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Sensitive Voltammetric Detection of Indomethacin Using TiO₂ Nanoparticle Modified Carbon Ionic Liquid Electrode

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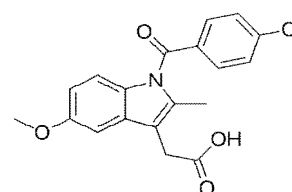
In this work, the TiO₂ nanoparticle modified carbon ionic liquid electrode (CILE) was employed as a sensitive sensor to investigate the electrochemical behavior of indomethacin (IND). This nanocomposite sensor has been fabricated by incorporation of TiO₂ nanoparticles and the ionic liquid 1-hexylpyridinium hexafluorophosphate (HPFP). The surface of the electrode was studied by scanning electron microscopy (SEM). Differential pulse voltammetry (DPV) was used for quantification of sub-micromolar amounts of IND. Electrochemical parameters of the electrode reaction of IND, including the electron transfer coefficient (α) and the electron-transfer number (n), were calculated by cyclic voltammetry (CV) methods. Under selected conditions, the anodic peak current was linear for the concentration of IND in the broad range of 1.0×10^{-7} - 1.0×10^{-4} M with the detection limit of 2.1×10^{-8} M. Moreover, the analytical performance of the proposed method for the determination of IND content in plasma samples was evaluated with good sensitivity and acceptable recoveries.

Keywords: Carbon ionic liquid electrode, Indomethacin, TiO₂ nanoparticle, Voltammetry

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently prescribed classes of drugs and adverse events related to their uses are common. Indomethacin (Scheme 1) {1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid} (IND) is a non-steroidal anti-inflammatory drug commonly used to reduce fever, pain, stiffness and swelling [1]. Although the overall risk of a serious gastrointestinal adverse event such as overt bleeding or perforation is relatively low, the high volume of NSAID in humans causes drowsiness, dizziness, severe headache, mental confusion, numbness of limbs, nausea, and vomiting, severe gastrointestinal bleeding, cerebral edema and cardiac arrest [2]. Hence, determining sensitive and selective analytical methods for measuring NSAID in pharmaceutical and biological samples is absolutely vital.

Several analytical techniques have been used for the



Scheme 1. Chemical structure of IND

determination of IND such as spectrophotometric [3-5], spectrofluorometric [6,7], chemiluminescence (CL) [8], high-performance liquid chromatography (HPLC) [9-11], and electroanalytical methods [12-14]. However, some of these methods include various disadvantages such as time-consuming, expensive apparatuses and pre-concentration procedure. In contrast to the traditional methods, electroanalytical techniques can be an appropriate selection because of good sensitivity, high selectivity, fast response time and low cost [14].

Nanoparticles have attracted much interest from researchers in the field of fabricating chemically modified

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electrodes and electrochemical biosensors. As a common semiconductor, TiO₂ nanoparticles have been applied in the development of different biosensors due to relatively good conductivity, larger surface area and higher catalytic activity [15,16]. Nano-TiO₂ can be combined with other compounds such as graphite and carbon nanotubes to provide the modified electrodes in the electrochemical analysis of some biologically important compounds, implying that they contain more active sites at the electrode surface. TiO₂ can also be used in wide potential windows, improving the stability of the electrode, thus increases the repeatability of the electrode response [17,18].

In the recent decade, carbon ionic liquid electrode (CILE) has received a particular much attention in electrochemistry [19,20] as a new sort of working electrode, providing a considerable rise in the electrochemical response of diverse electroactive substrates and decline the overpotentials of some organic substances [13]. In 2006, Maleki *et al.* studied the electrochemical performance of 1-octylpyridinium hexafluorophosphate based CILE, which illustrated better responses than the traditional carbon electrodes [14,21]. Some remarkable features of CILE are a wide potential window, relatively good ionic conductivity, renewable surface, low background current, provision of fast electron transfer rate and good anti-fouling properties [22,23]. Due to such unique characteristics, CILE can be used as an appropriate sensor in the electroanalytical analysis of various biological compounds.

In this work, we used ionic liquid (1-hexylpyridinium hexafluorophosphate) and TiO₂ nanoparticle for the fabrication of a modified electrode. This study aims at introduction of TiO₂/CILE as an excellent sensor in probing the IND electrochemical reactions. In addition, the application of differential pulse voltammetry (DPV) is investigated for highly sensitive quantification of IND drug. In general, the results demonstrate that TiO₂/CILE can be effectively applied to both purposes including the study of electrochemical behavior and the development of new analytical procedures for the determination of IND.

