

FULL PAPER

A nano-sensor based on screen printed electrode (SPE) for electro-chemical detection of vitamin B₉

Mohammad Reza Aflatoonian^{a,b} | Somayeh Tajik^{a,c,*} | Mehri-Saddat Ekrami-Kakhki^d | Behnaz Aflatoonian^c | Hadi Beitollahi^e

^aResearch Center for Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran

^bLeishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran

^cNeuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran

^dEsfarayen University of Technology, Esfarayen, Iran

^eEnvironment Department, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

***Corresponding Author:**

Somayeh Tajik

Email: Tajik_s1365@yahoo.com

Tel.: +98 (34) 32112794

The present study designed an electro-chemical instrument with high sensitivity and selectivity on the basis of a SPE modified with La⁺³/Co₃O₄ nano-cubes in order to analyze vitamin B₉. It was confirmed that this sensor possesses very good electron-moderating behaviors to oxidize vitamin B₉ in a 0.1 M phosphate buffer solution (PBS) (pH=7.0). In addition, according to optimal conditions (pH=7.0), a potential around 110 mV less positive than the one in the un-modified electrodes is required for vitamin B₉ oxidation at this electrode surface. This technique limit of determination for vitamin B₉ has been estimated to be 0.3 μM (S/N = 3). Responses have been linear in concentrations range between 1.0 and 600.0 μM. The modified electrode has been applied to detect vitamin B₉ in real specimens, which was accompanied by acceptable outputs. Experiments showed that La⁺³/Co₃O₄ nano-cubes will be valuable electrode materials providing a high ratio of surface to volume that enhances sensitivity. The study introduces a novel sensor with simpleness, quickness, sensitivity, and affordability features to quantify vitamin B₉.

KEYWORDS

Vitamin B₉; La⁺³/Co₃O₄ nano-cubes; screen printed electrode; modified electrode; voltammetry.

Introduction

The term "folic" in folic acid (FA) derives from the Latin word folium, meaning leaf, because it was found in the spinach leaf (1941). Moreover, FA is known as vitamin B₉, which makes it a part of vitamin-B complexes. The active form of FA is folate, which exists in food so that human body needs it for its function. FA does not become biologically activated unless it is transformed into an active form of folate [1]. If folic acid introduces into body through certain diets, it generally should be converted to the folate form for effective application. Food folates such as poly-glutamates and mono-glutamates (parent compound) are transformed into stomach to

dihydrofolate (DHF) and tetrahydrofolate (THF) and afterwards is converted into 5-methyl tetrahydrofolate using enzyme 5, 10 methyl-enetetrahydrofolate reductase (MTHFR). The active form of folic acid in plasma is 5-methyl THF [2,3]. This substance contributes to reactions of the single carbon transfer in metabolism, and is the precursor of active tetrahydro folic acid co-enzyme. FA has been explored in spinach for the first time. Its defect leads to the reduced growth in mammals and various types of anaemia and incapability for making purines and thymine needed to synthesize DNA. Folic acid is one of the potent agents to prevent cancers [4] *via* scavenging free radicals and anti-oxidant activities. It consists of the building

blocks of glutamic acid, p-amino-benzoic acid, and pterin. The superior name for folic acid and its derivatives is folate. Folic acid has been detected by electro-chemical techniques [5,6].

It is clear that the major basis of the electro-chemical methods is the analysis of the relationships between detection and current, voltages and resistance. Evolution of novel substances may lead to the building of novel electro-chemical sensors with higher sensitiveness and steadiness [7,8] that have benefits, such as rapid responses, excellent mobility, simple operations, affordability, greater precision, and lower limits of determinations in comparison to the HPLC-based procedures [9-11].

Alternatively, it has been found that nano-materials features are affected by their sizes and architecture, in particular, because of their surface instead of the nature of bulk atoms [12]. Extreme ratios of surface-to-volume in nano-materials offer these substances a number of benefits, including rapid reaction kinetic, and make them suitable alternatives for single-molecule determinations. Currently, researchers have concentrated on the evaluation of physical, electronic, and chemical properties of nanomaterials for sensor and catalytic uses [13-15].

