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Synergistic Signal Amplification Based on Ionic Liquid-$BaTiO_3$ Nanoparticle Carbon Paste Electrode for Sensitive Voltammetric Determination of Acetaminophen

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Monitoring the acetaminophen in biological samples and also in pharmaceutical formulations is important due to the concerns of public health care and drug safety. In this work a carbon paste electrode modified with $BaTiO_3$ nanoparticle (BTO NPs) and room-temperature ionic liquid (IL) (n-hexyl-3-methylimidazolium hexafluoro phosphate) was fabricated. The direct electro-oxidation behavior of acetaminophen (AC) was carefully studied by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronoamperometry (CHA) and the diffusion coefficient, D of acetaminophen at the BTOILCPE surface was estimated. The results exhibited a remarkable increase in the electron transfer rate and significant decrease in the overpotential for acetaminophen oxidation reaction in contrast to that on the bare carbon paste electrode (CPE). Under the optimal conditions using DPV, the oxidation peak current was linear to the acetaminophen concentration over the range of 1.0-600.0 µM with a detection limit of 0.46 µM. The proposed sensor was successfully applied in pharmaceutical and urine samples with satisfactory results.

Keywords: Acetaminophen, $BaTiO_3$ nanoparticles, Ionic liquid, Carbon paste electrode, Voltammetry

INTRODUCTION

Acetaminophen (N-acetyl-p-aminophenol, (AC)), is non-steroidal anti-inflammatory drug. It has been used comprehensively as a pharmaceutical pain reliever for patients who are susceptible to aspirin [1-3]. Using suitable doses, acetaminophen has an excellent safety profile, with about 90% of absorption by the organism and subsequently excretion via urine. The remaining 10% of acetaminophen is converted by the metabolism into N-acetyl-p-quinoneimine (NAPQI). Therefore, an overdose of acetaminophen can cause fatal circumstances in kidneys and liver such as renal failure and hepatic necrosis. Hence, monitoring the acetaminophen in biological samples and also in pharmaceutical formulations is important due to the concerns of public health care and drug safety [4-6]. Various analytical methods such as spectrophotometry, chromatography, colorimetry, Fourier transform infrared spectrometry, capillary electrophoresis, titrimetry and electrochemical methods have been employed to acetaminophen determination [7-13]. Commonly, these techniques are complicated, costly, time taking process, operated by highly skilled technicians and sometimes low sensitivity and selectivity [14-18].

Electrochemical methods mainly analyze the relationship between determine and current, voltage or resistance. As the new materials appear constantly, electrochemical sensors reveal much better sensitivity and stability, so that they have been developed fast and used widely in recent years [19-22]. Electrochemical sensors

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have the advantages of fast response, good portability, easy operation, low cost, more accurate with lower detection limits and they are easily adapted for real-time monitoring [23-26].

Carbon-pastes, prepared from graphite and a suitable water-immiscible binder, provide an appropriate medium for incorporating modifiers into electrodes. These electrodes offer advantages such as low background current, easy preparation and use, low cost, large potential window and simple surface renewal [27,28].

Room temperature ionic liquids (ILs) are generally regarded as the compounds entirely composed of bulky organic cations and relatively smaller inorganic anions that exist in the liquid state around room temperature. ILs have triggered a new genre for the development of novel electrode materials due to their amazing specific characteristics, such as wide electrochemical windows, high ionic conductivity, good solubility, low toxicity and volatility [29]. Carbon ionic liquid electrode (CILE) is a new type of working electrode, prepared by using IL as the binder and the modifier in the traditional CPE, has been widely applied in the electroanalytical community owing to its suitable properties [30].

It is widely recognized that properties of nanomaterials are rather dominated by their nanoscale architecture, particularly by properties of surface or interface atoms, than by atoms within the bulk of the material. An enormous surface to volume ratio of nanomaterials provides a platform for measurement of fast reaction kinetics and even for single-molecule detection with a superior sensing performance. In the field of electrochemistry, these materials are used to improve the electrochemical performance. Fabrication of the electrodes with these nanoparticles has shown large interest and wide application in bioscience [31-35]. The $BaTiO_3$ (BTO) nanoparticles used here are a well-known metal oxide with an ABO_3 perovskite structure, having (n-type) semiconducting and inherent piezoelectric properties. Its unique features, such as biocompatibility, second-harmonic generation (SHG), low dielectric constant, and high piezoelectric coefficient make it a potential candidate for applications in biosensing, bioimaging, and piezoelectric-based energy harvesting. Thus, it may be considered as an alternative metal catalyst support [36,37]. These nanoparticles increase the surface area of the electrode and facilitate in easy electrode reaction involving transfer of electrons. On the other hand, ionic liquids/nanomaterials modified electrodes can improve the quality of voltammetric sensors for trace and high sensitive determination of electroactive compounds in biological and pharmaceutical samples [38-40].

