DOI: 10.1002/elan.201400512

A Solid Film Electrode of Intermetallic $Ag_2Hg_{3.02}$ for the Direct Determination of Folic Acid in Fresh and Processed Fruit Juices

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Abstract: AgSIE was used for the direct analysis of folic acid (FA), with a detection limit and lower level of quantitation of 6.8×10^{-10} mol L^{-1} and 2.3×10^{-8} mol L^{-1}. The analysis in fresh and processed fruits was done without any sample pretreatment. In strawberry and acerola juices, FA concentration level values were below the method detection limit. FA was detectable in peach (77.7 ± 0.4 μg L^{-1} and 64.4 ± 0.5 μg L^{-1}), Persian lime (45.4 ± 0.7 μg L^{-1}), pineapple Hawaii (66.2 ± 0.4 μg L^{-1}), pear pineapple (35.3 ± 0.6 μg L^{-1}), cashew (54.4 ± 0.5 μg L^{-1}), passion fruit (73.2 ± 0.3 μg L^{-1}), and apple (84.4 ± 0.5 μg L^{-1}).

Keywords: Folic acid · Fruit juices · SWAdSV · Intermetallic $Ag_2Hg_{3.02}$ · SEM · XRD

1 Introduction

Folic acid (FA, Figure 1), is a water-soluble vitamin B complex known as vitamin B9, folacin or pteroylglutamic acid, and belongs to the group of folates [1].

It is commonly used in supplements and food fortification [2,3]. FA supplementation in the diet is needed as the daily consumption is 0.2 mg for adults and 0.4 mg recommended for pregnant women [4]. This vitamin can help prevent malformations in the brain and spinal marrow, a decrease of homocysteine and production of serotonin in the organism.

The popularity of electroanalytical methods has been increasing in recent few years due to various advantages over other analytical methods such as speed, sensitivity, low cost and the possibility, in many cases, of quantify a particular analyte without the necessity of sample chemical pretreatments.

It is well known from the literature that FA exhibits poor electroactivity on most common electrode material surface. However, some electroanalytical methodologies were already developed for FA analysis in pharmaceutical formulations using dropping mercury electrodes (DME) [5], static mercury drop electrode (SMDE) [6–8], and in estuary water using hanging drop mercury electrode (HDME) [9].

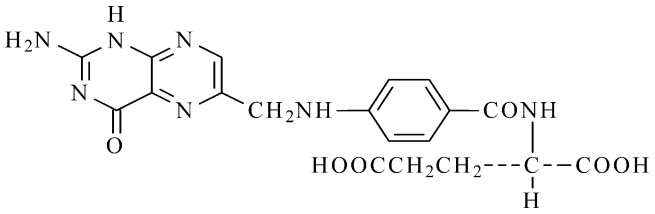

Fig. 1. Folic acid (FA) chemical structure

The potential risks of poisoning, contamination and disposal associated with the use of mercury have led to the development of modified electrodes for the determination of many compounds, including FA, using carbon fiber microelectrode [10], lead film electrode [11], MIP-sol-gel-modified PGE electrode [12], MIP – carbon composite fiber electrode [13], zirconia oxide (ZrO_2) nanoparticles-modified carbon paste electrode [14], modified carbon paste electrode with 2,2-[1,2buthanediylbis(nitriloethylidyne)]-bis and TiO_2 nanoparticles [15], carbon nanotubes paste electrode with ferrocenedicarboxylic

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acid [16], gold nanoparticles-modified carbon paste electrode (AuNPs/CPE) [17], ethynylferrocene modified carbon nanotubes paste electrode [18], carbon nanotube paste electrode (PBDCNPE) with 2,2′-[1,2-phenylenediylbis(nitrilomethylidene)]bis(4-hydroxyphenol) [19]. The detection limits obtained in these studies were in the level of 10^{-6} to 10^{-9} mol L^{-1} and sometimes the fabrication process associated with chemical modified electrodes is typically both time-consuming and laborious. For this reason, great attention has been paid to the development of solid amalgam electrodes due to their nearly nontoxic nature, easy preparation, mechanically stability, high durability, easy surface pretreatment, simple electrochemical regeneration and high sensitivity [20]. Solid amalgam electrodes have been used in many electroanalytical applications with good sensitivity and selectivity [20–22].

