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# Differential Pulse Voltammetric Determination of Folic Acid in the Presence of Ascorbic Acid using A Glassy Carbon Electrode Modified with Reduced Graphene Oxide

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**Abstract**- Reduced graphene oxide was estimated for the modification of a glassy carbon electrode. The fabricated electrode was employed for folic acid determination in 0.1 M KCl solution (pH 14) using cyclic, linear sweep, differential pulse voltammetric and chronoamperometric techniques. The modified sensor exhibits a high electro-catalytic activity towards FA oxidation in the presence of ascorbic acid. The anodic peak current (IP) of FA increased linearly with an increase in pH (12.6–14) and scan rate (20–500 mV/s) at the ERGO/GCE. Good linearity was obtained between IP and FA concentrations (3.01–7.23 µM) with the detection (LOD) and quantification (LOQ) limits are 4.68 and 15.6 nM, respectively. Under diffusion control, the diffusion coefficient was estimated to be 2.88×10−6 cm2/s at the ERGO/GCE. The fabricated sensor gives high selectivity, good sensitivity and excellent reproducibility. Thus, the proposed method could be applied to detect FA in pharmaceutical formulations and urine samples.

**Keywords-** Folic acid; Ascorbic acid; Modified GC electrode; Reduced graphene oxide; Differential pulse voltammetry; Chronoamperometry

## 1. INTRODUCTION

Vitamins are a class of organic molecules that are essential micronutrients in small quantities for human body metabolic activity [1]. Folic acid (FA) is an essential water-soluble vitamin that helps build healthy cells and important element for the haemapoietic system. Besides, FA acts as a co-factor in carbon transfer reactions and aids in amino acids metabolism. It plays a vital role in the synthesis of nucleotides, biosynthesis of purines and pyrimidines of DNA and cell replication [2-4]. FA (commonly called folate which is the form naturally occurring in the body), also known as vitamin $B_C$, vitamin $B_9$ or vitamin M is pteroyl-L-glutamic acid [5]. As a result of the inability of the body to produce FA, it must be supplied from foods such as fruits, vegetables, algae, broccoli, mushrooms, cabbage, nuts and fortified grains and pharmaceutical samples [6,7].

Another water-soluble vitamin essential in the human nutritional regimen and health care of human beings is ascorbic acid (AA). But, the enzymes necessary to synthesize AA do not exist. On the other hand, AA occurs naturally in fruits and vegetables. The biological activity as an antioxidant agent of vitamin C makes it useful in food, beverages and pharmaceutical applications [8,9]. Because of it participated in several human metabolic reactions [10]. Moreover, AA has widely been used for the treatment of influenza, mental diseases, infertility and cancer [11].

As a result, vitamins play a vital role in the human body, and their deficiency may cause often painful and potentially harmful diseases. Over the past decade, the deficiency of FA has closely lead to several disorders such as increases in the risk of cancer, gigantocytic anemia, leukopaenia, devolution of mentality psychosis, hypomethylation, cardiovascular disease, coronary heart disease, osteoporosis and Alzheimer's disease [12-15]. For these reasons, it must be control the concentration of folate in its various origins. On the other hand, a lack of FA during pregnancy is a marker of neural tube defects. Therefore, FA is a very essential nutrient vitamin for rapid cell growth for women planning pregnancy [16]. Therefore, FA supplementation can significantly reduce the occurrence and recurrence of neural tube defects [17]. Not this only, but also FA is usually used for the prevention or treatment of megaloblastic anemia during pregnancy [18].

The importance of FA in human health, methods for its determination and quantification in clinical, pharmaceutical and food samples have received increasing interest. Thus, several techniques have been developed for FA determination such as thermogravimetry [19], spectrophotometry [20], quartz crystal microbalance [21], flow injection chemiluminometry [22], enzyme linked immunosorbent assay (ELISAs) [23], capillary electrophoresis [24] and chromatography in various arrangements including liquid chromatography (LC) [25], high performance liquid chromatography (HPLC) [26], isotope dilution-liquid chromatography/tandem mass spectrometry [27] and microemulsion electrokinetic chromatography (MEEKC) [28]. Most of these methods offer excellent resolution and useful

information in terms of the identification and quantification of FA. Nevertheless, most of these methods generally require expensive reagents with complicated sample preparation, suffer from low sensitivities, consume a long time for analysis, nonspecific and laborious [29].

In this context, various electrochemical techniques have been used to determine biologically active molecules like FA. Compared with other electroanalytical methods, voltammetry is more desirable because it provides relatively low costs of instrumentation, high sensitivity, good stability, high accuracy and low detection limits [30–32]. But the unmodified electrodes used in voltammetry are poor in sensitivity and selectivity. On the other hand, chemically modified electrodes have attracted enormous interest in the field of electroanalytical chemistry in recent years [33–35]. This is because of their good selectivity, high stability, high sensitivity, low overpotential and high electron transfer rate in the determination of FA and other pharmaceutical compounds [36].

Recently, great attention has been paid to reduced graphene oxide (rGO) to employ as the electrode modification material [37,38] as a result of its unusual physical and chemical properties. The rGO is usually prepared by electrochemical reduction (a green and efficient process) of graphene oxide (GO). It's known that the rGO is hydrophobic because of the removal of most oxygen-containing functional groups [39]. GO is a single atomic plane of $sp^2$ hybridized carbon atoms [40]. Thus, GO has several advantages including a large surface area, readily dispersed in water, easily prepared, good electrocatalytic activity and high conductivity [41]. But GO showed electrical insulation and chemical reduction as documented. Therefore, for electroanalytical purposes, Go should be converted to rGO before applying.

Herein, simple and sensitive voltammetric methods, especially differential pulse voltammetry coupled with the unique properties of the rGO-modified GCE were utilized for the determination of FA with low limit of detection, high sensitivity and good selectivity. Besides, the analytical performance of ERGO/GCE was assessed for the determination of FA in the presence of AA. Additionally, the content of FA has been successfully detected in pharmaceutical and human urine samples.

## 2. EXPERIMENTAL SECTION

### 2.1. Chemical reagents and apparatus

FA (98%), AA (99%), graphene oxide powder (98%) and sulfuric acid ($H_2SO_4$, 99%) were purchased from Sigma-Aldrich. FA stock solution was prepared by dissolving an appropriate weight of FA substance in 0.1 M NaOH (El-Nasr Pharmaceutical Chemicals; Egypt) and kept in the dark under refrigeration (below 4 $^0$C) immediately after use. Stock solutions of 0.1 M KCl were prepared by dissolving the appropriate amount of KCl (BDH Chemicals Ltd; England) in 250-mL distilled water and used as the supporting electrolyte. Other chemicals and reagents used in the experiments were of analytical grade and used without any further

purification. All solutions were freshly prepared with double distilled water at room temperature.

