Electrochemical determination of vitamin B$_6$ (pyridoxine) by reformed carbon paste electrode with iron oxide nanoparticles

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Abstract
This work aims to introduce a simple high-performance sensitive electrochemical sensor of reformed carbon paste (CP) with iron oxide nanoparticles (IONs). It was developed to determine one of the most important drugs called vitamin B$_6$ in a medium of phosphate buffer (PBS) over pH ranging from 5.0 to 8.0. The electrochemical and surface characterization was achieved by different techniques comprising electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), differential pulse voltammetry (DPV), and scanning electron microscopy (SEM). The studied effects were, pH, buffer, interference, scan rate, accumulation time, and calibration plot. The sensitive electrode produced a linear calibration curve in a concentration range from 8.88 up to 1000.0 µM, with limits of detection and qualification estimated at 9.06 and 30.2 µM, respectively. Also, this method was established in human urine real samples and pharmaceutical drugs which have been shown a good result for vitamin B$_6$ detection.

Keywords Vitamin B$_6$ · Iron oxide NPs · Phosphate buffer · Urine · Centrum multivitamin

Introduction
Every day, our body produces muscles, skin, and bones; also, it sends thousands of nerve signals between body and brain. But to have the opportunity for doing all of this, some useful raw materials are needed by your body, which contains 30 or more vitamins, minerals, and certain nutrients that the body cannot manufacture in enough quantities by itself [1]. Vitamins are nutrients essential for doing a various important bodily function as the generation of energy and red blood cell productions for managing anemia. It needs our body about 13 vitamins; eight of them construct the water soluble of a group B vitamins. One of such things is vitamin B$_6$ (pyridoxine) which acts as antioxidant, aids in the metabolism of protein, helps in neurotransmitters and hemoglobin production [2, 3]; may improve mood by reducing depression symptoms [4–7]; may be useful in treating symptoms of PMS [8]; handles nausea during pregnancy [9], promotes eye health for preventing eye diseases [10–14]; reduces Alzheimer’s [15–17], heart disease [18–21], and cancer risks [22, 23]; and may treat inflammation associated with rheumatoid arthritis [24–27]. Vitamin B$_6$-rich foods are chickpeas, turkey, fish (salmon, tuna), starchy vegetables, potatoes, bananas, meats, organ meats, fortified cereals, and whole grains [28]. The RDA (recommended dietary allowance) is 1.7 mg and 1.5 mg for men and women over 50, respectively [2, 29, 30].

Several studies have examined numerous analytical approaches for detecting vitamin B$_6$ including HPLC, spectrophotometric methods, electrophoresis, and fluorimetric methods. All those methods show little flexibility and portability, and also, they are time consuming. Electrocatalytic methods have received great attention in present as they show simple procedure, high sensitivity, fast response, and good accuracy [31–35]. Carbon paste electrodes (CPEs) and chemically modified CPEs are well recognized due to their vast applicable potential window, chemical inertness, low residual background current, ease of fabrication, low cost, excellent stability, low detection limits, and safe disposability after the use with diverse sorts of modulators [31, 36, 37]. Various previous studies for electrochemical techniques used to detect vitamin B$_6$ were mentioned as the determination of vitamin B$_6$ by using vanadium(III) Schiff base complex modified GCE [38], determination of vitamin B$_6$ in preparation of pharmaceutical...
compounds by cyclic voltammetry with a copper(II) hexacyano-ferrate(III) modified CPE [39], voltammetric sensor for simultaneous determination of vitamin B₆ and vitamin C using ZrO₂ nanoparticle/ionic liquids CPE in food samples [40]. So, we can say that electrochemical sensors are ideal systems to monitor insert of biological compound using portable devices especially when using the low-cost carbon paste electrode doped with nanoparticle like iron oxide nanoparticles (IONs) in this study [36, 41–44].