Accordingly, it is important to create suitable conditions for the analysis of acetaminophen in biological fluids. In the present work, we describe the preparation of a new carbon paste electrode modified with $BaTiO_3$ nanoparticles and ionic liquid (BTOILCPE) and investigate its performance for the determination of acetaminophen. The proposed sensor showed good electrocatalytic effect on acetaminophen. BTOILCPE shows advantages in terms of reproducibility, and sensitivity. Eventually, we evaluate the analytical performance of the proposed sensor for acetaminophen determination in drug and urine samples.

EXPERIMENTAL

Apparatus and Chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrodes cell was used at 25 ± 1 ℃. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the BTOILCPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was employed for pH measurements.

All solutions were freshly prepared with double distilled water. Acetaminophen and all other reagents were of analytical grade and were obtained from Merck chemical company (Darmstadt, Germany). IL (n-hexyl-3-methylimidazolium hexafluoro phosphate) was purchased from Sigma Aldrich Co. The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

Synthesis of $BaTiO_3$ Nanoparticles

In a typical synthesis [41], the stoichiometric amounts of $Ba(NO_3)_2$ and glucose were dissolved in 15 ml of distilled water. Then, 0.2 g of tetrabutyl titanate

$[Ti(OCH_2CH_2CH_2CH_3)_4]$ was added to the above solution under constant stirring for 30 min. Afterwards, the final mixed solution was kept stirring to form a gel at 90 °C. Finally, the obtained product was placed in a conventional furnace in air atmosphere for 150 min at 700 °C. After thermal treatment, the system was allowed to cool to room temperature naturally, and the obtained precipitate was collected by filtration, and then washed with absolute ethanol and distilled water for several times. The final product was dried in vacuum at 80 °C for 3 h. A typical SEM of synthesized $BaTiO_3$ nanoparticles is shown in Fig. 1.

Preparation of the Modified Electrode

To obtain the best conditions in the preparation of the BTOILCPEs, we optimized the ratio of $BaTiO_3$ nanoparticles and IL. The results of our studied showed that the maximum peak current intensity of acetaminophen could be obtained at the surface of BTOILCPE with optimum ratio of $BaTiO_3$ nanoparticles and IL.

The BTOILCPEs were prepared by hand mixing 0.04 g of $BaTiO_3$ nanoparticles with 0.96 g graphite powder and 0.2 ml ionic liquid with a mortar and pestle. Then, ~0.7 ml of paraffin oil was added to the above mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (*ca*. 3.4 mm i.d. and 15 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, IL modified CPE (ILCPE) without $BaTiO_3$ nanoparticles, $BaTiO_3$ nanoparticles carbon paste electrode (BTOCPE) without IL, and unmodified CPE in the absence of both IL and $BaTiO_3$ nanoparticles were also prepared in the same way.

Preparation of the Real Samples

Five acetaminophen tablets (labeled 300 mg per tablet, Amin Company, Iran) were grinding. Then, the tablet solution was prepared by dissolving 300 mg of the powder in 25 ml water by ultrasonication. Then, different volumes of the diluted solution were transferred into a 25 ml volumetric flask and diluted to the mark with PBS (pH 7.0). The acetaminophen content was analyzed by the proposed method using the standard addition method.

Urine samples were stored in a refrigerator immediately after collection. 10 ml of the sample was 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 acetaminophen.

RESULTS AND DISCUSSION

Electrocatalytic Oxidation of Acetaminophen at BTOILCPEs

The electrochemical behavior of acetaminophen is dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the best electrocatalytic oxidation of acetaminophen. Thus, the electrochemical behavior of acetaminophen was studied in 0.1 M PBS in different pH values ($2.0 < pH < 9.0$) at the surface of BTOILCPE by CV. It was found that the electrocatalytic oxidation of acetaminophen at the surface of BTOILCPE was more favored under neutral conditions than in acidic or basic medium. This appears as a gradual growth in the anodic peak current of acetaminophen (Fig. 2). Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of acetaminophen oxidation at the surface of BTOILCPEs.

Figure 3 depicts the CV responses for the electro-oxidation of 200.0 μM acetaminophen at unmodified CPE (curve a), BTOCPE (curve b), BTOILCPE (curve c) and ILCPE (curve d).

