procedure assured the maintenance of the electrode surface in a reproducible and stable active state. The potential window up to $+1.2 \text{ V}$ in the electrochemical pre-treatment yields CoPC in the oxidized active state as was demonstrated by the experiment discussed below.

The electrochemical behavior of CoPC-CPE was investigated in an acetate buffer solution. An oxidation wave was clearly observed for the CoPC-CPE, Fig. 2A, which vanished after a few consecutive scans, demonstrating the one way conversion of Co(II)PC to Co(III)PC at that potential window. However, the reduced state of the CoPC could be promptly regenerated electrochemically by scanning the potential in the negative direction or by applying a fixed potential around -0.9 V for about a minute. Evidently, the reduced state could also be regenerated chemically.

It must be emphasized that, although citric acid is not electroactive in a bare CPE, a well defined electrocatalytic wave around 1.0 V can be clearly seen in a CoPC-CPE, Fig. 2B. Actually, the completely vanished CoPC oxidation wave reappears in the presence of citric acid, clearly demonstrating the existence of an electrocatalytic cycle (the electrochemically oxidized Co(III)PC is chemically reduced to Co(II)PC by the citric acid molecules) in the reaction mechanism. This electrochemical behavior makes CoPC

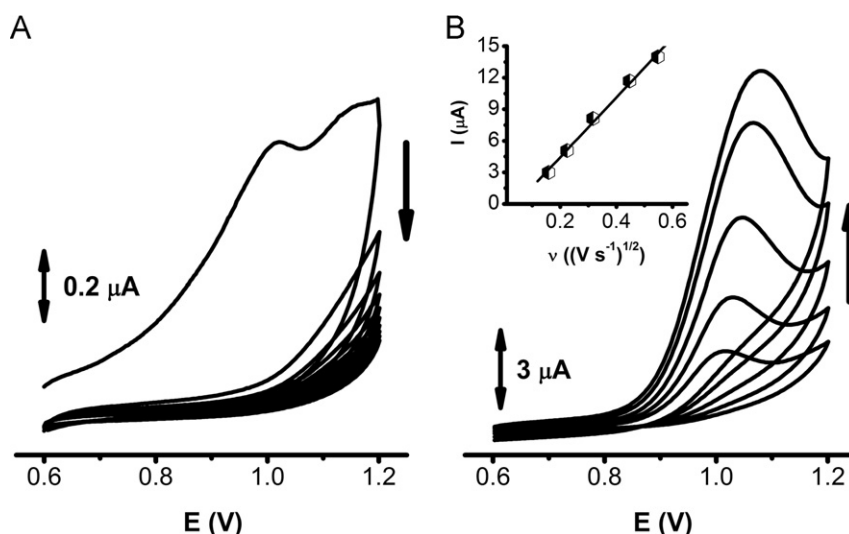


Fig. 2. (A) Consecutive cyclic voltammograms of CoPC-CPE in 0.1 mol L⁻¹ (pH=4.5) acetate buffer at 100 mV s⁻¹. (B) Cyclic voltammograms of 4.0 mmol L⁻¹ CA in 0.1 mol L⁻¹ (pH=4.5) acetate buffer at 25–300 mV s⁻¹.

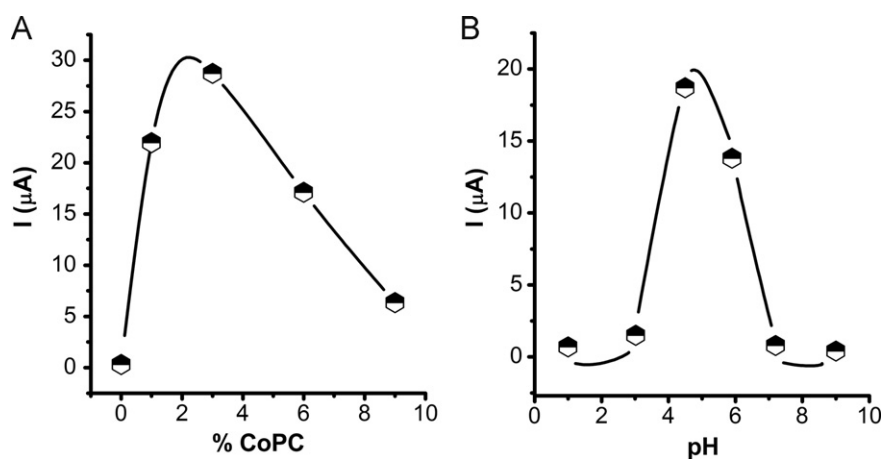


Fig. 3. Influence of (A) CoPC loading and (B) pH on the sensitivity of the voltammetric response.

an important electron mediator for electrocatalysis of citric acid and opens possibilities for developing electroanalytical methods for its determination. In addition, as can be seen in Fig. 2B, there is a linear dependence ($r^2=0.9955$) between peak current to square root of the scan rate, demonstrating that the process encompasses a mass transfer electrochemical process controlled by diffusion. All these electrochemical behavior is in accordance with what has been reported in the literature [25,26].