With regard to the respective electro-chemical functions, the materials were employed for modifying the electrode materials. Different fascinating benefits have been also confirmed in bio-science [16]. The existence of nano-particles increases surface areas of the electrode materials and makes easier the electron transfer and rate of reactions [17,18].

SPEs have been proposed in the 1990s for the first time. It has been recognized that these electrodes enjoy more reliable, reproducible, inexpensive, and possible mass manufacturing features [19-21]. They are flexible tools with probable uses in a variety of forms, in which different materials may be applied, and it is possible to simply modify it by various

biological specimens, such as enzyme, antibody, synthetic recognition components, and so forth [22-24].

It was demonstrated that higher over-potentials that enhance the background current and largely restrict detection limits are established by direct electro-chemical oxidation of vitamin B₉ through an unmodified electrode. Therefore, lanthanides based on the modified electrodes have been designed to determine vitamin B₉. As mentioned earlier, the main objective of the present study is to develop a modified electrode to detect vitamin B₉ sensitively *via* La⁺³/Co₃O₄ nano-cubes for augmenting the SPEs capabilities. The final electrode has been substantially used to determine vitamin B₉ in real specimens.

Experimental

Apparatus and chemicals

An Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) was applied for measuring electrochemicals. General Purpose Electrochemical System (GPES) software was employed to control conditions of experiments. The screen-printed electrode (DropSens, DRP-110, Spain) includes 3 main sections that contain a silver pseudo-reference electrode, a graphite working electrode, and graphite counter electrode. pH was measured by a Metrohm 710 pH meter.

Vitamin B₉ and all the remaining reagents had an analytical grade. They have been prepared *via* Merck (Darmstadt, Germany). Orthophosphoric acid and the related salts that were above the pH range of 2.0–9.0 were used for preparing the buffer solutions.

Synthesis of La⁺³/Co₃O₄ nanocubes

In a typical synthesis, 25 mL of ammonia solution (28-30%, Reagent Chemicals) was first mixed with ultra pure water (1/1, v/v). Then, 0.05 g of poly(vinylpyrrolidone) (PVP;

M.W. 58,000, Reagent Chemicals) was dissolved in the solution. After shaking for a few minutes, 0.364 g of cobalt nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) and 0.043 g of lanthanum nitrate hexahydrate ($\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$) were added into the reaction medium. After sonication, a brown transparent solution was obtained, which was then transferred into a 100 mL Teflon-lined stainless steel autoclave. The autoclave was kept in an electric oven at 180 °C for 6 h, after which the autoclave was taken out and cooled naturally to room temperature. After that, the black precipitate was harvested and washed with ultra-pure water several times *via* centrifugation. The product was then dried at 60 °C overnight, before being calcined at 300 °C for 2 h in air to be converted into $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes.

Preparation of the electrode

As seen below, $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes was applied to coat the bare screen printed electrode. Dispersion of 1 mg of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes with ultrasonication for 1 h was used for preparing a stock solution of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes in 1 mL of the aqueous solution, whereas 2 μL of aliquots of the $\text{La}^{3+}/\text{Co}_3\text{O}_4/\text{H}_2\text{O}$ suspension solution was cast on the carbon working electrodes. Then, it remained up to the time of evaporation of the solvent in room temperature.

Preparation of real samples

Samples of urine have been kept in a refrigerator directly after gathering. Ten millilitres of samples have been centrifuged for fifteen minutes at 2,000 rpm. The supernatant has been filtered by a 0.45 μm filter. Next, various volumes of solution has been transported into a 25 millilitres volumetric flask and diluted to the mark with PBS (pH= 7.0). This diluted urine samples were anaesthetized with different amounts of vitamin B₉. Content of vitamin B₉ have been

analyzed by the suggested procedure by employing the standard addition method.

