




Original scientific paper

## Electrochemical detection of folic acid using a modified screen printed electrode

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### Abstract

*In this work, an electrochemical sensor was established for the detection of folic acid based on Ni-BTC (BTC = benzene-1,3,5-tricarboxylic acid) metal-organic framework (MOF) modified screen-printed electrode (SPE). Electrochemical techniques: cyclic voltammetry (CV), differential pulse voltammetry (DPV), linear sweep voltammetry (LSV) and chronoamperometry (CHA) were used for the detection of folic acid at Ni-BTC MOF modified SPE. The results indicate that the as-prepared sensor has a good electrocatalytic effect on the detection of folic acid. This electrochemical sensor showed a dynamic linear response range from 0.08 to 635.0  $\mu\text{M}$  and the detection limit was estimated to be  $0.03 \pm 0.001 \mu\text{M}$ . Moreover, the feasibility of Ni-BTC MOF/SPE sensor to detect folic acid in real samples was also evaluated by the standard addition method.*

### Keywords

Electrochemical sensor; Ni-BTC metal-organic framework, voltammetry

### Introduction

Folic acid is the synthetic form of folate, vitamin B<sub>9</sub>, found in vitamin tablets and fortified foods, which acts as a coenzyme in one-carbon transfer reactions in the various human metabolic pathways [1,2]. Perhaps the most important role of folic acid is purine and pyrimidine nucleotide biosynthesis, necessary for the synthesis and replication of deoxyribonucleic acid (DNA) and thus differentiation of cells. It is also essential for the synthesis of methionine from homocysteine [3]. Promotion of the formation of red blood cells and thus prevention of anemia is another advantageous role of folic acid [4]. Folic acid has an important effect on the normal growth and development of the fetus, preventing congenital malformations, specifically spina bifida and anencephaly, referred to as neural tube defects (NTDs) [5]. Its deficiency may carry potential risks such as the development of cancer, megaloblastic anemia, cardiovascular disease, Alzheimer's disease, and psychiatric disorders [6-10]. In this sense, monitoring folic acid and its metabolites is highly desirable. Different analytical procedures are currently available for the determination of folic acid, as high-performance liquid

chromatography (HPLC) [11], ultra-violet (UV) spectrophotometric [12], flow injection luminescence [13] and capillary electrophoresis [14]. These methods are time-consuming, expensive and require complicated pre-treatment steps. On the other hand, the electrochemical methods are a very promising way for the determination of folic acid and other compounds due to easy fabrication, low cost and rapid analysis compared to other analytical methods [15-30].

Since the chemical analysis has recently focused on the design of portable, disposable, and low-cost instruments, screen-printed electrodes (SPEs) have become a quite widespread substitute for classic electrodes in electrochemical analytical measurements [31, 32]. SPE based on three-electrode systems is the best option to develop an electrochemical sensor with high sensitivity and selectivity [33-35].

In recent years, modified electrodes have had many applications in various fields, including sensing, electrocatalysis, fuel cells, batteries and supercapacitors [36-48]. Chemical modification of electrodes with various materials such as nanomaterials, single molecular, multi-molecular, ionic, and polymeric components provides electrocatalytic properties in terms of increasing active surface area and accelerating electron transfer kinetics, which is beneficial for electrochemical sensing applications [49-61].

Metal-organic frameworks (MOFs) constructed by metal ions as nodes and organic ligands as linkers are a new class of nano-sized polymeric and crystalline material. Due to their high porosity, structural diversity and open metal sites, MOFs possess tunable functionalities, adsorption affinity, and high surface area [62-69]. MOFs were demonstrated as a novel material to modify the electrode for the electrochemical application because of the electrochemical activity of the metal ions and the well-ordered porous skeleton [70-72]. Nickel-based MOFs such as Ni (BTC) have been broadly used in different applications due to the low cost and natural abundance of nickel [73].

In the present work, an electrochemical sensor with high sensitivity for measuring folic acid was developed based on the Ni-BTC modified SPE. Compared with unmodified SPE, the Ni-BTC modified SPE exhibited excellent electrochemical activity for the determination of folic acid. This sensor has been employed to measure folic acid in various real samples.

