La\textsuperscript{3+}-doped Co\textsubscript{3}O\textsubscript{4} Nanoflowers Modified Graphite Screen Printed Electrode for Electrochemical Sensing of Vitamin B\textsubscript{6}

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(Received 2 June 2018, Accepted 19 August 2018)

In this study, we combined the advantages of good conductivity, small size, large surface area, and the catalytic property of La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4} nanoflowers to fabricate an electrochemical sensor sensitive for determination of vitamin B\textsubscript{6} in real samples. La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4} nanoflowers were synthesized by a co-precipitation method that is a convenient, environment-friendly, and inexpensive process. The synthesized nanoflowers were characterized by SEM. A simple and sensitive sensor based on graphite screen printed electrode (GSPE) modified by La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4} nanoflowers was developed for the electrochemical determination of vitamin B\textsubscript{6}. The electrochemical behavior of vitamin B\textsubscript{6} was studied in 0.1 M phosphate buffer solution (PBS) using cyclic voltammetry (CV), chronoamperometry (CHA), and differential pulse voltammetry (DPV). The modified electrode (La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4}NFs/GSPE) showed excellent electrocatalytic activity and remarkable sensitivity towards the oxidation of vitamin B\textsubscript{6}. The fabricated sensor displayed good operating characteristics including low detection limit, and a wide linear dynamic range for the detection of vitamin B\textsubscript{6}. Using La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4}NFs/GSPE as the working electrode, a linear dynamic range between 1.0-600.0 μM and a limit of detection of 0.4 µM were obtained. Finally, reliability and accuracy of the proposed sensor were studied in real samples.

**Keywords:** La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4} Nanoflowers, Electrochemical sensor, Vitamin B\textsubscript{6}, Real samples, Modified electrode

**INTRODUCTION**

Vitamins are vital components for self-conservation, normal growth and elaboration of animal and human bodies. They are divided into water and fat-soluble vitamins [1].

Vitamin B\textsubscript{6} (Scheme 1) belongs to water soluble B complex vitamins group, commonly called pyridoxine [2]. Vitamin B\textsubscript{6} is a significant vitamin that aids in the formation of healthy red blood cells and supports the more vital physiological metabolism [3,4]. Vitamin B\textsubscript{6} is essential for many enzymes involved in the bio-metabolism of proteins, carbohydrates, amino acids and fats [5]. Vitamin B\textsubscript{6} also supports nervous and immune systems functions [6]. Furthermore, a vitamin B\textsubscript{6} deficiency can lead to anemia that resembles an iron deficiency anemia [7]. Due to its role in human body there is a need for fast, low cost and reliable analytical procedures for the selective and sensitive determination of vitamin B\textsubscript{6}.

Various methods such as spectrophotometry [8], chemiluminescence [9], liquid chromatography [10], high performance liquid chromatography [11] and
Electrochemical techniques [12-15] have been utilized to detect vitamin B₆. Some of these methods involve complicated operations, expensive instruments, and using large amounts of toxic organic solvents or need separation processes. Electrochemical detection of biological compounds is an alternative method that has attracted lots of attention due to its simplicity, low cost, high rate, selectivity, high sensitivity and good reproducibility [16-20].

However, the voltammetric response of biological compounds is not satisfactory at the bare electrodes for analytical applications because of slow heterogeneous electron transfer, and the direct electrochemical oxidation of biological compounds at high overpotential. Therefore, chemically modified electrodes (CMEs) are excellent approaches to reduce the overpotential and increase the sensitivity of determinations by introducing a modifier to the electrode [21-34].

Screen-printed electrodes (SPEs) have received great

Scheme 2. The proposed mechanism for the oxidation of vitamin B$_6$ at the La$^{3+}$/Co$_3$O$_4$/GSPE [49]

Fig. 3. CVs of a) La$^{3+}$/Co$_3$O$_4$/SPE and b) SPE in the presence of 100.0 µM vitamin B$_6$ at a pH 7.0. In all cases the scan rate was 50 mV s$^{-1}$.

attention for the on-site monitoring as well as fast speed, high efficiency, portability, low cost and small sample size. Therefore, the use of screen-printing technology in the serial production of disposable low-cost electrodes for the electrochemical determination of a wide range of substances is currently undergoing widespread growth [35-38].

Nowadays, the nanomaterials have been attracted much attention to modify the surface of electrodes for electrochemical detection of biological compounds due to their unique properties, such as large surface area, plenty
active sites, high electronic conductivity and good chemical stability [39,40].

The lanthanides represent a group of elements from La to Lu that shows very similar physical and chemical properties. Lanthanum (La) is one of the most important lanthanide elements. La ions own relatively low toxicity and extraordinary catalytic properties, thus, those ions can be used to construct environment-friendly sensors [41-43].

