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Original article

Electrochemical Behavior of Folic Acid at A Boron-Doped Diamond Electrode: Its Adsorptive Stripping Voltammetric Determination in Tablets

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The electrochemical properties of folic acid were investigated in pH range 1.0-9.0 by cyclic, linear sweep and adsorptive stripping voltammetry. The compound was irreversibly oxidized at an anodically pre-treated boron-doped diamond electrode in one or two oxidation steps, which are concentration- and/or pH-dependent. Using square-wave stripping mode, folic acid yielded well-defined voltammetric responses in both 0.1 M perchloric acid and 0.1 M Britton-Robinson buffer, pH 6.0 with limits of detection 0.035 µg/mL ($7.93\ 10^{-8}$ M) and 0.14 µg/mL ($3.2x10^{-7}$ M), respectively, after an accumulation of 120 s at open-circuit condition. Practical applicability of the newly developed approach was verified by the direct assays of tablet dosage forms.

Key words: Folic acid, Boron-doped diamond electrode, Cyclic voltammetry, Square-wave adsorptive stripping voltammetry, Determination, Tablet

Folik Asit'in Bor-katkılı Elmas Elektrot Üzerinde Elektrokimyasal Davranışı: Tabletlerden Adsorptif Sıyırma Voltametrisi ile Tayini

Folik asit'in elektrokimyasal özellikleri; pH 1.0-9.0 aralığında dönüşümlü voltametri, doğrusal taramalı voltametri ve adsorptif sıyırma voltametrisi ile incelenmiştir. Bileşik, anodik olarak ön-işlem görmüş bor-katkılı elmas elektrot üzerinde derişim- ve/veya pH-bağımlı bir ya da iki basamak halinde tersinmez olarak yükseltgenmektedir. 0.1 M perklorik asit ve 0.1 M Britton-Robinson tamponu (pH 6.0) içerisinde kare-dalga sıyırma formu kullanıldığında açık-devrede 120 s'lik biriktirme sonrası folik asit sırasıyla 0.035 µg/mL ($7.93\ 10^{-8}$ M) ve 0.14 µg/mL ($3.2x10^{-7}$ M) saptama sınırlarında iyi-belirlenmiş voltametrik yanıtlar vermiştir. Yeni geliştirilmiş olan tekniğin pratik uygulanabilirliği, tablet ilaç şeklinin doğrudan analiziyle kontrol edilmiştir.

Anahtar kelimeler: Folik asit, Bor-katkılı elmas elektrot, Dönüşümlü voltametri, Kare-dalga adsorptif sıyırma voltametrisi, Miktar tayini, Tablet

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INTRODUCTION

Folic acid (FA, *N*-[*p*-{[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino}benzoyl]-l-glutamic acid, as shown in Figure 1) belongs to the group of water-soluble B-vitamins. It is also known as vitamin B_9, vitamin B_c or folacin (in some cases is denoted as vitamin M). FA has long been recognized as part of the Vitamin B complex found in some enriched foods and vitamin pills. However, the role of this vitamin in maintaining good human health is far more important than its use as a vitamin and dietary supplement. In fact FA and its naturally occurring salts– folates (the anionic form) are essential compounds during periods of rapid cell division and growth, and highly effective in preventing birth-defects, anemia, cardiovascular and cerebrovascular diseases, and certain types of cancer (1-3). FA is itself not biologically active, but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver.

As a result of its importance in biological systems, there is an increasing need for developing methods for the measurement of FA in pharmaceutical, clinical and food samples. There have been several reports on the determination of FA either alone or in combination with other drugs, including the use of enzyme-linked immunosorbent assays (ELISAs) (4), chemiluminescence (5,6), microemulsion electrokinetic chromatography (7), spectrophotometry after coupling reaction with specific compounds (8), fluorimetry (6,9), high-performance liquid chromatography with ultra-violet, diode-array or electrochemical detection (10-12), liquid chromatography with tandem mass spectroscopy or with electrospray ionisation mass spectrometry (13,14), capillary electrophoresis (15), or biosensor-based determination (16). Most of the above mentioned methods offer very useful information in terms of identification and quantitation, excellent resolution and selectivity; however they are prone to many drawbacks, such as expensiveness, complicated and lengthy procedures.

Electrochemical methods, such as the voltammetric ones, offer certain advantages, such as the simplicity, fast response and offering sensitivity and dynamic range comparable to other analytical methods. Various voltammetric techniques have been proposed for analysis of FA individually or simultaneously in combination with other compounds because the molecule is electroactive at several electrodes. Although electrochemical behavior of FA was studied at first on mercury electrodes (17-21), a large number of papers in the literature involve the use of modified electrodes, such as carbon paste electrode chemically modified with palmitic or stearic acid (CM/CPE) (22), phosphomolybdic-polypyrrole film modified glassy carbon electrode (PMo_{12}-PPy/GCE)

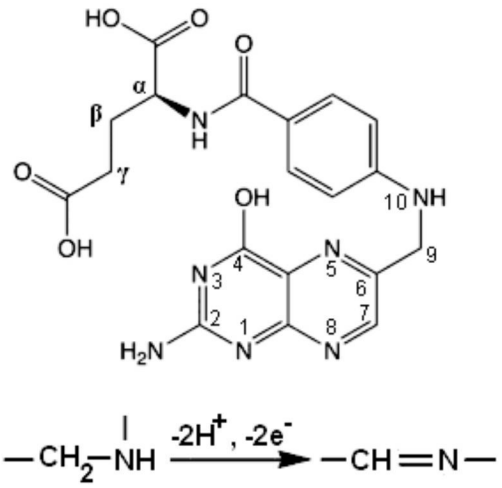

Figure 1. Chemical structure and oxidation of folic acid (FA) at C9–N10, which is reported to mimic biological oxidation (22).