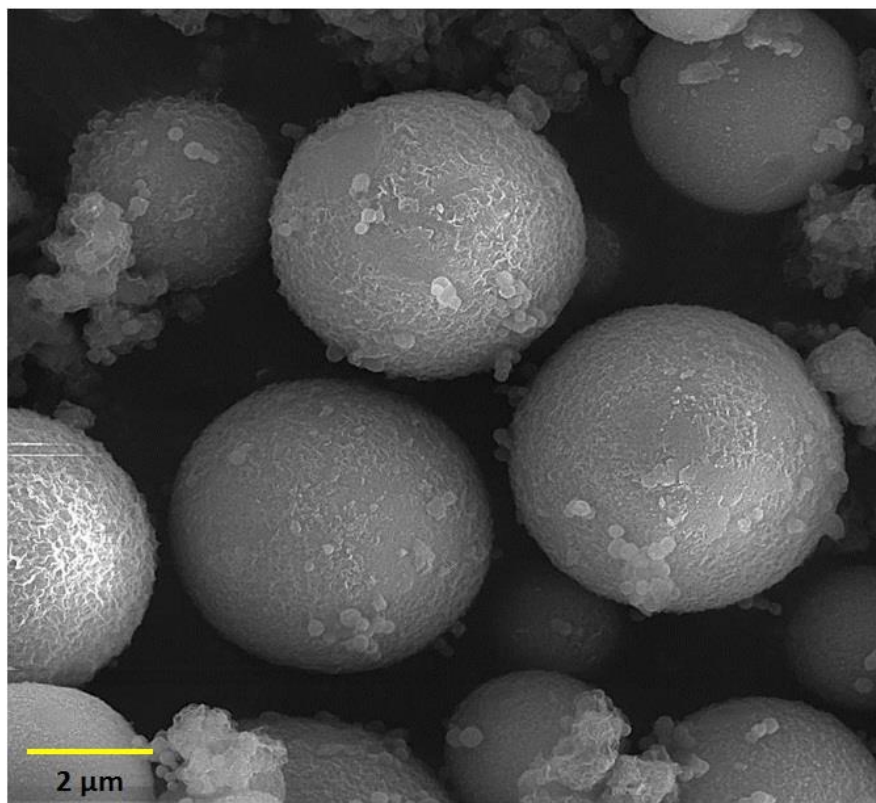
## Experimental

### *Apparatus and chemicals*

All electrochemical measurements were performed on a PGSTAT302N potentiostat/galvanostat Autolab and controlled with the general purpose electrochemical system (GPES) software. All experiments were carried out within a conventional three-electrode cell. The SPEs were purchased from DropSens (Spain) and consisted of a graphite working electrode, graphite counter electrode and Ag pseudo reference electrode. Solution pH values were determined using a 713 pH meter combined with a glass electrode (Metrohm, Switzerland). Folic acid and other chemicals used were analytical grade and were purchased from Merck.

### *Synthesis of Ni-BTC MOF*

For the synthesis of Ni-BTC MOF, 1.10 g of  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  and 0.44 g of BTC were added to methanol. The prepared mixture was stirred for 90 min at room temperature and then transferred to a Teflon-lined stainless steel autoclave and heated at 150 °C for 24 h. After the autoclave reached room temperature, the precipitate was separated by centrifuge and washed several times using methanol. Finally, the collected product (Ni-BTC MOF) was dried in an oven at 70 °C for 10h. The field emission-scanning electron microscopy (FE-SEM) image of the Ni-BTC MOF is shown in Figure 1.



**Figure 1.** FE-SEM image of Ni-BTC MOF

#### *Preparation of Ni-BTC MOF/SPE*

For modification of SPE by Ni-BTC MOF, 1 mg of Ni-BTC was dispersed in 1 mL of deionized water using an ultra-sonication bath for 20 min to get a homogeneous solution of 1 mg 1 mL<sup>-1</sup> of Ni-BTC. Then, 3 μL of Ni-BTC MOF suspension was drop cast on the surface of unmodified SPE and dried at room temperature to achieve Ni-BTC MOF/SPE.