Recently, electrodes modified with metal oxide nanostructures have been thoroughly investigated for the electrochemical determination of several biologically important analytes because of their interesting electrocatalytic properties. Among the various metal oxide nanoparticles, Co$_3$O$_4$ nanoparticles have recently gained more interest due to their biocompatibility, wide availability, low cost, earth abundance, good electrochemical properties, large surface area and good ability for promoting electron transfer reactions [44-46].

In this work, the preparation of an electrochemical sensor is described for the voltammetric determination of vitamin B$_6$. The sensor was designed by the modification of graphite screen printed electrodes (GSPE) with La$^{3+}$/Co$_3$O$_4$ nanoflower. The prepared modified electrode (La$^{3+}$/Co$_3$O$_4$/GSPE) showed very high performance in the electrochemical oxidation of vitamin B$_6$. The linear range and detection limit of vitamin B$_6$ were determined. Analytical application of the prepared electrode was tested by performing the determination of vitamin B$_6$ in real samples.

**EXPERIMENTAL**

**Chemicals and Apparatus**

An Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) was employed to perform the electrochemical experiments and the system was controlled using a general purpose electrochemical system software.

The screen-printed electrode (DropSens, DRP-110, Spain) consists of three conventional electrodes: graphite counter electrode, a silver pseudo-reference electrode, and an unmodified graphite working electrode. The pH was measured by a Metrohm 710 pH meter.

Vitamin B$_6$ and all other reagents were of analytical grade, and were purchased from Merck (Darmstadt, Germany). For the preparation of buffers, the orthophosphoric acid and its salts were used to provide the pH range of 2.0-9.0.

**Synthesis of La$^{3+}$/Co$_3$O$_4$ Nanoflowers**

All the chemicals used for the preparation of the nanopowders, namely cobalt acetate (Co(CH$_3$COO)$_2$.2H$_2$O), lanthanum nitrate (La(NO$_3$)$_3$.6H$_2$O), thiourea ((NH$_2$)$_2$CS) and ammonia (25% NH$_3$), were of analytical grade. All the precursors were dissolved in deionized water. During the preparation of the nano-powders, ammonia was used as a complexing agent. The La$^{3+}$-doped Co$_3$O$_4$ nanostructures were prepared by dissolving 0.46 mol of cobalt acetate in 80 ml of deionized water, 0.0046 mol of lanthanum nitrate in 80 ml of deionized water, 0.18 mol of thiourea in 80 ml of deionized water and lastly by adding 19.76 ml of ammonia in 80 ml of deionized water. The amount of solutions of cobalt acetate, thiourea and ammonia was held constant at a ratio of 1:1:1. Then, the cobalt acetate solution was added in a beaker in the reaction bath, followed by adding thiourea and lanthanum nitrate solution in the same reaction bath and the mixture was stirred for a few seconds. Lastly ammonia solution was added slowly into the mixture, while continuing stirring for 5 min. The temperature of the bath was then allowed to increase up to 80 $^\circ$C. After that the precipitates were formed and left overnight and filtered thereafter. The precipitates were then washed with ethanol. The obtained powders were dried at ambient conditions for several days.

**Preparation of the Electrode**

The bare screen-printed electrode was coated with La$^{3+}$/Co$_3$O$_4$ nanoflowers according to the following simple procedure. 1 mg La$^{3+}$/Co$_3$O$_4$ nanoflowers was dispersed in 1 ml aqueous solution within 45 min ultrasonication. Then, 5 µl of the prepared suspension was dropped on the surface of carbon working electrodes. It remains at room temperature until becomes dry.

**Preparation of Real Samples**

An ampoule of 1 ml of vitamin B$_6$ (concentration of vitamin B$_6$ of 50 mg ml$^{-1}$) was diluted to 10 ml with 0.1 M
phosphate buffer (PBS) (PH 7.0). Then, different volumes of the diluted solution were transferred into each of a series of 25 ml volumetric flasks and diluted to the mark with PBS. The vitamin B_{6} content was analyzed by the developed voltammetry procedure using the standard addition method.

Urine samples were stored in a refrigerator immediately after collection. Ten milliliters of the sample were centrifuged for 15 min at 2000 rpm. The supernatant was filtered out using a 0.45 μm filter. Then, different volumes of the solution were transferred into a 25 ml volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine sample was spiked with different amounts of vitamin B_{6}.

The vitamin B_{6} content were analyzed by the proposed method using the standard addition method.