(23), single-wall carbon nanotube modified glassy carbon electrode (SWNT/GCE) (24,25), multi-walled carbon nanotube modified gold (MWNT/GE), glassy carbon (MWNT/GCE) or paste (DWNT/PE or MWNT/PE) electrodes (26-29), calixarene-modified carbon paste electrode (CME-6) (30), lead film-coated glassy carbon electrode (PbFiE/GCE) (31), Ni-polymer modified carbon paste electrode (Ni/POA/CPE) (32), molecularly imprinted polymer–carbon composite fiber (MIP-fiber) (33,34), or –sol-gel-modified pencil graphite (MIP-sol-gel/PGE) (35) electrodes, mercury meniscus modified silver solid amalgam electrode (m-AgSAE) (36), TiO_2 (TNMCPE) or ZrO_2 (ZONMCPE) nanoparticles-modified carbon paste electrodes (37,38), nanostructured polyaniline doped with tungstophosphoric acid in carbon paste electrode (CPE-PANI/TPA) (39) and 2-mercaptobenzo-thiazole self-assembled gold electrode (MBT/SAM/Au) (40).

Boron-doped diamond (BDD) is emerging as a new and excellent carbon electrode material due to its outstanding electrochemical features: a wide working potential window in aqueous solutions (up to 3 V), low and stable background current, negligible adsorption of organic compounds and relative insensitivity to dissolved oxygen compared to the other electrode materials such as glassy carbon, platinum etc (41). These unique properties, together with the extreme robustness and high resistance to corrosion even in strong acidic media, recommend BDD as an excellent electrode material for several applications, especially in the field of electroanalytical chemistry. These fields of study have grown considerably in the past decade (42).

This paper reports on the coupling of adsorptive stripping voltammetric (AdSV) technique with the unique properties of the BDD electrode for the development and optimization of an analytical methodology for the determination of FA both in bulk form and in pharmaceutical preparations.

EXPERIMENTAL

Chemicals

Folic acid (FA) standard was purchased from Sigma. Tablet dosage forms containing the active compound were procured from commercial local pharmacies. Other reagents used were of analytical grade, and their solutions were prepared with deionised water further purified via a Milli-Q unit (Millipore).

Stock standard solutions (0.5-10 μg/mL FA) were prepared with 0.05 M NaOH aqueous solution, stored in dark bottles at 4 ^{0}C when not in use. The working solutions were prepared, just before use, by accurate dilution with a selected supporting electrolyte. Four different supporting electrolytes, namely perchloric acid ($HClO_4$, 0.1 M), acetate buffer (0.1 M, pH 4.8), Britton-Robinson buffer (BR, 0.1 M, pH 2-9), and phosphate buffer (0.1 M, pH 2.5 and 7.4) solutions were used.

Apparatus

All experiments of cyclic (CV), linear sweep (LSV) and square-wave adsorptive stripping (SW-AdSV) voltammetry were performed using a μAutolab type III electrochemical system (EcoChemie, The Netherlands) driven by the GPES 4.9 software. The potentiostat was connected to a personal computer. All SW voltammograms were smoothed using a Savicky and Golay algorithm and baseline-corrected by the moving average method (peak width of 0.01 V), using the software supplied with the equipment. A classical three-electrode cell of volume 10 mL was used with a platinum wire as an auxiliary electrode and an Ag/AgCl (3 M NaCl) electrode (Model RE-1, BAS, USA) as a reference electrode. The working electrode was a boron-doped diamond (BDD) working electrode (Windsor Scientific Ltd.; Ø: 3mm, diameter). In some cases a glassy carbon (GC, BAS; Ø: 3mm, diameter) electrode was also used as working electrode for comparison. Solution pH was measured using a WTW inoLab pH 720 meter with a combined electrode (glass-reference electrodes).

A procedure similar to that proposed in our previous work (43) was followed for the pre-treatment of BDD electrode. This electrode was firstly polarized in a 0.5 M H_2SO_4 by applying +3.0 V during 180 s; thus, the BDD surface was made predominantly oxygen-terminated. Afterwards, the electrode was pre-treated for 30 s under the same experimental conditions. In this study, the first anodic surface pre-treatment was daily performed before starting

the experimental work. The other step in the procedure was applied before each voltammetric experiment. The pre-treatment procedure was carried out in an independent electrochemical cell. GC electrode was polished manually with aqueous slurry of alumina powder (Ø: 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad), and then rinsed with deionised water thoroughly.

