ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2017**, *90*(2), 231–239
Published online: August 8, 2017
DOI: 10.5562/cca3162

HKD 1926

Sensitive Electrochemical Determination of Folic Acid Using *ex–situ* Prepared Bismuth Film Electrodes

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RECEIVED: Mary 12, 2017 * REVISED: June 30, 2017 * ACCEPTED: June 30, 2017

THIS PAPER IS DEDICATED TO PROF. MIRJANA METIKOŠ-HUKOVIĆ ON THE OCCASION OF HER BIRTHDAY

Abstract: The electrochemical behavior of folic acid (FA), at the electrochemically prepared ex situ bismuth film (BiF) on glassy carbon electrode, clearly indicates electrocatalytic nature of the prepared film toward FA reduction (at –0.55 V). Scanning electron microscopy is used for morphological characterization of the prepared BiF. Accordingly, we establishing an electrochemical procedure based on square wave cathodic stripping voltammetry, preceded by accumulation of FA on the BiF electrode (BiFE). This analytical method is optimized and its analytical performance is presented. This electrode displays a two linear response range: 0.1 to 1.0 μmol L^{-1} and 1.0–10.0 μmol L^{-1} with sensitivity of 20.10 μA μmol^{-1} L and 2.28 μA μmol^{-1} L, respectively. Developed method was validated in compliance with spectrophotometric method. Excellent recovery and standard deviation obtained with BiFE revealed great analytical potential of the proposed method which was applied for the determination of FA in pharmaceuticals formulation.

Keywords: bismuth film, folic acid, voltammetry, electroanalytical.

INTRODUCTION

FOLIC ACID (FA), N–(4–{[(2–amino–4–oxo–1,4–dihydropteridin–6–yl)methyl]amino} benzoyl)–L–glutamic acid, pteroyl–L–glutamic acid) belongs to the B–vitamin group also referred as vitamin M, vitamin B_9 (commonly called folate), vitamin B_c (or folacin). As it cannot be synthesized in body it must be supplied daily from the foods such as fruits, vegetables, mushrooms, algae, fortified grains, *etc.*[1] In food FA predominantly exist as polyglutamates, which have to be hydrolyzed in body to monoglutamates in order to be transported. Folate, as conjugate base of FA, represents active form which is incorporated in many metabolic pathways, mainly in carbon transfer reactions, such as amino acid interconversions and purine and pyrimidine biosynthesis.[2] Also, it has important role in homocysteine metabolism. However, synthetic B_9–vitamin (in dietary supplements or in fortified food) is in the form of folic acid, which is most stable form,[3] and in human body FA is transformed into its active form by the enzyme dihydrofolate reductase.

As consequence of low folates intake, numbers of health disorders were reported: neural tube defect, coronary heart diseases and osteoporosis, increased risk of breast and colorectal cancer, poor cognitive performance, hearing loss, anemia.

Although FA represents most stabile form among the group of "folates", it will be decomposed in the presence of the oxidizing and reducing agents, in alkaline or acidic medium, as well as it is exposed to UV light.[4]

Due to importance of FA in human health, methods for its determination have been received increasing interest Beside traditional microbiological methods, based on Lactobacillus rhamnosus, other analytical methods have been proposed for the detection and quantification of folic acid such as: high–performance liquid chromatography

(HPLC) combined with UV detection,[5,6,7,8] ion–pair based liquid chromatography (IP–LC) using mass spectroscopy,[9,10] capillary electrophoresis with mass spectrometry,[11] UV spectrophotometer,[12] flow injection chemiluminescence,[13] commercially available enzyme–linked immunosorbent assay (ELISA) test and fluorometric method.[14] However, some of the above mentioned methods are nonspecific and laborious, also needed harmful and expensive substances which in combination with long–time sample preparation and well–trained operators drastically increased the time and cost of analysis.

