ABSTRACT

Folic acid (FA) is a water soluble vitamin that exists in many natural species. The lack of FA causes some deficiencies in the human body, so finding a simple and sensitive method for determining the FA is important. One of the modern techniques which overcome the disadvantages of conventional determination methods is the sensors. Possibility of miniaturization, the development of microfabricated electrochemical (EC) sensors has resulted in high sensitivity, portability, improved performance and spatial resolution, low power consumption, and the opportunity for integration with other technologies made Micro-Electrical-Mechanical Systems-based EC sensors suitable to identify low concentration analytes and microorganisms in a variety of mediums.

Keywords: Biomaterials, folic acid, nanomaterials, selectivity, sensitivity, sensor

INTRODUCTION

Folate is yellowish-orange crystal powder that represents an essential nutrition component (important B Vitamin) in the human diet. It is found in kidneys and livers of animals, plants, mushrooms, algae, and incorporated in many metabolic pathways, mainly in carbon transfer reactions such as amino acid interconversions and purine and pyrimidine biosynthesis. A low folate intake has also been led to number of health disorders such as brain disorders such as depression, reduced cognition, Alzheimer’s disease and neural tube defect (NTD), coronary heart diseases; osteoporosis, increased risk of breast and colorectal cancer, poor cognitive performance, hearing loss, high homotype cysteine academia, and anemia. So, since 1998, the Food and Drug Administration in the USA has recommended the fortification of all cereal grain products produced from wheat, rice or maize with 140 μg folic acid (FA) per 100 g. As a result of this FA fortification, a 19% reduction of NTDs birth prevalence was occurred. Due to the important role of FA in human health, reliable, simple, quick, and effective determination methods of this vitamin have been received increasing interest.

Recently, various methods have been reported for the determination of FA, include high-performance liquid chromatography, colorimetry, microbial method, spectrophotometry, flow injection chemiluminescence, fluorometric method, and spectrophotometry, but some of them are nonspecific and laborious and do not allow an easily continuous monitoring because of their high cost, slow rate, need to well-trained operators, and need to step of extraction or sample pretreatment, in some cases, that increased the time and cost of analysis. Considering the disadvantages, there is a need to develop alternate techniques, which are rapid, accurate, and
reproducible and require no sample pretreatment. For real-time testing and online measurements in food industries, the sensors, specially nanosensors,\(^6\) may offer a fast, low cost, and disposable tool.\(^7\) Between different kinds of transducers, electrochemical (EC) transducer have many advantages such as the possibility to operate in turbid media, comparable instrumental sensitivity, and the possibility of miniaturization that lead to small sample volume need. Square wave voltammetry, chronopotentiometry, chronoamperometry, and differential pulse voltammetry (DPV) are modern electroanalytical techniques that have very low detection limits \((10^{-7} \text{ to } 10^{-9} \text{ M})\). In addition, the equipment required for EC analysis is simple and cheap in comparison with most other analytical techniques.\(^8\)

The interesting aspect of EC techniques is utilizing of the chemically modified electrode (CME) for sensitive and selective analytical applications. The electrode can work as a reactant to pump (reduction) or withdraw (oxidation) electron in the reaction, which cannot be expected in spectroscopic methods. To create the CME, a thin film of selected chemical is either bound or coated onto the electrode surface that leads to desirable properties on the electrode surface. The electrocatalytic property is one of these properties that have many advantages in electroanalytical chemistry.\(^9\)

This electrode is used as a sensor to sensing different materials [Figure 1].

One subgroup of sensors is biosensors that a biological group is immobilized on the electrode surface. The biological recognizers can be involved biocatalytic, bioaffinity, and hybrid receptors. The type of the biocomponent determines the degree of selectivity or specificity of the biosensor and is selected due to the characteristics of each sample and the type of physical magnitude to be measured.\(^{[8]}\) The creation of functional materials, devices, and systems through size control of matter at the 1–100 nm scale defined as nanotechnology. A wide variety of nanoscale materials with different sizes, shapes, and compositions are now available that have many desirable properties.\(^{[9]}\) Nanomaterials offer new platforms for developing a variety of advanced analytical technologies, including more sensitive and selective EC sensors for biomonitoring.\(^{[10]}\) Use of nanomaterials in sensors and biosensors can lead to the use of many new signal transduction technologies. Nanosensors, nanoprobe, and other nanosystems are revolutionizing the fields of chemical and biological analysis due to their size. Change in nanomaterials properties due to tailor the size and structure can cause to excellent prospects for designing novel sensing systems and enhancing the performance of the biochemical assay.\(^{[9]}\)

Up to now, different EC sensors based on different receptors had been used for FA determination that is reviewed in this study.

