

Electrochemical Determination of Vitamin A Based on a Novel Nanoalloy Modified Carbon Paste Electrode

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Preeclampsia has been proposed to occur after a partial breakdown in tolerance during fetal development due to maternal immune maladaptation. A few of the immunomodulatory features of the hormonal vitamin A system have the potential for enhancing immunity during pregnancy. This study proposed a new carbon paste electrode (CPE) that was modified using a nanoalloy (Pt:Co) room-temperature ionic liquid (RTIL) as an extremely sensitive sensor for the voltammetric detection of vitamin A in food specimens. The proposed sensor was found to be effective for vitamin A electro-oxidation in aqueous solutions. As an extremely sensitive electrochemical technique, square-wave voltammetry (SWV) was used for the detection of vitamin A (in submicromolar amounts). The linear response range and detection limit were found to be 0.1 μM to 100 μM and 0.04 μM , respectively. The developed sensor can be used for the detection of vitamin A in tablet and food specimens.

Keywords: Vitamin A; Ionic liquid; Nanoalloy; Electrochemical determination; Square wave voltammetry

1. INTRODUCTION

As a serious, pregnancy-specific disorder, preeclampsia has been reported to affect as many as 8% of all pregnant women [1, 2]. Preeclampsia can cause mortality of the mother and offspring, can lead to an increased risk for premature birth and fetal growth restriction, and cause a series of relevant short- and long-term health implications [3-5]. This disorder is considered to be basically multifactorial, and its progression can result from immunological and environmental factors as well as (a few) modifier genes. The main cause is linked to impaired placentation, and systemic maternal responses produce the clinical signs and symptoms of the disorder. Preeclampsia has been suggested to

occur after a breakdown in the tolerance during fetal development due to maternal immune maladaptation.

For women with certain autoimmune diseases, including rheumatoid arthritis and type 1 diabetes, an increase in the occurrence of preeclampsia is often observed, which indicates the potential of an immunogenetic association between these disorders [6, 7]. An increase in the intake of vitamin A as a potent immunomodulatory agent [8] has been suggested to reduce the risk of a few immunological diseases, such as autoimmune conditions [9, 10]. Pregnancy causes pronounced changes in the metabolism of vitamin A, and the active hormonal metabolite, 1,25(OH)₂D, has been suggested to be vital for cross-talk between the mother and the fetus [11]. This work shows that preeclampsia has a reputation for pronounced alterations in vitamin A metabolism compared with normal pregnancies. In addition, some supporting evidence supports the link between preeclampsia risk and the status of vitamin A.

Thus, a sensitive and fast technique for the detection of vitamin A in food specimens urgently requires development. Many strategies have been utilized for vitamin A detection, such as spectrophotometry [12, 13], chemiluminescence [14, 15], capillary electrophoresis [16-18], and high-performance liquid chromatography (HPLC) [19-23]. Unfortunately, an electrochemical technique for vitamin A detection has not been proposed. Electrochemical strategies are accurate, simple, and sensitive, as well as low cost with high dynamic ranges, and thus, they have been increasingly attractive for biological, pharmaceutical and environmental compound detection [24-29].

Recently, a novel modifier, RTILs, has been extensively used to prepare chemically modified electrodes [30-33]. This modifier is eco-friendly and possesses many other distinct electrochemical advantages, including broader electrochemical windows and greater ionic conductivities [30, 34-37]. Nanoparticles (NPs) have been increasingly attractive in a large number of fields, including biosensors, electrocatalysis, photocatalysis, and microelectronic devices [38]. Many nanomaterials, such as nanoparticles, nanowires and carbon nanotubes, are of great significance in scientific studies. Considering their varying compositions, shapes and sizes for bioanalytical utility, the synthesis and study of nanomaterials are of vital significance.

This work proposed the preparation and subsequent utilization of a new CPE modified by a Pt:Co nanoalloy based on the binder n-hexyl-3-methylimidazolium hexafluoro phosphate. We also studied the electrochemical performance of vitamin A using a Pt:Co/IL-modified CPE, an ionic liquid (IL)-modified CPE, a Pt:Co-modified CPE and a bare CPE. Compared to other CPEs, the Pt:Co/IL-modified CPE was the best considering its significant sensitivity and reversibility.