Nanomaterials combining electrochemical sensors for vitamin B₆ assurance are an amazing class of materials because of their special multi-functionality. Iron oxides (compounds) are a natural compound that could be synthesized easily in lab as a result of combination of iron and oxygen chemically. There are about 16 identified iron oxides including oxides, hydroxides, and oxide-hydroxides. These minerals are produced as a result of aqueous reactions under various pH and redox conditions. O, Fe, and/or OH are included in the basic composition, but differ in the valence of iron and the overall crystal structure [45–47]. IONs consist of maghemite (γ-Fe₂O₃) and/or magnetite (Fe₃O₄) particles with a ranging diameter from 1 to 100 nm.

Applications are found in drug delivery, magnetic data storage, biosensing, etc. [48–52]. Because of IONS’ super-paramagnetic properties, they had attracted a considerable interest, and hence, their potential for biomedical applications are arising from its nontoxicity and biocompatibility [53, 54]. Recently, IONs have attracted a great attention due to their unique properties like superparamagnetic, easy separation methodology, surface-to-volume ratio, and a greater surface area. Using a suitable surface chemistry, many chemical, biological, and physical methods have been adopted to synthesize magnetic NPs. Also, it exhibits great prospective in the fields of life sciences like agriculture, environment, and biomedicine [52, 54]. The convenience of using the iron oxide nanoparticle-modified carbon paste electrode (IONCPE) in sensing vitamin B₆ was checked using different voltammetric techniques. This proposed modification for vitamin B₆ detection has confirmed its utility as a sensitive, fast, and easily fabricated electrode with low cost. Both urine and centrum multivitamin samples showed an adequate response toward the newly proposed electrochemical sensor. Till now, no approved article has appeared to be dealing with the detection of vitamin B₆ using IONCPE near the biological pH.

**Experimental**

**Chemicals and reagents**

Vitamin B₆ in its pure form (≥ 99.0%) used to prepare its stock solution was purchased from Pharaonia Pharmaceuticals (Egypt). Sodium phosphate dibasic, sodium phosphate monobasic, ammonium hydroxide, sulfuric, hydrochloric, and phosphoric acids used in preparing 0.1 M supporting electrolytes were all purchased from Sigma-Aldrich. Graphite microparticles (<50 μm) was purchased from Merck, Darmstadt, Germany, and mixed with paraffin oil obtained from Aldrich, USA, to prepare CPE. Ferric chloride (FeCl₃) from El-Nasr Pharma and Ferrous sulphate (FeSO₄) from Riedl de Haen were used to prepare IONs.

Ascorbic acid from Merck and caffeine powder from Alpha Chemika (Mumbai, India) were used for the interference studies. Glucose, sucrose, starch, urea, and uric acid were obtained from MISR-Scientific Company. L-(+)-Ascorbic acid was brought from Alpha-Chem., India.

**Instruments and methodology**

The entire electrochemical experiments were done at room temperature in an ordinary 25.0 mL of three-electrode cell by applying the bare electrode or the IONCPE as working electrode (WE), a platinum rod as counter electrode (CE), and a saturated calomel electrode as reference electrode (RE).

This electrochemical cell was in connection with a Bio-logic SAS model SP-150 potentiostat supplied and a computer-controlled EC-Lab® electrochemical software, France, in order to perform all the electrochemical measurements including cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS).

EIS measurements were accomplished using 10 mV ac amplitude over a range of frequency of 100 mHz to 100 kHz. The EC-Lab® software was used to analyze and fit the data with a superlative equivalent model circuit. To determine the pH of the solutions, Adwa 1030 digital pH meter (Romania) was linked to a glass electrode and utilized. Analyzing the electrode’s surface morphology and composition were done using a scanning electron microscope (SEM) (Model Quanta 250 Field Emission Gun) attached with energy-dispersive X-ray (EDX) unit (FEI Company, Japan).

To reach a reproducible result, measurements were repeated for at least three times.