All electrochemical measurements were performed using EG&G Princeton applied research potentiostat/galvanostat model 263A (USA). A three electrodes cell (model K0264 micro-cell) was used for all electrochemical experiments. Where, a high purity platinum wire and an Ag/AgCl (saturated KCl) were used as the auxiliary and reference electrodes, respectively. Finally, a bare glassy carbon electrode (GCE, 2 mm) and ERGO/GCE were used as a working electrode. The pH values were adjusted through CyberScan pH 500 Meter (Euteoh-India).

### 2.2. Fabrication of the modified ERGO/GCE

Before electrodeposition, a mirror-shiny surface of a GCE was obtained by successively polishing the electrode with alumina powder (3 µm, Sigma-Aldrich) then rinsing with distilled water and ultrasonically cleaning with water/ethanol for 4 min. In addition, the GCE was electrochemically cleaned by cycling the electrode potential from -1.0 to 1.0 V (Vs. Ag/AgCl) in 1.0 M $H_2SO_4$ [42] till a stable voltammogram is obtained. The electrode was dried at room temperature after rinsed with redistilled water. The solid compound GO was transferred to the shiny surface of the GCE by abrasive immobilization [43]: about 1-3 mg of GO was placed on a coarse-grade filter paper. Then, the GCE is pressed onto the substance and rubbed over the material leading some compound of GO to adhere on the electrode surface. Finally, the modified ERGO/GCE was fabricated based on cyclic voltammetric reduction of GO according to literature [44–46] with some modifications. Where, the GO-modified GCE was immersed in 0.1 M PBS (pH 7.6) and was conditioned by cyclic sweeping from 0.5 to -1.7 V for 16 cycles.

### 2.3. Real samples preparation

FA tablets were purchased from a local pharmacy. Two tablets of FA pharmaceutical drug (500 µg folic acid per tablet) were accurately weighed, ground in a mortar to a fine and homogeneous powder, and then dissolved in 0.1 M NaOH solution (pH 14). In the end, the samples were filtered through a normal filter paper.

Urine samples of the healthy specimens were analyzed immediately after collection. Three milliliters of fresh samples were diluted to 250 mL with 0.1 M NaOH solution (pH 14). Then, filtered with a normal filter paper. The diluted urine samples were spiked with various amounts of FA in 0.1 M KCl solution.

## 3. RESULTS AND DISCUSSION

### 3.1. Preparation and electrochemical fabrication of the ERGO/GCE

In the last few years, electrochemical sensors based on rGO have received a lot of attention because of their unique properties including high surface area, good electrical conductivity and high mechanical properties [47].

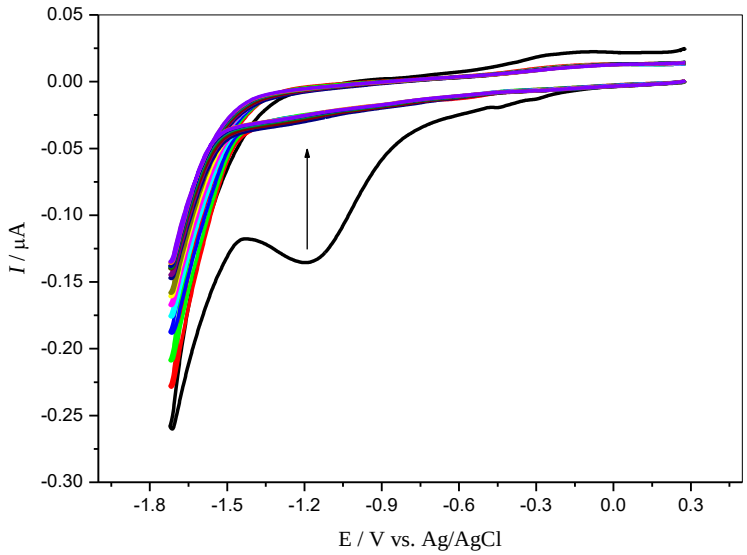
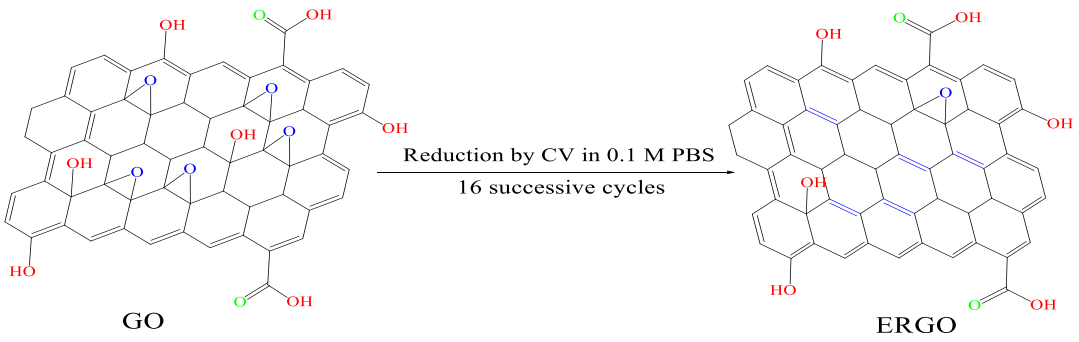



**Figure 1.** 16 successive CVs were performed in GO/GCE at 0.5 to -1.7 V *vs.* Ag/AgCl in 0.1 M PBS (pH 7.6) at a scan rate 100 mV/s

As a result, 16 successive CVs were performed in the potential range between 0.5 and -1.7 V for GO/GCE in 0.1 M PBS (pH 7.6) at a scan rate of 100 mV/s (Figure 1). As observed during the first potential scan, a high reduction peak appears at -1.19 V, which belong to oxygen functional groups at the GO surface [48,49] as illustrated in Scheme 1. With consecutive potential scans, this cathodic peak disappeared completely indicating the conducting reduced graphene oxide had been successfully deposited on the GCE surface. Where, is known that epoxy and hydroxyl groups are mostly located at the basal plane of the GO sheets, while carboxyl and carbonyl groups are present in the edges [50] that are too stable to be reduced by the potential scan [51].