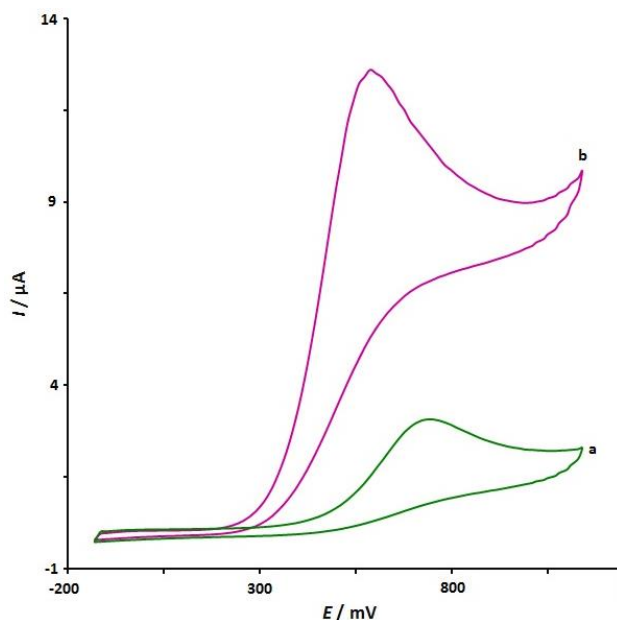
The surface area of Ni-BTC MOF/SPE and the bare SPE were obtained by CV using 1 mM K<sub>3</sub>Fe(CN)<sub>6</sub> at different scan rates. Using the Randles-Sevcik formula for Ni-BTC MOF/SPE, the electrode surface was found to be 0.116 cm<sup>2</sup> which was about 3.8 times greater than bare CPE.

## **Results and discussion**

#### *Electrochemical response of folic acid at different electrodes*

The effect pH value of electrolyte solution was investigated by DPV in 0.1 M phosphate buffer solution (PBS) at the pH range from 2.0 to 9.0 containing 50.0 μM folic acid on the Ni-BTC MOF/SPE surface. The oxidation peak current of folic acid reached a maximum value at pH 7.0, and therefore PBS with pH 7.0 was chosen as the optimum pH to detect folic acid.

Figure 2 displays the cyclic voltammograms of the folic acid at unmodified SPE (curve a) and Ni-BTC MOF/SPE (curve b), with the same concentration of 200.0 μM in 0.1 M PBS (pH 7.0). The anodic peak potential for the oxidation of folic acid at Ni-BTC MOF/SPE (curve b) is about 590 mV compared with 740 mV, for that on the unmodified SPGE (curve a). Similarly, when the oxidation of folic acid at the Ni-BTC MOF/SPE (curve b) and unmodified SPE (curve a) are compared, an extensive enhancement of the anodic peak current at Ni-BTC MOF/SPE, relative to the value obtained at the unmodified SPE (curve b), is observed. In other words, the results clearly indicate that the Ni-BTC MOF improves folic acid oxidation.



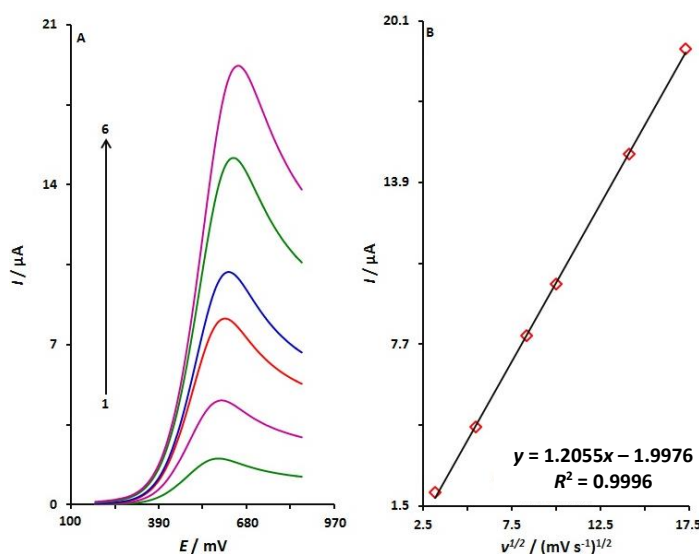
**Figure 2.** CV curves of unmodified SPE (curve a) and Ni-BTC MOF/SPE (curve b) in 0.1 M PBS containing 200.0 μM folic acid; scan rate: 50 mV s<sup>-1</sup>.