**DIFFERENT ELECTROCHEMICAL SENSORS**

O’Shea et al., reported the detection of FA at a bare carbon surface for the first time.\(^{[11]}\) They optimized an EC pretreatment regime for the determination of FA at cylindrical carbon fiber microelectrodes that gave rise to the higher faradic and capacitive currents because of the presence of new surface functionalities. A very well-defined reduction peak resulted in a sensor with the detection limit of \(1 \times 10^{-8} \text{ M}\) and a linear range of \(2 \times 10^{-8} \text{ M to } 1 \times 10^{-6} \text{ M}\) FA. In another study, a mercury meniscus modified silver solid amalgam electrode was used to investigate the voltammetric behavior of FA and folates using direct current voltammetry, DPV, and adsorptive stripping voltammetry by Bandzuchova et al.\(^{[12]}\) They could reach to detection limit about 0.5 nmol/L and the amounted relative standard deviation (RSD) was 4%. This sensor was a suitable instrument for the determination of FA in subnanomolar concentrations and could provide stable and reproducible responses during long-time measurements. A simple, sensitive, selective, and fast sensor based on molecularly imprinted polymers (MIPs) was developed for the ultra-trace level of FA detection by Prasad et al.\(^{[13]}\) MIPs are often electrically insulating materials because of the presence of diffusion barrier(s) between such MIP coating and the electrode surface. There is no direct path for the conduction of electrons from the binding sites to the electrode that restricted development of EC sensor. They could overcome these problems by combining insulating MIP and conducting carbon powder in consolidated phase. In this study, carbon particles are arranged orderly as a carbon strip assisting in the fast conduction of electrons from the binding sites to the transduction surface that could assure reliable results, without any cross-reactivity or matrix effect. The detection of FA with the MIP-fiber sensor was shown to be specific and quantitative (detection limit \(2 \times 10^{-10} \text{ g/L}, \text{RSD} = 1.3\%\),

![Figure 1: Schematic of typical electrochemical sensors elements](image)