**Scheme 1.** The electrochemical reduction of reduced graphene oxide

### 3.2. Electrocatalytic oxidation of FA at the surface of various electrodes

Figure 2 depicts CVs for the electro-oxidation of 4.55 µM FA at a bare GCE (curve a), the GO/GCE (curve b) and the ERGO/GCE (curve c) in 0.1 M KCl solution (pH 14) at a scan rate of 100 mV/s. As seen, an oxidation peak potential arises at +1.256 V for FA at the bare GCE (curve a) with the absence of any reduction peak on the reverse scan, suggesting that the oxidation reaction of FA is irreversible [52] as illustrated in Scheme 2 [53,54].

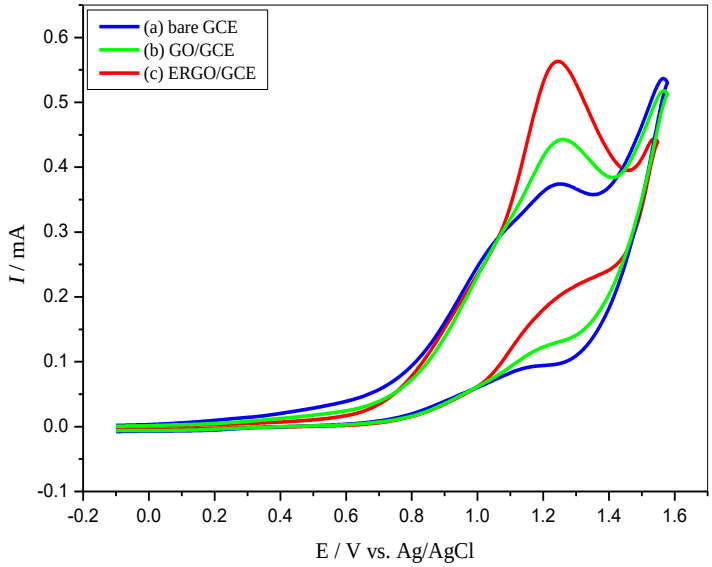
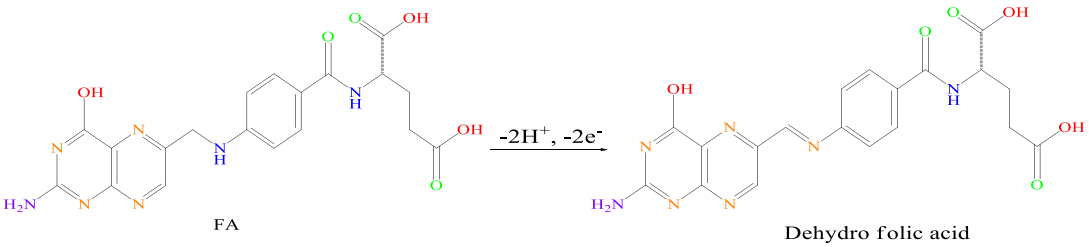



**Figure 2.** CVs of 4.55 µM FA in 0.1 M KCl solution of pH 14 at a bare GCE (a), the GO/GCE (b) and the ERGO/GCE (c) at a scan rate of 100 mV/s

While there is negative shifts to +1.255 V for the oxidation peak potential of FA at the Go/GCE (curve b) and the oxidation peak current slightly increases with 3-folds. This fact revealed that the GO/GCE can significantly catalyze the electrochemical oxidation of FA. Furthermore, the oxidation peak potential of FA negatively shifts to +1.246 V at the ERGO/GCE (curve c) and the anodic peak current obviously increases to 9-fold higher than that at the bare GCE. These results indicate excellent electrocatalytic activity of the ERGO/GCE toward the oxidation of FA compared to the bare GCE and the GO/GCE, because of the unique properties of the ERGO including good conductivity and numerous edge-plane, which constructed a novel conductive composite film. Thus, ERGO composite film highly increased the electroactive surface area and promoted the electron-transfer rate between the electrode and species in solution [55].




**Scheme 2.** Suggested oxidation mechanism of FA on the surface of ERGO/GCE

### 3.3. Effect of supporting electrolyte and pH at ERGO/GCE

The electrochemical oxidation of FA was tested in several supporting electrolytes such as phosphate buffer, acetate buffer, potassium nitrate and potassium chloride solution. A higher oxidation peak of FA with a better-defined shape was observed in 0.1 M potassium chloride solution.

The effect of pH value on peak current and peak potential was cautiously examined (Figure 3) for 4.55 µM FA at 100 mV/s using ERGO/GCE. Over the pH range from 12.6 to 14.0, the anodic peak current of FA linearly increased while its potential shifted gradually toward the positive potential side. This behavior is normally because of the solubility of FA is higher under alkaline conditions [56]. Inset Figure 3 shows a graph of oxidation peak potential values as a function of pH. The good linearity graph with $R^2$ = 0.9962 has a slope of 54.1 mV/pH is close to the theoretical value of 59 mV/pH, indicating that two electrons and two protons transfer reaction oxidation of FA [57,58].

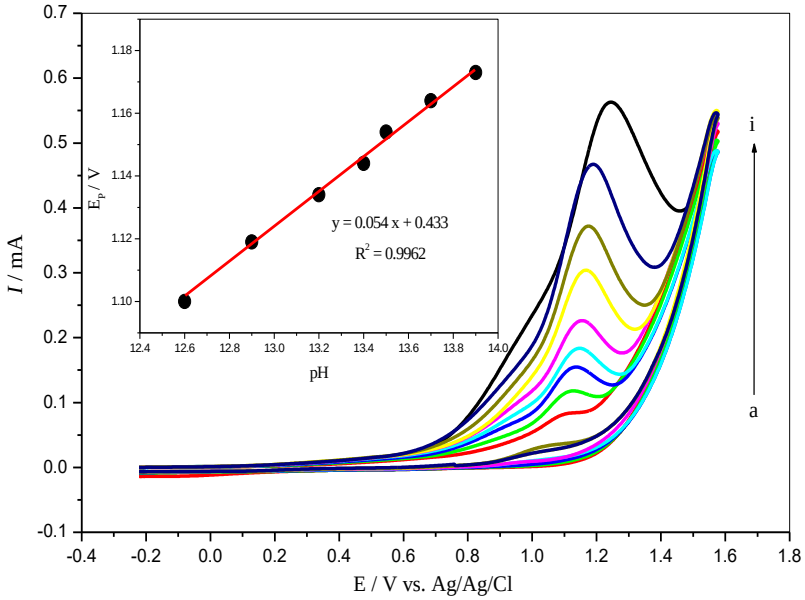



**Figure 3.** CVs of 4.55 µM FA at 100 mV/s on ERGO/GCE in 0.1 M KCl solution with different pH values: a→i: 12.6, 12.9, 13.2, 13.4, 13.5, 13.7, 13.9, 13.95 and 14. Inset plot of $E_p$ *vs.* pH from 12.6 to 13.9