*Influence of the scan rate on the results*

The voltammograms obtained using LSV were recorded at various scan rates to see the differences in the peak potential and current in 0.1 M PBS at pH 7.0 (100.0 μM folic acid) (Figure 3A). The scan rates were changed in the interval  $\nu = 10\text{-}300\text{ mV s}^{-1}$ . The voltammograms show that, with increasing scan rate, the peak current permanently increases, and the peak potential moves to more positive values. As can be seen in Figure 3B, the equation between the peak current and the square root of the scan rate for oxidation peak is given by equation (1):

$$I_{pa}(\text{Folic acid}) = 1.2055 \nu^{1/2} - 1.9976 \quad (R^2 = 0.9996) \tag{1}$$

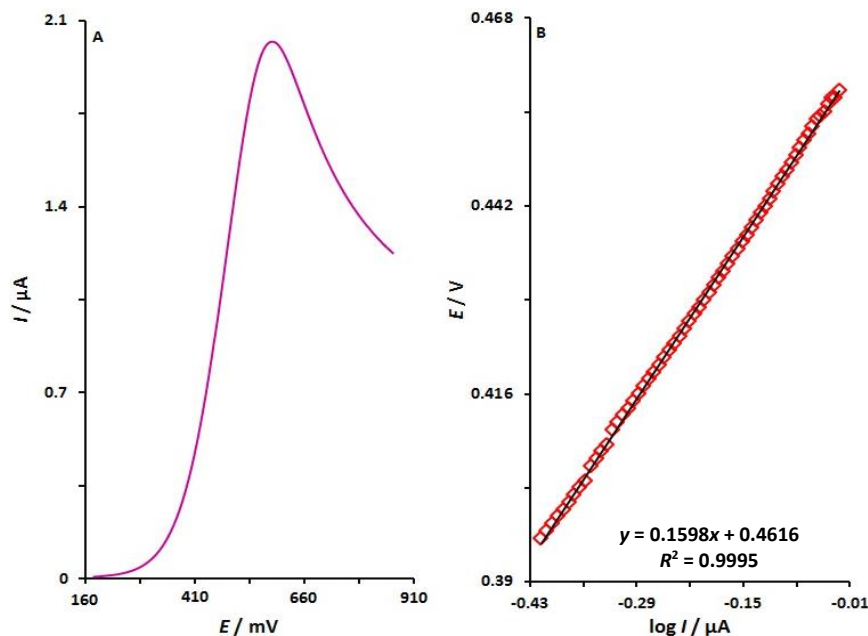
The linearity of  $I_{pa}$  vs.  $\nu^{1/2}$  graphs specified that the reaction is diffusion-controlled process.



**Figure 3.** A) LSV curves of 100.0 μM folic acid in 0.1 M PBS (pH 7.0) at a scan rate of 10 to 300 mV s<sup>-1</sup> at Ni-BTC MOF/SPE (1-6 refers to 10, 30, 70, 100, 200, and 300 mV s<sup>-1</sup>). B) Plot of the square root of the scan rate vs. the oxidation peak current of folic acid

In order to obtain some information on the rate-determining step, we drew a Tafel plot (Figure 4B) using the data from the rising part of the current-voltage curve recorded at a low scan rate of 10 mV s<sup>-1</sup>