Five vitamin B₉ pill (with the labling 100 mg per tablet, Tehran Chemie Pharmaceutical Co., Iran) have been ground. Afterwards, the pill solution has been prepared *via* dissolving 500 mg of powder in 25 mL water through ultra-sonication. Next, various volumes of the diluted solution has been transported in a 25 mL volumetric flask and diluted to the mark with PBS (pH= 7.0). The vitamin B₉ contents have been analyzed by the recommended technique *via* the standard addition method.

Result and discussion

Nanostructures characterization

Figure 1 displays the Fourier Transform Infrared (FT-IR) spectra of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes sample in the frequency range of 400–4000 cm^{-1} . The Fourier Transform Infrared spectrum of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes showed strong vibrational bands in the lower frequency regions (about 400–600 cm^{-1}), ascribable to the vibration of metal–O. The absorption seen at $\sim 3389 \text{ cm}^{-1}$ is thought to be due to the symmetric vibration of the -OH groups of the absorbed H_2O molecules. The peak at $\sim 1632 \text{ cm}^{-1}$ corresponds to O-H groups, again related to adsorbed H_2O molecules [25].

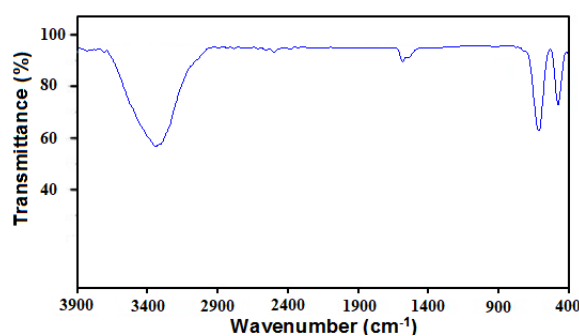


FIGURE 1 Fourier-transform infrared spectroscopy (FTIR) image of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes

The main characteristic diffraction peaks of the $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes are consistent with

the standard patterns of Co_3O_4 (JCPDS card No. 71-0816) with cubic spinel phase, suggesting that the doping of La will not change the backbone of pristine Co_3O_4 (Figure 2). As increase of La in the Co_3O_4 , the characteristic peak at $2\theta = 37.3^\circ$ become weaker and slightly shift to small-angle reflections with increase of La in the Co_3O_4 . The introduction of the La ions with f electronic and large atomic radiuses into the Co_3O_4 grain boundary causes the loss of atoms degree of order of Co_3O_4 , thereby contributing to the limited growth of the grain, grain refinement and the decrease of crystallinity [26].

The broadness of the diffraction peaks suggests the nano-sized nature of the product and the average crystallite size (t) of it was calculated using the Debye–Scherrer formula as 67.0 nm. $t = 0.9 \lambda / \beta \cos(\theta)$ where λ is the wavelength of the X-ray radiation (1.54056 Å for Cu lamp), θ is the diffraction angle and β is the full width at half-maximum (FWHM).

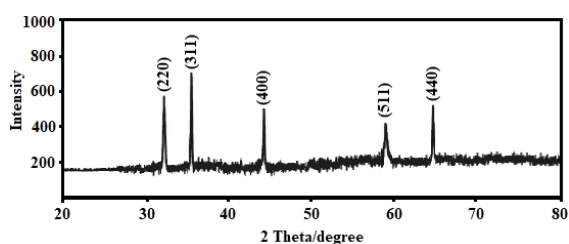


FIGURE 2 X-ray diffraction (XRD) pattern of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes

The morphology of the product was examined by scanning electron microscope (SEM). Figure 3 depicts the scanning electron microscope pictures of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanoparticles. From the graph, it was

observed that the nanoparticles, are nanocubes and they are seen as less than 70 nm.