2. EXPERIMENTS

2.1. Chemicals

All reagents were commercially available from Sigma Chemical Co. and were of analytical grade, except for the IL. The room temperature ionic liquid, n-hexyl-3-methylimidazolium hexafluoro phosphate, was ordered from Shanghai Chengjie Chemical Co. Ltd. Double-distilled water was used

for all experiments. The reagent (0.015 g) was dissolved in NaOH (0.1 mM) in a volumetric flask (100 mL) to obtain a folic acid stock solution. The phosphate buffer solution (PBS) was 0.1 M sodium dihydrogen phosphate and disodium monohydrogen phosphate plus sodium hydroxide with various pH values.

2.2. Synthesis of Pt:Co nanoparticles

1,2-Hexadecanediol (2.5 mM), 0.5 mM Co(acac)₂, and 0.5 mM Pt(acac)₂ were co-reduced after the addition of ethylene glycol (40 mL), 5 mM oleylamine and 5 mM oleic acid under N₂ flow at ambient temperature to produce monodisperse Pt:Co nanoparticles. Then, the mixture was heated at 100 °C for 0.5 h. The Co and Pt atoms were reduced after the addition of 1,2 hexadecanediol. Once the Pt and Co salts were reduced, the Co Pt nanoparticles were nucleated. This was followed by heating the mixed solution at the boiling point (180 °C) of ethylene glycol (heating rate: 5 °C) under reflux, and this temperature was maintained for 4 h to obtain a black solution. This solution was cooled to ambient temperature under N₂ flow. Afterwards, the mixed solution was mixed with ethanol (40 mL), and the black product was centrifuged to separate the precipitant and purify the product. The ethanol impurities were separated, and the black precipitant was dispersed in hexane after the addition of oleyl amine and oleic acid. The possible undispersed residue was removed after additional centrifugation at 8000 rpm for 10 min.

2.3. Preparation of the modified electrode

Graphite powder (0.85 g) was mixed with Pt:Co + paraffin (70/30, w/w) by hand to produce Pt:Co/CPE and mixed well for 40 min to obtain a uniformly wetted paste, which was subsequently packed into a glass tube. A copper wire was pushed down into the glass tube and into the back of the above mixture to achieve electrical contact. The excess paste was pushed out of the tube and was polished using weighing paper when necessary. Graphite powder (0.85 g), Pt:Co (0.15 g), liquid paraffin (0.70 g) and n-hexyl-3-methylimidazolium hexafluoro phosphate (0.3 g) were mixed together to synthesize Pt:Co/IL/CPE. A uniformly wetted paste was produced after mixing for 45 min. The Pt:Co/IL/CPE was finally synthesized after filling a portion of the paste into one glass tube as mentioned above.

2.4. Characterizations

Square-wave voltammetry, cyclic voltammetry and chronoamperometry measurements were performed using a μ -Autolab with PGSTAT (Eco Chemie, the Netherlands) operated on a PC using NOVA software. A traditional triple-electrode configuration was used. The working electrode was either an unmodified CPE, Pt:Co/IL/CPE, IL/CPE or Pt:Co/CPE, and the reference and auxiliary electrodes were Ag/AgCl/KCl_{sat} and a platinum wire, respectively. Liquid chromatography used as the comparison method, and the instrument was obtained from Waters (Milford, MA, USA). The

instrument consisted of a Model 600 E System Controller pump, a Model 700 Satellite Wisp injector and a Waters 996 Photodiode Array detector interfaced with an NEC PowerMate 386/33i personal computer and Millennium 2010 chromatogram measuring software (PDA). The column (150 x 3.9 mm LD) was a Nova-Pak C18 (4 μm , 60 \AA). The isocratic mobile phase was acetonitrile-tetrahydrofuran-water (55: 37: 8, VN) at a flow-rate of 1.5 mL min^{-1} . Detection was performed by scanning from 240–360 nm with an acquisition speed of 1 s and a resolution of 4.8 nm. Spectra were recorded after subtracting the solvent absorption. The optimum detection wavelength was 325 nm, corresponding to the absorption maximum for retinol acetate.

