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# Electrochemical behavior of folic acid on mercury meniscus modified silver solid amalgam electrode

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ABSTRACT

Voltammetric behavior of folic acid and folates has been investigated using direct current voltammetry, differential pulse voltammetry and adsorptive stripping differential pulse voltammetry at a mercury meniscus modified silver solid amalgam electrode (m-AgSAE). The optimum conditions have been found for their determination in a 1:9 mixture of methanol and aqueous acetate buffer, with the limit of detection about 0.5 nmol $L^{-1}$. RSD at this concentration level amounted to less than 4%. Practical applicability of the newly developed method was verified by analysis of three vitamin preparations and of two multivitamin juices.

## 1. Introduction

Folic acid (FA) (CAS: 59-30-3, pteroyl-L-glutamatic acid, folacin, Fig. 1) and its derivates, are very important essential compounds, which belong to the group of vitamins B – vitamin $B_9$ (in some cases is denoted as vitamin M). FA and folates (if not specified otherwise, it is not differentiated between "folic acid" and its salts – folates in this manuscript) are enzymatically transformed in the small intestine into their biologically active and very important form tetrahydrofolate ($H_4$-folate) [1]. This reaction is catalyzed by enzyme tetrahydrofolate dehydrogenase (dihydrofolate reductase, EC 1.5.1.3) [1,2]. $H_4$-folate participates in transferring one-carbon groups which are necessary for DNA and RNA biosynthesis, red blood cells formation and methylation processes in organism. $H_4$-folate also reduces homocysteine to cysteine [3,4]. Sufficient dietary intake of FA prevents neural tube defects (e.g., anencephaly or spina bifida) and certain types of anemia [1,5]. The protective effect of FA was also observed for treatment of some diseases like stroke, ischaemic heart disease [6] or colorectal cancer [7]. The recommended daily intake of FA is 0.2 mg (0.453 μmol) for adults and 0.4 mg (0.906 μmol) for pregnant women [8].

Many methods for the FA determination have been developed due to its biological significance. These methods include ELISA (enzyme-linked immunosorbent assays) [9], HPLC (high performance liquid chromatography) with UV [10,11] or diode array detection [12], liquid chromatography with tandem mass spectroscopy (LC/MS/MS) [13] or with electrospray ionisation mass spectrometry [14], microemulsion electrokinetic chromatography [15], capillary electrophoresis [16], spectrophotometry after coupling reaction with specific compounds [17] or biosensor-based determination [18].

Many electrochemical techniques have been developed, because FA is electrochemically active compound. Electrochemical behavior of FA was studied at first at mercury electrodes. Jacobsen and Bjørnsen applied a.c. polarography with DME (dropping mercury electrode) as a working electrode for the determination of FA in pharmaceutical preparations. FA provided a well defined wave in medium of acetate buffer (pH 5.5) at the DME [19]. Electrochemical behavior of FA using AdSV (adsorptive stripping voltammetry) at hanging mercury drop electrode (HMDE) as working electrode was also examined [20–22]. The limit of detection was $1 \times 10^{-11}$ mol $L^{-1}$ after 10 min accumulation [20]. Villamil et al. successfully used AdSV at HMDE for the determination of FA and riboflavin in vitamins preparations [23]. Cathodic stripping voltammetry in conjunction with HMDE was successfully applied for the determination of FA in sea water [24,25]. Besides HMDE, modified electrodes or carbon nanotube electrodes have been used recently. Voltammetric behavior of FA at glassy carbon electrode modified with phosphomolybdic-polypyrrole film ($PMo_{12}$-PPy/GC) is discussed in the paper [26]. This electrode

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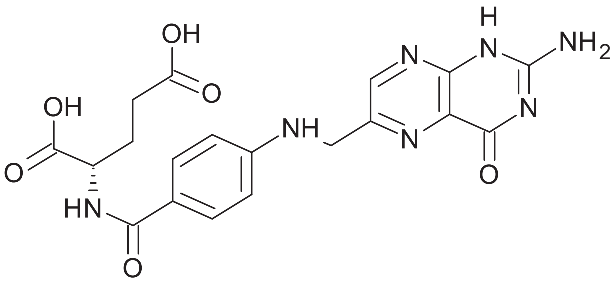

**Fig. 1.** Structure of folic acid (FA).

showed good sensitivity and stability for the determination of FA. The detection limit of $1.0 \times 10^{-10}$ mol L$^{-1}$ of FA was estimated [26]. Vaze et al. [27] studied possibilities of carbon paste electrode modified with *p-tert*-butyl-calix[n]arenes (where *n* = 4, 6 or 8) for the determination of FA in a variety of samples (serum, asparagus, spinach, oranges and multivitamin preparations). Carbon paste electrode modified with *p-tert*-butyl-calix[6]arene (CME-6) was found to be more sensitive than other two tested modifications. The detection limit of $1.24 \times 10^{-12}$ mol L$^{-1}$ was obtained using adsorptive stripping voltammetry at calixarene based chemically modified electrodes [27]. 2-Mercaptobenzothiazole self-assembled gold electrode (MBT/SAM/Au) was also used for voltammetric monitoring behavior of FA and its determination in pharmaceuticals. The detection limit of $4.0 \times 10^{-9}$ mol L$^{-1}$ was estimated using CV (cycling voltammetry) [28]. A single-walled carbon nanotube (SWNT) paste coated glassy carbon electrode (GCE), which had been prepared by using room temperature ionic liquid (1-octyl-3-methylimidazolium hexafluorophosphate, OMIMPF$_6$) as a binder was also used for studying electrochemical behavior of FA and its determination in real samples (wheat flour, fruit juice and milk). The detection limit of $1.0 \times 10^{-9}$ mol L$^{-1}$ was estimated [29].