**ION preparations**

A simple method for preparing IONs by preparing 50.0 mL of 0.2 M Fe⁺² salt solution (FeCl₃) and 50.0 mL of 0.1 M Fe⁺² (FeSO₄) in a proper cup and stirred for 30 min in water bath followed by heating. After that, NH₄OH was added drop wisely until pH 11.0 achieved and stirred further for 20.0 min in the same conditions, finally, let it to cool at room temperature.
IONCPE preparations

IONCPE sensor could be synthesized by hand blending 5.0 g of graphite powder with 3.0 mL of paraffin oil in a clean mortar for about 10 min to obtain a homogeneous paste [55]. After that, 0.05 g of IONs were chemically mixed with the acquired CP; the resulting mixture were then used to fill a hole of 3.0 mm diameter at a Teflon tube end to prepare the IONCPE. Finally, the electrode’s surface was refined and smoothed using a sand paper.

Sensor applications and preparation of real sample

Using two different samples (urine and Centrum multivitamin) and amount of vitamin B6 is being measured using a proper way.

Firstly, for detecting the Vitamin in urine samples we added 0.25 mL of urine to 100.0 mL, 0.1 M of PBS (pH 6.0), (1:400), in order to get rid of any matrix effect. After that we take 22.5 mL from this mixture in the electrochemical cell then make addition till we reach 25.0 mL wisely using vitamin B6 through the standard addition method.

Second, pharmacological samples were prepared by dissolving one tablet into 250.0 mL of distilled water. Then, 2.5 mL from the drug solution were added to 20.0 mL of 0.1 M of PBS (pH 6.0) and gather it with the electrochemical cell and make additions till reaching 25.0 mL wisely using vitamin B6.

Results and discussion

Characterizations of the surface

The surface of the modified electrode morphology was assigned using EDX and SEM analysis as presented in Fig. 1.

CP in Fig. 1A seems like a compact and opaque surface that enhances the good compatibility of graphite powder with the mineral oil and paste formation. The surface of the modified electrode was significantly different, exhibiting additional colony of well mixed iron nanoparticles with the CPE to give the new IONCPE in Fig. 1B. In the same manner, a further magnification for the electrode (Fig. 1C, D) shows the average size of the ION which is nearly 200 nm in size. The existence of iron, oxygen, and carbon peaks was confirmed by EDX data of the IONCPE surface as shown in Fig. 1E, indicating the well electrode preparation.

Electrochemical functioning of vitamin B6 at IONCPE modified sensor

To improve the measurement conditions, different types of buffer solutions such as 0.1 M of Britton–Robinson buffer (B-R), H2SO4, HCl, and 0.1 M of phosphate buffer solution (PBS) were tried out for using as supporting electrolytes. From Fig. 2A, we can see that there is approximately no peak in the case of HCl and H2SO4 solutions. On the other hand, using B-R buffer solution gives wide and broad peak, while PBS exhibited a sharp and clear peak, so making it the proper working solution.

In order to develop any electrochemical sensor, it is necessary to know its performance toward vitamin B6 compared with bare CPE. Checking the sensitivity and validation of using the IONCPE in detecting vitamin B6 was done by CV technique. Figure 2B illustrates the CVs of CPE and IONCPE in 0.1 M of PBS at (pH = 6.0) as supporting electrolyte containing 1.0 mM vitamin B6 with scan rate 0.05 Vs−1. Obviously now the oxidation of vitamin B6 on IONCPE is an irreversible reaction with clear separate oxidation peak appears at peak potential of about 0.78 V and brilliant peak current around 140.0 µA, which is approximately 9.3 times greater than the one of the bare electrode (15.0 µA).

Electrochemical impedance spectroscopy (EIS) studies including both Bode and Nyquist plots shown in Fig. 2C and D, respectively, is an effective tool to confirm the results of the CVs and assure that a higher electrocatalytic activity is attained by IONCPE towards the oxidation of 1.0 mM vitamin B6 than the bare CPE. The Nyquist plot is a semicircle part which indicates that the process is not mainly diffusion but charge transfer dependent. The best fit to the data done using an equivalent circuit shown in Fig. 2D inset with an acceptable error of an average 1%, where R1 represents the resistance of the solution; Q2 is “the constant phase element, CPE,” which related in parallel combination to R2, “the resistance of outer layers”; and C1 is “the double-layer capacitance,” which also combined parallel with R3, “the inner layer resistance” [56–60]. Due to surface roughness, an empirical exponent (α = 0 to 1) is proposed to monitor the deviation from a capacitive ideality behavior. For ideal capacitor, it is always corresponding to α = 1, whereas at α = 0.5, it becomes the CPE in Warburg component [61–66]. Table 1 introduces the greatest fitting values obtained from the equivalent circuit for the two electrodes’ impedance data, BCPE and IONCPE. The resistance value of the solution, R1, was almost constant for each electrode within the experimental errors limits. IONCPE demonstrate relatively greater capacitance values or lower impedance values compared to BCPE, indicating a more conducting behavior and to confirm the highest oxidation peak current that was achieved from CV results.

