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# Electrochemical determination of folic acid: A short review

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ABSTRACT

Folic acid (FA) is an electroactive compound of biological origin. It helps our body to produce and maintain healthy cells. It can significantly reduce the occurrence of neural tube defects and also prevents change in DNA structure. FA deficiency can lead to various health risks. Therefore, a sensitive, specific, and reproducible way of FA detection is essential. A number of analytical methods are in practice for the quantification of FA. However, electroanalytical methods are attracting much attention because of their advantage over conventional methods, as they are fast, simple, sensitive, and cost effective. Moreover, modification of electrodes offers control over size and morphology which allows miniaturization for applicability in portable electrochemical devices.



## 1. Introduction

Folic acid (FA) also called as pteroylglutamic acid (PteGlu), is a water soluble vitamin of B complex family. It is most commonly referred to as Vitamin $B_9$. The IUPAC name of folic acid is (2S)-2-[[4-[(2-amino-4-oxo-1H-pteridin-6-yl)methylamino]benzoyl]amino] pentanedioic acid [1]. Chemical structure of FA is shown in Fig. 1. Naturally occurring form of FA is called folate. FA is important for numerous human metabolic pathways. It is a key factor in the synthesis of Nucleic acid. FA along with vitamin $B_{12}$ promotes growth [2] and healthy red blood cells [3]. It is also reported to accelerate the cell division. It is an essential vitamin for healthy growth and development of foetus [4].

FA cannot be stored in the human body. Therefore, it's deficiency is one of the most common vitamin deficiencies. Regular intake of FA is essential for healthy living. Liver, dried beans, green leafy vegetables are all good sources of FA. Because of their low rate of consumption, FA deficiency can occur which may lead to a number of health problems in humans like megaloblastic anaemia and neural tube defects in developing foetuses [5], cancer and heart diseases [6]. To avoid these risk factors, the use of FA fortified dietary supplements or fortified food has been increasing rapidly [7]. However, it is also concerned that FA in excess can mask the vitamin $B_{12}$ deficiency symptoms which may lead to other health risks. These concerns have led the researchers to develop such analytical methods which can accurately measure the amount of FA in natural sources, fortified foods, and multivitamin preparations.

A number of analytical methods have been employed for the determination of FA in natural sources, folic acid fortified foods, and in pharmaceutical samples, viz. Thermogravimetry [8], Spectrophotometry [9], High performance Liquid Chromatography (HPLC) [10–12], HPLC coupled with Mass Spectroscopy [13], Colorimetric [14], Flow Injection Chemiluminescence [15,16], Fluorimetric [17], Spectrophotometry [18], and Electrophoresis [19] etc.

However, in most of the cases reported above, prior steps are required before the actual determination of FA. Nagaraja et al. [9] reported the use of iminodibenzyl, sodium molybdate–pyrocatechol and 3-aminophenol as a coupling reagent for the detection of FA. Zhang et al. [20] reported the use of complex peroxomonosulfate-cobalt(II) system for FA detection. In some cases, prior to folic acid detection, extraction was carried out from mixtures using other compounds [21]. Traditional methods of FA detection include microbiological assays which consist of several time consuming steps, requiring upto 48 h for developing assays [22]. Usually these methods use harsh and non-ideal internal conditions [8,23]. Furthermore, these methods are more expensive, complicated, and less sensitive.

However, electroanalytical approach is found to be excellent alternative for FA assaying. In recent years, electroanalytical methods are gaining importance because of their simple and low cost operations. These methods require relatively short analysis time. Among other analytical methods, electroanalytical ones are

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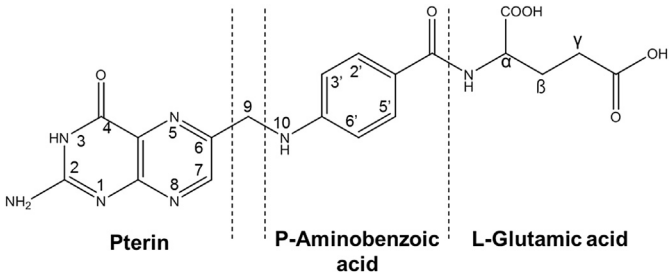


**Fig. 1.** Chemical structure of Folic acid.

highly sensitive and more accurate, making them ideal for applications in the field of pharmacy [24], food, and agriculture [25]. Well-defined electrochemical behaviour of biomolecules and drugs leads to the development of electro-chemical sensors and biosensors.

## 2. Structural aspects of folic acid

From the structural point of view, FA molecule comprises of bicyclic pterin moiety connected by a methylene bridge $C_{(9)}$—$N_{(10)}$ bond to p-aminobenzoic acid, which in turn is coupled to a molecule of L-glutamic acid through an α-peptide bond. A UV—Visible absorption spectra of FA shows two strong absorption bands in the spectral region 200—500 nm. The pterin moiety absorbs in the spectral region 280—320 nm and aminobenzoic acid absorbs in the spectral region 320—400 nm [26].

The term folic acid is commonly referred to pteroylmonoglutamic acid (PteGlu). In most of the natural food sources, FA is present in its reduced forms; 7,8- dihydropteroylmonoglutamic acid (DHF) or 5,6,7,8- tetrahydropteroylmonoglutamic acid (THF) [27]. Structure is shown in Fig. 2.