EXPERIMENTAL

Reagents and Solutions

IND was purchased from Aboureyhan Pharmaceutical

Company (Tehran, Iran), A stock solution of 1×10^{-3} M was made in double distilled water and little alkali was added to get a clear solution. TiO₂ nanoparticles (average particle size of 21 nm, Degussa P25, Aeroxide, Evonik Canada, Toronto) were used without further purification. 1-Hexylpyridinium hexafluorophosphate (HPFP), graphite powder, ascorbic acid, uric acid, glucose, potassium ferricyanide (K₃Fe(CN)₆), potassium dihydrogen phosphate, and dipotassium hydrogen phosphate were purchased from Merck and were used as received. 0.1 M phosphate buffer solutions (PBS), as supporting electrolyte, with various pH values were used. All the other chemicals in this study were of analytical reagent grade, and doubly distilled water was used in all the experiments. The drug-free plasma samples were kindly supplied by the Blood Transfusion Organization (Fars, Iran) and stored in the freezer until the assay.

Electrode Preparation

For fabrication of carbon ionic liquid electrode (CILE), graphite powder with ionic liquid (HPFP) at a ratio of 50/50 (w/w) was mixed thoroughly in a mortar to have a homogeneous carbon paste. Then, the resulting paste was packed firmly into the electrode cavity (1.8 mm i.d.) of a Teflon holder. To obtain further homogeneity in the composite and to decrease background current, the electrode was heated for 2 min in oven to a temperature more than the melting point of IL (m. p. ~ 65 °C) before use [24]. The electrical contact was created by a copper wire. Prior to each measurement, the electrode surface was renewed by pushing the extra of paste out of the tube and then smoothing on a clean paper to gain a new surface. The TiO₂ carbon ionic liquid electrode (TiO₂/CILE) was made according to our previous work [18] with the weighted amounts of graphite powder, IL and TiO₂ (40%:50%:10%, wt%), respectively. The TiO₂ carbon paste electrode (TiO₂/CPE) was prepared by mixing 60% graphite powder, 30% mineral oil and 10% TiO₂. The bare CPE was prepared in a similar way but without adding TiO₂ to the mixture.

Apparatus

Electrochemical measurements were performed with a conventional three-electrode cell, Autolab PGSTAT 12, potentiostat/galvanostat and GPES software, containing a

platinum wire as an auxiliary electrode, an Ag/AgCl/KCl (3 M) as a reference electrode and a working electrode. The working electrodes applied in this study were CILE, TiO₂/CILE, TiO₂/CPE and CPE. All experiments were typically performed at 25°C without removing the dissolved oxygen. Scanning electron microscopy (SEM) images were obtained using a KYKY EM-3200 (China).

RESULTS AND DISCUSSION

Surface Morphology and Structural Characterization of the Electrode

In order to investigate the surface morphology of the fabricated electrodes, scanning electron microscopy (SEM) images were taken from CPE (A) and TiO₂/CILE (B).

As can be seen in Fig. 1, TiO₂/CILE (B) which uses HPFP as a binder has clearly different morphology compared to the electrode uses mineral oil as a binder, which is in agreement with the literature [18,21,23]. The electrode containing HPFP represented uniform surface morphology and specific structure, illustrating that HPFP could fill up well the spaces between graphite nanoparticles.

Electrochemical Behavior of IND on the Surface of

To illuminate the electrocatalytic activity of the proposed electrode, it is essential to compare this electrode with other electrodes. Figure 2a shows the cyclic voltammograms of CPE (a), TiO₂/CPE (b), CILE (c) and TiO₂/CILE (d) in phosphate buffer (0.1 M pH 6.0), including 1 mM IND. No redox peaks were observed in the absence of IND at the surface of all examined electrodes (Fig. 2 inset). As can be seen, the oxidation peak at the CPE was weak and broad due to slow electron transfer, whereas the response of TiO₂/CILE was considerably improved. At the surface of CILE and TiO₂/CILE (curve c, d), there are two oxidation peaks at about +0.51 V (peak I), +0.79 V (peak II) and one reduction peak at about +0.04 V (Peak III), while the other two electrodes (curve a, b) presented only one anodic peak at about +0.61 V.