3.2. Modifier loading

To attain the optimal relationship between carbon powder and CoPC amounts in the modified carbon paste electrode (CPE), the percentage of modifier was investigated. Fig. 3A shows that the best signal to noise rate was verified at around 3% CoPC. For lower amounts of phthalocyanine, the number of active centers for oxidizing CA diminishes, while with higher percentages of CoPC, we can assume that the resistance inside the electrode starts to affect significantly the electrode performance.

3.3. Operational potential and pH

Important parameters affecting electroanalytical response were identified and adjusted for tuning the analytical response

of the proposed FIA method. The operational potential and pH are crucial for the analytical performance of the sensor for application to the proposed amperometric method. Fig. 3B shows the strong influence of the pH on the electrochemistry of citric acid and reveals an optimum sensitivity in the region of pH=4–5, in close agreement with the literature, highlighting what seems to be a reflection of the pKa values of the carboxylic groups in the citric acid [26]. Additionally, the operational potential is a function of pH. Thus, the acetate buffer (pH=4.5) was chosen as the supporting electrolyte and a potential of 0.95 V was chosen for the amperometric measurements since each represented an optimum compromise between sensitivity, signal stability and shield against some electroactive interferences.

3.4. Adjustment of the FIA approach

The applied potential, flow rate and sample volume were tuned up to improve the performance of the FIA system approach. A potential range from 0.80 to 1.10 V was investigated to identify the lowest effective value aiming to prevent interferences from electroactive species, and 0.95 V was chosen as a compromise between sensitivity and selectivity. A flow rate range from 1.0 to 4 mL min⁻¹ was investigated. No significant gain in sensitivity

was observed to flow rates higher than 2.5 mL min^{-1} for a fixed sample volume of $140 \text{ }\mu\text{L}$.

3.5. Interferences

Although ascorbic, tartaric, malic, oxalic and benzoic acids are pointed out as the main interferences in citric acid analysis in fruit juices and soft drinks, in the present approach only ascorbic acid showed a significant interference response at the working amperometric potential of 0.95 V with the proposed sensor. Thus, ascorbic acid was demonstrated as the main interference species for the determination of CA in the analysis of commercial citric juices using the method described above. The interference arises from the overlapping of the oxidation waves of both ascorbic acid and citric acid in the CoPC-CPE. Such a drawback was circumvented by submitting the samples to a pretreatment with the enzyme ascorbate oxidase to eliminate the ascorbic acid before analysis as we have described elsewhere [29]. Briefly, the ascorbic acid is readily destroyed by stirring an aliquot of the sample in the presence of thin slices of the cucumber mesocarp which is rich in ascorbate oxidase.

Table 1 compares the performance of the proposed amperometric FIA and SW approaches to some important methods reported in the literature. Despite their general satisfactory sensitivities, the chromatographic methods [9,10,13,30] are time consuming and the others cannot be considered practical, since they are very laborious [32–34] or involve enzymatic [14,15], chemiluminescence [17,18], photochemical [12], atomic absorption spectrometry [19], fluorimetry [31] and mass spectrometry [35] processes, not generally available in most laboratories. Therefore, it can be clearly seen that the proposed approaches compare advantageously to the previous ones, especially

to all the electroanalytical ones. A close review of the literature reveals that all the previously proposed FIA methods for the determination of CA [38] may also be considered as not practical. The simplest one seems to be a spectrophotometric method based on the discoloring of an iron(III) complex by citrate ions, which suffers from being subjected to numerous interferences [11].

3.6. Analytical application of the CoPC-CPE to real samples

SW voltammetry can be used to obtain a sensitive and selective quantification of CA. Fig. 4 displays SW voltammetric responses under optimized conditions of frequency, pulse height and step potential for increasing concentrations of CA at a CoPC-CPE. Well-defined SW voltammetric peaks are obtained for the oxidation of CA, with peak potentials at 1.0 V . The corresponding calibration plot (Inset of Fig. 4) shows a good linear relationship ($r^2=0.9993$) in the concentration range from 3.81 to $91.2 \text{ }\mu\text{mol L}^{-1}$. Under these conditions, the detection limit was calculated as $2.54 \text{ }\mu\text{mol L}^{-1}$ ($3 \sigma/S$, where σ is the standard deviation of the blank signal and S , the sensitivity) and the quantification limit as $8.46 \text{ }\mu\text{mol L}^{-1}$ ($10 \sigma/S$).