3. RESULTS AND DISCUSSION

The estimation of the active surface areas of the modified electrodes was achieved by monitoring the slope of the I_p versus $v^{1/2}$ plot for $\text{K}_4\text{Fe}(\text{CN})_6$ (at a certain concentration) according to the following Randles–Sevcik equation.

$$I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} v^{1/2} C_0$$

where I_{pa} is the anodic peak current, n is the electron transfer number, A is the surface area of the electrode, D_R is the diffusion coefficient, C_0 is the concentration of $\text{K}_4\text{Fe}(\text{CN})_6$, and v is the scan rate. The microscopic areas for the Pt:Co/IL-modified CPE, IL-modified CPE or Pt:Co-modified CPE and bare CPE were obtained based on the slope of the $I_{pa} - v^{1/2}$ relation for $\text{K}_4\text{Fe}(\text{CN})_6$ (1.0 mmol L^{-1}) (n equals 1 and D_R equals 7.6×10^{-6} cm^2/s) and were found to be 0.25, 0.17, 0.11 and 0.089 cm^2 , respectively. Therefore, the co-existence of ILs and Pt:Co was found to increase the active surface of the electrode.

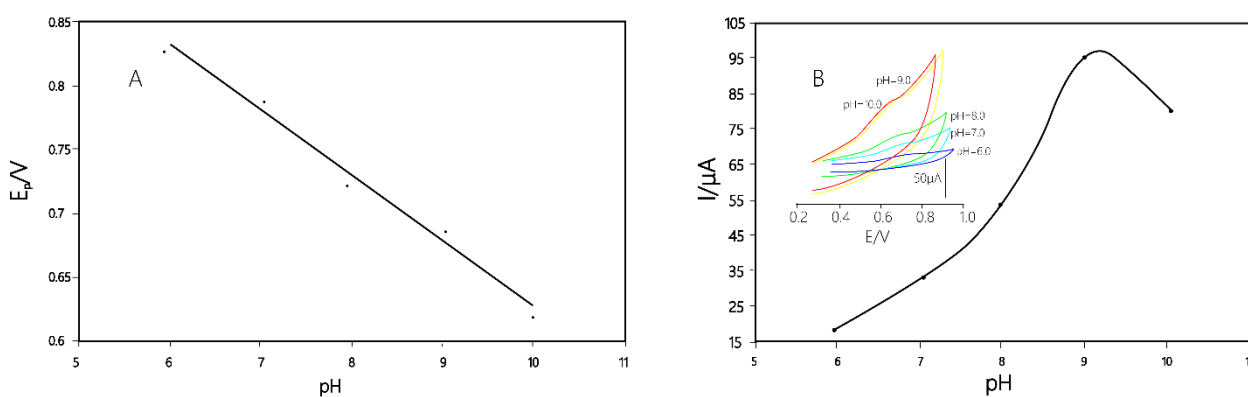


Figure 1. (A) Plot of the potential, E , vs. pH for the electro-oxidation of vitamin A on the Pt:Co/IL-modified CPE surface. (B) Current–pH profile for the electro-oxidation of vitamin A (0.45 mM) on the Pt:Co/IL-modified CPE. Effect of the pH on the cyclic voltammograms (CVs) of vitamin A on the modified electrode surface shown in the inset.

As shown in the inset of Figure 1, the voltammetric response of vitamin A in varying pH (6.0–10.0) solutions on the Pt:Co/IL-modified CPE surface was recorded. Figure 1A shows that the

potential (E) of the redox couple depended on the pH value, and the slope was -50.5 mV/pH unit at 25 °C. These data corresponded to the expected Nernstian value for a two-electron, two-proton electrochemical reaction. Thus, the modified electrode exhibited catalytic activity toward the oxidation of vitamin A. This further indicated the superiority of the Pt:Co/IL-modified CPE and indicated that the use of ILs and Pt:Co as modifiers facilitated the electron transfer between the Pt:Co/IL and the electrode [39]. As indicated in Figure 1B, the peak current reached its highest value at pH 9.0, which was then used in the following tests.