FA molecule exhibits a number of dissociable groups. The p*K* values of these dissociable groups have been estimated using different analytical techniques in a pH range <1 to 10. Due to poor solubility and self-aggregation of FA molecule in wide pH range, it is difficult to determine the dissociation constant of every dissociable group in FA molecule [28]. p*K* values reported in the literature are listed in Table 1.

## 3. Electrochemistry of folic acid

FA is an electrochemically active compound. Electro-reduction and electro-oxidation of FA has been studied extensively. It was first reported by Hrdý [30] and Asahi [31]. However, the analysis of reaction mechanism and the products obtained, was made more clearly by Kretzchmar and Jaenicke [32]. They reported the electrochemical reduction and oxidation of FA molecule in a pH range 1—12.

Electrochemical behaviour of FA has been studied extensively using cyclic voltammetry. It was observed that electrochemical reduction and oxidation of FA is strongly dependent upon pH. Three polarographic ($I_c$, $II_c$, $III_c$) waves were observed for the electrochemical reduction of FA at pH range 5—7 [32,33]. Half peak potentials for the waves ($I_c$, $II_c$, $III_c$) vary with respect to pH values. A typical cyclic voltammogram of FA at pH 5.2 is shown in Fig. 3.

A proposed reaction mechanism for the polarographic reduction of FA in acidic medium is shown in Fig. 4. It has been proposed that wave $I_c$ appears due to reversible reduction ($2e^-$, $2H^+$) of FA to 5, 8 dihydrofolic acid which undergoes tautomerization to give 7, 8 dihydrofolic acid. Wave $II_c$ appears due to reductive cleavage ($2e^-$, $2H^+$) of 7, 8 dihydro derivative of FA between the $C_{(9)}$ and $N_{(10)}$ positions. Wave $III_c$ is due to irreversible reduction of 7,8 dihydro-6-methyl pterin (IV) to 5, 6, 7, 8 tetrahydro-6-methyl pterin (V) [32]. Gurira et al. [34] reported for the first time the presence of three reduction waves ($I_c$, $II_c$, $III_c$) along with two prewaves associated with $I_c$ and $II_c$. They attributed the presence of these prewaves to the adsorption of FA at the electrode surface.

In basic conditions, presence of two waves ($I_c$ and $III_c$) was reported. A different mechanism was proposed as shown in Fig. 5. Firstly, FA showed reversible reduction ($2e^-$, $2H^+$) to give 5, 8 dihydrofolic acid which undergoes tautomerization. Experimental evidence showed the presence of 7,8 dihydro derivative (III) and 6, 7 dihydro derivative (IV). Second wave ($III_c$) appears due to reversible reduction ($2e^-$, $2H^+$) of later specie to 5, 6, 7, 8 tetrahydro derivative [32]. Here, it is important to note that tautomerization of 5, 8 dihydrofolic acid is a hydrogen catalysed process. Therefore, at higher pH values, further reduction of folic acid is prohibited [35,36]. A typical cyclic voltammogram of FA at pH 8.5 is shown in Fig. 6.

From literature, it is evident that the reaction mechanism for the first wave which corresponds to reversible reduction of folic acid to 5, 8 dihydrofolic acid does not change over the entire pH range [37]. Therefore, the first wave is of prime interest to study the electroanalytical behaviour of FA.

Electrochemical oxidation of FA has also been reported. A single wave was observed for anodic oxidation ($1e^-$, $1H^+$) of 5, 6, 7, 8-tetrahydrofolic acid (I) between pH 1 and 12. A typical cyclic voltammogram of FA is shown in Fig. 7.

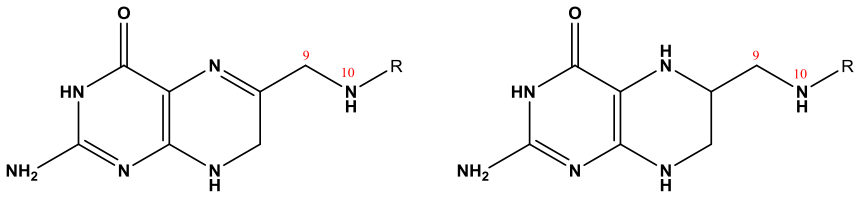


**Fig. 2.** Chemical structure of (right) 5,6,7,8-tetrahydropteroylmonoglutamic acid (THF) and (left) 7,8- dihydropteroylmonoglutamic acid (DHF).

**Table 1**
Estimated pK values for FA.

| p$K_1$ | p$K_2$ | p$K_3$ | p$K_4$ | | | Ref |
|---|---|---|---|---|---|---|
| $N_{(1)}H$ | α-COOH | γ-COOH | $N_{(3)}H$/CO | $N_{(10)}$ | $N_{(5)}$ | |
| 2.35 | | | 8.38 | 0.2 | <−1.5 | Poe 1977 [29] |
| 2.38 ± 0.04 | 3.46 ± 0.03 | 4.98 ± 0.03 | 8.08 ± 0.003 | | | Zoltan 2006 [28] |

Numbering system is the same as shown in Fig. 1.