FA was already analyzed in nutritional supplements and in two types of juice (artificial) using a meniscus modified silver solid amalgam electrode (m-AgSAE) [23] and a polished silver solid amalgam electrode (*p*-AgSAE) [24] with detection limits of 0.5×10^{-9} mol L^{-1} and 5.9×10^{-10} mol L^{-1}, respectively.

To the best of our knowledge, no work has been reported related to the electroanalysis of folic acid using a solid electrode film of intermetallic $Ag_2Hg_{3.02}$ (AgSIE) for the direct determination of folic acid, at trace levels, in fresh and processed fruit juices without any sample pretreatment.

2 Experimental

2.1 Instrumentation and Reagents

The voltammetric analyses were performed using a potentiostat/galvanostat AUTOLAB PGSTAT 30, in a conventional glass Pyrex cell with Teflon cap for electrodes insertion: working (AgSIE), reference (saturated calomel, SCE) and auxiliary (platinum wire, 1.0 cm^2).

A characterization of the electrode surface was performed using surface analyze techniques as SEM imaging, XRD and Mapping of elements. Mapping and SEM images were obtained on EDX LINK ANALYTICAL equipment, (Isis System Series 200), with a SiLi Pentafet detector coupled to an electron microscope. To obtain the XRD, a SIEMENS D-5000 Xray diffractometer (Siemens D5000, Karlsruhe, Germany) was used.

Folic acid (Merck) was purchased from M. CASSAB trade and Industry Ltd (Santo Amaro, SP, Brazil). All other reagents used were of analytical grade (Merck). Solutions and samples were prepared with de-ionized water (Millipore).

2.2 Procedures

2.2.1 Preparation of AgSIE Electrode

AgSIE electrode was fabricated by the insertion of silver powder particle (0.4 μm), under pressure, into a glass capillary (d_i = 3 mm, and l = 10 mm). A copper wire was placed into the powder to provide the electric contact. The formation of inter-metallic film $Ag_2Hg_{3.02}$ was obtained by physical contact with metallic mercury. Prior to each test, a pretreatment was done in two steps for the activation of electrode surface: a) $E_{applied}$ = −1.9 V for 60 s; b) cyclic voltammetry: $E_{initial}=E_{final}=-0.1$ V and $E_{reverse}=-1.5$ V, $v=100$ mV s^{-1}, $n=50$ cycles in KCl 0.2 mol L^{-1}. The AgSIE electrode surface characterization was done by SEM images, XRD patterns and Mapping analysis of elements (Hg and Ag).

2.2.2 Development of Electroanalytical Methodology

Square Wave Adsorptive Stripping Voltammetry (SWAdSV) was chosen for the development of the electroanalytical methodology for the quantification of folic acid due to its fast and sensitivity analysis. The experimental parameters of SWAdSV, frequency (f), pulse amplitude (a), scan increment (ΔE_i), accumulation time (t_{acc}) and accumulation potential (E_{acc}) were optimized to obtain the best conditions for FA determination. Analytical curves were constructed in the concentration range of 0.2 to 10.6×10^{-7} mol L^{-1} in 0.1 mol L^{-1} phosphate buffer solution (pH 5.6). SWAdSV voltammograms were obtained in triplicates.

The detection limit (DL) was calculated by the equation $DL=3\sigma/\theta$, as σ referring to the standard deviation of y-intercepts and θ the slope of the analytical curves. The lower level of quantitation ($LLOQ$) was the lowest standard concentration level obtained from the analytical curves [25]. The precision of the methodology was estimated in one day by repeatability ($n=5$) and between days by intermediate precision (5 different days) using 4.0×10^{-7} mol L^{-1} FA solution. The accuracy was checked through recovery experiments using pharmaceuticals.

2.2.3 Determination of FA in Pharmaceuticals

An FA tablet (5.0 mg) was dissolved in 50 mL of deionized water and filtered on quantitative filter paper (2.4 μm). To test recovery by standard addition of FA, the solution was diluted to C_{FA} = 52.3 μmol L^{-1} and 30 μL of this solution was used to obtain the square wave voltammograms in 10 mL phosphate buffer solution (0.1 mol L^{-1}, pH 5.6). The remaining voltammograms were obtained by successive additions of aliquots of 30 μL of FA standard solution (45.3 μmol L^{-1}). The quantity of FA recovered by the methodology was directly obtained by extrapolation from the linear regression of the I_p vs. C_{FA} curve ($n=5$).