As disposable tool for reliable, rapid, accurate, simple, fast and low–cost determination of FA, various electrochemical techniques have been introduced. Reported electrochemical techniques, used in determination of FA, together with various surface modification techniques and analytical performance were summarized by Mirmoghtadaie *et al.*[15] Review reveals that besides all electrochemical techniques, voltammetry (square wave, differential pulse or stripping) is commonly used electrochemical technique. Although using of hanging mercury drop electrode[16,17,18] or mercury meniscus modified silver solid amalgam electrode[19] have great advantage concerning possibility of preconcentration (adsorption) before stripping thus achieving very low limits of detection, toxicity as well as poor reproducibility limits this methods for further development and application. By using of an "environmentally friendly" electrode material – bismuth (mostly as electrodeposited bismuth film as it is well known resemblance in electrochemical behavior between Bi and Hg) in electrochemical "stripping" analysis,[20,21,22] it is possible to overcome issues related to mercury based materials. Although conventional and micro bismuth film electrodes have been widely used in electrochemical analysis of various organic compounds[23–37] their relevance for folic acid determination has been very poorly exploited. Only Ananthi *et al.*[38] reported determination of folic acid using glassy carbon electrode modified with an electrodeposited bismuth nanowires by square wave voltammetry. Authors used hydrogen bubbles (electrodeposition was performed at –0.1 V in acetate buffer pH 4.5) as a "stagnant template" for obtaining the bundles of dendritic compact nanowires of bismuth. This approach resulted in less negative reduction potential and higher electroreduction current, indicating excellent electrocatalytic nature of the prepared nanowires toward FA electroreduction, compared to that on the bare glassy carbon. Furthermore, determination of FA on glassy carbon can be precluded due to the surface fouling effect of the oxidized products of the ascorbic and uric acid as it was reported by Kalimuthu *et al.*[39] As most of biomolecules present in real sample (*e.g.* dopamine, uric acid, ascorbic acid) are easily oxidizable, direct reduction of FA represent potential path for resolution of interference problems. Results of our preliminary work[40] indicates that bismuth film prepared under controlled conditions can be used to resolve above mentioned problems.

Based on previously investigation[40] on the relevance of the prepared an *ex–situ* bismuth film at glassy carbon electrode (BiFE) for the determination of FA, in this work additional optimization of the parameters and procedure are presented. Film has been deposited in presence of a complexing agent (EDTA) to obtain arranged homogenous structure, confirmed with scanning electron microscopy (SEM). Such prepared BiFE was applied for selective adsorption (at chosen potential) of folic acid, followed by its reduction using square wave cathodic stripping voltammetry (SWCSV).

Developed method was validated and confirmed by proposed spectrophotometric method.[41] Developed method was applied in the determination of FA in real samples with excellent selectivity, reliability and accuracy.

EXPERIMENTAL

Material and Methods

All solutions were prepared from analytical grade chemicals and were used as received.

For electroanalytical determination: for the preparation of acetic buffer, sodium acetate and acetic acid all purchased from Kemika (Croatia) were prepared by dissolution in double distilled water. Stock solution of the bismuth nitrate (1×10^{-3} mol L^{-1} Bi(III)) was prepared by dissolution of 99.99 % $Bi(NO_3)_3\times5H_2O$ (Sigma–Aldrich, Inc., USA) in acetate buffer solution (0.1 mol L^{-1}; pH 4.5). The folic acid (ALFA AESAR, Ward Hill, MA, USA) solutions were prepared daily by dissolution of appropriate amount of the FA in acetate solution, previously deaerated with N_2.

For spectroscopic measurement: zinc powder, hydrochloric acid, amidosulfonic acid, sodium nitrite and sodium hydroxide all purchased from Kemika (Croatia). 3–aminophenol was obtained from Sigma Aldrich (St. Louis, USA), while potassium dihydrogen phosphate was purchased from Merck KGaA (Darmstadt, Germany). Folic acid (50 mg) was dissolved in 0.1 mol L^{-1} sodium hydroxide solution. This solution was reduced using zinc and concentrated hydrochloric acid to produce *p*–aminobenzoilglutamic acid (*p*–ABGA), filtered and diluted to 100 mL in a calibrated flask. The aliquot of folic acid (from 0 to 6 ppm FA) are transferred to 25 mL flask where added 2 mL of hydrochloric acid (5 mol L^{-1}), 1 mL of sodium nitrite (1 %), 1 mL amidosulfonic acid (4 %) and 5 mL 3–aminophenol (1 %). After obtaining a yellow–orange product, in flask where added 3 mL of hydrochloric acid (5 mol L^{-1}) and flasks is filled up to the mark. The solutions were freshly prepared with deionized water.

The food supplement (Folacin, Jadran – Galenic Laboratory (JGL), dietary supplement) was purchased from local drug store.

The standard electrochemical cell with saturated calomel electrode (SCE) as reference, Pt plate as auxiliary and 2 mm in diameter GCE (Metrohm, Herisau, Switzerland) or prepared BiFE as working electrode was used. The stripping measurements were carried out in deoxygenated solutions under pure nitrogen atmosphere. All experiments were carried out at 25 °C, controlled by thermostat (Huber CC1, Offenburg, Germany). All electrochemical measurements were carried out with potentiostat (Autolab PGSTAT 302N), connected to PC and driven by GPES 4.9 Software (Eco Chemie).