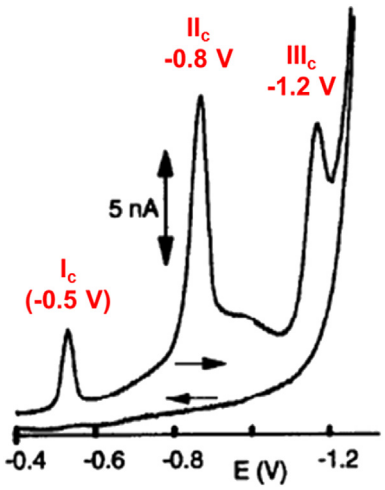


**Fig. 3.** Cyclic voltammogram of 100 nM folic acid recorded at hanging mercury drop electrode between the limits −0.4 and −1.3 V vs Ag/AgCl at pH 5.2 (pH was maintained using sodium acetate buffer). Figure reused with permission [36].

A reaction mechanism for anodic oxidation has also been reported and shown in Fig. 8. It showed that tetrahydrofolic acid oxidises initially to give a radical (II), which could further dimerize to III or oxidize to 6, 7 −dihydrofolic acid (IV) [32].

Electrochemical analysis of FA is most commonly based on oxidative or reductive cleavage of $C_{(9)}$—$N_{(10)}$ bond in the molecule. Kwee [35] reported the electroactivity of $C_{(9)}$—$N_{(10)}$ bond in FA. Studies were carried out using pulse polarography and cyclic voltammetry. Under acidic conditions, in dihydrofolic acid $C_{(9)}$—$N_{(10)}$ bond reduced electrochemically to give dihydro pterin derivative and p-aminobenzoylglutamic acid. However, oxidative cleavage of the $C_{(9)}$—$N_{(10)}$ bond was observed under aerobic conditions to give pterin derivative.

## 4. Adsorption behaviour of folic acid

FA molecule is reported to strongly adsorb on the surface of electrodes [33]. Jacobsen and Bjørnsem [38], during voltammetric study of folic acid, observed that the molecule is strongly adsorbed on the surface of electrode. Alvarez et al. [33] reported a change in the shape of cyclic voltammetry curves of FA with respect to its concentration. A change in the morphology of reduction waves at different concentration revealed the fact that not only the reactants but also the reduction products can strongly adsorb on to the surface of electrode. Various studies are reported in the literature to investigate the absorptive behaviour of folic acid molecule. This adsorption is commonly attributed to the phenyl ring of p-aminobenzoic acid moiety [39]. Gall and Berg [36] used cyclic voltammetry to study the electrochemical activity of FA and related

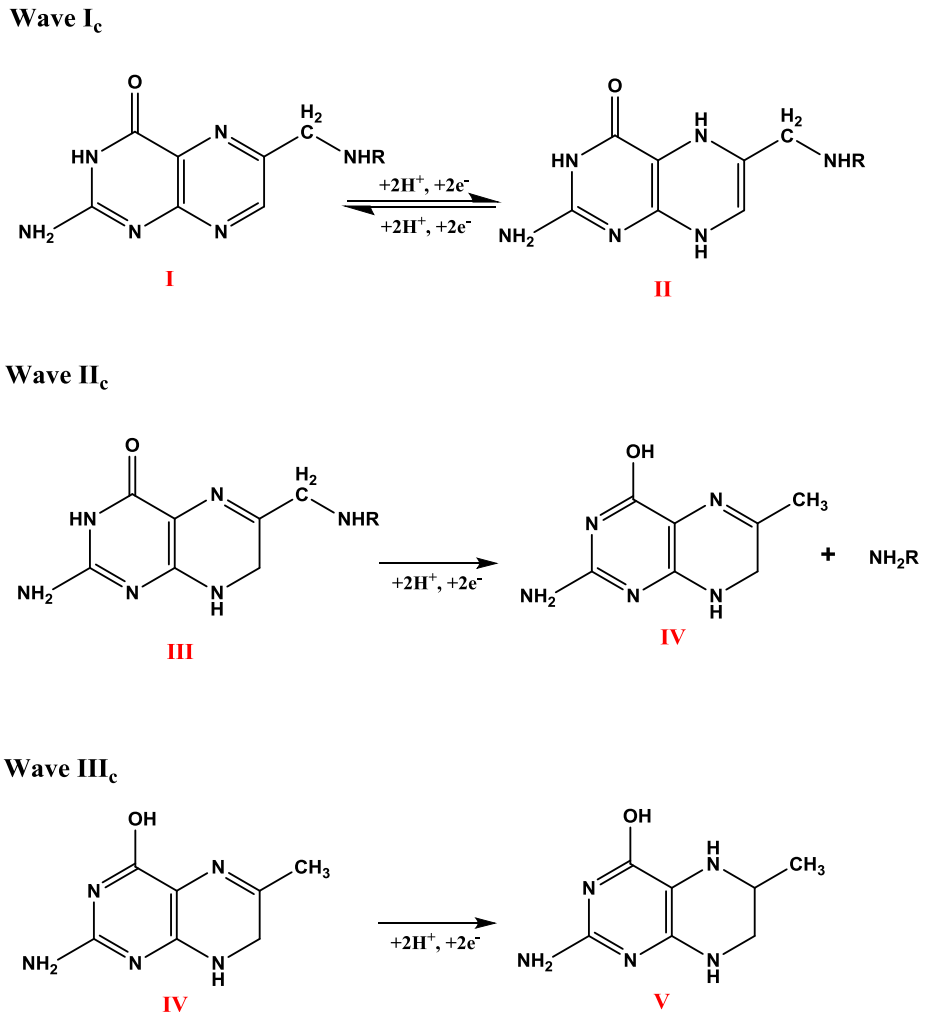


**Fig. 4.** Reaction scheme for the polarographic reduction of folic acid at pH 5–7 (Regenerated from G. Dryhurst [37] and Gurira et al. [34]).