Adsorptive stripping voltammetric procedure

The general procedure for stripping voltammetric analysis of FA was as follows: The three-electrode system was immersed in a voltammetric cell containing required aliquot of the FA working solutions and a selected supporting electrolyte at a desired pH. A selected accumulation potential was then applied to a BDD surface for a selected pre-concentration period, while the solution was stirred at 400 rpm. At the end of the accumulation period, the stirring was stopped and a 5 s rest period was allowed for the solution to become quiescent. Then, the voltammogram was recorded by scanning the potential toward to positive direction between +0.4 to +1.5 V using SW waveform.

The best instrumental parameters for SWV which was used for investigating the determination of FA were as follows: frequency, 100 Hz; pulse amplitude, 40 mV; scan increment, 10 mV. Successive measurements were carried out by repeating the above assay protocol on the working electrode. All measurements were performed in triplicate at laboratory temperature.

Sample preparation

Folbiol® tablets labeled as containing 5 mg FA was used for the present analytical applications. Ten tablets were weighed and the average mass per tablet was determined. The tablets were carefully grounded to a fine powder in a mortar with a pistil. An adequate amount of the resulting powder was weighed and transferred into a 100-mL calibrated dark flask, which was completed to the volume with 0.05 M NaOH. The content of the flask was sonicated for about 20 min to complete dissolution. The desired concentrations of FA were obtained by taking suitable aliquots of the clear supernatant liquor and diluting with BR buffer, pH 6.0. An aliquot volume of these solutions was added to BR buffer, pH 6.0 in the voltammetric cell and analyzed in the day of preparation according to the procedure developed for the pure electrolyte. The nominal content of the tablet amounts was calculated from the corresponding regression equations of previously plotted calibration curves.

RESULTS AND DISCUSSION

Investigation of the electrochemical behavior on the boron-doped diamond electrode

The electrochemical response of BDD electrode is strongly affected by the type of pre-treatment applied to the surface before experiments. Thus, this effect is big importance in the case of electroanalytical studies. Although BDD electrodes are known to be resistant to fouling, a preliminary conclusion indicated that slight fouling occurred at BDD electrode without pre-treatment during FA oxidation, and thus a way to restore the initial activity of the BDD electrode surface was necessary. Three different cleaning procedures were considered. First, the electrode was treated by mechanical cleaning (polishing manually with alumina (0.01 μm)/water slurries on felt pads). A second procedure consisted in a cathodic cleaning (-3.0 V for 180 s). Finally, the third procedure consisted in an anodic one (+3.0 V for 180 s). In order to decrease the background current, the acidic media of 0.5 M H_2SO_4 was used for both electrochemical cleanings. The anodic pre-treatment procedure was chosen; since it yielded a much better electrode response: more intense current signal, lower background current and higher reproducibility of the measurements. Furthermore, this pre-treatment was always preceded by an electrochemical cleaning procedure applying a shorter period (+3.0 V for 30 s) in between measurements in order to avoid fouling of the electrode surface as a consequence of the FA electrooxidation reaction.

Initial experiments using CV were performed without an accumulation step to characterize the voltammetric behavior of FA at the anodically pre-treated BDD within the range +0.4 to +1.5 V. The electrochemical behavior of the compound at BDD electrode using CV experiments at a scan rate of 100 mV/s yielded a single broad oxidation peak in more dilute solutions and/or at higher pHs

(in less acidic media). As solution acidity and concentration increased, FA oxidation resulted in the occurrence of two wave-shaped peaks, which indicates a two-step oxidation of the molecule. A representative cyclic voltammogram of 700 µg/mL (≈ $1.6x10^{-3}$ M, rather high concentration) FA in BR buffer at pH 6 (medium pH), with the half-peak potentials located at around +0.94 (I_1) and 1.15 (I_2) V, respectively, is shown in Figure 2. No reduction peak was observed in the negative scanning half-cycle, indicating the irreversible nature of the electrode process. As illustrated in Figure 2A, further potential cycles at the same BDD surface resulted in a decrease of the voltammetric response, which may be due to the desorption of FA molecule out of the electrode surface. This behavior indicated the interfacial adsorptive character of the FA onto the BDD electrode surface. For comparison the oxidation of FA was also obtained at GC electrode under identical experimental conditions (see Figure 2B) exhibiting two oxidation waves at ca. +0.73 and +0.99 V. As it can be observed from the experimental results, the background current for BDD electrode was lower than the one for GC electrode, which is ascribed to the low double layer capacitance of the surface of the former electrode (44). It is also seen that the accessible anodic potential limits of BDD electrode obtained from background voltammograms were higher in comparison with those for GC electrode by almost 0.2 V in this medum. The oxidation of FA took place at more positive potential at BDD electrode than its oxidation process at GC electrode. This observation seems to be similar to those of the previous reports (45-47), wherein, it was demonstrated that higher overpotential, indicating slower electron transfer kinetics, is required to oxidize the compounds on BDD electrode when compared to GC electrode. On the other hand, the usage of BDD electrode was proved to be much more sensitive, yielding current densities (obtained by subtracting the background currents from the recorded currents) considerably higher than those obtained by using GC electrode.