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\(\text{RSD}\) is the Relative Standard Deviation.
S/N = 3), in aqueous, blood serum, and pharmaceutical samples. In another study, an EC sensor was developed for the selective and quantitative recognition of FA, using a preanodized sol–gel coated pencil graphite (2B) electrode with imprinted polymer immobilized to its exterior surface. During preconcentration step at + 0.85 V (with respect to Ag/AgCl) and pH 2.5 in aqueous environment, FA is absorbed through mixed hydrophobically driven hydrogen bondings and iono interactions of pteridine and purely hydrogen bondings interactions of glutamic acid residue with modified electrode. The FA was selectively detected with a limit of detection (LOD) of 2.0 × 10^{-9} g/L, without any cross-reactivities and real matrix complications.\(^{14}\) Wan and Young measured FA with 2-mercaptobenzothiazole self-assembled gold electrode (MBT/SAM/Au). FA can bind strongly to MBT/SAM/Au.\(^ {15}\) The oxidation peak currents achieved on MBT/SAM/Au have a linear correlation with the logarithm of the content of FA in the range of 8.0 × 10^{-12} to 1.0 × 10^{-6} mol/L. The detection limit was 4.0 × 10^{-9} mol/L. The EC behavior of FA at the glassy carbon (GC) electrode that modified with Keggin-type phosphomolybdate (PMo12) doped polypyrrole (PPy) film (PMo12-PPy/GC electrode [CCE]) was studied by Guo et al. The reduction peak currents were directly proportional to the concentration of FA in the range of 1.0 × 10^{-8} to 1 × 10^{-7} M and a detection limit of 1.0 × 10^{-10} M of FA. This modified electrode presented excellent sensitivity and stability for the determination of FA.\(^ {16}\) An ordered mesoporous carbon (OMC) modified GCE was used by Yang et al. for FA determination. Due to the good characteristics of OMC, FA exhibited an enhanced EC response and lower reduction potential in the neutral solution. They observed that the peak current changes were linear with FA concentration changes in the range of 5.0 × 10^{-10} to 1.0 × 10^{-7} M with a lower detection limit of 6.0 × 10^{-12} M.\(^ {17}\) Ojani \(\text{et al.}\) prepared poly (O-anisidine) (POA) modified carbon paste electrode (POA/MCPE) with electropolymerization of O-aminophenol on CPE in the presence of sodium dodecyl sulfate. Ni/POA/CPE was prepared by open circuit accumulation of Ni (II) ions at the surface of POA/CPE that could catalyze the oxidation of FA via a surface layer mediated charge transfer. The catalytic oxidation peak current of FA was linearly dependent on its concentration, and a linear calibration curve was obtained in the range of 1 × 10^{-4} to 5 × 10^{-1} M with the LOD of 9.1 × 10^{-5} M. This sensor was used as simple, selective and precise voltammetric method for determination of FA in pharmaceutical preparations.\(^ {18}\) Voltammetric behavior of FA at calixarenes modified electrode was checked by Vaze and Srivastava. The current was found to be rectilinear with FA concentration in the range of 8.79 × 10^{-12} M to 1.93 × 10^{-9} M. The LOD obtained was found to be 1.24 × 10^{-12} M. This method was used for the FA determination in different samples such as serum, asparagus, spinach, oranges, and multivitamin preparations.\(^ {19}\) Kalimuthu and John demonstrated the selective determination of FA using electropolymerized film of 5-amino-2-mercapto-1,3,4-thiadiazole (p-AMT) modified GCE. They found bare GCE failed to determine the concentration of FA in the presence of ascorbic acid (AA) and uric acid (UA) due to the surface fouling caused by the oxidized products of AA and FA but, the p-AMT film modified electrode could separate the voltammetric signals of AA, UA, and FA and also higher oxidation current for these analytes were obtained. The amperometric current response had linearly correlation with FA concentration in the range of 1.0 × 10^{-8} to 8.0 × 10^{-4} M and the detection limit was found to be 2.3 × 10^{-10} M (S/N = 3). This modified electrode was successfully used for determining FA concentration in human blood serum samples.\(^ {20}\) The overoxidized PPy (OPPy)-modified carbon ceramic electrode was used to study EC behavior of FA. Its results showed linear response ranges for FA concentration from 7 × 10^{-6} to 5.5 × 10^{-5} M, 1 × 10^{-7} to 2.5 × 10^{-5} M, and from 4.9 × 10^{-7} to 7.8 × 10^{-6} M with detection limits of 1.8 × 10^{-6}, 3.1 × 10^{-8}, and 2.7 × 10^{-7} M for cyclic voltammetry (CV), DPV, and amperometric techniques, respectively. The OPPy-modified electrode had very high catalytic ability for electrooxidation of FA and this modified electrode exhibited excellent sensitivity and stability in the determination of FA in biological and other real samples.\(^ {21}\) An adsorptive stripping voltammetric procedure was used for FA determination at plated lead film electrode. The FA concentration and signal current had a linear correlation in the range of 2 × 10^{-9} to 5 × 10^{-6} mol/L. The detection limit was 7 × 10^{-10} mol/L, and the RSD for 2 × 10^{-8} mol/L of FA was 3.9%. This sensor was used for FA determinations in pharmaceutical preparations.\(^ {22}\) In another study, mechanochemical synthesized ZnO was used to modify the CPEs. They found that ZnO MCPE had an electrocatalytic activity toward the electroactive species like FA. By using the ZnO MCPE instead of bare CPE, they found an increase in electrocatalytic activity. The change in FA concentration led to change its anodic peak current linearly in the range of 0.01–0.16 Mm. The stability of electrode is good, and it could be rebuilt easily and on the other side it had remarkable sensitivity and selectivity.\(^ {23}\) Ananthi \(\text{et al.}\) (2015) reported electro-reduction of FA on electrodeposited bismuth nanowires on glassy carbon electrode (BiNWs/GCE). This electrode exhibited a high sensitivity for FA detection with LOD of 9.53 × 10^{-4} and limit of quantitation of 31.68 × 10^{-3} mol/L. In addition, suitable price and simplicity of BiNWs/GC sensor were other important properties that made it as an appropriate choice in EC analysis.\(^ {24}\)
DIFFERENT ELECTROCHEMICAL NANOSENSORS