What stands out from Fig. 2 is that the anodic peak currents of IND on both CILE and TiO₂/CILE were far higher than CPE and TiO₂/CPE. In addition, at the CILE

and TiO₂/CILE, the anodic peak potentials appeared at about +0.51 V and +0.79 V, whereas on the CPE and TiO₂/CPE, the peak potential was at about +0.61 V. These results correctly depict the electrocatalytic effect caused by the presence of IL used as a binder in both CILE and TiO₂/CILE and also amplified the sensitivity of these electrodes toward oxidation of IND. It is interesting to know that an increment in faradic currents at CILE and TiO₂/CILE in comparison with classic CPE and TiO₂/CPE is due to the good ionic conductivity of HPFP leading to a larger electroactive area. In addition, before electron transfer takes place, some part of polar reactant is transferred across IL/aqueous electrolyte and electrode reaction is performed at carbon/IL interface within CILE body. This is not possible at typical CPE with a nonpolar binder, as the electrode reaction occurs at the surface of carbon particles next to the electrolyte [25]. Furthermore, the higher and well-defined response of TiO₂/CILE compared by other tested electrodes is also because of the presence of TiO₂ nanoparticles, which possibly increased the conductivity, surface area, and electron transfer rate at the surface of the modified electrode [26]. To obtain the electroactive areas of TiO₂/CILE and CPE, K₃Fe(CN)₆ was used as a probe (Fig. S1). According to the Randles-Sevcik equation:

$$i_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C_0 \nu^{1/2} \quad (1)$$

where i_{pa} refers to the anodic peak current, n is the number of electrons transferred, A is the active surface area of the electrode surface (cm²), D is the diffusion coefficient of K₃Fe(CN)₆ in 0.1 M KCl (0.76×10^{-6} cm² s⁻¹) [27], C_0 is the concentration of K₃Fe(CN)₆, and ν is the scan rate (V s⁻¹). Then, from the slope of i_{pa} vs. $\nu^{1/2}$ plot, the electroactive area was calculated. Thus, the electrode surface areas of TiO₂/CILE and CPE were 0.034 and 0.023 cm², respectively, indicating that the surface area of TiO₂/CILE was nearly 1.5 times greater.

Effect of the Solution pH

In general, pH is one of the most important variables affecting the current and shape of voltammograms. Thus, the electro-oxidation of a solution containing 1 mM IND