compounds. They proposed that within the folic acid molecule, adsorption site is located between $C_{(9)}$—$N_{(10)}$ bond. They attributed the adsorption phenomenon to the phenyl ring of benzene due to available p-orbital electrons. Maali et al. [40] reported the presence of metastable absorbed layer of FA molecules at critical absorption density. Beyond this point, molecular rearrangement happens to accommodate higher FA concentration. An adsorption density of $3.1 \times 10^{-11}$ mol/cm$^2$ is reported for 0.5 μM folic acid with available surface area of 570 Å which supports the interaction of absorbed part of the folic acid molecule with the non-absorbed components [36]. Guo et al. [41] reported strong adsorption of FA at modified electrode. The adsorbed FA could not be removed even after extensive cycling of modified electrode in 0.01 M $H_2SO_4$.

Electroanalytical methods commonly employed for FA detection and to investigate its redox behaviour include cyclic voltammetry [24], differential pulse voltammetry [25], chronoamperometry [42], chronocoulometry [43], linear sweep voltammetry [44], square wave voltammetry [45] and adsorptive stripping voltammetry [46]. Because of the surface active properties of FA, adsorptive stripping voltammetry is considered as a most interesting technique for the quantification of FA at trace level. Luo [47] in 1986 claimed to use this technique for the first time for FA determination in pharmaceutical tablets and urine samples.

## 5. Electropolymerization of folic acid at the electrode surface

He and Zheng [48] reported for the first time the electrochemical immobilization of FA at carbon paste substrate. Electrochemical polymerization of FA was carried out by cyclic voltammetry technique. It was carried out by electropolymerization of FA molecule via chemically stable covalent linkage between nitrogen atom of the amine group from glutamic acid moiety and carbon paste electrode surface [49]. Electrodeposited poly (folic acid) film was reported to offer significant advantage including facile fabrication process, enhanced electrocatalytic ability, and improved physical stability toward electrochemical detection of biomolecule [48].