CV measurements were performed to determine the direct electrochemistry of vitamin A on the modified electrode surface. A characteristic CV for the varying electrodes in a pH 9.0 buffer solution is displayed in Figure 2. An electrochemical signal for vitamin A appeared on the bare CPE with an oxidation potential (E_{pa}) of 770 mV and an oxidation peak current (I_{pa}) of 18.6 μ A, whereas the oxidation potential (E_{pa}) and oxidation peak current (I_{pa}) were 760 mV and 22.3 μ A, respectively, for the Pt:Co-modified CPE. The slight peak potential variations suggested the slight catalytic activity of Pt:Co for the oxidization of vitamin A. Additionally, an oxidation peak was observed at 690 mV (peak current: 37.0 μ A) on the IL-modified CPE surface, suggesting an enhancement in the peak current and a decreased oxidation potential (plus a decrease in the overpotential) due to the ionic liquids (ILs) involved in the CPE. On the IL-modified CPE, an increase in the oxidation peak current was observed as the overpotential decreased. In contrast, on the Pt:Co/IL-modified CPE, an increase in the oxidation peak current to 50.8 μ A was observed as the oxidation peak potential reached 682 mV, which demonstrated the excellent electrocatalytic properties of the Pt:Co nanoalloy, which most likely resulted from the large surface-to-volume ratio, high electrical conductivity, favorable biocompatibility, excellent catalytic ability and surface reaction activity [40, 41]. As suggested by these results, the Pt:Co located on the IL surface significantly enhanced the electrochemical response, which was partially ascribed to the desirable Pt:Co features, including a high surface area, acceptable electrical conductivity and excellent chemical stability.

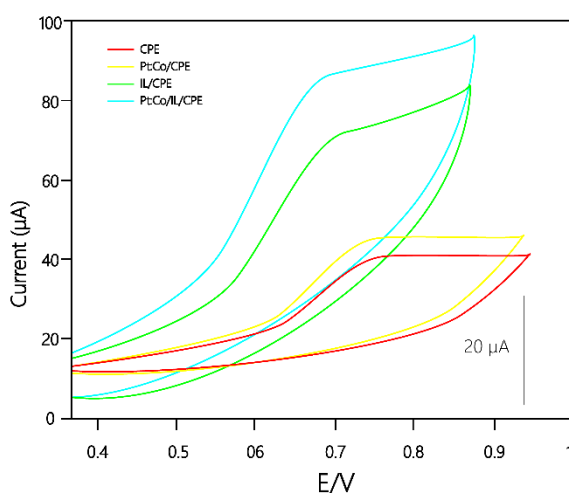


Figure 2. CVs of the bare CPE, Pt:Co-modified CPE, IL-modified CPE and Pt:Co/IL-modified CPE after adding vitamin A (0.3 mM) at pH 9.0.

The effect of the sweep rate (v) on the peak current (i_p) and peak potential (E_p) of vitamin A (0.4 mM) in PBS (pH 9) was investigated using the Pt:Co/IL-modified CPE in the range of 15–550 mV/s. A positive E_p shift appeared in the CVs (inset of Figure 3), confirming that the electro-oxidation was irreversible. The diffusion-controlled process was confirmed for the electro-oxidation of vitamin A on the Pt:Co/IL-modified CPE surface for the potential sweep rates in the studied range, which was indicated by the fact that the peak current (i_p) and $v^{1/2}$ in the equation below were linearly related.

$$I_p = 12.477v^{1/2} - 30.514$$

Additionally, the values of αn_α were obtained for the oxidation of vitamin A using the bare CPE and the Pt:Co/IL-modified CPE (pH 9.0) based on the equation below (n_α = the number of rate determining steps/involved electrons).

$$\alpha n_\alpha = 0.048 / (E_p - E_{p/2})$$

where $E_{p/2}$ represents the potential corresponding to $I_{p/2}$. The αn_α values for the Pt:Co/IL-modified CPE and bare CPE were 0.53 and 0.41, respectively, which indicated a reduction in the overpotential of vitamin A oxidation on the Pt:Co/IL-modified CPE surface along with a significant enhancement in the electron transfer rate. The increase in the I_{pa} values from the CV obtained using the Pt:Co/IL-modified CPE provided evidence for this result.