2.2.4 Determination of FA in Fruit Juices

Samples of fruit juices (fresh and processed) were obtained from the local market and maintained under refrigeration prior to use. The fresh juices (pulp) used in this work were: peach, persian lime, pineapple Hawaii, pearl pineapple, acerola (*Barbados cherry*), apple (*fuji*). The processed juices were: peach, strawberry, cashew,

and passion fruit (yellow). The samples were prepared as follows: **1** – *fresh* (50 g pulp/water 100 mL) were liquefied and subsequently centrifuged for 40 min at 1,500 rpm and filtered through quantitative filter paper (2.4 μm); **2** – processed juices were centrifuged and filtered without prior dilution. The pH of filtrates (fresh and processed juices) was adjusted to 5.6 by the addition of a KOH solution 0.1 mol L^{-1}. Samples were immediately analyzed by the developed electroanalytical methodology. Samples were diluted (1:1, v/v) in phosphate buffer solution (0.1 mol L^{-1}, pH 5.6). To find out the concentration of FA in fresh and processed juices, recovery experiments were done by standard addition method to effectively compensate for any matrix interferences from fruit juices samples. SWAdSV voltammograms for fruit samples were first obtained under optimized conditions. The remaining voltammograms were obtained by successive additions of aliquots of 30 μL of FA standard solution (45.3 μmol L^{-1}). The quantity of FA found in each juice sample was directly obtained by extrapolation from the linear regression of the I_p vs. C_{FA} curve ($n=5$).

3 Results and Discussion

3.1 Characterization of AgSIE Electrode

AgSIE electrode was used in this work to minimize the generation of hazardous mercury waste to meet the concept of "green chemistry". SEM images, XRD and SEM/EDS mapping for the elements Ag and Hg for the characterization of AgSIE surface were presented in Figure 2. SEM image (Figure 2a) shows a rough substrate of crystals of an intermetallic compound $Ag_2Hg_{3.02}$ [26], of relatively homogeneous dimensions. The 200X magnification allowed the visualization of a surface with gray coloration characteristic of rough surfaces. The homogeneity of the particles and the homogeneity in the distribution of the elements Ag and Hg on the surface were confirmed by the results of mapping for both elements (Figures 2b,c). The distribution of Hg atoms (white dots, Figure 2b) and Ag (white dots, Figure 2c) on the substrate revels that Hg atoms are the major component of the intermetallic compound.

Figure 2d shows the X-ray diffraction pattern obtained for the film formed on the electrode surface. A considerable collection of reflections for the range of $30° \leq 2\theta \leq 70°$ can be observed and the set of reflections allowed the