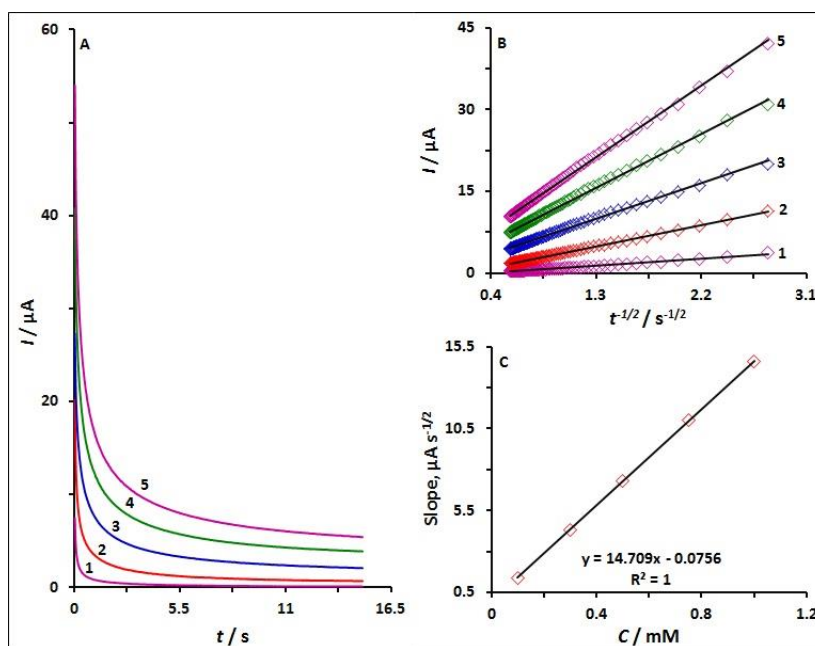
(Figure 4A) for 100.0  $\mu\text{M}$  folic acid. The linearity of the  $E$  versus  $\log I$  plot implies the intervention of the kinetics of the electrode process. The slope of this plot can be used to estimate the number of electrons transferred in the rate-determining step. According to Figure 4B, the Tafel slope for the linear part of the plot was estimated to be equal to 0.1598 V. The value of the Tafel slope indicates that one-electron transfer process is the rate-limiting step, assuming a transfer coefficient ( $\alpha$ ) of about 0.63.



**Figure 4.** A) LSV response for 100.0  $\mu\text{M}$  folic acid with  $10 \text{ mVs}^{-1}$  scan rate; B) Tafel plot derived from the rising part of the corresponding voltammogram

### Chronoamperometric analysis

Chronoamperometric measurements of folic acid at Ni-BTC MOF/SPE were carried out by setting the working electrode potential at 0.65 V for the various concentrations of folic acid in 0.1 M PBS (pH 7.0) (Figure 5A).



**Figure 5.** A) The chronoamperograms obtained at Ni-BTC MOF/SPE in 0.1 M PBS at pH of 7.0 for different concentrations of folic acid (1-5 refers to: 0.1, 0.3, 0.5, 0.75, and 1.0 mM). B) The  $I$  plot versus  $t^{-1/2}$  observed by chronoamperograms 1-5. C) The slope plot of the straight line vs. concentration of folic acid

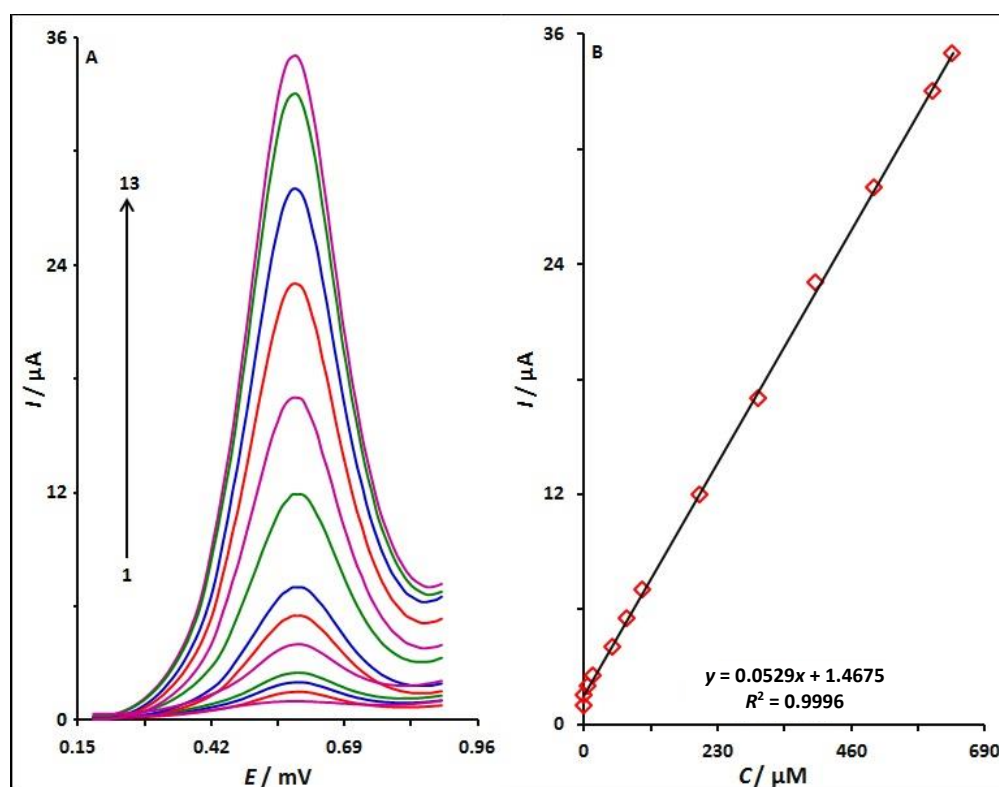
For an electroactive material (folic acid in this case) with a diffusion coefficient  $D$ , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [74]. Experimental plots of  $I$  vs.  $t^{-1/2}$  were employed, with the best fits for different concentrations of folic acid (Figure 5B). The slopes of the resulting straight lines were then plotted vs. folic acid concentration (Figure 5C). From the resulting slope and Cottrell equation, the mean value of the  $D$  was found to be  $1.85 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ .