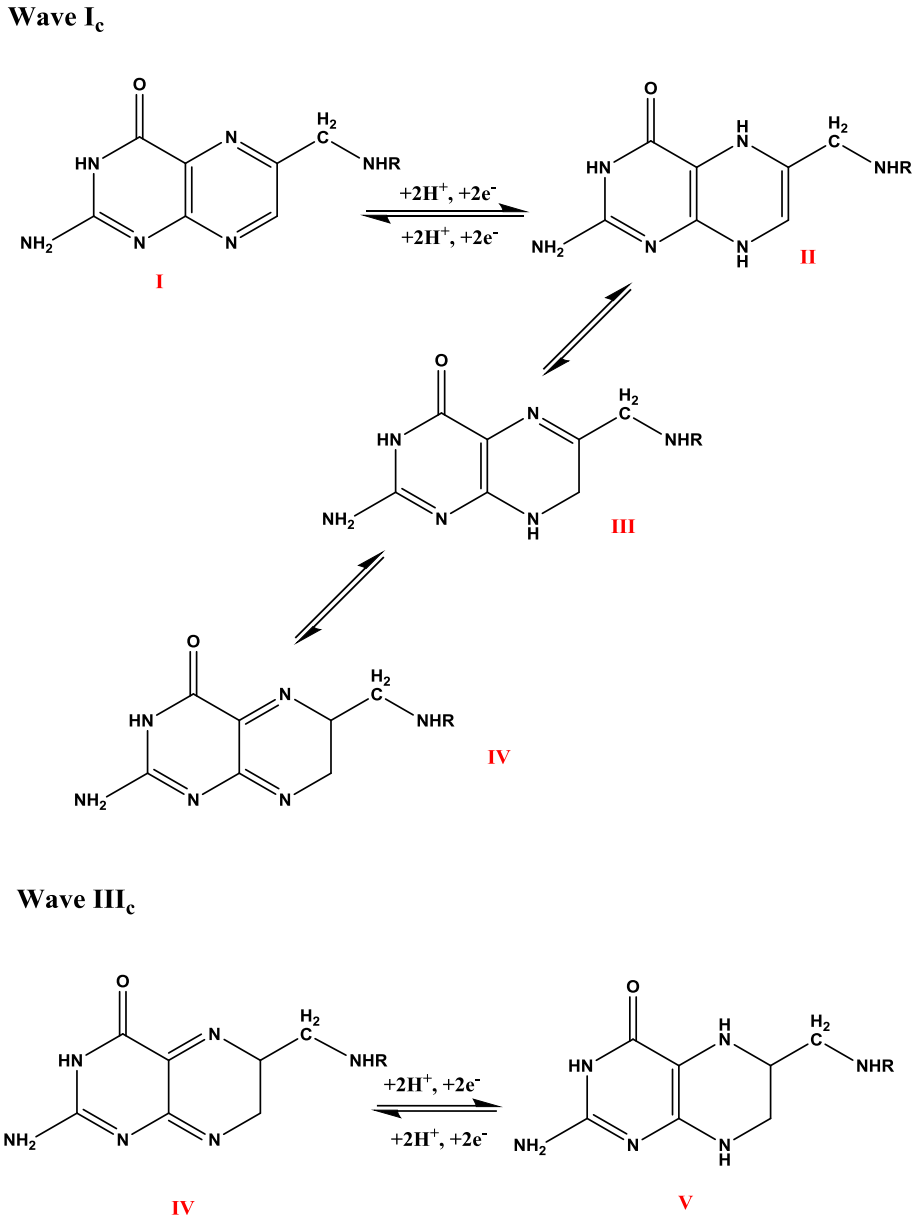


**Fig. 5.** Reaction scheme for the polarographic reduction of folic acid at pH 9 (Regenerated from G. Dryhurst [37] and Gurira et al. [34]).

## 6. Factors affecting the adsorptive stripping behaviour of folic acid

Different factors affecting the adsorptive stripping behaviour of folic acid have been demonstrated in the literature. Electrochemical response of FA is directly affected by a change in pH of electrolyte medium, preconcentration step, presence of surface active impurities in the electrolyte medium, and scanning modes. These factors are discussed in detail below.

### 6.1. Effect of buffer pH

Adsorptive stripping behaviour of FA is strongly affected by a change in pH of an electrolyte medium. In order to investigate the effect of pH, electrochemical behaviour of FA has been studied in the presence of buffer solutions of different compositions like phosphate buffers, acetate buffers, borate buffers, and Britton-Robinson buffers etc. as discussed below. Effect of pH on cathodic peak potentials is summarized in Table 2.

Peak potential were reported to vary linearly with the pH range 2–10. In same electrolytic medium, with the increase in pH, peak potential shifts toward more negative potential values and vice a versa [50]. Most pronounced signals for the reduction peaks were obtained at pH 5. However, it was observed that buffer solution composition had no significant influence on stripping current [33]. In the studies reported above, an enhanced electrochemical response was observed in the presence of supportive electrolytes. However, an interesting finding was reported by Guo et al. that voltammetric response of FA recorded at phosphomolybdic-polypyrrole film modified electrode was actually weakened in the presence of buffers as supporting electrolyte. In this study, Britton-Robinson buffers of range $2 < pH < 8$ and $H_2SO_4 + Na_2SO_4$ of range $0.7 < pH < 2.6$ were used [41].

### 6.2. Preconcentration step

Due to the adsorption behaviour of FA at the electrode surface, preconcentration time and potential plays critical role in the absorptive studies of FA. During preconcentration step electrodes are held in the FA solution for specific period of time at a specific potential value (However, in some cases an open circuit was the choice). A detailed discussion is given below.

Luo [47] in 1986 demonstrated the importance of preconcentration time during voltammetric studies. It was pointed out that the cathodic peaks currents were increased when static mercury drop electrode was held at −0.3 V for extended time period before the actual scanning. The detection limit was estimated to be $1.0 \times 10^{-10}$ M for 5 min preconcentration time. A detection limit of $1.0 \times 10^{-11}$ M was reported by Alvarez et al. [33] using 10 min of preconcentration time. Preconcentration times of 8 min and 2 min, were also reported for the quantification of FA at the $10^{-8}$ and $10^{-5}$ M levels respectively [51]. 7 min of preconcentration time is reported for 1 μM FA solution using multi-walled carbon nanotube-modified gold electrode [43]. A detection limit of $2 \times 10^{-10}$ M of folic acid was obtained using preconcentration time of 3 min [52]. Ananthi et al. [53] reported to achieve a detection limit of $9.53 \times 10^{-9}$ M of FA with a preconcentration time of 4 min using Bismuth nanowires-modified electrode. At phosphomolybdic-polypyrrole film modified electrode, slower current observed due to prolonged immersion time [41]. Effect of preconcentration time has also been studied for anodic oxidation of folic acid. Wang et al. [54] reported an increase in anodic current with respect to an increase in the preconcentration time and equilibrium was achieved at 7.5 min of preconcentration time. Concentration of the bulk solution can directly affect the preconcentration time. Villamil et al. [46] reported that growth rate becomes faster as the concentration of the bulk solution increases.

Different studies reported above, show that accumulation time needs to be optimized with respect to the concentration of FA solution and are also dependent upon the type of electrode used for investigation of electrochemical behaviour of FA.

Effect of preconcentration potential on the voltammetric response of FA has also been demonstrated. A range of preconcentration potentials were tested for $5 \times 10^{-8}$ M FA in 0.1 M sulfuric acid, highest cathodic peak currents were obtained at a preconcentration potential of −0.20 V vs Ag/AgCl [47]. In another study, it was reported that cathodic peak currents remain unaffected by changes in preconcentration potential. The system was examined for range +0.1 to −0.6 V vs standard calomel electrode while pH 5 was maintained using Britton-Robinson buffers of constant ionic strength or sodium acetate buffers [33]. Same findings for anodic oxidation of FA were reported by Wang et al. [54]. Electrochemical behaviour remained unaffected when studied within potential range −0.5 to +0.5 V vs standard calomel electrode. Under these circumstances, an open circuit could be the choice of FA molecule accumulation at the electrode surface.