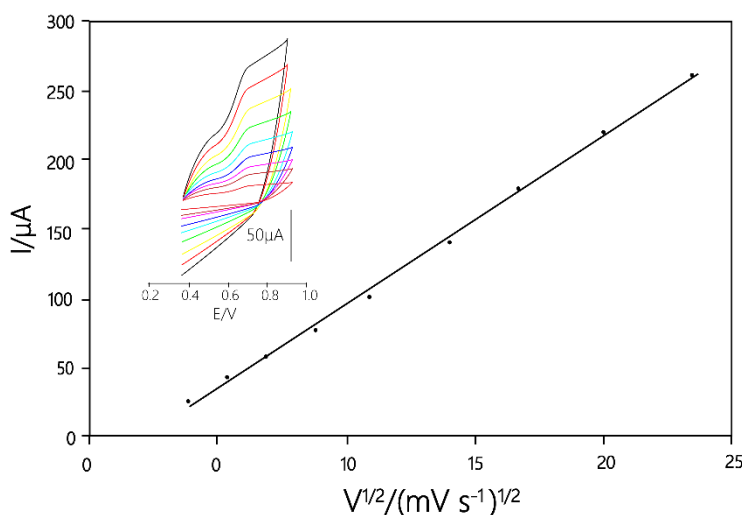


Figure 3. Plot of I_{pa} versus $v^{1/2}$ for the oxidation of vitamin A on the Pt:Co/IL-modified CPE. CVs of folic acid using the Pt:Co/IL-modified CPE in a phosphate buffer (0.1 M, pH 9.0) at different scan rates are shown in the inset.

The electro-oxidation of vitamin A on the Pt:Co/IL-modified CPE was studied using chronoamperometry and other electrochemical techniques. Figure 4 shows the chronoamperometric assays of vitamin A at varying concentrations using the Pt:Co/IL-modified CPE. The slopes of the obtained straight lines were plotted against the concentration of vitamin A, and the resultant slopes were used in the Cottrell equation [42]. With respect to an electroactive material, such as vitamin A, with a diffusion coefficient of D , the current for the electrochemical reaction (at a mass transport limited rate) is described by the Cottrell equation.

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

Under diffusion control, a plot of I versus $t^{-1/2}$ is linear. The value of D could be calculated from the slope, and its mean value was $1.25 \times 10^{-5} \text{ cm}^2/\text{s}$.

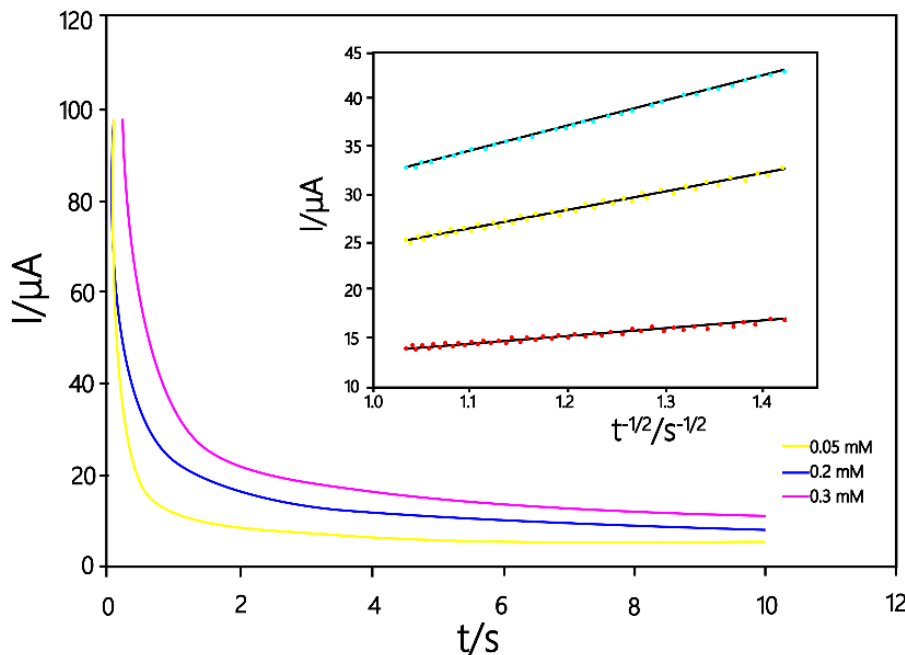


Figure 4. Chronoamperograms obtained using the Pt:Co/IL-modified CPE after adding vitamin A (0.05, 0.2 and 0.3 mM) in a pH 9.0 buffer solution. Cottrell's plot for the data from the chronoamperograms is shown in the inset.

Compared with the CV measurement, the SWV assays exhibited enhanced current sensitivity and were selected for vitamin A detection, as indicated in Figure 5A. Figure 5B and C show two linear sections to characterize the plot of the peak current vs. the vitamin A concentration. The slopes were 0.4102 and 0.1120 $\mu\text{A}/\mu\text{M L}$, and the concentration ranges were 0.1–100.0 μM and 100.0–500.0 μM , respectively. The second linear section showed a sensitivity decrease, which was ascribed to kinetic limitations. The calibration profiles exhibited slope variations, which were ascribed to the varying activity of the electrode surface upon using the analyte at high and low concentrations. Specifically, a great decrease in sensitivity was observed when the vitamin A concentration was high, which was ascribed to a smaller number of active sites (*vs.* the total number of analyte molecules) primarily at the electrode surface. In contrast, the high slope of the first calibration profile was observed when the vitamin A concentration was low, which was ascribed to the increased number of active sites (*vs.* the total number of analyte molecules). As indicated in the result analysis, the limit of detection (LOD) was 0.04 μM for vitamin A. The sensing performance of the proposed sensor was compared with recently reported sensors, as shown in Table 1.