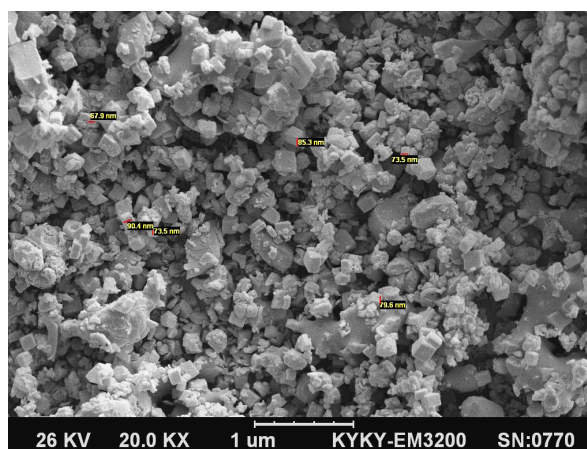


FIGURE 3 Scanning electron microscopic (SEM) image of La^{3+} -doped Co_3O_4 nanocubes

Electrochemical behaviour of vitamin B₉ at the surface of various electrodes

The electrochemical behaviour of vitamin B₉ depends on the pH value of the aqueous solution (Figure 4). Thus, it is essential to optimize the solution pH in order to gain more useful results for electro-oxidation of vitamin B₉. Therefore, vitamin B₉ electrochemical behaviour was examined in 0.1 M PBS at distinct pH numbers (2.0–9.0) at $\text{La}^{3+}/\text{Co}_3\text{O}_4/\text{SPE}$ surface by voltammetry. The results indicated more advantageousness of neutral conditions for vitamin B₉ electro-oxidation at $\text{La}^{3+}/\text{Co}_3\text{O}_4/\text{SPE}$ surface in comparison to the basic or acidic medium, because the obtained current was more than other pHs. Here, pH 7.0 was selected as an optimal pH for vitamin B₉ electro-oxidation at $\text{La}^{3+}/\text{Co}_3\text{O}_4/\text{SPE}$ surface.

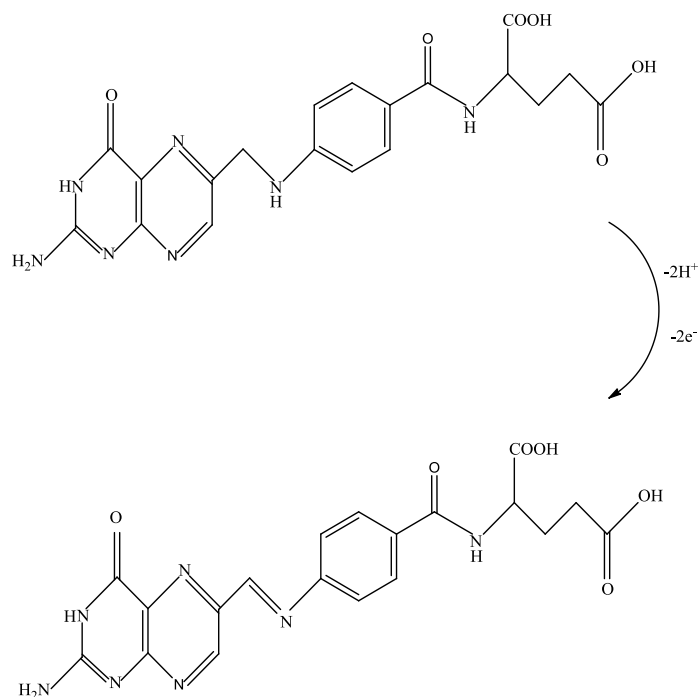


FIGURE 4 Electrochemical oxidation of vitamin B₉

Figure 5 shows responses of CV to electro-oxidation of 100.0 μM vitamin B₉ at the unmodified SPE (curve b) and La³⁺/Co₃O₄/SPE (curve a). The peak potential occurs at 620 mV due to vitamin B₉ oxidation, which is around 110 mV more negative than the unchanged SPE. Furthermore, La³⁺/Co₃O₄/SPE exhibits

very high anodic peak currents for vitamin B₉ oxidation than that of the unchanged SPE. This showed a significant improvement of the electrode performance toward vitamin B₉ oxidation by changing the constant SPE with La³⁺/Co₃O₄ nanocubes.