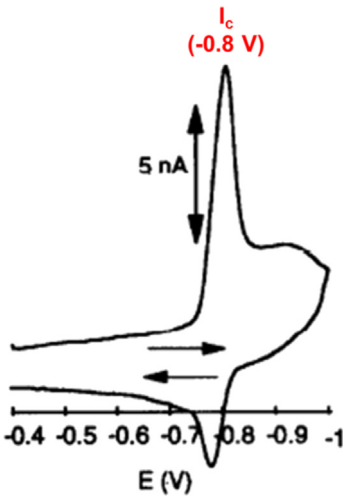


**Fig. 6.** Cyclic voltammogram of 100 nM folic acid recorded at hanging mercury drop electrode between the limits −0.4 and −1.0 V vs Ag/AgCl at pH 8.5 (pH was maintained using Borate buffer). Figure reused with permission [36].

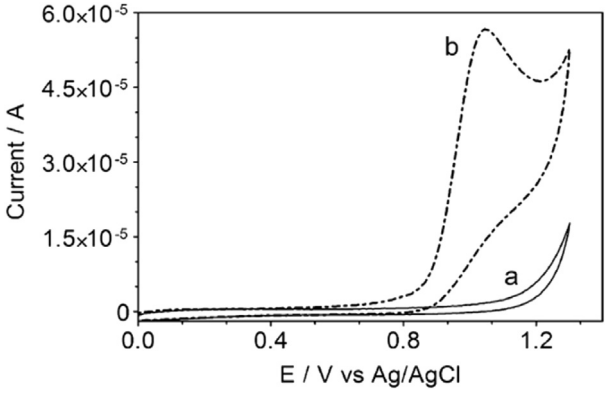


**Fig. 7.** Cyclic voltammogram of 0 (a) and 20 (b) μM folic acid recorded at carbon paste electrode between the limits 0.0 and 1.3 V vs Ag/AgCl in the presence of 0.1 M KCl solution. Figure reused with permission [24].

### 6.3. Effect of scanning modes

Different stripping modes such as direct current stripping, differential pulse, and normal pulse were used. Differential pulse mode gave better signal/noise ratio as compare to normal pulse mode [47]. In another study, linear sweep voltammetry, differential pulse voltammetry, and square wave voltammetry techniques were compared. Highest sensitivity for FA detection at lower reduction potentials was observed using square wave voltammetry technique [53]. At phosphomolybdic-polypyrrole film modified electrode, differential pulse voltammetry offered greater sensitivity toward FA reduction compared to cyclic voltammetry [41].

### 6.4. Influence of surface active impurities

In stripping voltammetry, presence of surface active agents can greatly influence the adsorption phenomenon of FA and can limit the application of this method. Influence of many foreign species was investigated and reported in various studies. Adsorptive behaviour of FA was observed in the presence of sodium lauryl sulphate, cetyltrimethylammonium bromide, and Triton X-100. A decrease in the stripping peak currents was observed in the presence of these surfactants. Triton X-100 was reported to completely suppress the peak signals at a concentration of 2 ppm which was attributed to the full coverage of the electrode surface by the surfactant [33]. However, an increase in reduction peak current of FA was reported in the presence of humic acid upto certain concentration limit [36].

FA molecule exhibits chelating properties. The pterin moiety along with carboxylic group of the glutamic acid is reported to interact with different metal ions ($Cd^{+2}$, $Cu^{+2}$, $Ni^{+2}$, $Co^{+2}$) [55–57]. The complex formation can exert a distinct influence on the electrochemical determination of FA molecule. A number of articles are published on the electrochemical behaviour of FA in the presence of