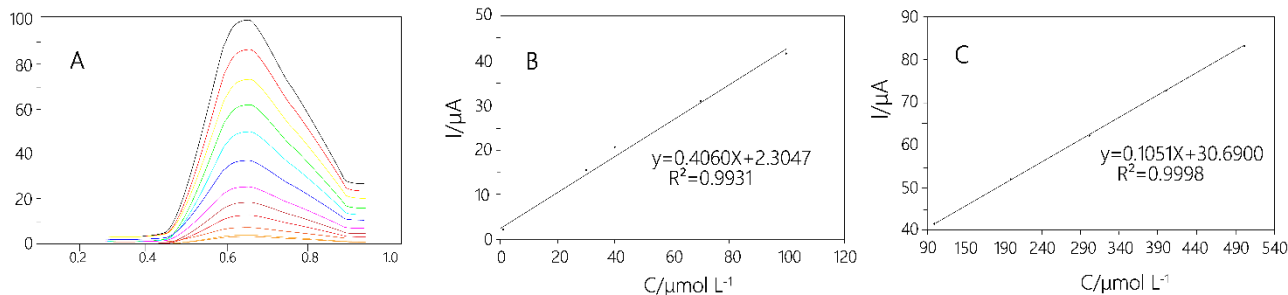


Figure 5. (A) SWVs of vitamin A at varying concentrations (0.1, 1.0, 10.0, 20.0, 30.0, 40.0, 70.0, 100.0, 200.0, 300.0, 400.0 and 500.0 μM), corresponding to those from inner to outer. Plots of the electrocatalytic peak current as a function of the vitamin A concentration in the range of (B) 0.1–100.0 μM and (C) 100.0–500.0 μM.

Table 1. Performance comparison of the Pt:Co/IL modified CPE and other vitamin A determination methods.

Method	Linear range	Detection limit	Reference
High pressure liquid chromatography	—	0.04 mM	[43]
RP-HPLC/UV	—	0.1 μM	[19]
Spectrophotometric detection	2.0–10 μM	1 μM	[44]
Pt:Co/IL modified CPE	0.1-500 μg/mL.	0.04 μM	This work

CV assays were performed to study the stability and repeatability of the Pt:Co/IL-modified CPE for vitamin A (10.0 μM) measurements, and the relative standard deviation (RSD) was 0.91% for seven successive measurements. The RSD for seven measurements was 1.9% using five varying electrodes. The decorated electrode retained 98.2% and 93.5% of its initial response after a week and 40 days, respectively, using an electrode stored in the laboratory in the dark at ambient temperature, which confirmed the applicability of the Pt:Co/IL-modified CPE with acceptable reproducibility and stability for vitamin A detection.

Table 2. Detection of vitamin A using real specimens.

Sample	Detection (μM)	Added (μM)	Found (μM)	Reference method (μM)
Cabbage 1	3.51	20	23.88	24.05
Cabbage 2	7.52	50	56.91	57.40
Tablet 1	154	50	202.5	206.3
Tablet 2	202	100	298.3	304.7

The developed technique was used to detect vitamin A in vegetable and tablet specimens to assess its analytical utility. The concentration of vitamin A in the specimens was measured using the standard addition method. A comparison between a previous technique and the developed technique is

described herein. Table 2 shows the corresponding results, which confirmed the efficiency of the decorated electrode for the detection of vitamin A in real specimens.

4. CONCLUSIONS

This work proposed the synthesis and utility of Pt:Co nanoelectrocatalysts as extremely sensitive sensors for voltammetric vitamin A detection using an ionic liquid carbon paste electrode. The Pt:Co/IL-modified CPE showed a decreased overpotential for the oxidation of vitamin A in the oxidation peak current compared to a conventional CPE. Finally, the developed sensor showed favorable results for the detection of vitamin A in tablet and food specimens.

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