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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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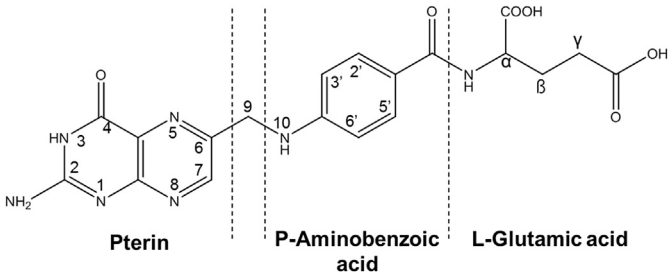

Fig. 1. Chemical structure of Folic acid.

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.

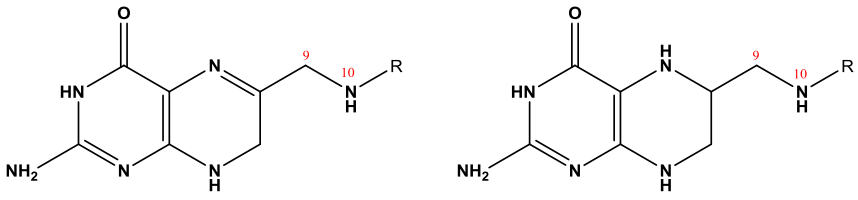

Fig. 2. Chemical structure of (right) 5,6,7,8-tetrahydropteroylmonoglutamic acid (THF) and (left) 7,8- dihydropteroylmonoglutamic acid (DHF).

Table 1
Estimated pK values for FA.

pK_1	pK_2	pK_3	pK_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.