### 3.4. Effect of scan rate at ERGO/GCE

To understand the electrocatalytic process nature, the effect of varying scan rates on the electrochemical oxidation of 4.55 µM FA in 0.1 M KCl solution (pH 14) at the ERGO/GCE was investigated using CV. As can be seen in Figure 4, an increase in the peak current of FA with increasing the scan rates from 20 to 500 mV/s. Additionally, the oxidation peak potential shifted to more positive values, which confirm the irreversibility of the oxidation reaction of FA at the ERGO/GCE. Furthermore, over the scan rate range of 20–500 mV/s, a linear dependence (Figure 4, inset) of the anodic peak current ($I_p$) on the square root of the scan rate ($\nu^{1/2}$) with the equation: $I_P = 0.047\ \nu^{1/2} - 0.025$ and the correlation coefficient value $R^2 = 0.9958$, suggesting that the oxidation processes are diffusion-controlled [59, 60].

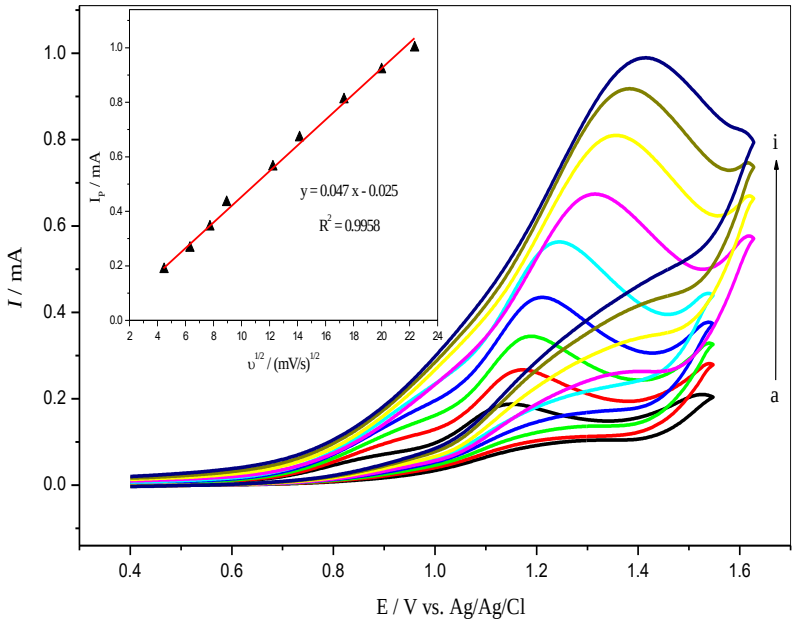



**Figure 4.** CVs of 4.55 µM FA in 0.2 M KCl solution (pH 14) at ERGO/GCE with different scan rates (10-100 mVs: a→i: 20, 40, 60, 80, 150, 200, 300, 400 and 500 mV/s. Inset plot of $I_p$ vs. square root of ν

Tafel plot can be drawn from the data of the Tafel region of the CV that was recorded at a scan rate 100 mV/s for FA as shown in Figure 5. Its known that the Tafel plot is affected by electron transfer kinetics between the substrate (FA) and the ERGO/GCE. Inset Figure 5 displays the Tafel plot with the linear equation E (V) = 0.46 log *I* (µA) – 0.085 and ($R^2$ = 0.9911). The slope of the Tafel plot can be used to estimate the charge transfer coefficient (α) and obtained as 0.13 for FA.

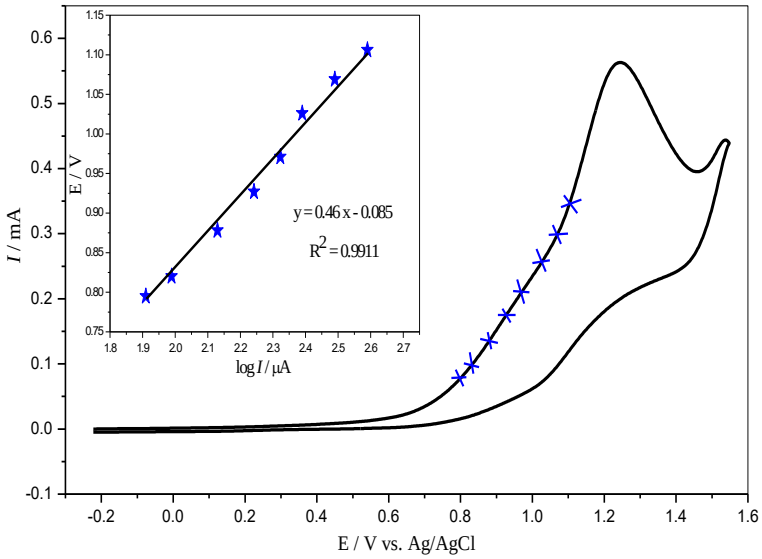



**Figure 5.** CV of 4.55 µM FA in 0.2 M KCl solution (pH 14) at ERGO/GCE with a scan rate 100 mV/s. The points are the data used in the Tafel region. The inset shows the Tafel plot derived from the CV curve

### 3.5. Effect of FA concentration at ERGO/GCE

The DPV method was employed to estimate the electrochemical oxidation of FA in 0.1 M KCl solution (pH 14) at various concentrations ranging from 3.01 to 7.23 µM using the ERGO/GCE as observed in Figure 6 (scan rate: 20 mV/s, Pulse high: 25 mV; Pulse width: 50 ms). As clearly shown in Figure 6, the anodic peak current increased proportionally with the FA concentration and the peak potential shifted slightly toward positive values. A highly linear calibration curve, I*p* (µA) = 9.83 C (µM) –13.22 ($R^2$ = 0.9998) was obtained when plotting of the anodic peak current versus FA concentration (Figure 6, inset).