As it is seen, while the anodic peak potential for acetaminophen oxidation at the BTOCPE, and unmodified CPE are 590 and 620 mV, respectively, the corresponding potential at ILCPE and BTOILCPE is ~420 mV. These results indicate that IL can act as a good mediator and peak potential for acetaminophen oxidation at the ILCPE and BTOILCPE shift by ~170 mV and 200 mV toward negative values compared to BTOCPE and unmodified CPE. However, BTOILCPE shows much higher anodic peak current for the oxidation of acetaminophen compared to ILCPE, indicating that the combination of $BaTiO_3$

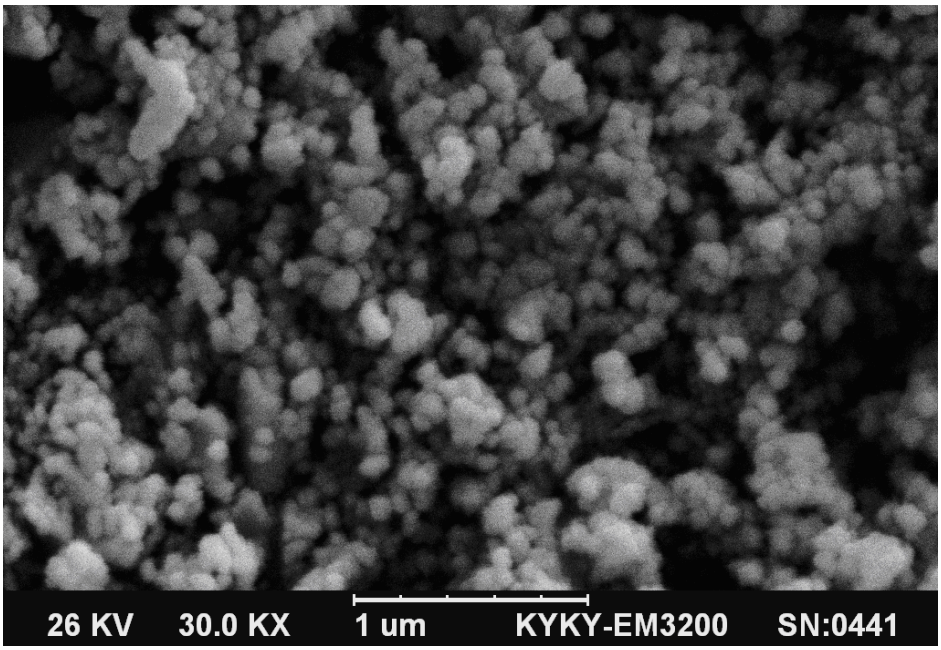

Fig. 1. SEM image of $BaTiO_3$ nanoparticles.

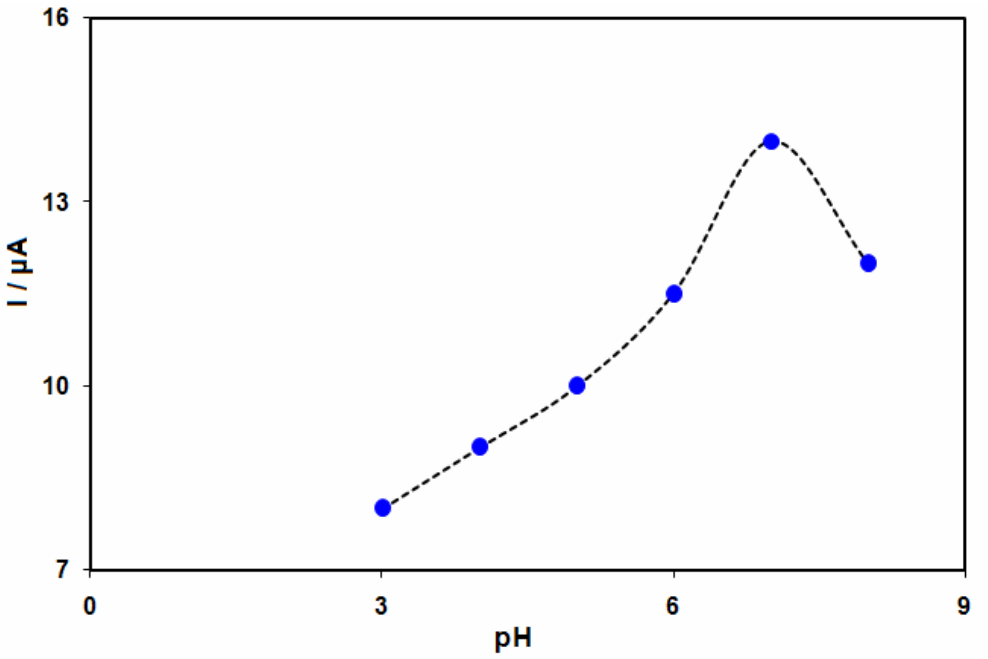

Fig. 2. Plot of I_p *vs*. pH (pHs: 3, 4, 5, 6, 7, 8) at the surface of BTOILCPE in 0.1 M PBS containing 0.2 mM acetaminophen.