The EC behavior of FA was investigated on carbon nanotube modified electrode with CV and square wave stripping voltammetry by Jiang et al. They found a good linearity between the peak current of FA and its concentration in the range of 3.00 × 10⁻⁷ to 8.00 × 10⁻⁵ M and the detection limit was 1.34 × 10⁻⁷ M. Wei et al. used CV, chronoamperometry and chronocoulometry to study voltammetric behavior of FA at a multi-walled carbon nanotube (MWCNT) modified gold electrode. They found the peak current changed linearly with FA concentration in the range of 2.0 × 10⁻⁸ M to 1.0 × 10⁻⁶ M, and the detection limit was 1.0 × 10⁻⁸. This method was applied for the determination of FA in drug tablets with recovery amounted about 95.9–96.9%. They also examined the influence of folding a Nafion layer on the gold electrode before deposition of MWNTs that resulted in giving the better response to FA and suppressing the interference by some foreign species. In another study GCE and indium tin oxide, the electrode was used for immobilization of electrochemically active composite film which was contained MWCNTs and poly (brilliant cresyl blue) (PBCB). The presence of MWCNTs in the composite film (MWCNTs-PBCB) enhanced the surface coverage concentration of PBCB and exhibited enhanced electrocatalytic activity toward the biochemical compound Vitamin B9 (FA). The LOD of FA at MWCNTs-PBCB film was 7.6 × 10⁻⁵ M. The DPV and selectivity studies revealed the sensor efficiency for FA determination in a real sample. Wang et al. used single-wall carbon nanotubes (SWNTs) to modify the surface of a GCE to determine FA with CV and linear sweep voltammetry. They observed linearity between reduction peak current and FA concentration over the range of 1 × 10⁻⁸ to 1 × 10⁻⁴ mol/L with a detection limit of 1 × 10⁻⁹ mol/L after 5 min accumulation. Their film electrode provided an efficient way for eliminating interferences from some inorganic and organic species in the solution and high sensitivity, selectivity and stability of the film electrode made suitable tool for simple and rapid determination of FA in tablets. Xiao et al. coated GCE with SWNT paste using room temperature ionic liquid (such as 1-octyl-3-methylimidazolium hexafluorophosphate, OMIMPF₆) as a binder. Mnoch et al. studied EC oxidation of FA at CuOnanoleaves (CuONs) on MWCNTs/GCE nanocomposite film modified electrode. They synthesized CuONs by alcoholic reduction of Cu (II) chloride in the presence of poly (diallyldimethylammonium chloride). CV was used to characterize the EC performance of the CuONs/MWCNTs/GCE nanocomposite modified electrode. This modified electrode exhibited good ability toward the electrocatalytic oxidation of FA. The sensitivity of 3.35 μA/μM, detection limits of 15.2 × 10⁻⁹ M and linear response range of 1 × 10⁻⁴ to 9 × 10⁻⁷ M for this modified electrode led to efficient way for determination of FA. Mirmoghtadaie et al. developed a novel activated gold electrode based on modification with gold nanoparticles by applying a high potential in the presence of sodium hydroxide. They used this electrode for measuring FA in real samples such as FA tablets, wheat flour, fortified wheat flour, and spinach. A good linear correlation was found between the peak current and concentration of FA in the range of 1.0 × 10⁻⁸ to 1.0 × 10⁻⁶ mol/L with a detection limit of 7.50 × 10⁻⁹ mol/L. Their proposed method is highly selective and sensitive to FA. The α-Fe₂O₃ nanofibers modified GCE studied for FA determination by Maiyalagan et al. Bare GCE failed to determine the concentration of FA in the presence of a higher concentration of AA because of the surface fouling of the oxidized products of AA and FA. Using α-Fe₂O₃ nanofibers modified GCE resolved this problem. The amperometric current response was linearly dependent on FA concentration in the range of 6.0 × 10⁻⁸ to 6.0 × 10⁻⁴ M, and the experimental detection limit was 6.0 × 10⁻⁸ M.