Furthermore, from the slope of the calibration curve, the detection (LOD) and quantification (LOQ) limits were estimated using the following equations [61,62]:

$$LOD = 3\ S/M \text{ and } LOD = 10\ S/M$$

Where, S is the standard deviation of 9 blank measurements and M is the slope of the calibration plot. LOD and LOQ were found to be 4.68 and 15.6 nM, respectively, and are comparable with values reported in other literature and given Table 1. Thus, the performance of the modified electrode exhibited high sensitivity, relatively wide linear range, and low limit of detection toward FA determination.

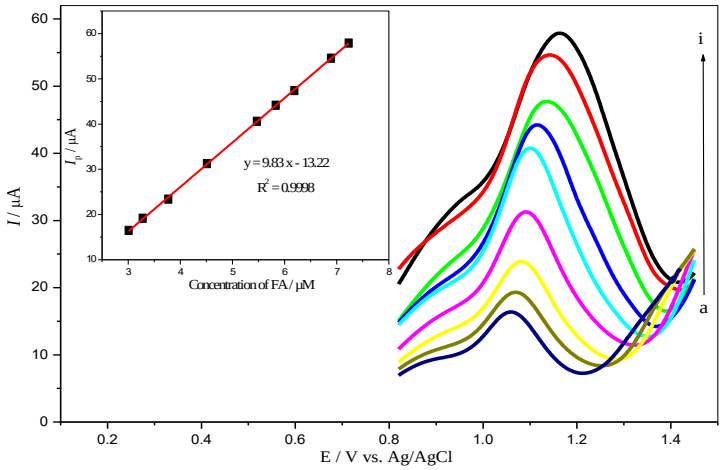



**Figure 6.** DPVs at ERGO/GCE in 0.1 M KCl solution (pH 14) containing different concentrations of FA (a→i: 3.01, 3.28, 3.77, 4.51, 5.47, 5.83, 6.19, 6.89 and 7.23 µM; scan rate 20 mV/s, Pulse high 25 mV; Pulse width: 50 ms. Inset: plot of the peak current as a function of FA concentration

**Table 1.** Comparison of the limit of detection of the ERGO/GCE with other modified electrodes for the determination of FA

| Electrode | Technique | pH | Linear range (µM) | LOD (µM) | Reference |
|---|---|---|---|---|---|
| $ZrO_2$ nanoparticles/CPE | DPV | 7.0 | 20–2500 | 9.86 | [63] |
| Mercury film electrode | DPV | 7.1 | 0.13–1, 2–10 | 0.014 | [64] |
| ZnO/Carbon nanotubes/CPE | SWV | 7.0 | 3–700 | 1.0 | [65] |
| Au nanoparticles/Au electrode | DPV | 14 | 0.01−1.0 | 0.0075 | [66] |
| MWCNTs/Au electrode | CA and CC | 2.5 | 0.02–1 | 0.01 | [67] |
| Hydroquinone derivates/CPE | CV and DPV | 7.0 | 200–3200 | 25 | [68] |
| Mesoporous carbon/graphiteE | CV and DPV | 7.0 | 5.0–2000 | 0.7 | [69] |
| MWCNTs/FDCA/CPE | DPV | 9.0 | 4.6–152 | 1.1 | [70] |
| GCE/f-MWCNT-Ni(OH)$_2$-$Si_4Pic^+Cl^-$ | DPV | 7.0 | 0.5–26 | 0.095 | [71] |
| ERGO/GCE | DPV | 14.0 | 3.03–7.28 | 0.00468 | This work |

CPE: carbon paste electrode, MWCNTs: Multiwall carbon nanotubes and FDCA: ferrocenedicarboxylic acid. $Si_4Pic^+Cl^-$: an inorganic ionic silsesquioxane polymer

### 3.6. Chronoamperometric measurements

A useful electrochemical method for determining the diffusion coefficient of an electroactive material (FA, in this case) is chronoamperometry. Figure 7 shows the

chronoamperometric measurements for various concentrations of FA in 0.1 M KCl solution (pH 14) at the ERGO/GCE. These current-time profiles were obtained by setting the working electrode potential at 0 V (first potential step) and 1. 7 V vs. Ag/AgCl (second potential step). The current observed for the electrochemical reaction of FA under mass transport limited conditions is described by the Cottrell equation [72]:

$$I = nFAD^{1/2}C_b/ \pi^{-1/2} t^{-1/2}$$

where n is the number of electrons transferred (2), F is the Faraday constant (96,485 C/mol), A is the surface area of the working electrode ($cm^2$), D is the diffusion coefficient ($cm^2/s$) and $C_b$ is the bulk concentration (M).

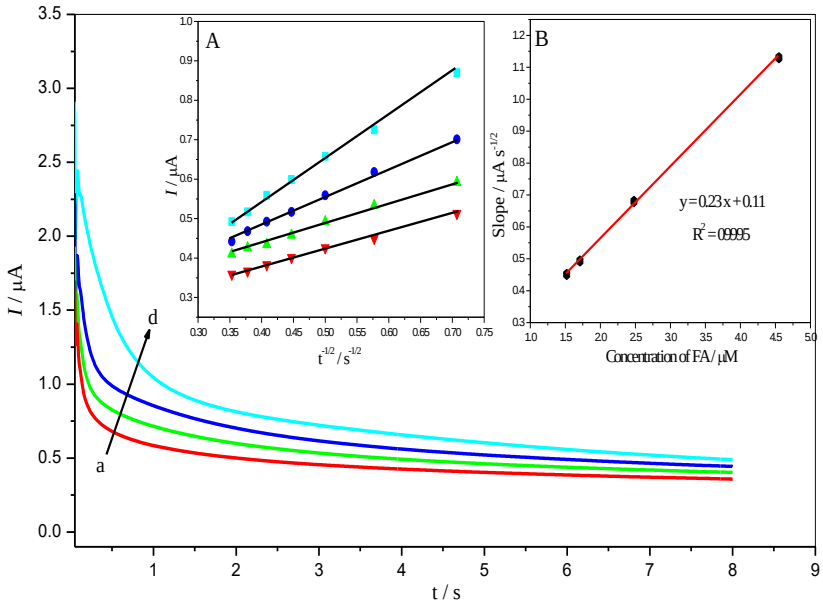



**Figure 7.** Chronoamperograms obtained at the ERGO/GCE in 0.1 M KCl solution (pH 14) for different concentration of FA: a→d: 1.52, 1.71, 2.48 and 4.55 μM

Under diffusion control, good linearities were obtained from the experimental plots of *I* vs. $t^{-1/2}$ for different concentrations of FA as depicted inset in Figure 7(A). The slopes of the resulting straight lines were then plotted vs. the FA concentration as can be seen inset Figure 7(B). From the resulting slope and based on the Cottrell equation, D was estimated to be $2.88\times10^{-6}$ $cm^2/s$.