Effect of solution pH

The CV technique revealed the effect of changing the pH of the solution on the electro-oxidation of vitamin B6 at a concentration of 1.0 mM in 0.1 M PBS (pH 5.0–7.0) at
IONCPE (Fig. 3A). It is clear that the electro-oxidation of vitamin B₆ is pH dependent due to the complex distribution of species resulting from acid base and hydration equilibria. In an acidic environment (pH 5.0), the current peak expands and begins to disappear, which can be attributed to the protonation of the vitamin B₆ molecule, which is electroactive only in its unprotonated form. As the pH
increases, an optimal peak appears and for this work so that the optimal operating pH has been set to be 6.0.

The pH influence on the current peak (Fig. 3A, inset) shows that the current increases as the pH rises from 5.0 to 6.0, then drops as the pH rises, confirming the process of vitamin B₆ protonation in an acidic media and becoming electro-inactive.

Also, the peak current was decreasing with increased solution pH and almost highest in the pH 6.0 as it is expected that the vitamin is in an anionic form only in very strong basic solution (pH > 9.0) due to the deprotonating of the (−OH) group bonded to the pyridinic ring. This may also cause to a decrease in the peak current. On the other hand, decreases of anodic peak current in the high basic pH may also be attributed to the changes in electrostatic interaction between vitamin B₆ and IONs and the changes in electrode reaction rates. Also, the influence of changing pH of PBS was studied using Nyquist and Bode plots (Fig. 3B). It gives the same tendency as CVs. It shows that the lowest value of impedance is obtained with lowest semicircle diameter for pH = 6.0 and the highest value of conductivity.

The change of the anodic peak potential $E_p$ for the oxidation of vitamin B₆ as a function of pH is demonstrated

### Table 1

| R₁ + (Q₂/R₂) + (C₃/R₃) | Value       | BCPE | IONCPE |
|------------------------|-------------|------|--------|
| R₁ (kΩ)                | 0.109       | 0.089|
| Q₂ (µF)                | 47.65       | 105.7|
| α                      | 0.809       | 0.737|
| R₂ (kΩ)                | 0.389       | 0.132|
| C₃ (µF)                | 3.98 x 10⁻⁶ | 0.652 x 10⁻³ |
| R₃ (kΩ)                | 0.194       | 21.70|

Fig. 2 A CVs of 1.0 mM vitamin B₆ at scan rate 0.05 V s⁻¹ using four different supporting electrolytes. B CVs of 1.0 mM vitamin B₆ at BCPE and IONCPE in 0.1 M PBS (pH 6.0) and scan rate 0.05 V s⁻¹.

C, D EIS (in the form of Bode and Nyquist plots, respectively) technique at 1.0 mM vitamin B₆ and in 0.1 M PBS (pH 6.0). D (inset) The most optimum equivalent circuit
in Fig. 3C. The anodic $E_p$ shifted negatively with enlarging pH value according to a linear relation between pH and the potential peak corresponding to the following equation:

$$E_p (V) = 1.01 - 0.022 \text{pH} \ (r^2 = 0.930)$$

(1)

With a proton-transfer step in the electro-oxidation of vitamin B$_6$, the anodic peak potential dependence on pH is demonstrated. The slope (0.022 V/pH) differs from the ideal Nernstian slope (0.059 V/pH) at 25 °C, indicating that the number of transferred electrons and protons are not equal, which could be due to the oxidation mechanism’s complexity.