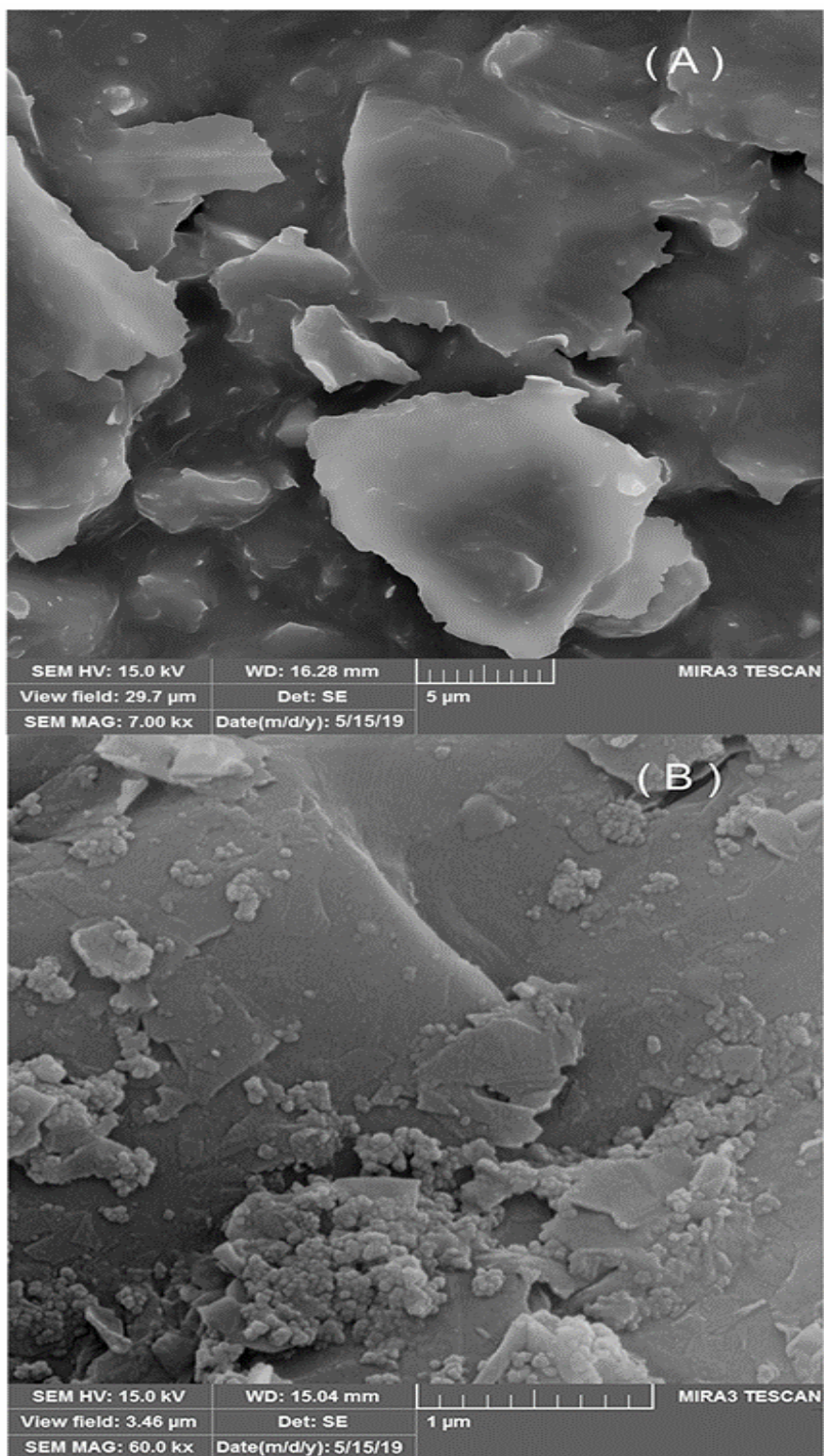


Fig. 1. Scanning electron microscopy of CPE (A) and TiO₂/CILE (B) surfaces.

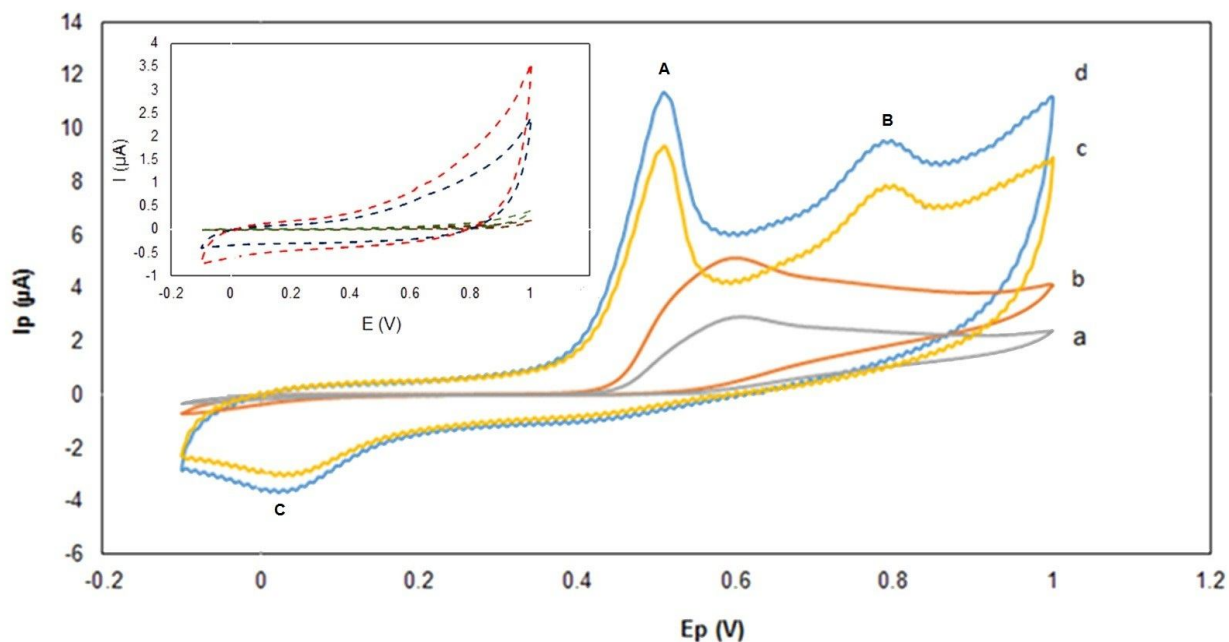


Fig. 2. Cyclic voltammograms of different electrodes in PBS with pH 6 at a scan rate of 100 mV s^{-1} using CPE (a) TiO_2/CPE (b) CILE (c) and TiO_2/CILE (d) in the presence of 1 mM IND. The inset shows the voltammograms of corresponding electrodes in the absence of IND.