**RESULTS AND DISCUSSION**

**Nanostructures Characterization**

The main characteristic diffraction peaks of the La^{3+}/Co_{3}O_{4} nanoflowers are consistent with the standard patterns of Co_{3}O_{4} (JCPDS card No. 71-0816) with cubic spinel phase, suggesting that the doping of La will not change the backbone of pristine Co_{3}O_{4} (Fig. 1). With increasing La in Co_{3}O_{4}, the characteristic peak at 2θ = 37.2° becomes weaker and slightly shifts to smaller angles. The
Introduction of the La ions with f electronic and large atomic radiiues into the $\text{Co}_3\text{O}_4$ grain boundary causes the loss of atoms degree of order of $\text{Co}_3\text{O}_4$, thereby contributing to the limited growth of the grain, grain refinement and the decrease of crystallinity [47].

The broadness of the diffraction peaks suggests the nano-sized nature of the product that its average crystallite size ($t$) was calculated using the Debye-Scherrer formula as $37.0 \text{ nm. } t = 0.9 \lambda \beta \cos(\theta)$, where $\lambda$ is the wavelength of the X-ray radiation (1.54056 Å for Cu lamp), $\theta$ is the diffraction angle and $\beta$ is the full width at half-maximum (FWHM) [48].

The morphology of the product was examined by SEM (Fig. 2). The SEM picture of the nanoflowers shows the formation of nanoflowers with the petals in the nanorod shape, and less than 40 nm in size.

Electrochemical Profile of the Analytes on La$^{3+}$/Co$_3$O$_4$/GSPE

To study the electrochemical behaviour of vitamin B$_6$ that is pH-dependent (Scheme 2), the optimized pH value is required to achieve the accurate results. Using the modified electrodes at various pH values ranging from 2.0-9.0 revealed that the best results for electro-oxidation of vitamin B$_6$ occur at pH = 7.0. The obtained cyclic voltammograms

in the presence of 100.0 μM vitamin B$_6$ using La$^{3+}$/Co$_3$O$_4$/GSPE the (Curve a) and bare GSPE (Curve b) are shown in Fig. 3. According to CV results, the maximum oxidation of vitamin B$_6$ on the La$^{3+}$/Co$_3$O$_4$/GSPE occurs at 750 mV that is about 100 mV more negative than that of unmodified GSPE.

**Effect of Potential Scan Rate on the Results**

The study of the effect of potential scan rates on the oxidation currents of vitamin B$_6$, shown in Fig. 4, indicates that increasing the scan rate leads to enhanced oxidation peak current. In addition, there is a linear relationship between Ip and the square root of the potential scan rate ($ν^{1/2}$) demonstrating that the oxidation procedure of analyte is in the control of diffusion. The Tafel curve of analyte was plotted applying the data from the rising sections (i.e. the Tafel regions) of the current-voltage curve obtained at 10 mV s$^{-1}$ (Fig. 5). The kinetics of electron transfer in the electrode reactions, influence on the Tafel regions of the current potential curve. The Tafel slope was obtained 0.1 V in this case. Using this slope, charge transfer coefficient of $α = 0.41$ was obtained [50].
Chronoamperometric Analysis

The analysis of chronoamperometry for vitamin B\textsubscript{6} samples was performed by the use of La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4}/GSPE vs. Ag/AgCl/KCl (3.0 M) at 0.8 V. The Chronoamperometric results of different concentrations of vitamin B\textsubscript{6} sample in PBS (pH 7.0) are demonstrated in Fig. 6. The Cottrell equation for chronoamperometric analysis of electroactive moieties under mass transfer limited conditions is as follows [50]:

\[ I = nFAD^{1/2}C_b^{1/2}t^{-1/2} \]

where D represents the diffusion coefficient (cm\textsuperscript{2}s\textsuperscript{-1}), and \( C_b \) is the applied bulk concentration (mol cm\textsuperscript{-3}). Experimental results of I vs. \( t^{1/2} \) were plotted in Fig. 6A, with the best fits for different concentrations of vitamin B\textsubscript{6}. The resulted slopes corresponding to straight lines in Fig. 6A, were then plotted against the concentration of vitamin B\textsubscript{6} (Fig. 6B). On the basis of the resulting slope and Cottrell equation, the mean value of D was determined to be 5.4 \times 10^{-6} cm\textsuperscript{2}/s.