The above mentioned solid modified electrodes or carbon nanotube electrodes exhibit high sensitivity of FA determination, but their construction and preparation for measurement are often rather complicated, the prices of modifying substances are usually higher (e.g., calix[n]arenes, SWNT) and their surface stabilities and reproducibilities are relatively lower in comparison with amalgam electrodes, which applicability for this purpose is described in this manuscript. More precisely, the utilization of m-AgSAE (mercury meniscus modified silver solid amalgam electrode) for FA determination is discussed in this paper.

Voltammetry and amperometry belong among the most frequently used electroanalytical methods. They are especially suitable for large scale environmental monitoring of electrochemically active pollutants in various types of matrices, because they are inexpensive, extremely sensitive, suitable for speciation and they present an independent alternative to so far prevalent spectrometric and separation techniques [30]. Nevertheless at present, the number of used polarographic, voltammetric and similar devices, utilized in commercial laboratories, is relatively lower in comparison with spectral and other analytical devices (atomic absorption spectroscopy, ICP-MS etc.). The crucial and fundamental problem in their practical application is the choice of a suitable working electrode [31]. The main reason consists in traditional electrode material, i.e., mercury. Strict ecological and safety rules introduced in the world as well as popular prejudices, fears and faults essentially complicate the use of mercury or liquid mercury containing electrodes (including hanging mercury drop electrode (HMDE)).

The research is focused on the material, which could successfully and adequately replace the liquid mercury, however, which would be non-toxic, friendly toward the environment and thus compatible with the concept of so called “green analytical chemistry”. In consequence of this situation, attention has been recently devoted to the development of solid or paste electrodes (carbon paste electrodes [32], solid composite electrodes [33–37], and many others). Several years ago new types of electrodes based on amalgamation of soft metal powder (MeSAE – metal solid amalgam electrodes) were designed by Prague research group [38]. At the same time, the Trondheim research group developed a solid dental amalgam electrode, which was prepared simply by placing the dental amalgam paste in a cavity of the electrode holder [39]. So far, this group focuses on the analysis of heavy metals [39,40]. The electrodes prepared by the Prague group feature a variety of metals used for amalgam preparation (e.g., silver, copper, gold, and thallium). They can be used either as mercury-free electrodes after polishing of solid amalgam disc (p-MeSAE) or after modification of their surfaces by mercury film (MF-MeSAE) or mercury meniscus (m-MeSAE). The meniscus covering the m-AgSAE is stable; pH value does not affect its dissolution inside the working potential window. The results, achieved using these solid amalgam electrodes are fully comparable with those obtained using HMDE or DME [41–47]. Although the solid amalgam electrodes do not reach the quality of HMDE, in many cases they approach it, as it was proved by a variety of analytical applications, including the voltammetric determination of heavy metal cations and many inorganic anions as well as organic species (e.g., [48]). The value of the hydrogen overvoltage at amalgam electrodes is as high as at liquid mercury. Therefore, the field of their application is similar to this of HMDE or DME (e.g., [31,41–46,48–55]). Such universality and easiness of their surface renovation and regeneration belong to advantages of amalgam electrodes.

The reaction mechanism of FA at mercury electrode has been studied in detail (e.g., [21,22,56]). All available sources conclude that the reduction process consists of three distinct steps in acidic media and only a single reduction step in alkaline media [56]. In acidic medium the reduction process can be described as follows: the molecule of FA is converted to a transient 5,8-dihydro-FA, some of which tautomerizes to 7,8-dihydro-FA. The remainder undergoes a proton dependent non-electrochemical cleavage of the para-aminobenzoylglutamate side chain. The second step involves electrochemical cleavage of the C(9)–N(10) chemical bond of the 7,8-dihydro-FA while the final reduction converts the 6-methyl-7,8-dihydropterin derivative generated in the second step to a 6-methyl-5,6,7,8-tetrahydropterin. In alkaline solutions, a single $2e^-/2H^+$ reduction step is observed only, because the proton dependent tautomerization process is slow [56]. On the other hand, in anodic way of polarization only one oxidation step was observed in acidic medium as well as in alkaline media. It was concluded that this peak corresponds to the oxidation of dihydro-FA, originated in the first reduction step [56].

## 2. Experimental

### 2.1. Materials

All chemicals used for preparation of the supporting electrolyte, standard solutions and other stock solutions were of p.a. purity. Britton–Robinson (B–R) buffer of pH value from 3 to 12 was prepared from alkaline component 0.2 mol L$^{-1}$ NaOH (Lachema, Brno, Czech Republic) and acidic component consisting of 0.04 mol L$^{-1}$ $H_3PO_4$, 0.04 mol L$^{-1}$ $H_3BO_3$ and 0.04 mol L$^{-1}$ $CH_3COOH$ (Lachema, Brno, Czech Republic). 0.5 mol L$^{-1}$ acetate buffer of pH 5 was prepared by mixing the stock solutions of acetic acid and sodium acetate (Lachema, Brno, Czech Republic). 0.01 mol L$^{-1}$ borate buffer was prepared by mixing the stock solutions of boric acid and sodium tetraborate (borax) (Lachema, Brno, Czech Republic). Methanol was purchased from the Penta Com-