### 3.7. Stability and reproducibility investigation

One of the most important properties of the modified electrode is long-term stability. Therefore, the LSV technique has been employed to investigate the stability of the ERGO/GCE for the determination of 9.1 μM FA in 0.1M of KCl solution at pH 14. It was found that

relatively no loss of electroactivity of the ERGO/GCE was found for the continuous cyclical sweep for 5 h. Also, the modified electrode was not deteriorated even for more than two weeks, indicating its high stability in the determination of FA. Furthermore, 6 repetitive measurements were carried out using the same ERGO/GCE for an identical solution containing 4.55 µM of FA in 0.1 M KCl solution (pH 14). The relative standard deviation (RSD) was 1.67 %, which indicates the excellent reproducibility of the ERGO/GCE.

### 3.8. Simultaneous determination of FA and AA

Herein, the ERGO/GCE was used for the determination of 4.55 µM FA and 4.55 mM AA in 0.1 M KCl solution at pH 14 and a scan rate of 100 mV/s as displayed in Figure 8(A). As can be seen, two well-defined voltammetric peaks observed at –0.40 and +1.16 V for AA and FA, respectively, with potential differences of 1.56 V vs. Ag/AgCl. Thus, the precise determination of FA in the presence of AA is possible at the ERGO/GCE.

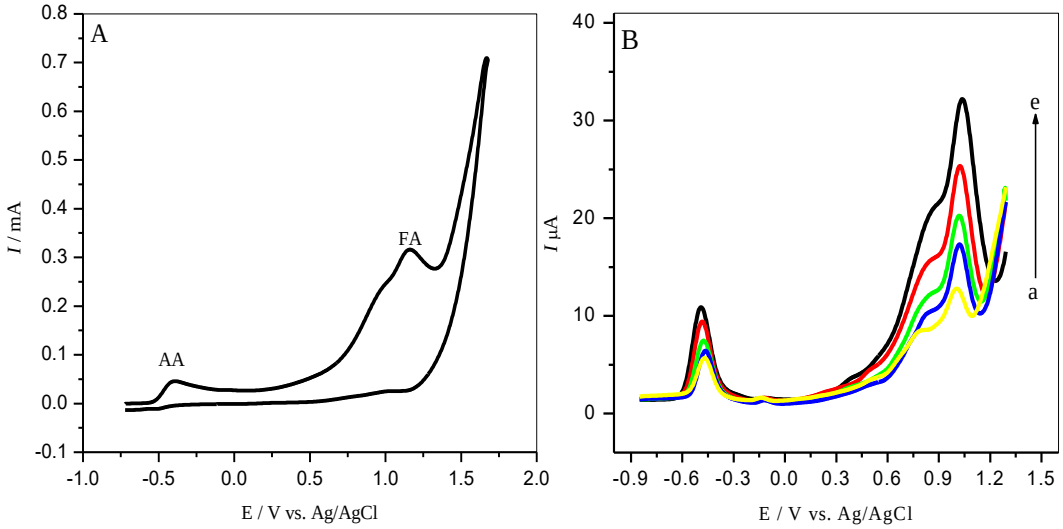



**Figure 8.** (A) CV of the homogeneous solution of 4.55 µM FA and 4.55 mM AA in 0.1 M of KCl solution (pH 14) at scan rate 100 mV/s on the ERGO/GCE. (B) DPVs obtained for the simultaneous concentration increment of FA and AA in 0.1 M of KCl solution (pH 14) at the ERGO/GCE. FA concentrations a→e: 1.52, 1.82, 2.27, 3.03 and 4.55 µM, while AA concentrations a→e: 1.52, 1.82, 2.27, 3.03 and 4.55 mM; scan rate 20 mV/s, Pulse high 25 mV; Pulse width: 50 ms

In addition, Figure 8(B) shows the DPVs were obtained from the different concentrations of FA and AA at the ERGO/GCE. As can be seen, the peaks current increased linearly with the simultaneous concentration increment of FA and AA, indicating the sensing ability of the modified electrode toward the determination of FA in the presence of AA.

### 3.9. Interference study

The effect of some foreign species on the determination of 4.55 µM FA in 0.1 M of KCl solution (pH 14) was also investigated using LSV at ERGO/GCE. It was performed by the addition of the interfering substance to the FA solution under the optimized conditions and the results are shown in Table 2. Thus, interference under the same condition showed no effect on the current response. Therefore, the ERGO/GCE exhibits good selectivity for the determination of FA.

**Table 2.** Interference of some foreign substances for 9.1 µM of FA at ERGO/GCE

| Interferents | Folds |
|---|---|
| $Na^+$, $K^+$, $Cu^{2+}$, $Zn^{2+}$, $NO_3^-$, $SO_4^{2-}$ and $Cl^-$ | 2500 |
| Glucose, sucrose and starch | 2000 |
| Alanine and cysteine | 1000 |
| Citric acid, tartaric acid and maleic acid | 1000 |
| P-Nitrophenol | 500 |

### 3.10. Analysis of real samples

With negligible background current, the DPV was applied for the simultaneous determination of the FA content in commercial tablets because of its high sensitivity and selectivity [73].

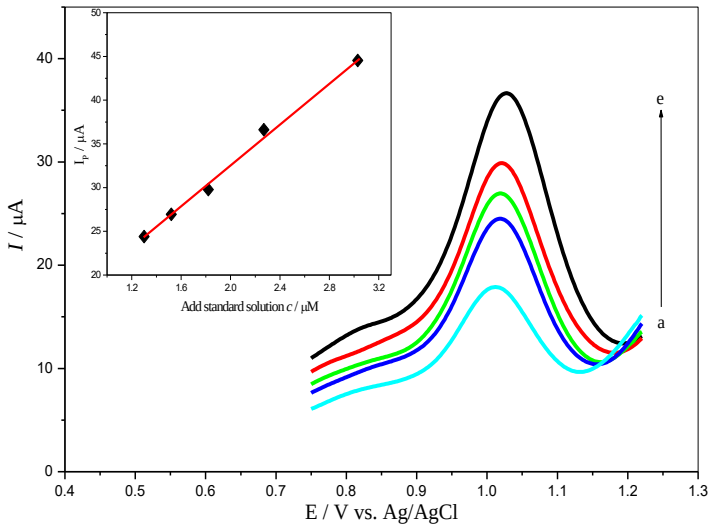



**Figure 9.** DPVs of recovery studies of FA in 0.1 M of KCl solution (pH 14) at ERGO/GCE; concentration of FA standard solution added: a→e: 1.3, 1.52, 1.82, 2.27 and 3.03 µM

Last but not the least, the applicability of the fabricated electrode was tested in spiked human urine samples as a biological fluid. Diluted urine samples were spiked with various known concentrations of FA (Figure 9) and the recovery of the analyte was determined as shown in Table 3. As can be seen, the recoveries for the determination of FA added to urine samples at the ERGO/GCE are in the range of 95.3% to 108.6%, declaring that this novel electrode is effective and reliable.