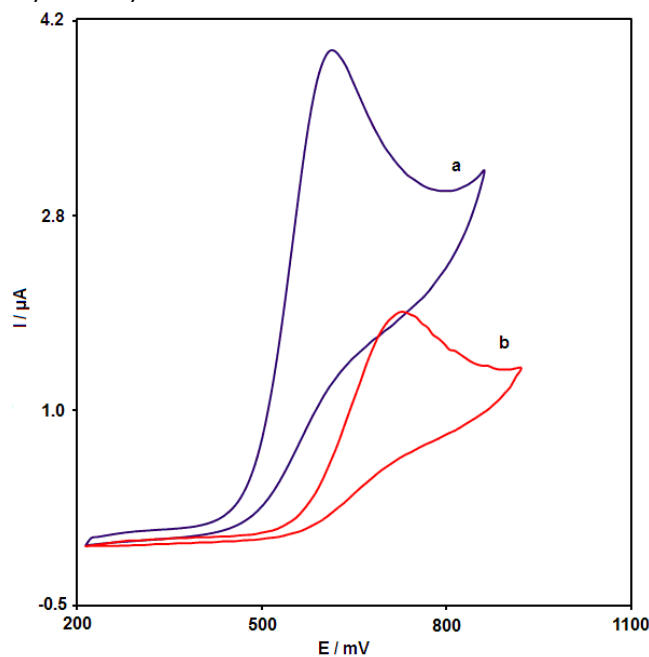


FIGURE 5 CVs of a) La³⁺/Co₃O₄ nanocubes /SPE and b) unmodified SPE in the presence of 100.0 μM of vitamin B₉ at pH 7.0. In all cases, the scan rate was 50 mV s⁻¹

Effect of scan rate

Researchers investigated the impact of the rates of potential scan on vitamin B₉ oxidation current (Figure 6). Findings indicated induction of enhancement in the current of the peak by the increased potential scan rate. Additionally, diffusion in oxidation process is monitored, as inferred by the linear dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) for vitamin B₉ (Figure 6A) [27].

Data of the ascending section of the current-voltage curve, which has been

registered at a scan rate of 10 mVs⁻¹ for vitamin B₉, was used for drawing Tafel plot (Figure 6B). Electron transfer kinetics between La³⁺/Co₃O₄/SPE and substrate (vitamin B₉) affect these sections of voltammogram that are called Tafel region. The study achieved Tafel slope of 0.1194 mV. This finding is compatible with the engagement of one electron at the rate that determines the electrode process phase [27], providing that charge transfer coefficients $\alpha = 0.5$ for vitamin B₉.