The influence of scan rate on the oxidation of FA at the BDD electrode was checked by LSV in BR buffer, pH 6. The voltammetric curves for relatively lower concentration of 20 µg/mL (≈ $4.5x10^{-5}$ M) of FA (in case of single oxidation step) carried out for the increased values of scan rate (v) in the range of 100–600 mV/s gave rise to an anodic peak with intensities (i_p) that showed a linear increase with the scan rate, followed the relationship: i_p (µA) = 0.038 v (mV/s) + 7.274, r = 0.995). This suggests that the electrode reaction at the BDD electrode is controlled by the adsorption process.

The adsorption phenomenon of FA can be used as an effective pre-concentration step prior to actual voltammetric quantification of analyte. The AdSV response of FA at BDD electrode was examined using SW excitation waveform, which combines good sensitivity with high speed, and reduces problems with poisoning of the electrode surface. As a consequence, further work was dedicated towards studying the influence of acidity and nature of the

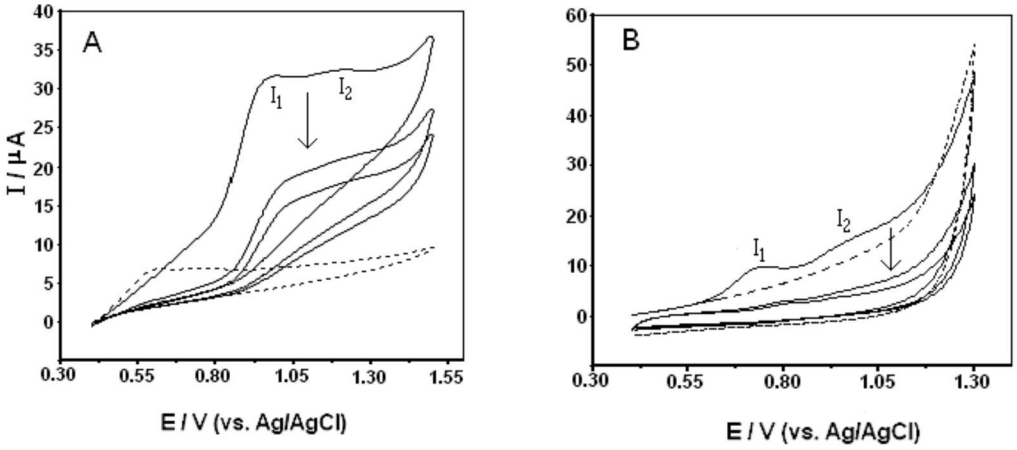

Figure 2. The repetitive cyclic voltammograms of 700 µg/mL (*ca.* $1.6x10^{-3}$ M) FA solutions in BR buffer pH 6.0 at BDD (A) and GC (B) electrodes. Scan rate, 100 mV/s. Dashed lines represent background current.

supporting electrolyte using SW-AdSV approach. As a consequence, further work was dedicated towards studying the influence of acidity and nature of the supporting electrolyte using SW-AdSV approach. In Figure 3A, this parameter was established within the pH range 2.0-9.0 of BR buffer by carrying out stripping measurement on 20 μg/mL FA solution, with an open-circuit mode at 120 s. Bearing in mind that aqueous alkaline solutions change the morphology of the BDD surface resulting in surface degradation (48), any measurement beyond pH>9 were avoided. Under the strong acidic condition (at pH value of 2.0), two distinct anodic peaks were seen at +0.84 and 1.01 V with peak currents of 2.08 and 1.20 μA, respectively. The presence of secondary process observed at more positive potential became less distinct as the acidity was decreased. When the experiments were performed at pH 5.0, the first peak became predominant, while the second peak changed into a shoulder and was not detected above pH ≥ 6.0 (in neutral and alkaline solutions). For a solution pH of 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, oxidation peak potentials for the first peak were +0.84, 0.81, 0.85, 0.89, 0.89, 0.89, 0.91 and 1.00 V, respectively, with the peak currents of 2.08, 0.62, 0.89, 1.11, 0.71, 0.55, 0.50 and 0.50 μA. Figure 3B depicts the SW voltammograms in various supporting electrolytes. Using 0.1 M $HClO_4$, phosphate buffer pH 2.5, acetate buffer pH 4.8 and phosphate buffer 7.4, anodic peak potentials of first peak +0.85, 0.81, 0.89 and 0.91 V were obtained, respectively, together with the decrease of the anodic peak currents with different degrees (2.55, 1.01, 0.73 and 0.51 μA), which are in agreement with the results in BR buffer. The evolution of the peak potential with pH shows four almost linear segments, the first between pH 2.0 and 3.0, the second between pH 3.0 and 4.0, the third between pH 4.0 and 5.0, and the last between 8.0 and 9.0. According to the very recent observations of Wu et al., (49) the solubility of FA is higher at alkaline and strong acidic surroundings than the solubility at weak acidic conditions. In the investigations by Wu et al., (49) and Poe (50), the authors concluded that FA has six acidic dissociation constants due to its several ionic forms in aqueous electrolytes. The pK_a values are reported to be pK_{a1} = -1.5 (N5), pK_{a2} = 0.2 (N10), pK_{a3} = 2.35 (N1), pK_{a4} = 3.46 (α-COOH), pK_{a5} = 4.56 (β-COOH) and pK_{a6} = 8.38 (N3). In very strong acidic condition, the protonated form of FA predominates in the supporting electrolytes due to the protonation of nitrogen atoms and carboxyl groups in the molecule. When solution pH is around 2.5, predominantly neutral species is involved. At about pH>5, two carboxyls of FA turn to predominantly their anionic forms. Under the