**Table 3.** Determination of FA in commercial tablets samples using ERGO/GCE (n = 3)

| Sample | FA Added (μM) | Founded (μM) | Accuracy (RE) | Recovery (%) |
|---|---|---|---|---|
| Tablet | 0 | 1.02 | – | – |
| | 0.5 | 1.64 | ±0.09 | 109.3 |
| | 1.5 | 2.65 | ±0.06 | 106.0 |
| | 2.0 | 3.21 | ±0.07 | 107.0 |
| Urine | 0 | – | – | – |
| | 1.5 | 1.63 | ±0.08 | 108.6 |
| | 2.5 | 2.43 | ±0.03 | 97.2 |
| | 3.0 | 2.86 | ±0.04 | 95.3 |

## 4. CONCLUSION

In this study, the ERGO/GCE was fabricated by a simple and sensitive electrochemical reduction and exhibits high selectivity and electro-catalytic activity toward FA in the presence of AA. Several kinetic parameters including charge transfer coefficient (α), diffusion coefficient (D), limit of detection (LOD) and limit of quantification (LOQ) were estimated. Finally, the modified electrode could fruitfully pertain for the detection of FA in real samples because of their unique properties such as high sensitivity, stability and reproducibility.

## REFERENCES

[1] G. F. M. Ball, Vitamins-Their Role in the Human Body. John Wiley & Sons, UK (2008).
[2] M. Dietrich1, C. J. P. Brown, and G. Block, J. Am. Coll. Nutr. 24 (2005) 266.
[3] B.T. Zhang, L. Zhao, and J.M. Lin, Talanta 74 (2008) 1154.
[4] B. Unnikrishnan, Y.L. Yang, and S.M. Chen, Int. J. Electrochem. Sci. 6 (2011) 3224.
[5] H. K. Mitchell, E. E. Snell, and R. J. Williams, J. Amer. Chem. Soc. 63 (1988) 1941.

[6] World Health Organization, Food and Agricultural Organization of the United Nations: Vitamin and mineral requirements in human nutrition, 2nd ed. Geneva (2004) 289.
[7] R. Shashanka, D. Chaira, and B. K. Swamy, Int. J. Electrochem. Sci. 10 (2015) 5586.
[8] D. Banan, W. T. Tan, Y. Sulaiman, M. F. Yusri, M. Zidan, and S. Ab Ghani, Int. J. Electrochem. Sci. 8 (2013) 12519.
[9] B. N. Olana, S. A. Kitte, and T. R. Soreta, J. Serb. Chem. Soc. 80 (2015) 1161.
[10] L. Zhang, Z. Wang, Y. Xia, G. Kai, W. Chen, and K. Tang, Crit. Rev. Biotechnol. 27 (2007) 173.
[11] O. Arrigoni, and M. C. De Tullio, Biochim. Biophys. Acta 1569 (2002) 1.
[12] K. S. Woo, P. Chook, Y. I. Lolin, J. E. Sanderson, C. Metreweli, and D. S. Celermajer, J. Am. Coll. Cardiol. 34 (1999) 2002.
[13] E. Reynolds, Vitamin B12, folic acid, and the nervous system. Lancet Neurol. 5 (2006) 949.
[14] P. Kalimuthu, and S. A. John, Biosens. Bioelectron. 24 (2009) 3575.
[15] A. Tyagi, and A. Penzkofer, Chem. Phys. 367 (2010) 83.
[16] H. S. Strandler, Determination of Folate for Food Composition Data, Development and Improvement of Analytical Methods, Dissertation, Swedish University (2012).
[17] E. Gujska, and A. Kuncewicz, Eur. Food Res. Technol. 221(2005) 208.
[18] F. J. Al-Shammary, K. A. Al-Rashood, N. A. Mian, and M.S. Mian, Anal. Profiles Drug Sub. 19 (1990) 221.
[19] A. Vora, A. Riga, D. Dollimore, and K. S. Alexander, Thermochim. Acta 392 (2002) 209.
[20] M. R. Shishehbore, A. Sheibani, and A. Haghdost, Spectrochim. Acta A 81 (2011) 304.
[21] D. C. Apodaca, R. B. Pernites, R. R. Ponnapati, F. R. Del Mundo, and R. C. Advincula, ACS Appl. Mater. Inter. 3 (2011)191.
[22] B.T. Zhang, L. Zhao, and J. M. Lin, Talanta 74 (2008) 1154.
[23] D. Hoegger, P. Morier, C. Vollet, D. Heini, F. Reymond, and J. S. Rossier, Anal. Bioanal. Chem. 387 (2007) 267.
[24] S. Zhao, H. Yuan, C. Xie, and D. Xiao, J. Chromatogr. A 1107 (2006) 290.
[25] B. C. Nelson, K. E. Sharpless, and L. C. Sander, J. Chromatogr. A 1135 (2006) 203.
[26] A. Rodríguez-Bernaldo de Quirós, C. Castro de Ron, J. López-Hernández, and M. A. LageYusty, J. Chromatogr. A 1032 (2004) 135.
[27] M. Jung, B. Kim, D. W. Boo, and H. Y. So, Bull. Korean Chem. Soc. 28 (2007) 745.
[28] M. S. Aurora-Prado, C. A. Silva, M. F. Tavares, and K. D. Altria, J. Chromatogr. A 1051 (2004) 291.
[29] U. B. Trivedia, D. Lakshminarayana, I. L. Kotharib, P. B. Patela and C. J. Panch, Sens. Actuators B, 136 (2009) 45.