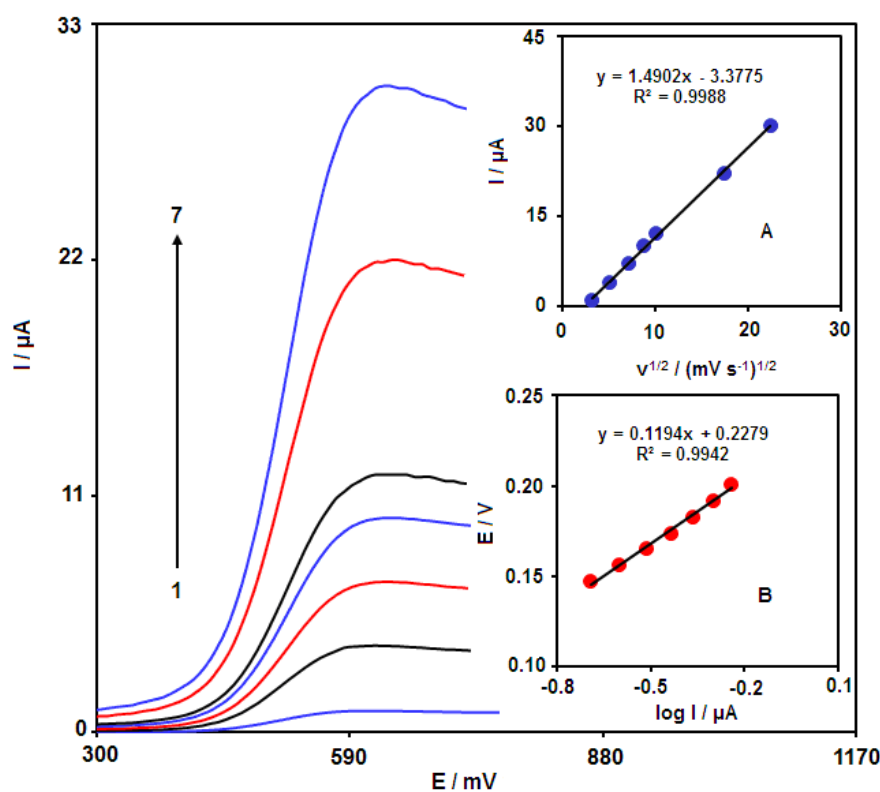


FIGURE 6 LSVs of La³⁺/Co₃O₄ nanocubes /SPE in 0.1 M PBS (pH 7.0) containing 200.0 μM of vitamin B₉ at various scan rates; numbers 1-7 correspond to 10, 25, 50, 75, 100, 300 and 500 mV s⁻¹, respectively. Inset: (A) Variation of anodic peak current vs. square root of scan rate. (B) Variation anodic peak potential vs. log v

Chronoamperometric measurements

Chronoamperometric measurements of vitamin B₉ at La³⁺/Co₃O₄/SPE were conducted by adjusting the working electrode potential at 670 mV for different concentrations of vitamin B₉ (Figure 7) in PBS (pH 7.0). For electroactive materials (vitamin B₉ in this

case) with a diffusion coefficient of D , the Cottrell equation describes current seen for electrochemical reaction at the mass transport limited condition [27]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2}$$

where D and C_b respectively represent diffusion coefficient (cm² s⁻¹) and bulk

concentration (mol cm^{-3}). Experimental plots of I versus $t^{-1/2}$ were used with the best fits for various concentrations of vitamin B₉ (Figure 7A). Then, the resultant straight lines slopes were drawn against vitamin B₉ concentrations

(Figure 7B). According to the resultant slope and the Cottrell equation, mean values of D were $2.75 \times 10^{-5} \text{ cm}^2/\text{s}$ for vitamin B₉. This value is comparable with some previous reports ($9.749 \times 10^{-5} \text{ cm}^2/\text{s}$ [28]).