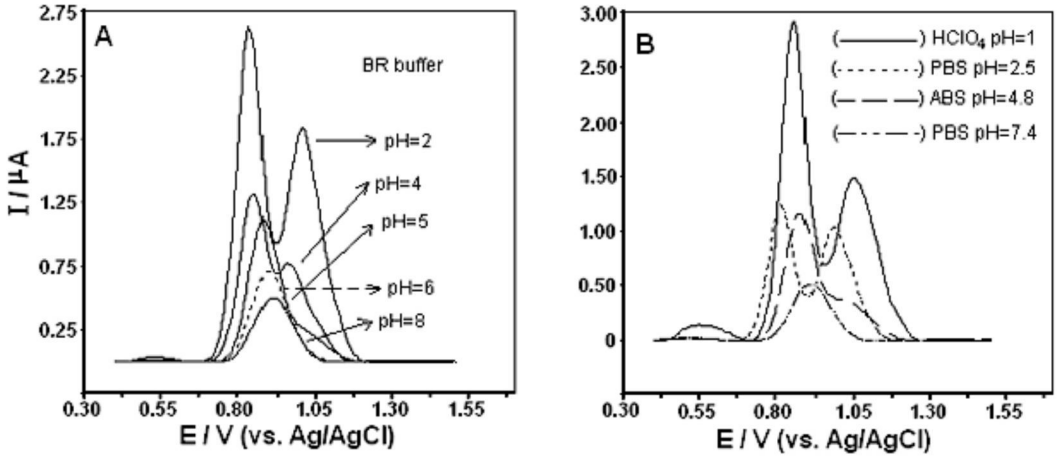

Figure 3. The stripping voltammograms of 20 μg/mL (*ca.* $4.5x10^{-5}$ M) FA solutions in BR buffer pH at different pHs (A), and in various supporting electrolytes (B). Pre-concentration period, 120s at open circuit condition; SWV parameters: frequency, 25 Hz; scan increment, 8 mV; pulse amplitude, 30 mV. ABS: acetate buffer solution, PBS: phosphate buffer solution.

strong alkaline condition (pH> 9.5), the amount of the uncharged FA in the aqueous solution is negligible because of both deprotonation of carboxyl groups and amide ionization at N(3). It should be pointed out, as previously reported by several workers (41) that after strong anodic polarization process at very high anodic potentials BDD surface becomes hydrophilic (negative) due to the formation of carbon-oxygen functionalities. Based on this fact, the variation of electrostatic interaction (from attraction to repulsion) between different charged FA molecules in different pH values (from strong acidic to alkaline region) and the negative surface charge of BDD electrode may explain why the response of FA decreases and peak potentials become slightly more positive by raising the solution pH. On the other hand, the intersection points of the first process are close to the pK_a values of FA from pK_{a3} to pK_{a6}, and it can be explained by changes in protonation of the acid-base functions in the molecule. However, the pH-independent zone in between pH 5.0 and 8.0 mean that there are no proton transfer steps before the electron transfer rate-determining step. Previous investigations (25, 26, 30, 34, 38) have addressed the electrochemical behavior of FA, and proposed an irreversible two-electron pH-dependent reaction for its oxidation in aqueous solutions. In the present paper, the electrochemical mechanism underlying such an electron transfer was beyond the scope of this study. However, the main oxidation peak served as the analytical response for FA determination. There are two possible processes of analytical use; one is the first oxidative peak in strong acidic media such as 0.1 M $HClO_4$, due to its highest sensitivity and sufficient separation from the second one to quantify, and the other is the single oxidative peak in 0.1 M BR buffer at pH 6.0, with relatively better current response and peak morphology, and lower background signal than the others obtained at higher pHs. Thus, these solutions were selected for further experiments.

Pre-concentration of the analyzed compound on the surface of BDD electrode is one of the essential conditions for highly sensitive determinations. Next, the attention was turned