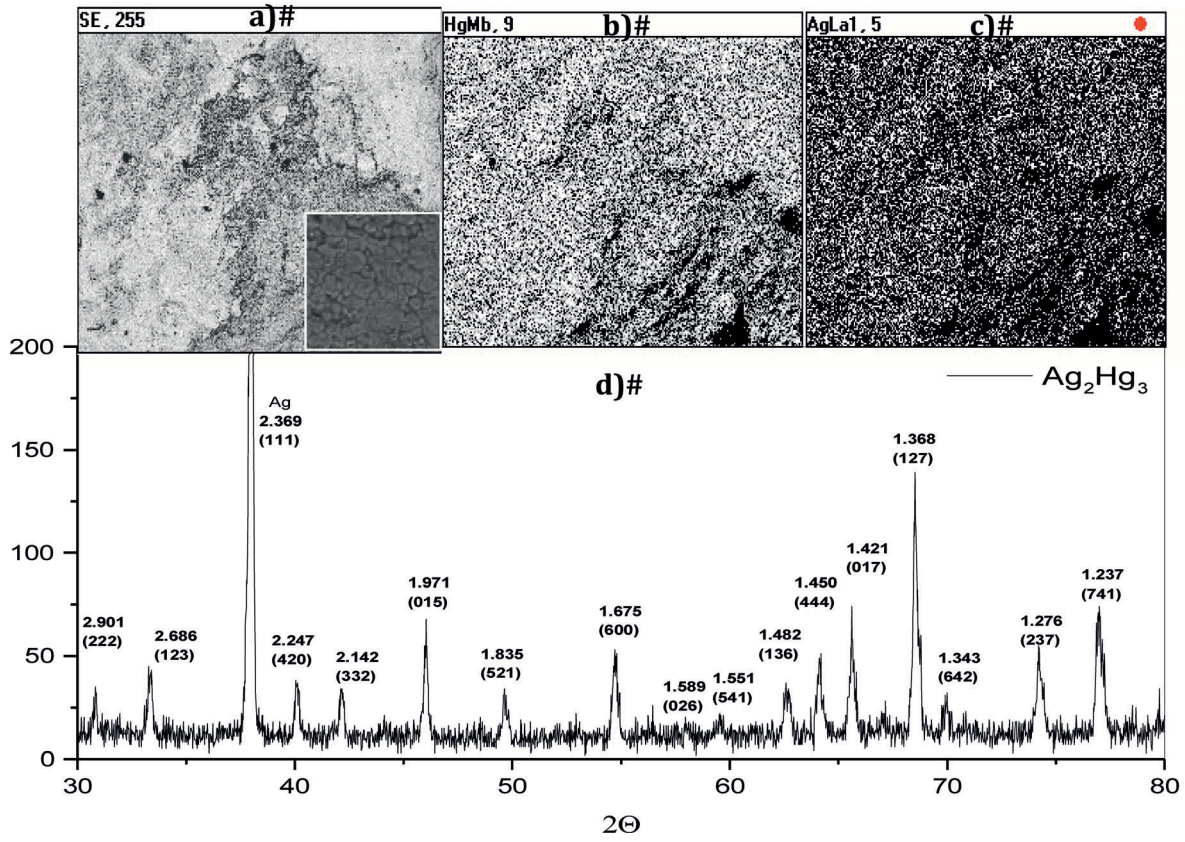

Fig. 2. a) SEM image of the surface of the electrode (500X); b) Mapping of elementary Hg(0); c) Mapping of elementary Ag(0); d) XRD patterns of the intermetallic film surface ($Ag_2Hg_{3.02}$). Inset: SEM image magnification: 5000X. XRD pattern reference data were obtained from: ICDD – PDF Data File No. 01-072-8408.

identification of the phase: $Ag_2Hg_{3.02}$. Reflections characteristics of liquid Hg were not observed.

There is no special storage for the $Ag_2Hg_{3.02}$ electrode if it is constantly used and the surface activation is done daily before the experiments. The electrode was only kept inside the amalgam reservoir during extended periods of non-utilization (more than one month). In general, the electrode maintains its perfect repeatability and reproducibility for at least 6 months of daily measurements. The electrode lifetime is only limited by accidental mechanical damages, as already mentioned in literature [27]. The regeneration of $Ag_2Hg_{3.02}$ electrode was easily to perform ($E_{applied} = -1.9$ V for 60 s) before each measurement.

3.2 Reversibility and Optimization of Experimental Parameters

FA SWAdSV voltammograms presented a reduction peak at −0.6 V vs. E_{sce} with characteristics of a reversible process controlled by the adsorption of the reagent on the AgSIE surface.

Figure 3 presents the current components (direct, reverse and total) for the reduction of FA using SWAdSV technique. It is evident that the total current has an additional contribution of reverse peak and therefore is interesting for analytical applications due to a gain in the sensitivity.

The effect of pH on the FA reduction peak was studied in the range of 3 to 8.5.

Figure 4 shows the SWV voltammograms obtained for FA ($C_{FA} = 0.7$ μmol L^{-1}) in 0.1 mol L^{-1} phosphate buffer solution. Folic acid presents high solubility in pH 5 or above. In acid medium, however, this vitamin presents low solubility and may precipitate. Therefore, the best pH interval to analyze folic acid is around 5 to 12. The highest reduction peak was obtained in pH 5.6, which was chosen for analytical application. The cathodic peak shifted to more negative potentials with the increasing of pH value (ΔE_p/pH = 66.0 mV/pH), suggesting the participation of equal number of protons and electrons on the reduction of FA.