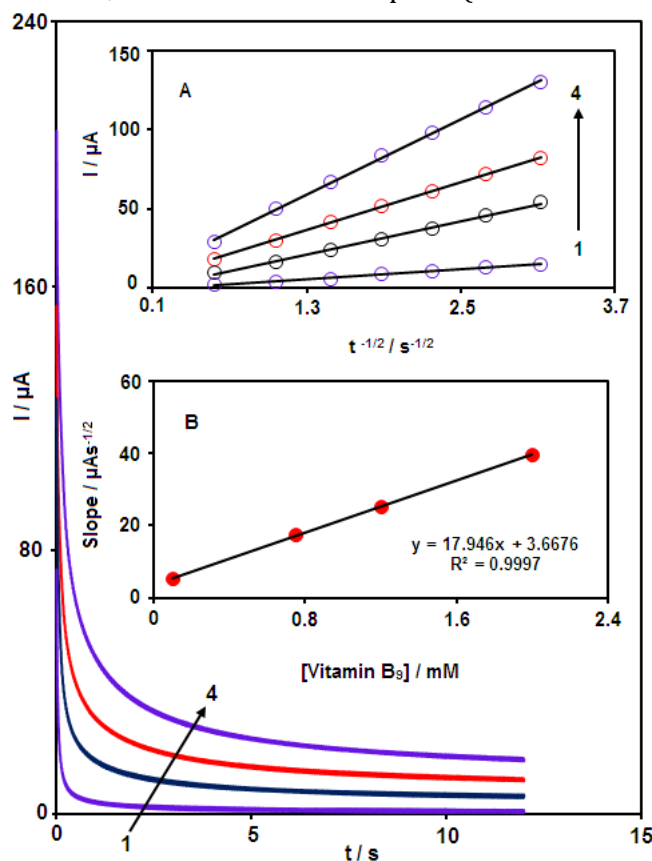


FIGURE 7 Chronoamperograms obtained at $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes/SPE in 0.1 M PBS (pH 7.0) for different concentrations of vitamin B₉. The numbers 1-4 correspond to 0.1, 0.75, 1.2 and 2.0 mM of vitamin B₉. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1-4. (B) Plot of the slope of the straight lines against vitamin B₉ concentrations

Calibration plot and limit of detection

The electro-peak currents of vitamin B₉ at $\text{La}^{3+}/\text{Co}_3\text{O}_4$ /SPE surface can be applied to define vitamin B₉ in thoxidation e solution. Since the increased sensitivity and more suitable properties for analytical utilizations are considered as the benefits of differential pulse voltammetry (DPV), $\text{La}^{3+}/\text{Co}_3\text{O}_4$ /SPE in 0.1 M PBS consisting of different distinct concentrations of vitamin B₉ was used to conduct DPV experiments (Figure 8) (Initial

potential= 440 mV, End potential=700 mV, Step potential=0.01 V and pulse amplitude=0.025 V). It was found that the electrocatalytic peak currents of vitamin B₉ oxidation at $\text{La}^{3+}/\text{Co}_3\text{O}_4$ /SPE surface linearly depended on vitamin B₉ concentrations above the range of 1.0-600.0 μM (with a correlation coefficient of 0.9993), while determination limit (3σ) was achieved to be 0.3 μM . These values are comparable with the values obtained by other researchers (Table 1).

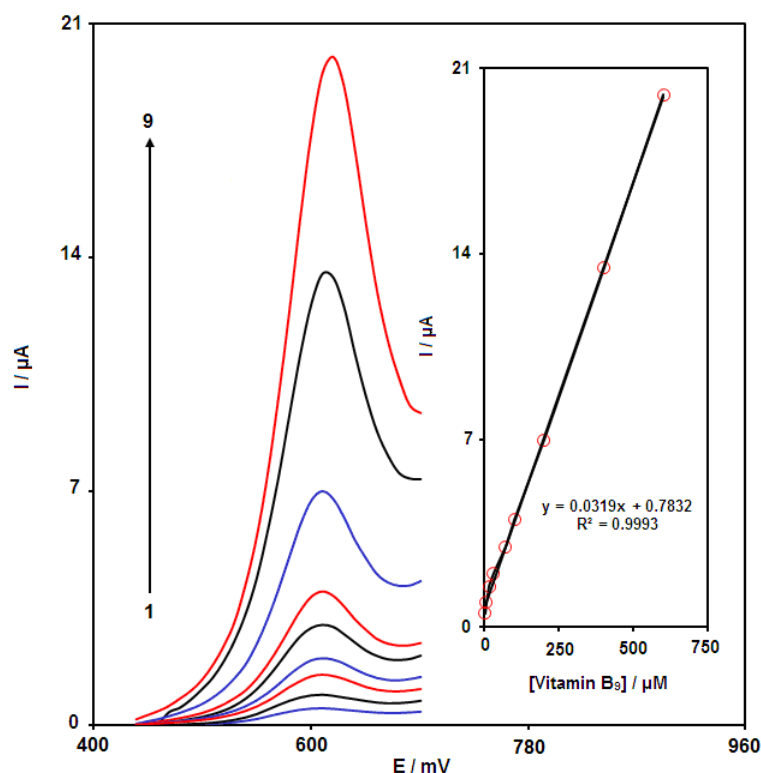


FIGURE 8 DPVs of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes /SPE in 0.1 M PBS (pH 7.0) containing different concentrations of vitamin B_9 . Numbers 1-9 correspond to 1.0, 5.0, 15.0, 30.0, 70.0, 100.0, 200.0, 400.0 and 600.0 μM of vitamin B_9 . The inset shows the plot of the peak current as a function of the vitamin B_9 concentration in the range of 1.0-600.0 μM