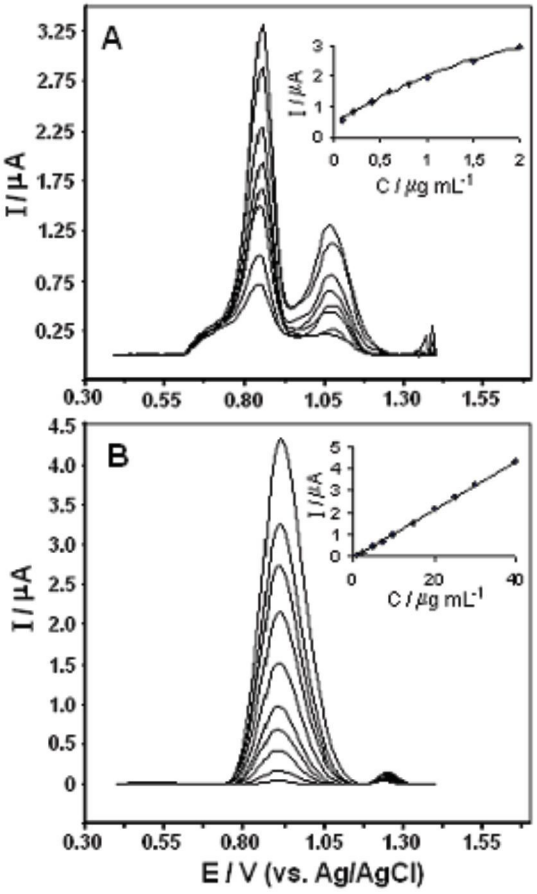

Figure 4. The stripping voltammograms in 0.1 M $HClO_4$ (A), and in 0.1 M BR buffer pH 6.0 (B) containing different concentrations of FA (from inner to outer: 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 and 1.0, 2.5, 5.0, 7.5, 10, 15, 20, 25, 30, 40 µg/mL in 0.1 M $HClO_4$ and 0.1 M BR buffer, pH 6.0, respectively). Calibration graphs for FA are showing in the insets. Pre-concentration period, 120s at open circuit condition; SWV parameters: frequency, 100 Hz; scan increment, 10 mV; pulse amplitude, 40 mV.

to the effect of pre-concentration/stripping conditions, such as accumulation potential and time (data not shown). The accumulation potential on the stripping peaks was evaluated at open-circuit condition or at a potential range from +0.1 to 0.2 V for a pre-concentration period of 120 s in stirred μg/mL FA solution. Similar values of peak current were obtained in all cases. Since the baseline were distorted in the range +0.1 V and +0.2 V, so the accumulation in the rest of experiments was adopted under open-circuit. The influence of the accumulation time upon the analytical signal was examined in the range 30-300 s. The current increased linearly with accumulation time till 120 s beyond which the peak current started to decrease, indicating that electrode surface becomes saturated with the analyte molecules. Therefore, this accumulation time was selected for all the AdSV experiments.

The SW response markedly depends on the parameters of the excitement signal. In order to obtain the maximum development of the SW-AdSV peak current, the various instrumental conditions (square-wave frequency, 25 Hz $\leq f \leq$ 125 Hz; pulse amplitude, 10mV $\leq a \leq$ 50 mV; and scan increment, 2mV $\leq \Delta E_s \leq$ 14mV) were studied for 20 μg/mL FA in selected electrolytes following pre-concentration for 120 s under open-circuit. The variation in the f values shown that its increase promoted an increase in the peak current due to the increase in the effective scan rate. However the background current and noise ware also increased at f values higher than 100 Hz. This was attributed to the greater contribution of the capacitive current at higher frequencies. The voltammetric responses for FA determination as a function of variation in a demonstrated that peak current values increased upon increase of this parameter. However, the best peak morphology and sharper one was obtained at 40 mV. In addition, at higher values of 10 mV, an increase in ΔE_s resulted in a decrease in peak current. To account for the results, in subsequent experiments, values of f = 100 Hz, a = 40mV, and ΔE_s = 10 mV were adopted.

Analytical applications

Under application of the above mentioned optimized experimental parameters, SW stripping voltammograms at different concentrations of FA were recorded to estimate the analytical characteristics of the developed method (Figure 4). For this, aliquots from the FA standard solution were consecutively added to the electrochemical cell and the SWV responses at potentials of +0.85 and +0.91 V in 0.1 M $HClO_4$ and BR buffer pH 6.0, respectively, were evaluated for each addition. By analyzing the inset in Figure 4, one can conclude that the respective analytical curves presented a good linearity in the ranges of concentration from 0.1 to 2.0 μg/mL ($2.3x10^{-7}$ M - $4.5x10^{-6}$ M) and 1.0 to 40 μg/mL ($2.3x10^{-6}$ M - $9.0x10^{-5}$ M) in 0.1 M $HClO_4$ and BR buffer, pH 6.0, respectively. The corresponding calibration equations are:

i_p/μA =0.646 + 1.226[C/(μg/mL)] (r =0.988, n =8) (in 0.1 M $HClO_4$)

i_p/μA= –0.117+0.112[C/(μg/mL)](r= 0.999, n = 10) (in BR buffer pH 6.0)

where i_p is the adsorptive stripping peak current, C FA concentration, r the correlation coefficient and n the number of experiments.

From the data obtained by the analytical curves, the detection (LOD) and quantification (LOQ) limits were calculated using the formulas 3 s/m and 10 s/m, respectively, where s is the standard deviation of the response (blank) (seven runs), and m the slope of the calibration plot. LOD of 0.035 μg/mL ($7.9x10^{-8}$ M) and 0.14 μg/mL ($3.2x10^{-7}$ M), and LOQ of 0.117 μg/mL ($2.7x10^{-7}$ M) and 0.47 μg/mL ($1.1x10^{-6}$ M) were achieved in 0.1 M $HClO_4$, and BR buffer pH 6.0, respectively.

Although the sensitivity in terms of quantitation range and LOD is approximately ten times lower in BR buffer pH 6.0 than that reached in 0.1 M $HClO_4$, this disadvantage seems to be less important due to higher levels of FA in pharmaceutical formulation. Moreover, the correlation coefficient (r) obtained at pH 6.0 was found to be higher than that obtained in strongly acidic medium. It is also important to underline that analysis of FA is not an easy task in the presence of strong acidic environment because of its lower stability on exposure to light under these conditions. As explained above, the protonated species (pK_{a3} = 2.35) of FA molecule predominantly forms in this medium which undergoes photolytic degradation. The rate of photodegradation of FA is high within

Table 1. Comparison of the efficiency of the bare BDD electrode with literature modified electrodes for FA determination.