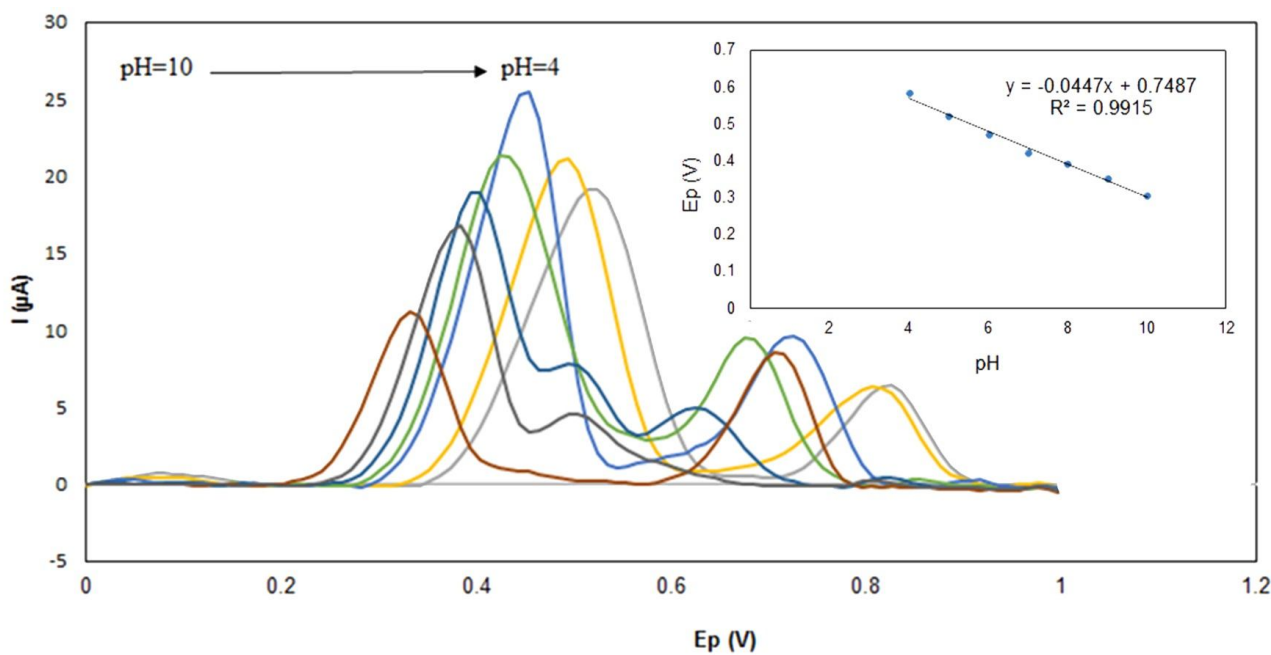


Fig. 3. Effect of pH on the differential pulse voltammograms recorded for a solution of 1 mM IND in PBS 0.1. The pH values: 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0. Inset: The plot of anodic peak potentials *versus* pH.