The usefulness of the CoPC-CPE as an electrochemical sensor for CA determination was illustrated by applying the proposed FIA method to the analysis of five different commercial fruit juices. Fig. 5 shows two typical series of the flow injection response peaks of the standards (1–5), before and after the analysis of five samples (A–E). Under these optimized conditions, a selective FIA amperometric method with a good linear response ranging from 0.25 to 15.0 mmol L^{-1} ($r^2=0.9979$) and an estimated detection limit of $0.117 \text{ mmol L}^{-1}$ were easily achieved for a rapid and reliable determination of citric acid (65 samples per hour).

Table 1
Comparison of citric acid determination methodologies.

Technique	Sample	Detection limit (mol L^{-1})	Linear range (mol L^{-1})	Interferences	Ref.
Ion chromatography	Pharmaceuticals	3.13×10^{-7}	$(10.4\text{--}5205) \times 10^{-7}$	No data	[30]
HPLC-UV	Pulps and fruit juices	8.90×10^{-3}	Up to 4.17×10^{-3}	No data	[9]
HPLC-UV	Wines	2.57×10^{-5}	$(5.21\text{--}104) \times 10^{-5}$	No interferences	[10]
HPLC-Potentiometry	Not specified	1×10^{-6}	$(1\text{--}100) \times 10^{-5}$	No data	[13]
Fluorimetry	Human, rabbit and rat blood and plasma	No data	$(2.60\text{--}364) \times 10^{-5}$	No Data	[31]
Spectrophotometry	Orange and lemon soft drinks	No data	$(4\text{--}30) \times 10^{-5}$	Glucose, malic, phosphoric, succinic, oxalic, acetic and tartaric acids	[32]
Spectrophotometry	Canned, soft drinks and juices	5.21×10^{-6}	$(5.21\text{--}104) \times 10^{-6}$	No data	[15]
Spectrophotometry	Soft drinks and pharmaceuticals	4.83×10^{-4}	$(7.79\text{--}250) \times 10^{-4}$	No data	[33]
Spectrophotometry	Fruit juices and soft drinks	2.60×10^{-6}	$(5.21\text{--}6.25) \times 10^{-6}$	Ascorbic acid	[12]
Spectrophotometry	Beverages and pharmaceutical formulations	5.00×10^{-6}	$(0\text{--}5.21) \times 10^{-3}$	No interferences	[34]
Chemiluminescence	Pharmaceuticals and soft drinks	No data	$(2\text{--}1000) \times 10^{-7}$	Cu^{2+} , Mn^{2+} , Cr^{3+} , Ni^{2+} , Co^{2+} , oxalate, tartrate, lactate and ascorbic acid	[17]
Chemiluminescence	Human urine and orange juice	3.0×10^{-8}	$(3.0\text{--}600) \times 10^{-8}$	No interferences	[18]
Atomic absorption spectrometry	Soda, juices and sweet drinks	3.64×10^{-6}	$(10.4\text{--}208) \times 10^{-6}$	Tartaric acid	[19]
Mass spectrometry	Fermentation media	5.21×10^{-9}	$5.21 \times (10^{-8}\text{--}10^{-1})$	No interferences	[35]
Cyclic voltammetry	Not specified	1.17×10^{-4}	$(1\text{--}9) \times 10^{-3}$	No data	[27]
Potentiometry	Juices and isotonic drink	3.0×10^{-6}	$(7\text{--}700) \times 10^{-5}$	Ascorbic and benzoic acids	[3]
Potentiometry	Wines	No data	$(5.21\text{--}260) \times 10^{-4}$	Oxalic acid	[36]
Enzymatic-Amperometry	Fruit, juices and sport drinks	4×10^{-6}	$(1.5\text{--}50) \times 10^{-5}$	Malic acid	[14]
Polarography	Orange and lemon soft drinks	No data	$(2\text{--}100) \times 10^{-4}$	No interferences	[32]
Cyclic voltammetry	Pharmaceuticals, fruits and juices	2×10^{-4}	$(2\text{--}2000) \times 10^{-3}$	EDTA and oxalic acid	[26]
Potentiometry	Soft drinks	No data	$(1\text{--}10) \times 10^{-3}$	No interferences	[37]
Enzymatic-Amperometry	Fruits	2×10^{-5}	$(1\text{--}10) \times 10^{-4}$	No data	[16]
Square wave voltammetry	Fruit juices	2.54×10^{-6}	$(3.81\text{--}91.2) \times 10^{-6}$	Ascorbic acid	This work
Amperometry	Fruit juices	1.64×10^{-4}	$(2.5\text{--}150) \times 10^{-4}$	Ascorbic acid	This work