Electrode	Linear working range (M)	LOD (M)	Medium	Remarks	Ref.
PMo_{12}-PPy/GCE	$1x10^{-8}$–$1x10^{-7}$	$1x10^{-10}$	0.01 M H_2SO_4	Cathodic range	(23)
SWNT/GCE	$1x10^{-8}$–$1x10^{-4}$	$1x10^{-9}$	pH 5.5	Cathodic range	(24)
SWNT/GCE	$2x10^{-9}$–$4x10^{-6}$	$1x10^{-9}$	pH 5.5	Anodic range	(25)
MWNT/GE	$2x10^{-8}$–$1x10^{-6}$	$4x10^{-9}$	pH 2.5	Anodic range	(26)
MWNT/GCE	$3x10^{-7}$–$8x10^{-5}$	$1.34x10^{-7}$	pH 6.4	Cathodic range	(27)
CME-6	$8.8x10^{-12}$–$1.9x10^{-9}$	$1.24x10^{-12}$	pH 4.0	Anodic range	(30)
PbFiE/GCE	$2x10^{-9}$–$5x10^{-8}$	$7x10^{-10}$	pH 5.6	Cathodic range	(31)
Ni/POA/CPE	$1x10^{-4}$–$5x10^{-3}$	$9.1x10^{-5}$	0.1 M NaOH	Anodic range	(32)
m-AgSAE	$5x10^{-9}$ – $2.5x10^{-8}$	$5x10^{-10}$	pH 5.5	Cathodic range	(36)
MIP-fiber	$1.35x10^{-9}$–$8.7x10^{-9}$	$4.53x10^{-10}$	pH 7.8	Anodic range	(34)
TNMCPE	$1.4x10^{-4}$–$2.3x10^{-4}$	Not given	pH 7.0	Anodic range Simultaneously with ascorbic acid and uric acid	(37)
ZONMCPE	$2x1x10^{-5}$–$2.5x10^{-3}$	$9.86x10^{-6}$	pH 7.0	Anodic range Simultaneously with epinephrine and acetaminophen	(38)
DWNT/PE	$1.5x10^{-5}$–$8x10^{-4}$	$3x10^{-7}$	pH 7.0	Anodic range Simultaneously with epinephrine and uric acid	(28)
MWNT/PE	$4.6x10^{-6}$-$1.52x10^{-4}$	$1.1x10^{-6}$	pH 9.0	Anodic range Simultaneously with 6-thioguanine	(29)
(CPE-PANI/TPA	$2.0x10^{-6}$-$2.1x10^{-3}$	3.0 $x10^{-7}$	pH 7.0	Anodic range Simultaneously with norepinephrine and acetaminophen	(39)
BDD	$2.3x10^{-7}$-$4.5x10^{-6}$	7.93 10^{-8}	0.1 M $HClO_4$	Anodic range	present work
BDD	$2.3x10^{-6}$-$9.0x10^{-5}$	$3.2x10^{-7}$	pH 6.0	Anodic range	present work

pH 2.0-4.0 and gradually decreased on moving from the acid to the alkaline region because of formation of deprotonated species. When solution pH $\geq$ 9.0, existence of mesomer stabilized anion is probably much less susceptible to the photodegradation process. Thus, a pH 6.0-7.0 appears to be a better choice to achieve optimum stability on exposure to light (51). Taking into account the obtained results and stability of FA solutions, only the peak in 0.1 M BR buffer solution at pH 6 was studied in detail in the following measurements with the aim of its analytical application in pharmaceutical samples.

It is worth to compare the determination of FA on BDD electrode with other voltammetric methods. Majority of the reported papers are based on modified electrodes. The linear range, LOD, and the pH values of supporting electrolytes for bare BDD electrode presented

in this work were compared with the reported modified electrodes and were given in Table 1. This shows that although BDD electrode exhibits a more sensitive response than some solid modified electrodes or carbon nanotube electrodes reported earlier, for many others more improved LOD values have been found. However, the disadvantage of these types of electrodes is in their preparation. In most cases, the processes of modifying bare electrodes are often complicated, time-consuming and inconvenient, and the prices of modifying substances are usually high. Furthermore, the surface stability and reproducibility of these electrodes are not always good. Based on the above, the simplicity of present methodology enables its use without requiring a procedure for modification of the electrode surface, in addition to sufficient analytical sensitivity for application to pharmaceutical formulation.

In order to determine the precision of the determinations, standard solutions of FA (µg/mL) were analyzed ten times within the same day (intra-day variation) and on four different days (inter-day variation). The relative standard deviations (RSD) were calculated to be 2.40 and 4.15% for intra-day and inter-day repeatability, respectively, which are acceptable for practical applications.

It is noteworthy to underline once again that FA is only soluble and stable in dilute alkaline solution and dissolves but is unstable in acid medium (49-52). To study the stability of stock alkaline solutions of FA, they were kept in refrigerator for at least 7 days and the current response remained almost unchanged. All working solutions (from the acid to alkaline region) used for the validation experiments were freshly prepared, protected from light and used within 10 h.