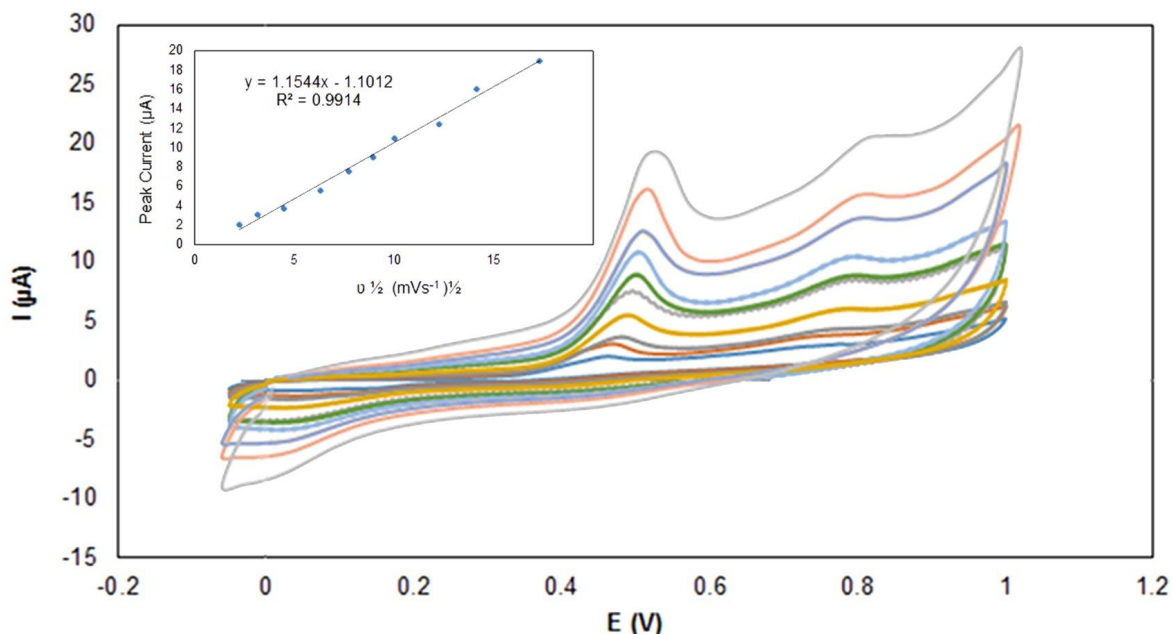


Fig. 4. Cyclic voltammograms of 1 mM IND in PBS 0.1 M with pH 6 using TiO₂/CILE recorded at various potential sweep rates over the range of (5-300 mV s⁻¹). Inset: The plot of anodic peak current *versus* square root of scan rate.

was investigated over the pH range of 4.0-10.0 using differential pulse voltammetry (Fig. 3). The results showed that the peak current reached a maximum value at phosphate buffer with pH 6.0. Accordingly, pH 6 was selected as the optimal pH value. The results also showed the anodic peak potentials shifted toward less positive potentials with an increase in pH of the solution, indicating the proton participation in the overall electrode reaction. To gain better insight into the mechanism of electrocatalytic oxidation of IND, the plot of E_{pa} vs. pH was drawn (Fig. 3 insets), following the linear equation:

$$E_{pa} \text{ (V)} = 0.7487 - 0.0447\text{pH}; \text{ and } R^2 = 0.991 \quad (2)$$

A linear correlation seen in this figure in the range of pH from 4-10, with the slope of 0.0447 V/pH, indicates the equal number of electrons and protons involved in the oxidation process according to the following equation [28],

$$0.0592(h/n) \text{ V/pH} \quad (3)$$

where n and h are the numbers of electrons and protons participated in the electrode process, respectively. It seems acceptable that the mechanism of IND oxidation includes the participation of the equal number of electrons and protons, which is in agreement with the proposed mechanism for IND oxidation by previously reported studies [13,14].

Sweep Rate Effect Studies

To obtain more information about the type of mass transport and reaction mechanism, the effect of scan rates on the electrooxidation of IND at the TiO₂/CILE was investigated using cyclic voltammetry over the range of 5-300 mV s⁻¹ (Fig. 4). As shown in Fig. 4, when the scan rate increased, the peak currents also increased and the anodic peak potentials shifted positively. The anodic peak current also exhibited a linear relationship with the square root of scan rate ($v^{1/2}$) at the mentioned range (Fig. 4 inset). The regression equation was $i_p = 1.1544 v^{1/2} - 1.1012$; and $R^2 = 0.9914$, illustrating a diffusional-controlled process of IND on TiO₂/CILE. Furthermore, the plot of log anodic