For microscopic study, after electrodeposition, BiFEs were rinsed carefully in redistilled water, shortly dried in N_2 atmosphere and then transferred to a microscope chamber. The surface morphology of the BiFEs was studied on Vega II LSH (TS 5130 LS) scanning electron microscope (Japan Electron Optics Laboratory, Japan). Obtained image was quantified using ImageJ Program (Rasband, U.S. NIH, Bethesda, Maryland).

A Varian, Cary, UV Visible spectrophotometer with 1.0 cm matched cells was used for all spectroscopic measurements. Spectroscopic measurements were performed according procedure reported by Nagaraja *et al.*[41]

Our previous studies[40] have revealed that the optimum potential used for electrodeposition of bismuth on glassy carbon electrode is –0.9 V *vs.* SCE during 600 s. The procedure was carried out ex situ in quiescence solution of acetate buffer 0.1 mol L^{-1} (pH 4.5) containing bismuth and EDTA, in equal concentrations amounting 1×10^{-3} mol L^{-1}.

For electroanalytical determination of FA the best parameters were established by optimization as it is presented in Results and Discussion.

Solution of food supplement (Folacin) was prepared as follows: tablet which contains 5 mg (according to declaration) of active substance – folic acid was dissolved in appropriate amount of water. Such solution was used in further determinations. The exact concentration of this solution was determined by spectrophotometric method according to Nagaraja *et al.*[41]

RESULTS AND DISCUSSION

SEM Study of Electrodeposited Bismuth Film

Detail optimization of the preparation procedure for obtaining BiF with satisfying analytical purpose, together with its electrochemical characterization by electrochemical impedance spectroscopy and morphology of the bismuth film electrodeposited on to glassy carbon electrode were presented by Vladislavić *et. al*.[40] The micrograph of the optimized BiF, obtained by SEM, revealed that deposited bismuth particles form crystals characterized with large flakes–like dendritic structure (see Figure 1A). The part of formed film was evaluated with *ImageJ* software. Film thickness (δ) was assessed by measuring the *zy* or *zx* planes of SEM image (Figure 1.A) and by assessing the cross–sectional region of each *z–stack* image using the ''*Plot Profile*'' (Figure 1.B).[42]

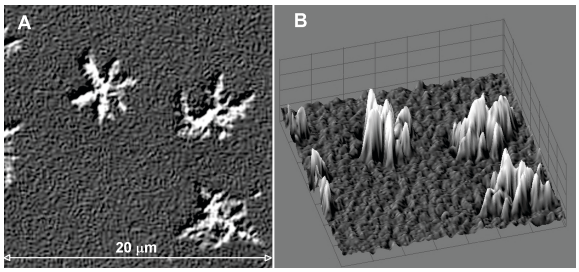

Figure 1. (A) part of SEM image at magnification 3000× of the bismuth particles of film formed in 0.1 mol L^{-1} acetate buffer solution (pH 4.5) containing 1.0×10^{-3} mol L^{-1} Bi(III) and 1.0×10^{-3} mol L^{-1} EDTA at –0.9 V for 60 s; (B) cross–sectional view generated from SEM image.

This approach reveals that the flakes–like bismuth particles have approximately 6 μm in diameter and about 10 μm in heights. These particles are randomly distributed over the entire surface (with average thickness of 2 μm) that consisted from deposited bismuth. The BiFE with such obtained morphology was applied for electrochemical determination of FA.

Electrochemical Behavior of FA at Prepared BiFEs

One of the advantages of BiF is high hydrogen overvoltage, which allows good operating cathodic potential. For getting insight of electrochemical behavior of the FA at bare GCE and BiFE, the cyclic voltammetry measurements were performed in wide potential window. However, in anodic branch (around 0 V vs. SCE) intensive dissolution of BiF was occurred (not shown), which is not plausible if considering mechanism of FA determination presented by Le Gall and van den Berg[18] and Ananthi *et al*.[38] According to these observations, electrochemical behavior of FA at BiFE and GCE were investigated in potential window between –0.35 V and –0.9 V, in acetate buffer solution. Obtained voltammograms are presented in Figure 2A. As it can be seen, significant changes in cyclic voltammogram in the presence of FA can not be observed. In contrast, voltammetric response of the BiFE in the presence of FA revealed well defined reduction peak around –0.55 V, which clearly indicate electrocatalytic nature of the prepared BiF toward FA reduction.