The effects of some substances commonly found with FA in pharmaceutical, clinical and/or food samples on the electrochemical oxidation of 10 µg/mL FA in BR buffer pH 6.0 were evaluated on the BDD electrode. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ±5% for the determination of FA. The results showed that 100-fold of glucose, 50-fold of thiamine hydrochloride and nicotinamide, 500-fold Ca^{2+}, Mg^{2+}, K^{+}, and 100-fold Fe^{2+}, Cu^{2+} had almost no influences on the peak current and potentials of FA. That is because some of the above

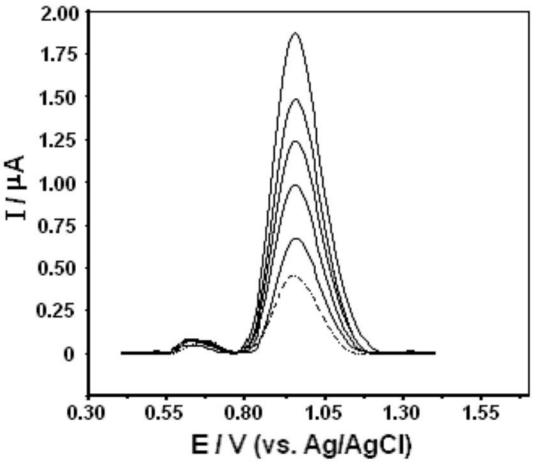

Figure 5. The stripping voltammograms in 0.1 M BR buffer pH 6.0 obtained for the determination of FA in tablet samples. A diluted sample (dashed line) and sample spiked at a FA levels (from inner to outer: 2.5, 5, 7.5 10 and 12.5 µg/mL, respectively). Other operating conditions as indicated in Figure 4.

Table 2. Results obtained for FA determination and recovery studies in Folbiol® tablets.

Labeled value (mg)	Found value[a](mg)	RSD (%)	Bias (%)	Added (µg/mL)	Found[a] (µg/mL)	Recovery (%)	RSD (%)	Bias (%)
5.00	4.68	2.6	6.4	5.00	5.07	101.4	2.7	-1.4
				7.50	7.39	98.5	2.3	1.5
				10.00	9.51	95.1	2.7	4.9

[a]Average of three measurements

substrates are nonelectroactive in the potential window studied or the oxidation peaks have a good separation between the electroactive substrates and FA. The presence of ascorbic acid (physiological interferent) results in peak widening probably due to the proximity of ascorbic acid oxidation peak to that of FA. In the case of FA formulations containing ascorbic acid, the stripping step in clean electrolyte by using medium exchange technique could be used for eliminating interference from ascorbic acid. On the other hand, uric acid (physiological interferent) did significantly interfere with the current response. The proposed method may be used if its selectivity could be improved using a simple preliminary reaction, including elimination of uric acid before the quantification of FA. Moreover, this is not a problem in case of analyzing pharmaceutical samples.

The applicability of the BDD electrode for SW-AdSV determination of FA was verified by analysis of pharmaceutical samples (Folbiol® tablets). The analyzed solutions were prepared as it was described above (in Section 2.4), without any sample extraction, evaporation or filtration, and after adequate dilutions. The dilute real samples were almost similar to aqueous sample in behavior (Figure 5, dashed line). It was found the mean value of 3.51 μg/mL of FA in the measurement cell. Taking into account the successive dilutions of the sample, FA content was calculated to be 4.68 mg per tablet, which approximates the label value of 5.00 mg per tablet declared by producer. The precision of the analysis performed was good (RSD = 2.6%) The bias was around 6% when compared to the label value which is considered as the true value (Table 2).

In order to know whether the common excipients and filling materials present in the analyzed tablets show any interference with the analysis, the recovery experiments were carried out adding standard FA solutions (2.5-12.5 μg/mL) prepared in supporting electrolyte to 10 mL of sample solution in voltammetric cell and voltammetric responses were evaluated (Figure 5, solid lines). Recovery of FA was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure FA. As demonstrated in Table 2, the recovery studies allowed concluding that the matrix effect did not present any significant interference.

CONCLUSIONS

As stated in the introduction, the main goal of this work is to throw a more light upon the electrochemical behavior of FA in the case of using anodically pre-treated BDD electrode. A SW-AdSV procedure developed and validated in this study was simple, rapid, precise and accurate, being applicable directly to the routine quality control of pharmaceutical formulation after dissolution of their samples, dispensing any use of organic reagents or expensive apparatus.

Obviously, such low detection limits still are not sufficient for most clinical applications in real samples (e.g. normal level of 0.0151 ± 0.0045 μg/mL in human blood serum); however they give hope for future improvement. The experimental data obtained at BDD electrode might also be used for the development of liquid chromatography or capillary electrophoresis with electrochemical detection.

Finally, it should be mentioned once again that, to the best of our knowledge, no literature data were found on the electrochemical oxidation of FA using bare electrodes, except in two earlier works dealing with its differential pulse voltammetric determination using glassy carbon electrode (53) and carbon fiber microelectrode (54).

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