[30] H. Yaghoubian, H. Karimi-Maleh, M. A. Khalilzadeh, and F. Karimi, J. Serb. Chem. Soc. 74 (2009) 1443.
[31] R. E. Sabzi, A. Hasanzadeh, K. Ghasemlu, and P. Heravi, J. Serb. Chem. Soc. 72 (2007) 993.
[32] H. Yang, B. Lu, B. Qi, and L. Guo, J. Electroanal. Chem. 660 (2011) 2.
[33] K. H. Xue, F. F. Tao, and W. Xu, J. Electroanal. Chem. 578 (2005) 323.
[34] P. Prabhua, R. S. Babua, and S. S. Narayanan, Colloids Surf. B 87 (2011) 103.
[35] M. Kumar, and K. B. E. Swamy, J. Chem. Eng. Res. 2 (2014) 121.
[36] D. Manoj, D. Ranjith Kumar, and J. Santhanalakshmi, Appl Nanosci 2 (2012) 223.
[37] B. Devadas, M. Rajkumar, S. M. Chen, and R. Saraswathi, Int. J. Electrochem. Sci. 7 (2012) 3339.
[38] Q. He, J. Liu, X. Liu, Y. Xia, G. Li, P. Deng, and D. Chen, Molecules 23 (2018) 2130.
[39] H. Wang, Q. Hao, X. Yang, L. Lu, and X. Wang, Electrochem. Commun. 11 (2009) 1158.
[40] M. Sivakumar, M. Sakthivel, S. M. Chen, K. Pandi, T. W. Chen, and M. C. Yu, Int. J. Electrochem. Sci. 12 (2017) 4835.
[41] Y. Yang, M. Kang, S. Fang, M. Wang, L. He, J. Zhao, H. Zhang and Z. Zhang, Sens. Actuators B Chem. 214 (2015) 69.
[42] M. A. Kamyabi, O. Narimani, and H. H. Monfared, J. Electroanal. Chem. 644 (2010) 67.
[43] A. Salimi, A. Noorbakhsh, and S. Soltanian, Electroanalysis 18 (2006) 703.
[44] S. Cheemalapati, S. Palanisamy, and S. M. Chen, Int. J. Electrochem. Sci. 8 (2013) 3953.
[45] L. Chen, Y. Tang, K. Wang, C. Liu, S. Luo, Electrochem. Commun. 13 (2011) 133.
[46] D. Zhang, X. Ouyang, W. Ma, L. Li, and Y. Zhang, Electroanalysis 28 (2016) 312.
[47] A. O. Idris, N. Mabuba, D. Nkosi, N. Maxakato, and O. A. Arotiba, Int. J. Environ. Anal. Chem. 97 (2017) 534.
[48] V. Mani, B. Devadas, and S. M. Chen, Biosens. Bioelectron. 41 (2013) 309.
[49] S. Palanisamy, A.T. Ezhil Vilian, and S.M. Chen, Int. J. Electrochem. Sci. 7 (2012) 2153.
[50] D. C. Marcano, D. V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, L. B. Alemany, W. Lu, and J. M. Tour, ACS Nano 4 (2010) 4806.
[51] L. Chen, Y. Tang, K. Wang, C. Liu, and S. Luo, Electrochem. Commun. 13 (2011) 133.
[52] B. B. Prasad, M. P. TIwari, R. Madhuri, and P. S. Sharma, Anal. Chim. Acta 662 (2010) 14.
[53] M. Arvand, and M. Dehsaraei, Mater. Sci. Eng. C 33 (2013) 3474.
[54] T. Maiyalagan, J. Sundaramurthy, P.S. Kumar, P. Kannan, M. Opallo, and S. Ramakrishna, Analyst 138 (2013) 1779.

[55] J. Wang, S. Yang, D. Guo, P. Yu, D. Li, J. Ye, and L. Mao, Electrochem. Commun. 11 (2009) 1892.
[56] Z. Wu, X. Li, C. Hou, and Y. Qian, J. Chem. Eng. Data 55 (2010) 3958.
[57] U. Chandra, B. E. K. Swamy, O. Gilbert, and B. S. Sherigara, Electrochim. Acta 55 (2010) 7166.
[58] R. N. Adam, Electrochemistry at Solid Electrodes. Marcel Dekker, New York 73 (1996) 1098.
[59] P. Kalimuthu, and S. A. John, Biosens. Bioelectron. 24 (2009) 3575.
[60] B. Unnikrishnan, Y. L. Yang, and S.M. Chen, Int. J. Electrochem. Sci. 6 (2011) 3224.
[61] S. Chitravathi, B.E.K. Swamy, G. P. Mamatha, and B.S. Sherigara, Chem. Sens. 3 (2013) 5.
[62] K. R. Mahanthesha, B.E.K. Swamy, U. Chandra, Sathish Reddy, and K. V. Pai, Chem. Sens. 4 (2014) 10.
[63] M. Mazloum-Ardakani, H. Beitollahi, M. K. Amini, F. Mirkhalaf, and M. Abdollahi-Alibeik, Sens. Actuators B 151 (2010) 243.
[64] P. A. M. Farias, M. de Castro Rezende, and J. C. Moreira, IOSR J. Pharm. 2 (2012) 302.
[65] J. B. Raoof, N. Teymoori, M. A. Khalilzadeh, and R. Ojani, Mater. Sci. Eng. C 47 (2015) 77.
[66] L. Mirmoghtadaie, A. A. Ensafi, M. Kadivar, M. Shahedi, and M. R. Ganjali, Int. J. Electrochem. Sci. 8 (2013) 3755.
[67] S. Wei, F. Zhao, Z. Xu, and B. Zeng, Microchim. Acta 152 (2006) 285.
[68] M. Mazloum-Ardakani, H. Beitollahi, M. A. Sheikh-Mohseni, H. Naeimi, and N. Taghavinia, Appl. Catal. A 378 (2010) 195.
[69] M. Mazloum-Ardakani, M. A. Sheikh-Mohseni, M. Abdollahi-Alibeik, and A. Benvidi, Sens. Actuator B 380 (2012) 171.
[70] A. A. Ensafi, and H. Karimi-Maleh, J. Electroanal. Chem. 640 (2010) 75.
[71] J. P. Winiarski, R. Rampanelli, J. C. Bassani, D. Z. Mezalira, and C. L. Jost, J. Food Compos. Anal. 92 (2020) 103511.
[72] A. J. Bard, L. R., Faulkner, Electrochemical Methods, Fundamentals and Applications, 2nd ed., Wiley, New York (2001).
[73] S. B. Tanuja, B. E. K. Swamy, and K. V. Pai, J. Electroanal. Chem. 798 (2017) 17.