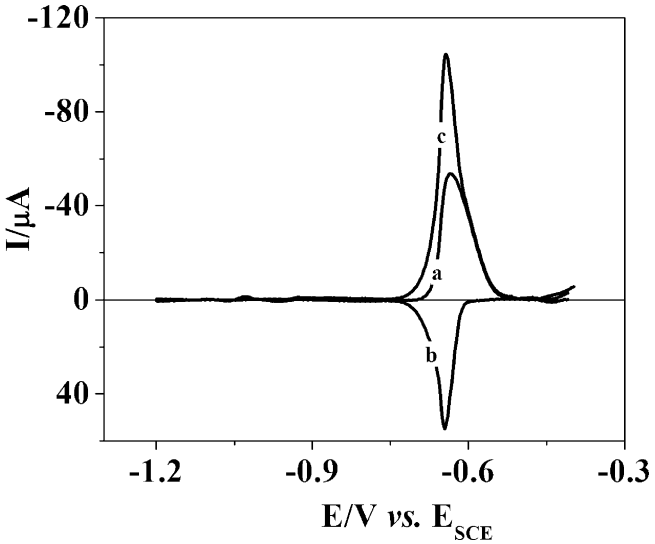

Fig. 3. SWV curves for FA (1.1 μmol L^{-1}) in 0.1 mol L^{-1} phosphate buffer solution (pH 5.6) at AgSIE electrode. (a) Direct current; (b) Reverse current; (c) Total current. $f = 200$ s^{-1}, $a = 50$ mV, $\Delta E_i = 2$ mV, $t_{acc} = 30$ s and $E_{acc} = -100$ mV.

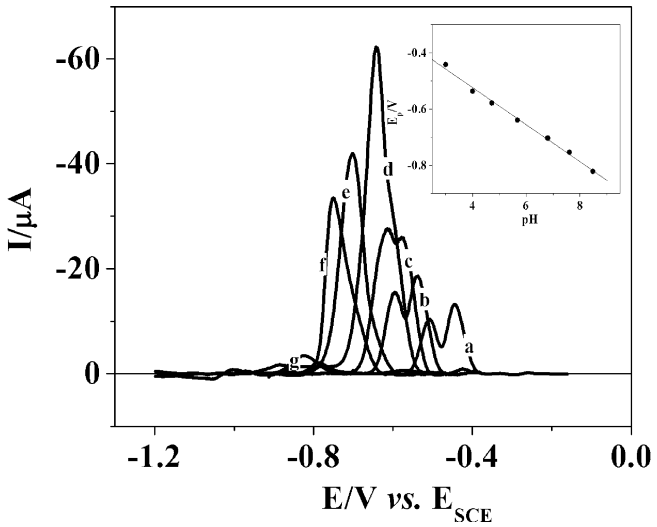

Fig. 4. SWV curves for FA (0.7 μmol L^{-1}) in 0.10 mol L^{-1} phosphate buffer solution at AgSIE electrode. pH (a) 3.0; (b) 4.0; (c) 4.7; (d) 5.6; (e) 6.8; (f) 7.6; (g) 8.5. Inset: E_p vs. pH curve (ΔE_p/ΔpH = 0.66 V/pH, $R^2 = 0.997$). $f = 200$ s^{-1}, $a = 50$ mV, $\Delta E_i = 2$ mV, $t_{acc} = 30$ s and $E_{acc} = -100$ mV.

An estimation of electrons involved in the reduction of FA was done by mathematical analysis of the SWAdSV peak. The peak half-width can be correlated to the number of exchanged electrons by the following equation [28]:

$$W_{1/2} = 3.5\ RT/nF$$

Where F is the Faraday constant (C mol^{-1}), n is the number of exchanged electrons, T is the temperature (K). The obtained value ($n = 2.1$) indicated that 2 electrons and 2 protons participated on the reduction of each FA molecule, which is in agreement with the result obtained by Den Berg and Le Gall [9].

The next step for the development of FA electroanalytical methodology was the optimization of SWAdSV instrumental parameters (frequency, amplitude, scan increment, accumulation time and potential) in phosphate buffer (0.1 mol L^{-1} and pH 5.6).

The accumulation time and potential were investigated in the range of 0 to 90 s and 0 to 600 mV, respectively. The highest FA reduction peak was obtained when an accumulation potential of −100 mV was applied for 30 s.