DIFFERENT ELECTROCHEMICAL BIOSENSORS

An EC DNA biosensor was proposed by Mirmoghtadaie et al. as a screening device for the rapid analysis of FA using a salmon sperm ds-DNA modified pencil graphite electrode. At first, they optimized immobilization of the ds-DNA on pencil graphite electrode using response surface methodology. Then the binding of FA with immobilized DNA at a pencil graphite electrode was studied through verifying the EC signal of adenine. FA was measured in the range of 1.0 × 10⁻⁷ to 1.0 × 10⁻⁵ mol/L with a detection limit of 1.06 × 10⁻⁸ mol/L. This biosensor was successfully used to the selective measurement of FA in different real samples. For the first time, Lermo et al. developed an indirect competitive immunoassay-based strategy for FA determination with EC magneto sensors. They immobilized a protein conjugate BSA-FA on the tosyl-activated magnetic bead with a covalent bond. Further competition between FA in the food sample and FA immobilized on the magnetic bead for the specific antibody was occurred that followed by the reaction with a secondary antibody conjugated with hors eradish peroxidase (anti-IgG). Then, the modified magnetic beads were easily sorbed by a magneto sensor made of magneto graphite–epoxy composite which was also used as the transducer for the EC detection. They compared EC immunoassay strategy with a magneto-ELISA method using the same immune reagents that resulted in similar detection limits. The detection limit was found to be 1.31 × 10⁻¹⁰ mol/L for skimmed milk.
SELECTIVITY OF DIFFERENT ELECTROCHEMICAL SENSOR IN FOLIC ACID DETERMINATION

The effect of potential interferences compounds on the performance of the different sensors in FA determination was studied in some researches with substances that were chosen from the group of compounds commonly found with FA in different samples.

Comparison between different results [Table 1] shows that using biomaterials such as DNA can increase the selectivity of EC sensors. FA has specific interaction with the salmon sperm ds-DNA because of an electroactive NH$_2$ group of FA. The redox behavior of original ds-DNA immobilized PGE exhibited two oxidation processes of adenine and guanine residues that is changed in interaction with electroactive compounds. The interaction of FA with the ds-DNA leads to decrease in the initial signal height of the adenine.$^{[32]}$

CONCLUSIONS

The detection and identification of food nutrients continued based on conventional techniques. The need for a more rapid, reliable, specific, and sensitive method of detecting a target analyte, at low cost, is the focus of a great deal of research. Sensor technology has the potential to speed up the detection, increase specificity and sensitivity, and enable high-throughput analysis. In respect to other transducing principles, EC techniques are much easier to use and allow the miniaturization for the integration of handheld devices. Study on different FA EC sensors shows lower detection limit than some conventional methods, and no additional process are needed for extraction and purification. Comparison between different EC sensors revealed that utilizing nanomaterials can lead to a decrease in detection limit and increase in selectivity of FA measurement to some extent. The combination of various nanomaterials into different composites because of exploring their synergistic effect is recommended. Furthermore, the incorporation of biomaterials in sensor making caused to huge increase in sensor selectivity. Therefore, it seems that simultaneous using of nonmaterials and biomaterials can lead to more sensitive and more selective EC sensor.

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Table 1: Comparison between different sensor selectivity

| Folic acid sensor | Interference (tolerance limit) | References |
|------------------|--------------------------------|------------|
|                  | B$_1$ | B$_2$ | B$_3$ | B$_4$ | C |
| MWCNT/GCE        | -     | -     | -     | -     | C | Jiang et al., 2009$^{[31]}$
| SWCNT/GCE        | -     | -     | -     | -     | 100 | Xiao et al., 2008$^{[27]}$
| Calixarenes/CPE  | 10    | 10    | 10    | 1     | Vaze and Srivastava, 2007$^{[14]}$
| MWCNT/AuE        | 50    | 50    | 1     | -     | 50 | Wei et al., 2006$^{[26]}$
| DNA/PGE          | 500   | 500   | 1000  | 1000  | 1000 | Mimoghaddaie et al., 2013$^{[1]}$

B$_1$=Thiamine, B$_2$=Riboflavin, B$_3$=Niacin, B$_4$=Pyridoxine hydrochloride, C=Ascorbic acid, GCE=Glassy carbon electrode, MWCNT=Multi-walled carbon nanotube, SWCNT=Single-wall carbon nanotube, CPE=Carbon paste electrode, PGE=Pencil graphite electrode.
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