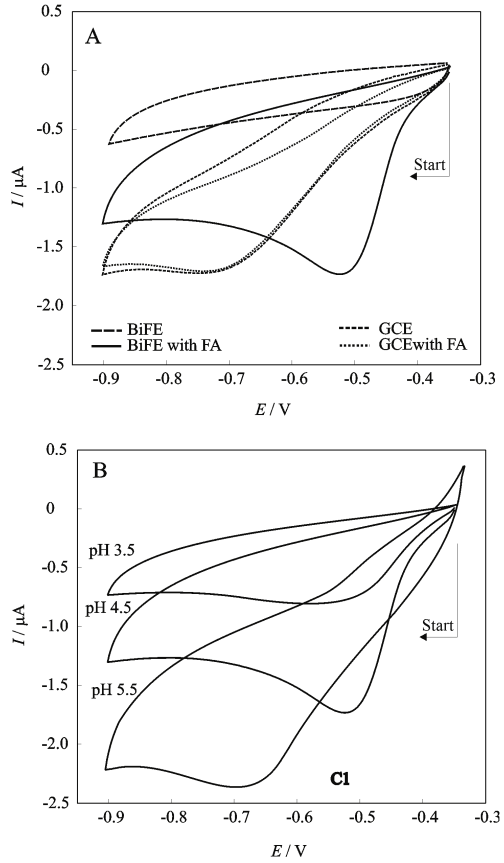

Figure 2. Cyclic voltammograms obtained at: (A) GCE and BiFE in 0.1 mol L^{-1} acetate buffer solution (pH 4.5) in absence and in presence of 1.0×10^{-4} mol L^{-1} FA and (B) BiFE in acetate buffer solutions with different pH in presence of 1.0×10^{-4} mol L^{-1} FA; (scan rate 25 mV s^{-1}).

The observed shift of the reduction potential to the less negative values and the increase of FA reduction current on BiFE *vs.* GCE clearly indicate the electrocatalytic nature of BiFE for this reaction. Such behavior was also observed with BiNWs / GC, and was attributed to high surface / volume ratio of the electrode together with a uniform pore size that facilitates the fast kinetics of the reduction of FA.[38]

A proposed reaction mechanism for the reduction of FA in acetic buffer solution is shown in Figure 3, due to reversible reduction of FA to 5,8-dihydrofolic acid, followed by tautomerization to give 7,8-dihydrofolic acid. [17,18]

As it previously reported, pterin part of folic acid can produce a reduction peak at potentials between –0.5 V and –0.8 V, depending on pH, where glutamic acid (part of folic acid) is electroinactive. Based on this information, obtained reduction peak at –0.55 V can be attributed to the reduction of nitrogen from pteridine according previously proposed reaction.

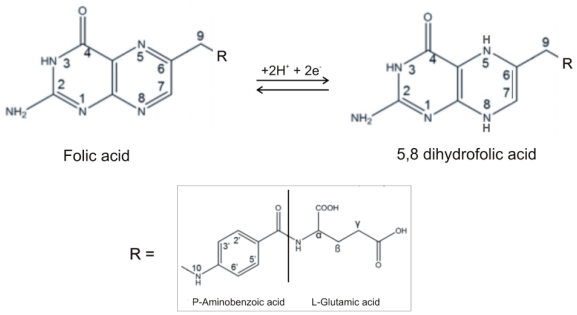

Figure 3. Reaction scheme for the electrochemical reduction of folic acid at pH 4.5.

By increasing of pH (Figure 2B) reduction peak of folic acid (C1) increases and shifts toward more negative potentials, which is expected if considering previously proposed reaction.[18] In addition to voltammograms recorded at pH 3.5 and 4.5, voltammogram recorded at pH 5.5 show increase of oxidation current, probably owning to improved dissolution of the bismuth film.[33] Although higher cathodic current can be observed at pH 5.5, the reduction signal attributed to cathodic reduction of FA is more pronounced at pH 4.5. Thus, for optimum pH value 4.5 was chosen.

Optimization of SWCSV Procedure and Quantitative Utility

The SWCSV procedure has been established by monitoring the influence of applied potential increment (ΔE_s), frequency (f), and pulse height (ΔE_p), accumulation potential (E_{acc}) and accumulation time (t_{acc}) on peak currents (I_p) and obtained results of the optimization are presented in Figure 4.