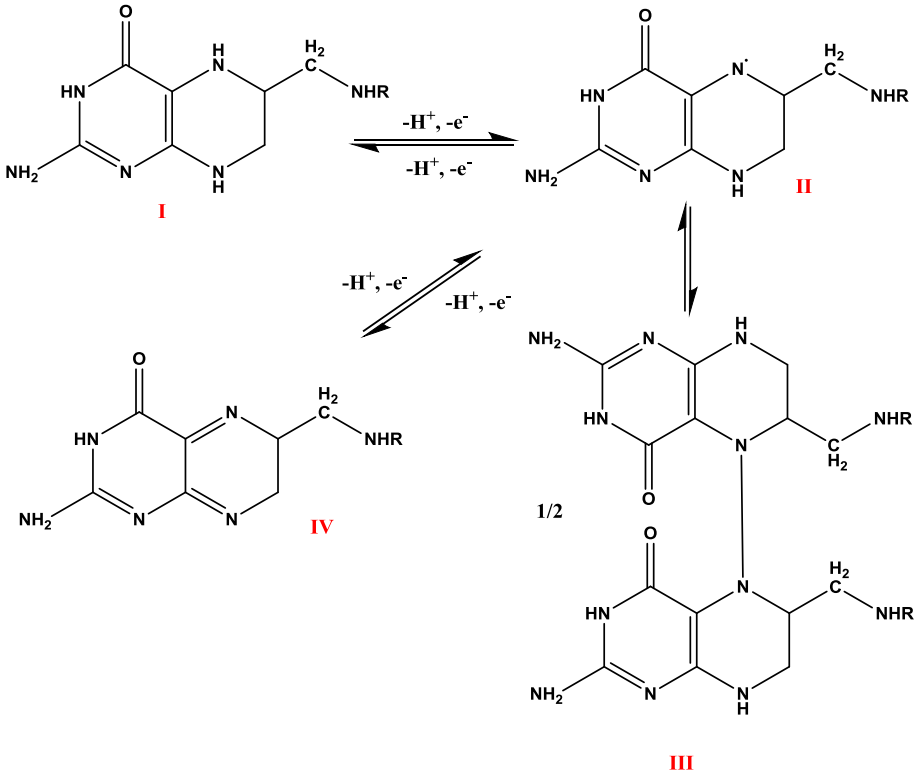


**Fig. 8.** Reaction scheme for the anodic oxidation of folic acid at pH 6.8 (Regenerated from G. Dryhurst [37]).

**Table 2**
Effect of pH on electrochemical reduction of Folic acid.

| Electrolyte medium | pH | FA (μM) | Cathodic peak potentials | | | | Working electrode | Reference electrode | Ref |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1st (V) | 2nd (V) | 3rd (V) | 4th (V) | | | |
| 0.1 M $H_2SO_4$ | <1 | 30 | −0.55 | −0.83 | _ | _ | SMDE[a] | Ag/AgCl | Luo 1986 [47] |
| Britton Robinson buffer | 3.5 | 100 | −0.54[c] | −0.9[c] | −1.21[c] | _ | HMDE[b] | Ag/AgCl | Gurira et al., 1992 [34] |
| $NaClO_4$ | 4.0 | 10 | −0.045 | −0.55 | −0.85 | −1.0 | HMDE | Ag/AgCl | Szczepaniak and Ren 1994 [50] |
| Britton Robinson buffer | 5 | 4.0 | −0.65 | −1.05 | −1.29 | _ | SMDE | Standard Calomel electrode | Alvarez et al., 1987 [33] |
| Britton Robinson buffer | 5 | 100 | −0.65 | −1.10 | −1.23 | _ | HMDE | Ag/AgCl | Gurira et al.,1992 [34] |
| Sodium Acetate buffer | 5.2 | 0.1 | −0.5 | −0.8 | −1.2 | _ | HMDE | Ag/AgCl | Gal and Berg 1993 [36] |
| Britton Robinson buffer | 7.0 | 100 | −0.73 | −1.14 | −1.4 | _ | HMDE | Ag/AgCl | Gurira et al.,1992 [34] |
| Phosphate Buffer | 7.1 | 2.26 | −0.57 | −0.75 | −1.18 | −1.39 | HMDE | Ag/AgCl | Farias et al., 2012 [51] |
| Borate Buffer | 8.5 | 0.1 | −0.85 | _ | _ | _ | HMDE | Ag/AgCl | Gal and Berg 1993 [36] |

[a] HMDE, Hanging Mercury Drop Electrode.
[b] SMDE, Static Mercury Drop Electrode.
[c] Values estimated using Fig. 3 given in ref Gurira et al., 1992 [34].

different metal ions. Interestingly, it was found by Farias [51] that presence of divalent metal cations such as $Cu^{+2}$ and $Fe^{+2}$ do not interfere in the determination of FA up to certain levels of concentration. A study on FA electro-reduction was also reported in the presence of inorganic metal ions ($K^{+1}$, $Na^{+1}$, $Zn^{+2}$). It was observed that reduction peak current for FA did not change in the presence of these ions even at higher concentrations [53]. In another study it is reported that cathodic peak heights for $3.4 \times 10^{-9}$ M solution of FA remained unchanged upon addition of riboflavin, pyridoxine hydrochloride, thiamine hydrochloride, calcium pantothenate, and cyanocobalamin (concentration range of 10–50 times the concentration of FA). However, peak widening was observed in the presence of ascorbic acid [25]. Adsorptive stripping response of FA has also been demonstrated in the presence of other vitamins such as riboflavin which do have adsorptive capability like FA. Well defined adsorptive peaks for FA and riboflavin were observed at −0.55 and −0.29 V vs Ag/AgCl respectively thus making it possible for FA determination in multivitamin preparations [46].

Interference studies were also carried out during anodic oxidation of FA. A number of foreign species were introduced into the FA system ($5 \times 10^{-6}$ M). Anodic peak currents were reported to be unchanged upon addition of high concentration of $Na^{+}$, $K^{+}$, $NO^{-3}$, and $Cl^{-1}$, $SO_4^{-2}$, $Br^{-1}$, $NH_4^{+1}$, $Ca^{+2}$, and $Mg^{+2}$, glycine, valine, lucine, threonine, and alanine [54].

## 7. Anodic behaviour of folic acid molecule

A few studies are reported regarding the anodic oxidation of FA. Araujo et al. [24] reported anodic behaviour of folic acid at graphite paste electrode. A single well defined anodic peak appeared during cyclic voltammetry and differential pulse voltammetry. The detection limit was estimated to be $8.11 \times 10^{-6}$ M. Anodic response of FA was greatly influenced by a change in pH. Oxidation behaviour of FA was studied at nanosized gold and graphene modified electrode in the pH range 4–7. Enhanced peak currents were observed in less acidic environment up to pH 5.5 and deformation of peaks were reported beyond this pH [54]. Linear sweep voltammetry has also been employed to study the electroxidation of FA. Taherkhani et al. [45] reported the presence of a well-defined oxidation wave at pH 9 in a linear sweep voltammogram. Well defined anodic behaviour shows the applicability of this technique for the folic acid determination.