The frequency was varied from 40 to 200 s^{-1}. The good linearity ($R = 0.997$) obtained between I_p and $f^{1/2}$ confirmed the reversible nature of FA reduction process, which is in accordance with SWV theory [29]. Frequencies higher than 200 s^{-1} were also used, however a distor-

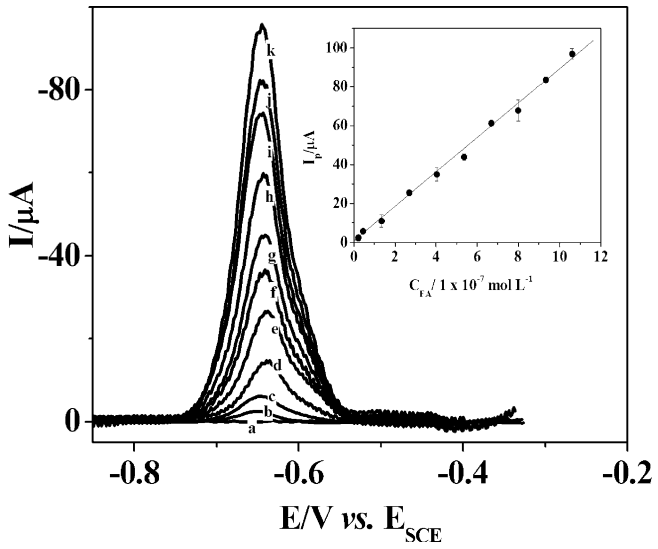

Fig. 5. SWV curves for different FA concentrations in 0.10 mol L^{-1} phosphate buffer solution (pH 5.6) at AgSIE electrode: (a) blank, (b) 2×10^{-2}, (c) 4×10^{-2}, (d), 0.1, (e) 0.3, (f) 0.4, (g) 0.5, (h) 0.7, (i) 0.8, (j) 0.9 and (k) 1.1 μmol L^{-1}. $f=200\ s^{-1}$, $a=50$ mV, $\Delta E_i=2$ mV, $t_{acc}=30$ s and $E_{acc}=-100$ mV. Inset: Analytical curve ($R^2=0.998$).

tion of SWV signal was obtained and for this reason a frequency of 200 s^{-1} was adopted for analytical applications. The effect of pulse amplitude (a) and the scan increment (ΔE_i) on the FA reduction peak were evaluated in the range of 10 to 80 mV and 1 to 10 mV, respectively. The highest peak current was obtained when both parameters were 50 mV and 2 mV.

3.3 Electroanalytical Methodology

After the optimization of experimental parameters, analytical curves were constructed in the range of 0.2 to 10.6×10^{-7} mol L^{-1} (Figure 5).

The linear regression equation for the analytical curve was I_p (μA) $=(8.3\times10^{-7}\pm7.1\times10^{-8})+(88.6\pm1.5)\times C_{FA}$, with a correlation coefficient of 0.998. The detection limit (DL) and the lower level of quantitation ($LLOQ$) were equal to 6.8×10^{-10} mol L^{-1} and 2.3×10^{-9} mol L^{-1}. The analytical sensitivity was $S=8.3\times10^{-7}$ μA/μmol L^{-1}. The precision ($n=5$) of the methodology was checked in terms of repeatability (2.8%) and intermediate precision (4.7%) at the FA concentration level of 4.0×10^{-7} mol L^{-1}.

The regeneration of AgSIE electrode was quite fast and easy. The stability of the electrode depends only on its cleaning and activation procedures.

3.4 Determination of FA in Pharmaceutical Sample

The accuracy of the developed methodology was checked by the recovery experiments of FA in pharmaceutical formulation using standard addition method after five repeated experiments. The mean recovery was 98.4±2.7% (4.9±0.1 mg), which is in accordance with the declared amount (5.0 mg). The acceptance criteria employed was $95\le R\%\le105$ [30], demonstrating the accuracy of the method.

The determination of FA in tablets confirmed that AgSIE/SWAdSV methodology is simple and rapid for the routine analysis. The developed methodology was also applied to the analysis of FA in more complex matrices, such as fresh fruit and processed fruit juices, in order to evaluate the possibility to monitor this vitamin in real samples.