**Effect of scan rate**

The scan rate effect on the anodic peak current of 1.0 mM vitamin B$_6$ in 0.1 M PBS was demonstrated using the CV technique (Fig. 4A). We can see that increasing the scanning rate in a very wide range from 0.01 to 2.0 Vs$^{-1}$ increases the peak current and also shifts the peak potential to more positive values, ensuring irreversible electrochemical oxidation [67].

A linear relationship between the anodic peak current and the square root of the scan rate (Fig. 4B) asserts the presence of a diffusion-controlled mechanism and represented as:
1 3

Also, a linear relationship between \( \log(I) \) versus \( \log(\nu) \) with slope of 0.37 (Fig. 4C) indicates an adsorption-controlled mechanism which reflects that CPEs are excellent for adsorbing particles on its active surface through the electrode reaction, due to their surface passivation. So overall, it is a mixed diffusion–adsorption mechanism.

The kinetic parameters from Fig. 4D are determined using the Laviron model (Eq. 4), which represents a linear relationship between the peak potential and the logarithm of scan rate and is represented as follows [68]:

\[
I(\mu A) = 1.78 \nu^{1/2} + 7.12; \quad r^2 = 0.97 \text{ for BCPE} \tag{2}
\]

\[
I(\mu A) = 13.25 \nu^{1/2} + 43.86; \quad r^2 = 0.99 \text{ for IONCPE} \tag{3}
\]

Also, a linear relationship between \( \log(I) \) versus \( \log(\nu) \) with slope of 0.37 (Fig. 4C) indicates an adsorption-controlled mechanism which reflects that CPEs are excellent for adsorbing particles on its active surface through the electrode reaction, due to their surface passivation. So overall, it is a mixed diffusion–adsorption mechanism.

The kinetic parameters from Fig. 4D are determined using the Laviron model (Eq. 4), which represents a linear relationship between the peak potential and the logarithm of scan rate and is represented as follows [68]:

\[
E_{pa} = E^0 + 2.3 RT/[(1 - \alpha)nF] \times \log \nu
\]

\[
E_{pa}(V) = 0.67 + 0.08 \log \nu(Vs^{-1}) \quad (r^2 = 0.984) \tag{4}
\]

where \( \alpha \) is the electron-transfer coefficient, which for irreversible processes is around 0.4–0.6 [69] and \( n \) is the number of electrons exchanged in the electro-oxidation reaction, which for vitamin \( \text{B}_6 \) was calculated from Eq. 4 to be approximately two electrons [70].

The active surface area of IONCPE was assigned using the CV technique in 1.0 mM \( \text{K}_4\text{Fe(CN)}_6 \) and 0.1 M KCl as an electrolyte. By applying the Randles–Svćik equation (Eq. 5) [58, 71]:

\[
I_{pa} = (2.69 \times 10^5)n^{3/2}ACD^{1/2}\nu^{1/2}
\]
where $I_{pa}$ is the anodic peak current ($A$), $n$ is the number of transferred electrons through the redox reaction and is equal to 1, $A$ is the electro-active area of the electrode ($cm^2$), $C$ is the concentration of $K_4Fe(CN)_6$ (mol cm$^{-3}$), $D$ is the diffusion coefficient = $7.6 \times 10^{-6}$ (cm$^2$ s$^{-1}$), and $v$ is the scan rate (Vs$^{-1}$). For the BCPE, the area is calculated to be 0.069 cm$^2$. For the IONCPE, the average electro-active area is 0.439 cm$^2$.

The electro-oxidation mechanism of pyridoxine may proceed with the formation of the aldehyde (pyridoxal) and the subsequent oxidation to pyridoxic acid, according to the scheme reported in Scheme 1. In some cases, only an overall process was observed because pyridoxal is present as hemiacetal form but the free aldehyde (the electroactive one) is negligible, and then a previous chemical transformation at the electrode is necessary on the time scale of the voltammetric experiment [70].