## 8. Modification of working electrode

Due to the importance of FA, development of such devices is highly desirable which are equally applicable for FA quantification in pharmaceutical preparation, biological, and agricultural samples. It is also desirable that the devices should be simple, portable, easy to miniaturize while maintaining high accuracy, sensitivity, and reproducibility. Efforts are being made to design electrochemical sensors for the quantification of biomolecules [58]. Electrode modification is a modern and versatile concept, attracting much attention in the field of electrochemistry. Modified electrodes can exhibit high selectivity and sensitivity toward FA determination. Electrode modification can be achieved chemically using thin film or particles of some conducting material or by decorating the electrode surface with nanostructured particles, carbon nanotubes, nanowires, nanohorns, and nanocomposites. A number of studies are reported in the literature. Some of them are summarized in the Table 3.

Comparison between different results reported in Table 3 shows that modification of electrode can increase the selectivity and sensitivity of electrochemical methods used for the detection of FA in different samples. It is also observed that modification of electrode is mostly carried out at glassy carbon electrodes or graphite powder electrodes. Maiyalagan et al. [62] reported that bare glassy carbon electrode were unable to detect FA in the presence of higher concentration of an interfering specie. However, modification of the electrode with α-$Fe_2O_3$ nanofibers not only resolved the issue but an enhanced voltammetric response was also observed. From comparison, it is also evident that a very low detection ($1.06 \times 10^{-5}$) of FA was reported for DNA modified-pencil graphite electrode [61].

## 9. Summary

Due to the co-existence of FA with other biomolecules/nutrients, its selective and sensitive determination is of major concern. Folic acid detection and identification has been carried out using

**Table 3**
Comparison of analytic parameters for modified electrodes used for folic acid determination.

| Modified electrode | Electroanalytical techniques employed | pH | Linear dynamic range (μM) | Detection limit (nM) | Samples analysed |
|---|---|---|---|---|---|
| Gold nanoparticle and graphene modified carbon ionic liquid electrode [54] | CV[a], DPV[b] | 4.0 −7.0 | 0.01–50 | 2.7 | Pharmaceutical preparation |
| Mn doped $SnO_2$ nanoparticles (NPs) modified glassy carbon electrode [59] | EIS[c], CV and SWV[d] | 6.0 | 1–500 | 38 | pharmaceutical and urine samples |
| Carbon nanohorns supported interwoven titanate nanotubes [60] | LSV[e], CV and Amperometry | 7.0 | 0.0001–50 | 2500 | Folic acid tablets and oats |
| ZnO nanoparticle modified ionic liquid- carbon paste electrode [45] | CV, chronoamperometry, and SWV | 9.0 | 0.05–550 | 10 | Urine, apple juice, pharmaceutical tablets |
| DNA modified-pencil graphite electrode [61] | DPV | 4.8 | 0.1–10.0 | $1.06 \times 10^{-5}$ | Fortified wheat flour, spinach, pharmaceutical tablets |
| Chemically modified Carbon paste electrode modified ZnO/Carbon nanotubes nanocomposite electrode [42] | SWV | 7.0 | 3.0–700 | 1000.0 | Urine, human blood, pharmaceutical tablets |
| Multi-walled carbon nanotube modified Gold electrode [43] | CV, Chronoamperometry, and Chronocoulometry | 2.5 | 0.02–1.0 | 10 | Pharmaceutical tablets |
| Calixarene coated graphite powder [25] | DPVand Chronocoulometry | 4.0 | $8.79 \times 10^{-6}$ $-1.93 \times 10^{-3}$ | $1.24 \times 10^{-3}$ | Serum, asparagus, spinach, oranges, multivitamin |
| Bismuth nanowires modified glassy carbon electrode [53] | CV, SWV, LSV, and DPV | 4.5 | 0.01–0.15 | 9.53 | pharmaceutical tablets |
| α-$Fe_2O_3$ nanofiber modified glassy carbon electrode [62] | CV, DPV, Amperometry | 7.2 | 0.06–60 | 0.112 | Human blood serum |

[a] CV, Cyclic Voltammetry.
[b] DPV, Differential Pulse Voltammetry.
[c] EIS, Electrochemical Impedance Spectroscopy.
[d] SWV, Square Wave Voltammetry.
[e] LSV, Linear Sweep Voltammetry.

conventional methods. In the recent years, there is a need for fast, highly sensitive, more specific, and cost effective method of detection. Electrochemical techniques are much more sensitive and easy to use, and offers low detection limit as compare to conventional methods. Comparison of different electroanalytical methods shows that adsorptive stripping voltammetry is the most common choice for folic acid quantification. In recent years, potentiostatic electro-reduction and electro-oxidation of folic acid on modified electrodes has been studied extensively. It is also evident that modified electrodes offer better electrochemical performance with high sensitivity and selectivity. Furthermore, tailoring the size and morphology of modified electrodes allows the miniaturization of electrochemical devices which may lead to more sensitive and selective determination of FA.

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