3.5 Determination of FA in Fresh and Processed Fruits

Figures 6a and b show the SWV voltammograms and their recovery curves for the processed peach and fresh

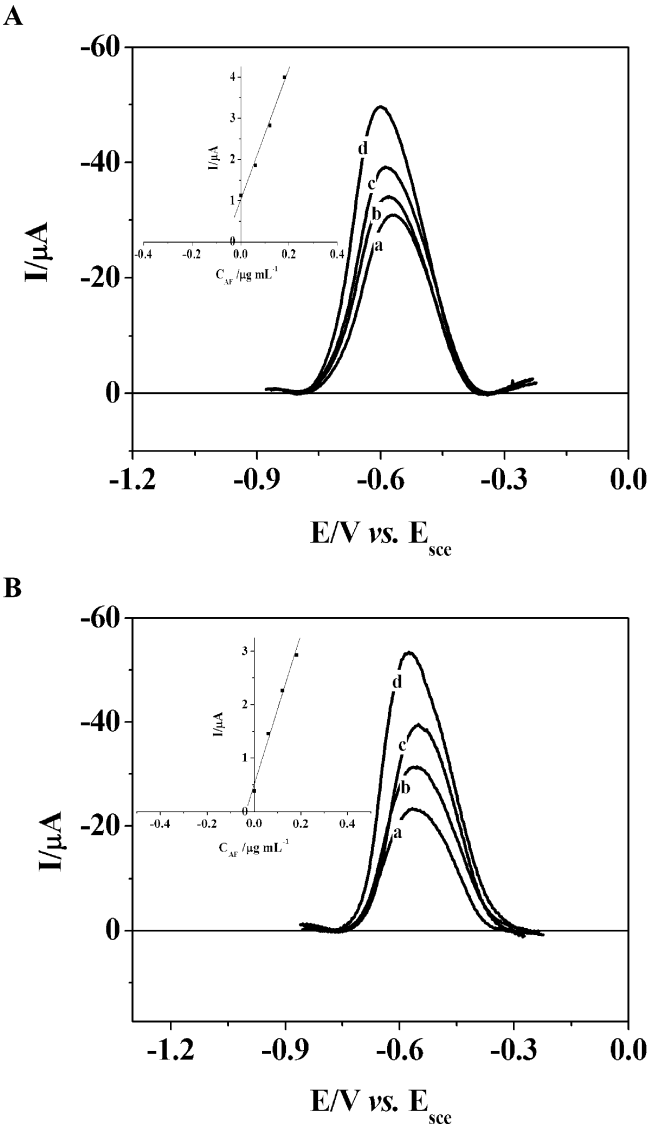

Fig. 6. SWV curves mixture of fruit juice with 0.1 mol L^{-1} phosphate buffer solution (pH 5.6), 1:1 (v/v): A) Industrialized Peach Juice (Inset: I vs. C_{FA}, $y=1.0+15.9x$, $R^2=0.994$); B) Fresh Pineapple Hawaii Juice (Inset: I vs. C_{FA}, $y=1.1+17.1x$, $R^2=0.994$). Electrode AgSIE: $f=200\ s^{-1}$, $a=50$ mV, $\Delta E_i=2$ mV, $t_{acc}=30$ s, $E_{acc}=-100$ mV.

Table 1. Concentrations of folic acid found in fresh and processed fruit juices.

Fruit juice	R^2	FA concentration ($\mu g L^{-1}$)
Peach (N)	0.996	77.7 ± 0.4
Peach (NI)	0.994	64.4 ± 0.5
Persian lime (N)	0.991	45.4 ± 0.7
Pineapple Hawaii (N)	0.994	66.2 ± 0.4
Pearl Pineapple (N)	0.993	35.3 ± 0.6
Strawberry (NI)	0.997	$\leq LD$
Cashew (NI)	0.983	54.4 ± 0.5
Acerola (N)	0.997	$\leq LD$
Passion fruit (NI)	0.999	73.2 ± 0.3
Apple (N)	0.996	84.4 ± 0.5

(N) Fresh fruit juice. (NI) Processed fresh fruit juice.

Table 2. Comparison of the efficiency of modified metal electrodes or not, using in the determination of folic acid.