The influence of accumulation potential on to reduction current was monitored in the potential range where no reduction of FA or / and dissolution of the BiF take place. As it can be seen, maximum of the cathodic peak current was obtained at –0.4 V. Decrease of the reduction current, at more negative accumulation potential of –0.4 V, can be attributed to the process of reduction of the FA but of the small extent. The increase of the cathodic current with prolonged accumulation time can be observed till 600 s, after changes in current were negligible. According to above, for analytical measurements, folic acid was accumulated for 120 s in a stirred solution, followed by accumulation for 60 s in quiescent solutions under nitrogen atmosphere. Effect of ΔE_s on cathodic current was examined in dependence of the frequency. At frequencies above 30 Hz signal was strongly influenced by background noise. Also, at high frequencies, shift of the reduction potential toward negative values was observed. Analytical signal at high ΔE_s values (at

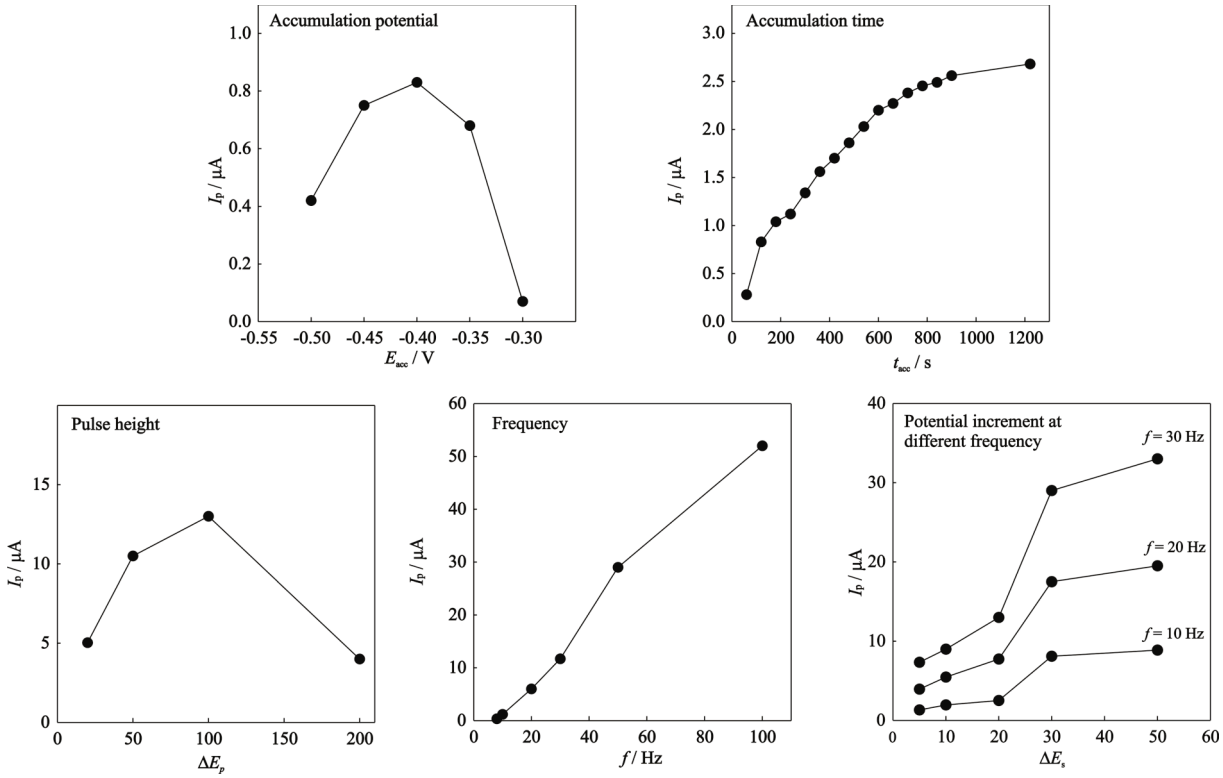

Figure 4. Effect of the electrochemical parameters on reduction peak current for SWCSV on BiFE in 0.1 mol L^{-1} acetate buffer solution (pH 4.5) containing 1.0 μmol L^{-1} FA. Optimizing time and potential accumulation was carried out with f =10 Hz, ΔE_s = 20 mV and ΔE_p = 100 mV.