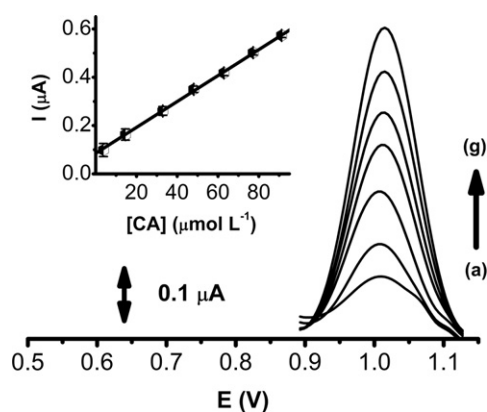


Fig. 4. SW voltammograms (with base line correction) for CA in acetate buffer (pH=4.5) on a CoPC-CPE: (a) 3.81, (b) 14.6, (c) 32.9, (d) 48.1 (e) 62.9 (f) 77.2 (g) 91.2 $\mu\text{mol L}^{-1}$. ($f=20$ Hz, pulse height=25 mV and $\Delta E=2$ mV). Inset: Corresponding analytical curve.

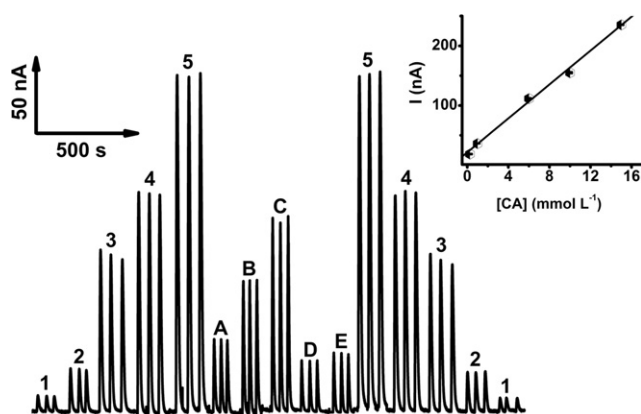


Fig. 5. FIA signals for standard solutions: (1) 0.25, (2) 1.00, (3) 6.00, (4) 10.0 and (5) 15.0 mmol L^{-1} and samples of fruit juices (A–E).

Table 2

Citric acid concentrations in five commercial citric juices ($n=3$).

Sample	Citric acid concentration (mmol L^{-1})		Relative error (%)
	HPLC comparative method	FIA proposed method	
A	13.93 ± 0.12	13.59 ± 0.055	–2.42
B	27.28 ± 0.25	27.79 ± 0.14	1.86
C	16.93 ± 0.11	16.83 ± 0.40	–0.57
D	15.39 ± 0.022	15.39 ± 0.12	0.00
E	22.05 ± 0.50	22.44 ± 0.13	1.78

The optimized FIA approach was employed to estimate the amounts of CA in various samples. The concentration values found for CA were in agreement with those obtained using an HPLC method [9], used as a comparative method, as can be seen in Table 2, demonstrating the reliability of the proposed method, since there are no relevant statistical differences between the two approaches under 95% of confidence.

4. Conclusions

The oxidation of citric acid on a CoPC-CPE surface is well suited for analytical applications of the modified electrode to be used as a sensor for voltammetric and amperometric assays of

citric acid. The electrode is applicable to flow analysis systems. A very simple FIA assembly, using the CoPC-CPE as the working electrode in a wall-jet electrochemical detector cell configuration enables a simple and rapid method (65 samples per hour) for determination of CA in the millimolar range with precision. The method can be applied for the analysis of fruit juices. The sensor can also be applied for the determination of CA in a low micromolar range with precision and selectivity in batch analysis by using SW voltammetry. So, the proposed approaches present advantages in comparison to the voltammetric ones described in the literature, with important characteristics such as lower detection limits, broader linear response ranges, robustness of the sensor for many determinations, ease of preparation and renewability of the electrode surface. In addition, the physical stability of the electrode makes it applicable in flow analysis systems. The results obtained by FIA compares well with the ones obtained by HPLC.

It should be emphasized the sensor requires low maintenance and presents high durability, since it can be stored at room temperature and has a lifetime of several months. Such characteristics make this sensor very promising for routine applications.

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