Tech	Electrode	Sample	DL ($mol L^{-1}$)	Reference
SWAdSV	C/Pb (red)	P	7.0×10^{-10}	[11]
DPV	ZONMCPE (oxi)	P	9.8×10^{-6}	[14]
DPV	BQTMCPE (oxi)	P	3.0×10^{-7}	[15]
DPAdSV	*m*-AgSAE (red)	P/FJ	0.50×10^{-9}	[23]
DPAdSV	*p*-AgSAE (red)	P/FJ	5.9×10^{-10}	[24]
CV	MBT/SAM/Au (oxi)	P	4.0×10^{-9}	[33]
CV	C/Au (oxi)	P	1.0×10^{-8}	[34]
CV	Ni/POA/CPE (oxi)	P	9.1×10^{-5}	[35]
DPAdSV	HMDE (red)	P	1.4×10^{-8}	[36]
SWAdSV	AgSIE (red)	P/FJ	6.8×10^{-10}	This work

P: Pharmaceutical. FJ: Fruit juice.

Hawaiian pineapple, respectively. Table 1 presents the concentration of FA found in each fruit samples (fresh and processed). The obtained results pointed out to the potential use of the developed electroanalytical methodology (SWAdSV/AgSIE) for the directly analysis of FA in real samples without the necessity of any sample pretreatment. The AgSIE electrode can be successfully used due to its sensitivity (comparable to HDME electrode) and also to the no generation of toxic waste.

FA was already analyzed in nutritional supplements and in two types of fortified juice (artificial) using a meniscus modified silver solid amalgam electrode (m-AgSAE) [23], a polished silver solid amalgam electrode (*p*-AgSAE) [24] and with a single-walled nanotube-ionic liquid paste electrode [31]. There is only one work that determined FA in fresh orange juice (28.2 ± 1.3) µg/100 g, $RSD = 1.3\,\%$) without fortification using a calixarene based chemically modified electrode [32].

Table 2 shows a comparison between the results obtained in this study with those already published in the literature for the determination of FA in pharmaceuticals and fruit juices [11,14,15,23,24,33–36].

The DL obtained in this work is in the same concentration level of Bandzuchová et al. [23,24] using a meniscus or a polished silver solid amalgam electrode, suggesting that Ag-Hg intermetallic electrodes presents high sensitivity for the electroanalysis of FA and therefore can be used in many electroanalytical determinations to replace mercury electrodes (avoid the generation of toxic waste) and also provide a good alternative to the utilization of chemical modified electrodes (sometimes associated with time-consuming and laborious fabrication process).

To the best of our knowledge, this is the first time that an amalgam electrode (AgSIE) was used for the directly analysis of FA in no fortified fresh and processed fruits with good sensitivity, without serious interference from sample matrix and no generation of waste (solvents for extraction). These results pointed out to the potential use of electroanalytical methods in routine analysis of pharmaceutical and environmental compounds.

4 Conclusions

The surface of AgSIE was characterized as being constituted effectively by a solid film of intermetallic $Ag_2Hg_{3.02}$ in the absence of liquid mercury.

The developed electroanalytical methodology is selective and sensitive to low concentrations of folic acid ($LD = 6.8 \times 10^{-10}$ mol L^{-1}), fast and costless, for the routine analysis of folic acid in pharmaceuticals and in fresh processed fruit juices without interferences

The speed of the analytical method was effectively established, when considered the pretreatment proposed for samples of fresh fruits and processed fruit and for pharmaceutical sample: liquefaction of solid sample (only for fresh fruits), centrifugation and filtration, followed by measurement. This fact, combined with others reported above, becomes evident when comparing the method proposed in this paper to other chromatographic and spectrophotometric methods already described in literature, where preparative steps are extensive and generate toxic residuals and usually involve high cost analyses.

The utilization of the developed methodology minimizes the quantities of toxic chemicals, taking into consideration the principles of "green chemistry", using minimal reagent consumption in sample preparation. In addition, the intermetallic solid electrode ($Ag_2Hg_{3,02}$) is stable and was used instead of a mercury electrode, minimizing the formation of substances with environmental toxicity such as organometallic (methyl mercury, dimethyl mercury, ethyl mercury chloride), among others.

Acknowledgements

The authors wish to thank *CAPES*, *CNPq*, *University of Macau Multi-Year Research Grant (MYRG) and Science and Technology Development Fund (FDCT 063/2013/A2)*.

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Received: September 14, 2014
Accepted: October 21, 2014
Published online: December 8, 2014