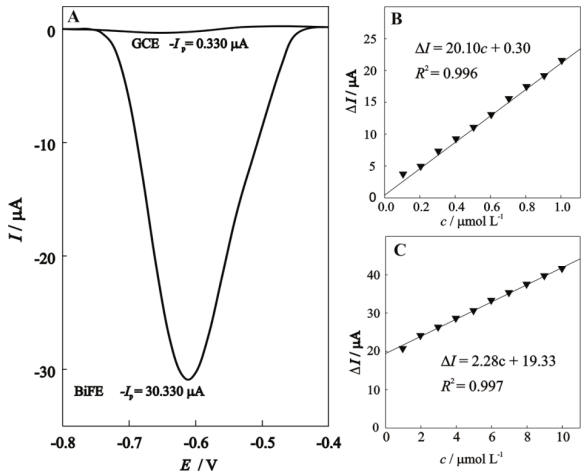

Figure 5. (A) SWCSVs with baseline correction, recorded at GCE and BiFE in 0.1 mol L^{-1} acetate buffer solution (pH 4.5) in the presence of 5.0 μmol L^{-1} FA; (B) calibration plot derived by subtracting background current from corresponding voltammograms for concentrations range 0.1–1.0 μmol L^{-1}; (C) calibration plot derived by subtracting background current from corresponding voltammograms for concentration range 1.0–10.0 μmol L^{-1}.

30 Hz) suffered from the lack of reproducibility and precision (due to the inability for obtaining sufficient number of points). According above, for working frequency 30 Hz, together with ΔE_s = 20 mV were chosen. Thus, all subsequent SWCSV were carried out with: E_{acc} = –0.4 V, t_{acc} = 180 s, f = 30 Hz, ΔE_s = 20 mV and ΔE_p = 100 mV.

In the Figure 5.A comparable SWCS voltammograms of the reduction of folic acid at the bare GCE and BiFE are shown. As it can be seen, at optimized condition, BiFE shows high sensitivity for FA compared to bare GCE. This difference in electrochemical behavior can be attributed to the lack of the accumulation (physical adsorption) of FA onto GCE. Also, slightly shift of the reduction potential of the FA (on BiFE) toward negative values, compared to the reduction potential observed by cyclic voltammetry (see Figure 2.), can be attributed to the kinetic limitation of the FA reduction at optimized parameters of SWCSV.

Figure 5.B and 5.C represent calibration plots derived by subtracting background current from corresponding voltammograms (not shown).

As it can be seen from Figure 5B and 5C, two different linear ranges were obtained, both with good linearity (R^2 = 0.996 and 0.997) and with sensitivity of 20.10 μA μmol^{-1} L and 2.28 μA μmol^{-1} L for concentrations

ranges 0.1–1.0 μmol L^{-1} and 1.0–10.0 μmol L^{-1}, respectively. The calculated limit of detection (LOD) of FA, based on the 3σ criterion,[44] obtained from the slope of the analytical curve, was 0.001 μmol L^{-1}. The limit of detection is given as LOD = 3 × SD / *b*, where SD is the standard deviation of 10 measurements of a blank solution and *b* is the

Table 1. Comparison of the analytical performances, reported for the electrochemical determination of FA with our work.