Calibration Plot and Limit of Detection

The peak current of vitamin B\textsubscript{6} oxidation at the surface of La\textsuperscript{3+}/Co\textsubscript{3}O\textsubscript{4}/GSPE can be used for determination of vitamin B\textsubscript{6} in the solution (Fig. 7). Hence, differential pulse

Table 1. Comparison of the Efficiency of some Modified Electrodes Reported for the Detection of Vitamin B$_6$

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Modifier</th>
<th>Method</th>
<th>LDR (M)</th>
<th>LOD (M)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glassy carbon electrode</td>
<td>Carbon nanotubes mixed with ionic liquid crystal and CuO nanoparticles</td>
<td>Voltammetry</td>
<td>$5.0 \times 10^{-7}$-$5.0 \times 10^{-5}$</td>
<td>$3.09 \times 10^{-9}$</td>
<td>[1]</td>
</tr>
<tr>
<td>Boron-doped diamond electrode</td>
<td>-</td>
<td>Voltammetry</td>
<td>$7.0 \times 10^{-6}$-$4.7 \times 10^{-5}$</td>
<td>$3.76 \times 10^{-6}$</td>
<td>[2]</td>
</tr>
<tr>
<td>Glassy carbon electrode</td>
<td>Silver doped poly(L-arginine)</td>
<td>Voltammetry</td>
<td>$1.0 \times 10^{-3}$-$3.0 \times 10^{-3}$</td>
<td>$5.0 \times 10^{-6}$</td>
<td>[6]</td>
</tr>
<tr>
<td>Glassy carbon electrode</td>
<td>Au-CuO/MWCNTs</td>
<td>Voltammetry</td>
<td>$7.9 \times 10^{-7}$-$1.84 \times 10^{-5}$</td>
<td>$1.5 \times 10^{-7}$</td>
<td>[7]</td>
</tr>
<tr>
<td>Screen printed electrode</td>
<td>Ru nanoparticles-MWCNTs</td>
<td>Voltammetry</td>
<td>$2.6 \times 10^{-6}$-$2.0 \times 10^{-4}$</td>
<td>$0.8 \times 10^{-6}$</td>
<td>[14]</td>
</tr>
<tr>
<td>Screen printed electrode</td>
<td>La$^{3+}$/Co$_3$O$_4$ Nanoflowers</td>
<td>Voltammetry</td>
<td>$1.0 \times 10^{-6}$-$6.0 \times 10^{-4}$</td>
<td>$4.0 \times 10^{-7}$</td>
<td>This work</td>
</tr>
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</table>

Table 2. Determination of Vitamin B$_6$ in Real Samples. Concentrations are in μM (n = 5)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked</th>
<th>Found</th>
<th>Recovery (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.0</td>
<td>-</td>
<td>-</td>
<td>3.2</td>
</tr>
<tr>
<td>Vitamin B$_6$ ampoule</td>
<td>2.5</td>
<td>7.4</td>
<td>98.7</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>10.1</td>
<td>101.0</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>12.4</td>
<td>99.2</td>
<td>2.3</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urine</td>
<td>10.0</td>
<td>9.8</td>
<td>98.0</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>15.5</td>
<td>103.3</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>20.1</td>
<td>100.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>
voltammetry (DPV) experiments were carried out for different concentrations of vitamin B$_6$. The oxidation peak currents of vitamin B$_6$ at the electrode surface were proportional to the concentration of the vitamin B$_6$ in the range of 1.0-600.0 µM. The detection limit (3σ) of vitamin B$_6$ was found to be 0.4 µM. Table 1 shows a comparison of analytical properties for the detection of vitamin B$_6$ at the prepared electrode in this work and various electrodes.

Interferences Study

The influence of various substances as compounds potentially interfering in determination of vitamin B$_6$ was studied under optimum conditions with 30.0 µM vitamin B$_6$ at pH 7.0. The potentially interfering substances were chosen from the group of substances commonly found with vitamin B$_6$ in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of interfering substance causing an error less than ±5% in determination of vitamin B$_6$. According to the results, glucose, sucrose, lactose, fructose, citric acid, methanol, ethanol, Mg$^{2+}$, SO$_4^{2-}$, Al$^{3+}$, NH$_4^+$, Fe$^{2+}$, Fe$^{3+}$, CO$_3^{2-}$, Cl$^-$ or F$^-$, alanine, methionine, phenylalanine, glycine, folic acid (vitamin B$_9$), saturated starch solution, urea, dopamine, ascorbic acid, epinephrine and norepinephrine did not interfere in determination of vitamin B$_6$.

Analysis of Real Samples

In order to evaluate the analytical applicability of vitamin B$_6$, the proposed method was also applied for determination of vitamin B$_6$ in vitamin B$_6$ ampoule and urine samples, using a standard addition method. The results for determination of the vitamin B$_6$ in real samples are given in Table 2. Satisfactory recoveries of the experimental results were found for vitamin B$_6$. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

CONCLUSIONS

La$^{3+}$/Co$_2$O$_4$ nanoflowers were synthesized and used for modification of GSPE. The modified electrode exhibited good electrochemical sensor applications for vitamin B$_6$ determination. The proposed method showed good sensitivity and a low detection limit (0.4 µM) for vitamin B$_6$ determination. In addition, the good recovery results were applicable to the real sample analysis.

REFERENCES

La3+ doped Co3O4 Nanoflowers Modified Graphite Screen