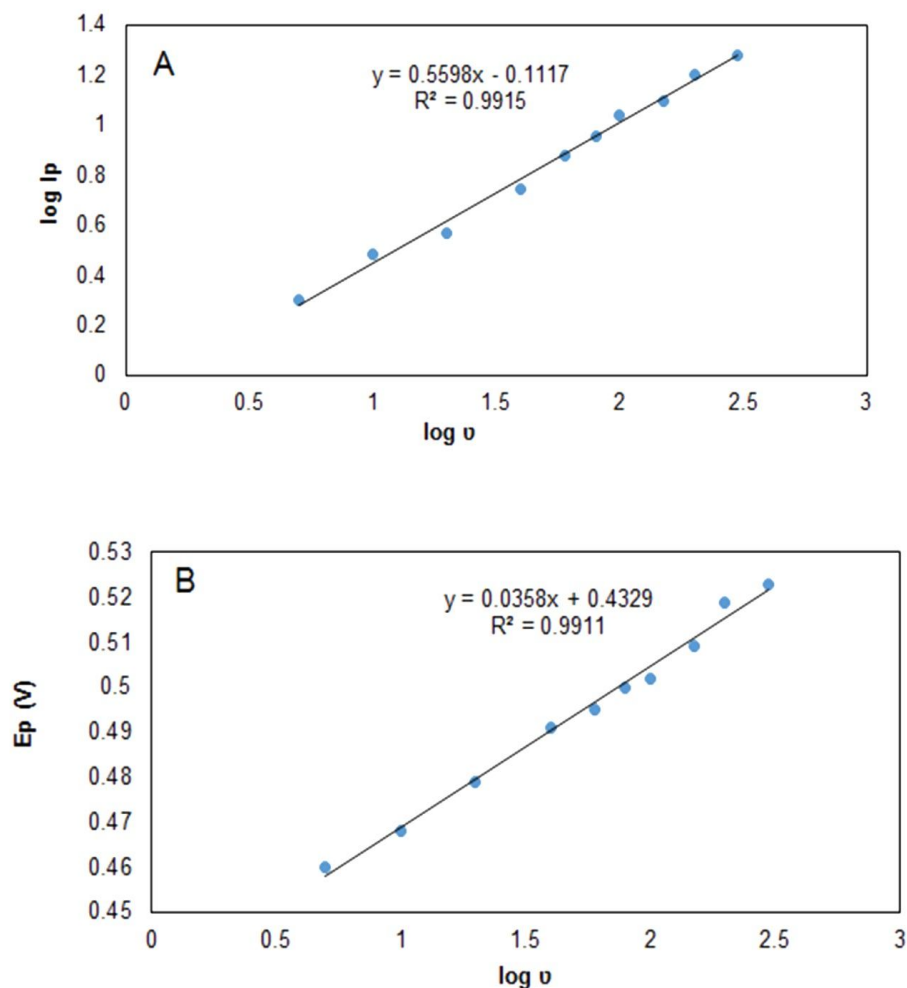


Fig. 5. (A) the linear dependence of log peak current to log sweep rate in the range of 5-300 mV s^{-1} , and (B) the plot of peak potential versus log sweep rate for 1 mM IND in PBS 0.1 M with pH 6.

peak current versus log sweep rate was also linear (Fig. 5A) over the mentioned range (5-300 mV s^{-1}). For an ideal reaction of surface species, a slope of 0.5 is expected and the slope of 0.55 is very close to the expected value for a diffusional-controlled process [29].

Looking at the details, regarding Fig. 5B, the anodic peak potential (E_{pa}) and the logarithm of the scan rate also showed a linear correlation, following the equation:

$$E_{pa} \text{ (V)} = 0.0358 \log v + 0.4329; \text{ and } R^2 = 0.9911 \quad (4)$$

Tafel slope (b) can be easily calculated from the slope of

E_p vs. $\log v$ using Eq. (5) [30]:

$$E_{pa} = b/2 \log v + \text{constant} \quad (5)$$

Tafel slope was calculated to be 71.4 mV. Using the number of electrons involved during IND and the Tafel slope, the value of transfer coefficient (α) was determined according to following Eq. (6) [31]:

$$b = (2.303RT)/[(1-\alpha)nF] \quad (6)$$

the value of α calculated was 0.59.