Methods	Modification	pH	Linear range/ μmol L^{-1}	LOD / μmol L^{-1}	Samples analysed	Reference
AC–AdSV	Static mercury drop electrode	5.0	1×10^{-2}–5×10^{-5}	2×10^{-6}	N/A	[16]
DPP DPASV	Carbon paste electrode modified with palmitic/stearic acid	7.4	6×10^{-3}–600	4×10^{-3}	with Pb and Cd	[47]
CV and LSV	Singlewall carbon nanotubes /glassy carbon electrode	5.5	0.01–100	1×10^{-3}	N/A	[48]
CA and CC	Multiwall carbon nanotubes/Au	2.5	0.02–1	0.01	Ph. Form.	[49]
DPV	(PMo_{12}) doped polypyrrole film	2.0	0.01–0.1	1×10^{-4}	N/A	[50]
ASV	In situ lead film electrode/glassy carbon electrode	5.6	2×10^{-3}–0.08	7×10^{-4}	Ph. Form.	[51]
DPASV	Calixarene modified carbon paste electrode	4.0	8.79×10^{-6} –$\times10^{-3}$	1.2×10^{-6}	vegetables and fruits	[52]
DPV	Singlewall carbon nanotubes electrode with ionic liquid paste	5.5	2×10^{-3}–4.0	1×10^{-3}	wheat flour, fruit juices, milk	[53]
DPCSV	Graphite pencil electrode/Molecularly imprinted polymer–immobilized sol–gel–modified	7.8	5×10^{-3}– 0.156 μg mL^{-1}	3.6×10^{-6}	blood serum	[54]
DPV	Carbon paste electrode with ZrO_2 nanoparticles	7.0	20–2500	9.86	mixture of FA, norepinerin, paracetamol	[55]
CV and DPV	Carbon paste electrode modified with hydroquinone derivates	7.0	200–3200	25	mixture of FA, norepinerin, paracetamol	[56]
DPV	Mercury free Ag amalgam electrode	5.0	1×10^{-3}–4.0	588×10^{-6}	Ph. Form.	[57]
CV	Multiwall carbon nanotubes /polivinilsulfonic acid on glassy carbon electrode	7.0	53–1700	N/A	N/A	[58]
CV and DPV	Mesoporous carbon/graphite electrode	7.0	5.0–2000	0.7	mixture of FA, norepinerin, paracetamol	[59]
Voltammetry	Carbon nanotube paste modified with ferrocen carboxylic acid	5.0	0.10–750	65×10^{-3}	urine	[60]
DPV	Mercury film electrode	7.1	0.13–1 2–10	14×10^{-3}	Ph. Form.	[61]
DPV	Au electrode modified with Au nanoparticles	14	0.01–1.0	7.5×10^{-3}	Ph. Form, flour, spinach	[62]
CV and DPV	Glassy carbon electrode modified with PMo_{12}/PPy/GR	2.0	0.001–0.2	3.3×10^{-4}	N/A	[63]
CV and SWCSV	Boron doper diamond electrode	1.0 6.0	0.23–4.5 2.3–90	0.0793 0.32	Ph. Form.	[64]
CV, CA and SWV	ZnO nanoparticle modified ionic liquid– carbon paste electrode	9.0	0.05–550	0.01	Ph. Form, urine, apple juice	[65]
CV and SWCSV	Bismuth nanowires/glassy carbon electrode	4.5	0.01–0.15	9.53×10^{-3}	Ph. Form.	[38]
SWV	Chemically modified Carbon paste electrode modified ZnO/Carbon nanotubes nanocomposite electrode	7.0	3–700	1	Ph. Form. urine, human blood,	[66]
LSV, CV, A	Carbon nanohorns supported interwoven titanate nanotubes	6.0	1×10^{-4}–50	25×10^{-6}	Ph. Form., oats	[67]
EIS, CV, and SWV	Mn doped SnO_2 nanoparticles (NPs) modified glassy carbon electrode	7.0	1–500	0.038	Ph. Form	[68]
CV and SWCSV	Bismuth film/glassy carbon electrode deposited with EDTA	4.5	0.1–10.0	1×10^{-3}	Ph. Form	This work

Ph. Form. – pharmaceutical formulations AdSV: adsorptive stripping voltammetry; CA: chronoamperometry; SWCSV: square wave cathodic stripping voltammetry; LSV: Linear Sweep Voltammetry; EIS: Electrochemical Impedance Spectroscopy; SWV: square wave voltammetry; CV: cyclic voltammetry; DPV: differential pulse voltammetry.

analytical sensitivity. This value is comparable with values reported by other researchers for determination of FA at the surface of chemically modified electrodes (Table 1).

The excellent reproducibility of this method has been determined by the measurement of the current responses toward FA on three formed BiFEs. Also, relative standard deviation of ten consequent measurements, in solutions containing 2.0 μmol L^{-1}, was 1.4 %.

The change in slope, with linearity preserving, can be attributed to the increased amount of folic acid accumulated at electrode surface, resulting in saturation of electrode surface, as it was reported for the determination of cysteine on Hg.[45,33] However, this will not take effect on analytical capabilities since these ranges are well defined and reproducible, although some authors[46] suggest employing of shorter accumulation time at higher analyte concentrations.

The determination of FA was found to be strongly affected in the presence of glutathione, cysteine and *N*–acetyl cysteine owing to presence of thiol group in these molecules, which is known to have high affinity for bismuth.[33,40] SWCS voltammograms (not shown), in the presence of these thiols, exhibit wider, less defined reduction peak, obviously as a consequence of simultaneous reduction of presented species on BiFE. However, this can be prevented by manipulation of accumulation time and accumulation potential of FA. Also, no interferences were observed in the presence of the substances that can be found in pharmaceutical formulations: lactose monohydrate, microcrystalline cellulose, magnesium stearate, crospovidone and povidone.