The slope in Fig. 4B equals $(2.69 \times 10^5) n^{3/2} ACD^{1/2}$ according to the Randles–Sevcik equation and by substituting with the electrode area; the diffusion coefficients of both CPE and IONCPE were estimated to be $8.0 \times 10^{-5}$ and $25.1 \times 10^{-2}$ cm$^2$ s$^{-1}$, respectively. It is obvious that using ION as a modifier has facilitated the diffusion of vitamin B$_6$ molecules through the electrolyte as well as increasing the active surface area by a factor of 6.9.

**Effect of accumulation time**

In order to examine the response of IONCPE, CVs for 1.0 mM vitamin B$_6$ in 0.1 M of PBS solution (pH 7.4) were recorded over different time intervals (Fig. 5). A rapid increase in the anodic peak currents with the immersion time of sensor occurs, till reach maximum at 5.0 min, then decrease again till a stable plateau at high times above 25.0 min.

**Calibration curve study**

In order to validate the sensitivity of IONCPE for vitamin B$_6$ electrochemical determination, a linear relation between the anodic current peak and a different

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**Scheme 1** The suggested oxidation mechanism

**Fig. 5** The obtained CVs using the same IONCPE in 0.1 M PBS pH 6.0 containing 1.0 mM vitamin B$_6$ at increasing times (0–35.0 min). Inset: Variation of anodic peak current ($I_{pa}$) obtained for successive analyses at increasing times (0–35.0 min)
concentrations of vitamin B₆ (Fig. 6) can be represented by the linear equation:

\[ I(\mu A) = 0.95 + 0.114 C (\mu M); r^2 = 0.987 \]

Figure 6 inset shows the corresponding differential pulse curves for increasing vitamin B₆ concentrations (from 8.88 up to 1000.0 µM) in 0.1 M of PBS (pH 6.0) and scan rate 0.01 V s⁻¹ using IONCPE. Limit of quantification (LOQ) and limit of detection (LOD) were calculated by the following equations [72]:

\[ \text{LOQ} = 10 s/m \]  \hspace{1cm} (6)

\[ \text{LOD} = 3 s/m \]  \hspace{1cm} (7)

They were found to be 30.2 and 9.06 µM, respectively, which enhanced the electrode sensitivity. The reproducibility of the suggested electrode was controlled five times under the same terms by repeated measurements resulting in a relative standard deviation (RSD) of 3.7%.

Comparison with several electrochemical sensors already described for vitamin B₆ detection is given in Table 2. However, those methods are using expensive or toxic chemicals besides the difficulty in fabrication compared to the proposed modified electrode in this work. So, this method has confirmed its reliability and sensitivity for vitamin B₆ detection with a relatively low detection limit and a good selectivity.

**Commercial samples analysis**

Vitamin B₆ was detected in actual samples such as urine and pharmaceuticals to identify the application of the new sensor through DPV, by spiking the samples with standard concentrations of vitamin B₆ using the standard addition method.

Figure 7 shows the calibration curve for using the IONCP sensor with baby urine samples, which gave a straight line in a concentration range of 8–1000 µM. From the equation of the calibration curve (\( I_{pa} (\mu A) = 0.057 C (M) + 10.135 \)), the concentration of vitamin B₆ in urine samples was calculated. The correlation coefficient was \( r^2 = 0.966 \), the LOD was 52.63 µM, and the LOQ was 175.44 µM. To guarantee that the suggested approach is validated in urine samples, Table 3 shows the suggested technique of detection of vitamin B₆ for four distinct concentrations on the curve exhibits accuracy and precision; each of these was done five times.

Results are shown in Table 3, confirming that the modified electrode can be used for estimating vitamin B₆ in pharmaceutical samples with satisfactory recoveries for every sample between 98.03 and 102.0% with RSDs of 1.1–3.7%. Every measurement of the oxidation current peak was accomplished with average of five replicate measurements determining the amount in the actual samples of vitamin B₆.
Interfering materials, sensor selectivity, reproducibility, and long-term stability