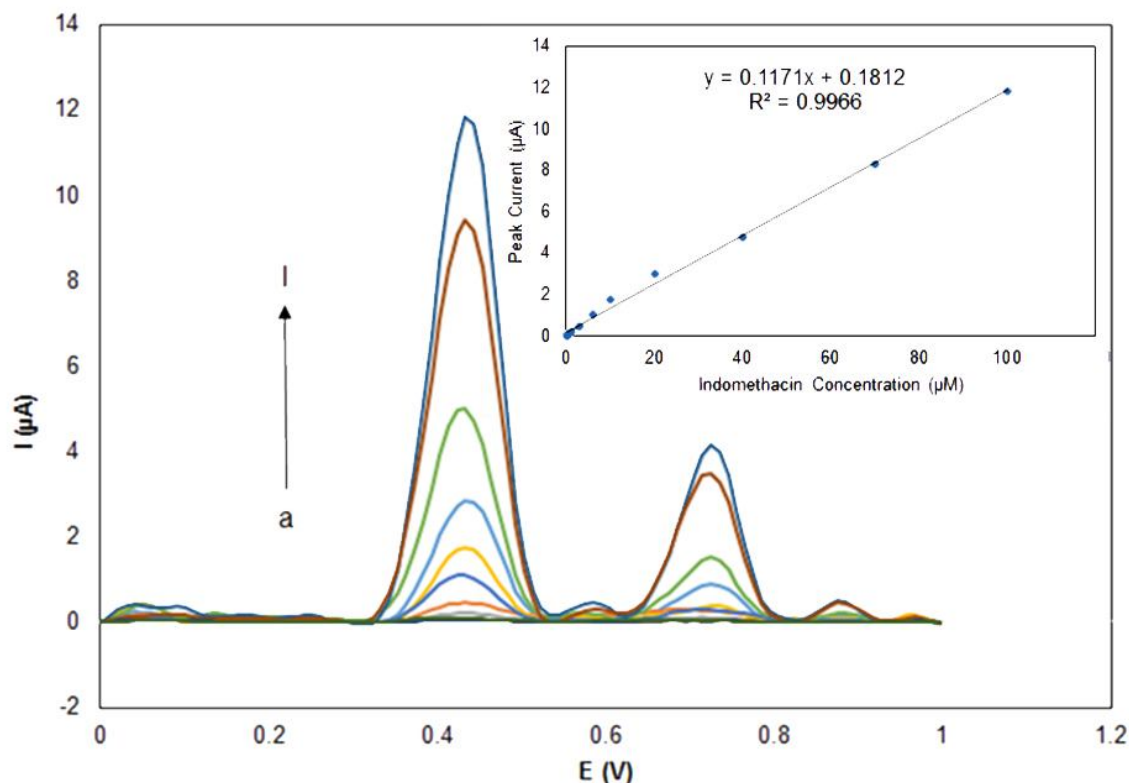


Fig. 6. (A) Differential pulse voltammograms for various concentrations of IND (a) 0.1 (b) 0.2 (c) 0.3 (d) 0.5 (e) 1 (f) 3 (g) 6 (h) 10 (i) 20 (j) 40 (k) 70 (l) 100 μM , at TiO_2/CILE in 0.1M PBS (pH = 6). Inset: Dependence of peak currents on the concentration of IND.

Table 1. Recovery Study of IND in Human Blood Plasma Sample. (N = 3)

No.	IND added (μM)	IND found (μM)	Recovery (%)
1	0	ND ^a	-
2	2	1.91(\pm 0.14)	95.5
3	4	4.03(\pm 0.18)	100.75
4	6	5.84(\pm 0.15)	97.3

^aNot detected.

Differential Pulse Voltammetry of IND at TiO_2/CILE

Under the optimal experimental parameters, the

calibration curve for IND was characterized by DPV. Figure 6 illustrates the differential pulse voltammograms recorded for a series of IND solution with different

concentrations in PBS 0.1 M (pH = 6) and the respective calibration curve (inset). It can be seen that there is good linearity between i_p value and IND concentrations, covering the range of 1.0×10^{-7} - 1.0×10^{-4} M. The linear regression equation was expressed as:

$$i_p (\mu\text{A}) = 0.1171C_{\text{IND}} (\mu\text{M}) + 0.1812 \quad (R^2 = 0.9966) \quad (7)$$

Using the calculated parameters from the analytical curve, the limit of detection (LOD) was obtained as $\text{LOD} = 3S_b/m$, where S_b is the standard deviation of blank ($n = 7$) and m is the slope of the calibration curve. Under the given conditions, the estimated LOD of IND was to be 2.1×10^{-8} M.

Interference Studies

In order to evaluate the anti-interference ability of the present method for IND determination, the effect of various species especially biological compounds commonly existing in the body fluids including glucose and ascorbic acid were examined. The tolerance limit was described as the maximum concentration ratio of interfere/IND caused an error of less than $\pm 5.0\%$ for the quantification of IND. In the presence of 50 μM IND, the results exhibited that 15-fold excess of glucose and 12-fold excess of ascorbic acid did not interfere with the analysis of IND.

Real sample Preparation

In order to evaluate the ability of the proposed method for the analysis of real samples, it was applied for the determination of IND content in human plasma. First of all, a 10 ml human blood plasma sample was deproteinized by adding 2 ml of 10% (w/w) trichloroacetic acid for preparing the plasma sample. After that, the solution was centrifuged and diluted 10 times with 0.1 M PBS with a pH of 6.0. Finally, the proper amount of this diluted sample was transferred to the electrochemical cell for the determination of IND. The results are presented in Table 1.

CONCLUSIONS

This work describes the ability of the TiO_2 carbon ionic liquid electrode (TiO_2/CILE) as a sensitive analytical sensor for electrochemical quantification of IND. The electrode

illustrated a better response than previously reported electrodes, making it an appropriate sensor for practical applications. We have also reported a proper analytical methodology, based on differential pulse voltammetry which demonstrated the highest performance due to the electrocatalytic effect of the proposed electrode.

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