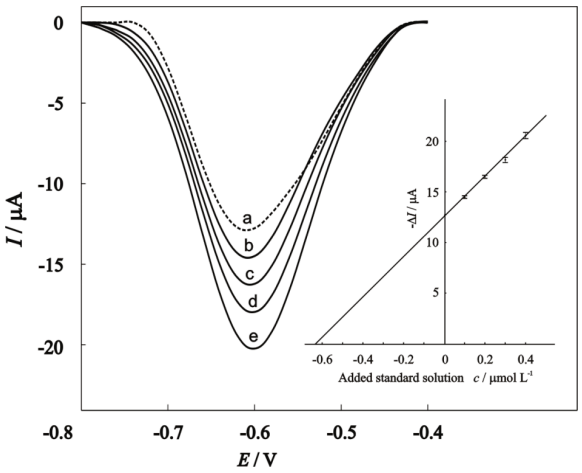

Figure 6. Analytical determination and recovery studies of FA in 0.1 mol L^{-1} acetate buffer solution (pH 4.5) at BiFE under optimized conditions for food supplement (Folacin) solution. Concentration of FA standard solution added: (a) supplement solution, (b) 0.10 μmol L^{-1} (c) 0.20 μmol L^{-1} (d) 0.30 μmol L^{-1} (e) 0.40 μmol L^{-1}.

Obtained analytical performance by proposed method are comparable with values reported by other research groups (see Table 1). As it can be seen, the presented method showed satisfactory analytical performance. Although this method can not be recommended for determination of FA in serum (concentration of folic acid in human serum is around 0.10–0.01 μmol L^{-1}), in further experiment (see Analytical application) we proved that it is suitable as analytical tool for determination of FA in pharmaceutical formulations.

Analytical Application in Pharmaceutical Sample

The analytical performance of the prepared BiFE was evaluated by determination of folic acid in Folacin tablets. In voltammetric cell 95 mL of acetic buffer solution was spiked with 5 mL of supplement solution. The concentration of FA in such prepared solution, determined with adopted spectrophotometric method,[41] was 0.63 μmol L^{-1}.

Electroanalytical determination of FA and recovery experiments was performed using established SWCSV procedure by standard addition method (concentration step of FA standard solution was 0.1 μmol L^{-1}). The resulting voltammograms showed well–defined reduction stripping peaks and the standard addition plots were linear, as can be seen in Figure 6. The concentration of the folic acid was calculated from difference of the obtained peak currents (Figure 6). Four additions of FA standard solution to the sample yielded recoveries in the range of 95.5 to 103.0 %.

Additionally, the pharmaceutical formulations containing FA were independently analyzed with adopted spectrophotometric method.[41] The comparative results obtained by these methods are given in Table 2.

Using the proposed method relative standard deviation (RSD) of the mean of three determinations of FA sample was lower than 3.0 %. Also, the relative error between methods, within 97 % of confidence level, indicated that proposed method can be successfully applied for FA determination in food supplement.

Table 2. Reproducibility of proposed electrochemical method and its comparison with adopted spectrophotometric method.

Tablets	Labeled value / mg $tablet^{-1}$	Spectrophotometric Analysis	Proposed Electrochemical Method	Relative errors / %
		Found / mg $tablet^{-1}$		
1	5.000	5.020	4.967	1.056
2	5.000	5.021	4.888	2.649
3	5.000	5.022	5.170	2.947

|Relative error| = [(SWCSV – Spectrophotometric) / Spectrophotometric] × 100 %.

CONCLUSION

The BiF formed in acetate buffer, in the presence of EDTA at optimized condition, show electrocatalytical nature toward folic acid reduction which can be attributed to the reduction of nitrogen from pteridine part.

This behavior was utilized to establish a novel electrochemical procedure for quantitative determination of FA. The method is based on selective physical adsorption of FA onto BiF followed by its reduction using SWCSV.

Analytical calibration curve was characterized with two linear ranges: 0.1 μmol L^{-1} up to 1.0 μmol L^{-1} and 1.0 μmol L^{-1} up to 10.0 μmol L^{-1} with linearity (R^2) of 0.996 and 0.997, respectively. Obtained detection limit was 0.001 μmol L^{-1}, with relative standard deviation of 1.4 %.

Analysis of the authentic samples containing FA, showed no interference from additives and excipients presented in pharmaceutical formulations. Consequently, a proposed method was successively applied for the analysis of folic acid in tablets with satisfactory recoveries (from 95.5 to 103.0 %).

Adopted spectrophotometric method was used to validate proposed method. The obtained results show a satisfactory matching in the selected ranges.

Due to a non–toxic character of BiFE, simple procedure and analytical performances, this method is advantageous when compared with other reported studies.

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