To assess the impact of different interfering substances on the detection of vitamin B6 by IONCPE, a constant concentration of vitamin B6 (500 µM) was spiked with the same and doubled concentrations of different compounds, including sucrose, glucose, starch, urea, uric acid, and vitamins such as ascorbic acid (B6), thiamine hydrochloride (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), biotin (B8), folic acid (B9), and cyanocobalamin (B12). Vitamin B6 has been studied on the voltammetric response as presented in Table 4. B1, B2, B3, B5, and B8 resulted not to be electroactive in the reported system (within the explored potential range and in the chosen supporting electrolyte). Also, other vitamins such as ascorbic acid (vitamin C) were examined for selection of the suggested technique for vitamin B6. By using the same experimental conditions, CV measurements were performed and the sensor could detect both of them at separate peak potentials of 1.13 V for vitamin B6 and 0.42 V for vitamin C.

![Fig. 7](image-url) The calibration plot of vitamin B6 in PBS (pH 6.0) using IONCPE in urine real sample. Inset: The corresponding curves for increasing vitamin B6 concentrations using DPV at step potential of 0.004 V, modulation amplitude of 0.025 V and scan rate 0.01 Vs⁻¹.

### Table 2 Comparison of IONCPE to other analytical and electrochemical techniques in the literature

| Sensor name                                                                 | Technique                        | LOD (µM) | LDR (M)                  | References |
|----------------------------------------------------------------------------|----------------------------------|----------|--------------------------|------------|
| Amperometric electrochemical detection                                    | Micellar electrokinetic chromatography | 1.0      | 2.5 × 10⁻⁶–1.0 × 10⁻³    | [73]       |
| Electrochemically pretreated glassy carbon electrode                      | CV                               | 0.8      | 7.5 × 10⁻⁵–7.5 × 10⁻²   | [74]       |
| Glassy carbon electrode                                                    | CV                               | 37.0     |                          | [75]       |
| Pencil graphite electrode                                                 | CV                               | 2.81     |                          | [76]       |
| Glassy carbon electrodes                                                  | DPV                              | 0.05     |                          | [77]       |
| High performance liquid chromatography                                     | HPLC-RP method                   | 0.03     |                          | [78]       |
| MWCNTs-Mn³⁺salen                                                          | DPV                              | 0.42     |                          | [79]       |
| Coulometric electrochemical and ultraviolet detection                     | HPLC                             | 0.013    | 2.4 × 10⁻⁶–1.7 × 10⁻⁵   | [80]       |
| Multiwall carbon nanotube modified carbon-ceramic electrode              | CV                               | 0.41     | 1.2 × 10⁻⁶–6.9 × 10⁻⁴   | [81]       |
| Glassy carbon electrode modified with chromium(III) hexacyanoferrate(II) | CV                               | 0.35     | 1.3 × 10⁻⁶–1.3 × 10⁻⁵   | [82]       |
| Glassy carbon electrode                                                   | DPV                              | 0.1      | 3 × 10⁻⁷–2 × 10⁻⁴       | [83]       |
| Carbon-based electrode with ION                                            | DPV                              | 9.06     | 8.88 × 10⁻⁶–1 × 10⁻³    | This work |

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For reproducibility of IONCPE validation in terms of RSD, 5 sequential voltammetric determinations for 50 μM of vitamin B₆ have been carried out without evident vitamin B₆ peak current alteration with RSD value of 2.4%. This guarantees the accuracy of the investigated electrode. The long-term stability of the proposed procedure was assessed by keeping IONCPE in a fridge (4 °C) for a week. Then, a voltammetric measurement for vitamin B₆ was carried out, revealing a current response at 98% of the value obtained after new preparation immediately reflects high electrode storage stability.

**Conclusion**

A new selective and sensitive electrochemical sensor with excellent performance for vitamin B₆ was constructed on the basis of the modification of CPE with IONs. Several electrochemical methods were used to optimize the circumstances of measurement to achieve the best sensitive determination of vitamin B₆. The newly prepared sensor showed its efficiency in detection with a wide linear dynamic range of 8.88–1000 μM of vitamin B₆ and LOD of 9.06 μM. The proposed method was simple, easy, affordable, and sufficiently sensitive for detecting vitamin B₆ in pharmacological and baby urine real samples under physiological conditions precisely.
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