TABLE 1 Comparison of the efficiency of some modified electrodes used in detection of vitamin B_9

Method	Electrode	Modifier	Linear range (μM)	Detection limit (μM)	Ref
Voltammetry	Glassy carbon	MWCNT	0.3-80.0	0.13	[28]
Voltammetry	Carbon paste	MWCNT	4.6-152.0	1.1	[29]
Voltammetry	Carbon paste	(DEDE) and NiO/CNTs nanocomposite	3.0-550.0	0.9	[30]
Voltammetry	Carbon paste	Ruthenium(II) Complex-ZnO/CNTs Nanocomposite	3.0-700.0	1.0	[31]
Voltammetry	Carbon paste	ZrO ₂ nanoparticles	20.0-2500.0	9.86	[32]
Voltammetry	Screen printed	$\text{La}^{3+}/\text{Co}_3\text{O}_4$	1.0-600.0	0.3	This work

TABLE 2 Determination of vitamin B₉ in real samples. All the concentrations are in μM (n=5)

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Urine	0	-	-	-
	5.0	4.9	98.0	3.2
	10.0	10.3	103.0	2.1
	15.0	15.3	102.0	1.9
	20.0	19.9	99.5	2.8
Tablet	0	7.0	-	2.7
	2.5	9.3	97.9	1.9
	7.5	15.0	103.4	3.4
	12.5	19.8	101.5	2.4
	17.5	24.3	99.2	2.6

Real sample analysis

The method illustrated above was used to evaluate $\text{La}^{3+}/\text{Co}_3\text{O}_4$ /SPE usability for determining vitamin B₉ in real samples in order to determine vitamin B₉ in tablet and urine samples. Therefore, the standard addition technique was applied. Table 2 reports the results. Acceptable recoveries of vitamin B₉ were observed, and reproducible results were shown with regard to the mean relative standard deviation (R.S.D.).

The repeatability and stability of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes /SPE

The long-term stability of the $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes /SPE was tested over a 3-week period. When CVs were recorded after the modified electrode was stored in an atmosphere at room temperature, the peak potential for vitamin B₉ oxidation was unchanged and the current signals showed only less than 2.2 % decrease of the initial response. The antifouling properties of modified electrode towards vitamin B₉ oxidation and its oxidation products were investigated by recording the cyclic voltammograms of modified electrode before and after using in the presence of vitamin B₉. Cyclic voltammograms were recorded in the presence of vitamin B₉ after having cycled the potential for 15 cycles at a scan rate 50 mV s^{-1} . The peak potentials were unchanged to positive values and the currents decreased by

less than 2.3 %. Therefore, at the surface of $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nanocubes /SPE, not only the sensitivity increases, but the fouling effect of the analyte and its oxidation product also decreases.

Conclusion

This study aimed to develop the screen-printed electrode modified with $\text{La}^{3+}/\text{Co}_3\text{O}_4$ nano-cubes and use it as a new sensor for detecting vitamin B₉ with higher sensitivity and selectivity. Findings showed the designed instrument functions with features of portability, affordability, and disposability to determine vitamin B₉. Moreover, limit of detection for vitamin B₉ has been estimated as $0.3 \mu\text{M}$. In general, it has been viewed a very good device for analytic procedures in the clinic where determining the levels of traces of vitamin B₉ will be necessary.

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Orcid:

Somayeh Tajik: <https://orcid.org/0000-0002-1151-5515>

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