#### Calibration curve, linear range and detection limit

Figure 6A shows the electrochemical detection of folic acid by using DPV at various concentrations using Ni-BTC MOF/SPE (Step potential = 0.01 V and pulse amplitude = 0.025 V). As seen in Figure 6B, the oxidation peak current of folic acid linear with a folic acid concentration in the concentration range of 0.08 – 635.0  $\mu\text{M}$ . The regression equation of the calibration graph is given by Eq. (2):

$$I_{pa} (\text{Folic acid}) = 0.0529 C_{\text{Folic acid}} + 1.4675 \quad (R^2 = 0.9996) \quad (2)$$

The limit of detection value was calculated to be  $0.03 \pm 0.001 \mu\text{M}$ . It is better than some recent report in determination of folic acid using SPE [75].



**Figure 6.** DPV responses of folic acid on Ni-BTC MOF/SPE at different folic acid concentrations (1-13 refer to: 0.08, 1.0, 7.5, 15.0, 50.0, 75.0, 100.0, 200.0, 300.0, 400.0, 500.0, 600.0, and 635.0  $\mu\text{M}$ ) in 0.1 M PBS (pH 7.0). Inset: The relationship between the oxidation peak currents and folic acid concentration

#### Analytical application

The determination of folic acid in real samples such as folic acid tablets and urine was performed using Ni-BTC MOF/SPE sensor. The concentration values of folic acid were calculated by the standard addition method. The results are summarized in Table 1, the recovery is between 96.0 and 102.7 %, and the relative standard deviations (RSDs) are all less than or equal to 3.3 %. The experimental results confirmed that the Ni-BTC MOF/SPE sensor has a great potential for analytical application.

**Table 1.** Determining folic acid in real samples by using Ni-BTC MOF/SPE (n=5)

Sample	Concentration, $\mu\text{M}$		Recovery, %	RSD, %
	Spiked	Found		
Folic acid tablet	0	4.0	-	3.3
	2.5	6.4	98.5	1.9
	3.5	7.7	102.7	2.4
Urine	0	-	-	-
	5.0	5.1	102.0	2.2
	7.5	7.2	96.0	2.9

## Conclusion

In this work, we developed a Ni-BTC MOF modified SPE as an electrochemical sensing platform for the detection of folic acid. According to voltammetric results, the prepared sensor showed an excellent performance toward folic acid oxidation. Under an optimized condition, the prepared electrochemical sensor displayed a low detection limit of  $0.03 \pm 0.001 \mu\text{M}$  and a broad linear range of  $0.08$  to  $635.0 \mu\text{M}$  for folic acid. Furthermore, the Ni-BTC MOF/SPE was also tested for its ability to detect folic acid in real samples giving excellent recoveries.

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