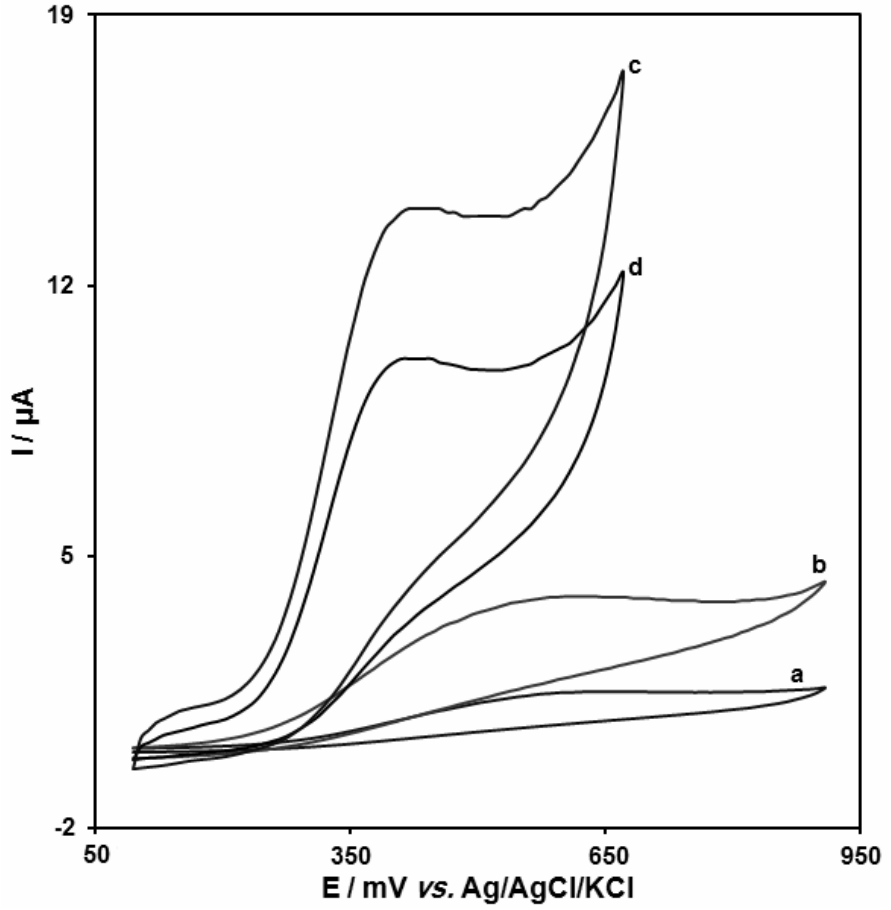

Fig. 3. CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0) containing 0.2 mM acetaminophen; (b) BTOCPE in 0.1 M PBS (pH 7.0) containing 0.2 mM acetaminophen; (c) BTOILCPE in 0.1 M PBS (pH 7.0) containing 0.2 mM acetaminophen and (d) ILCPE in 0.1 M PBS (pH 7.0) containing 0.2 mM acetaminophen.

nanoparticles and the ionic liquid has significantly improved the performance of the electrode toward acetaminophen oxidation.

The effect of potential scan rate on the electrocatalytic oxidation of acetaminophen at the BTOILCPE was investigated by linear sweep voltammetry (LSV) (Fig. 4). As can be seen in Fig. 4, the oxidation peak potential is shifted to the more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) *vs.* the square root of scan rate ($\nu^{1/2}$) was found to be linear in the range of 10-300 mV s^{-1}, suggesting that at sufficient overpotential the process is diffusion rather than surface controlled (Fig. 4 inset) [42].

Chronoamperometric Measurements

Chronoamperometric measurements of acetaminophen at BTOILCPE were carried out by setting the working electrode potential at 0.5 V for the various concentrations of acetaminophen in PBS (pH 7.0) (Fig. 5). For an electroactive material (acetaminophen in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [42];

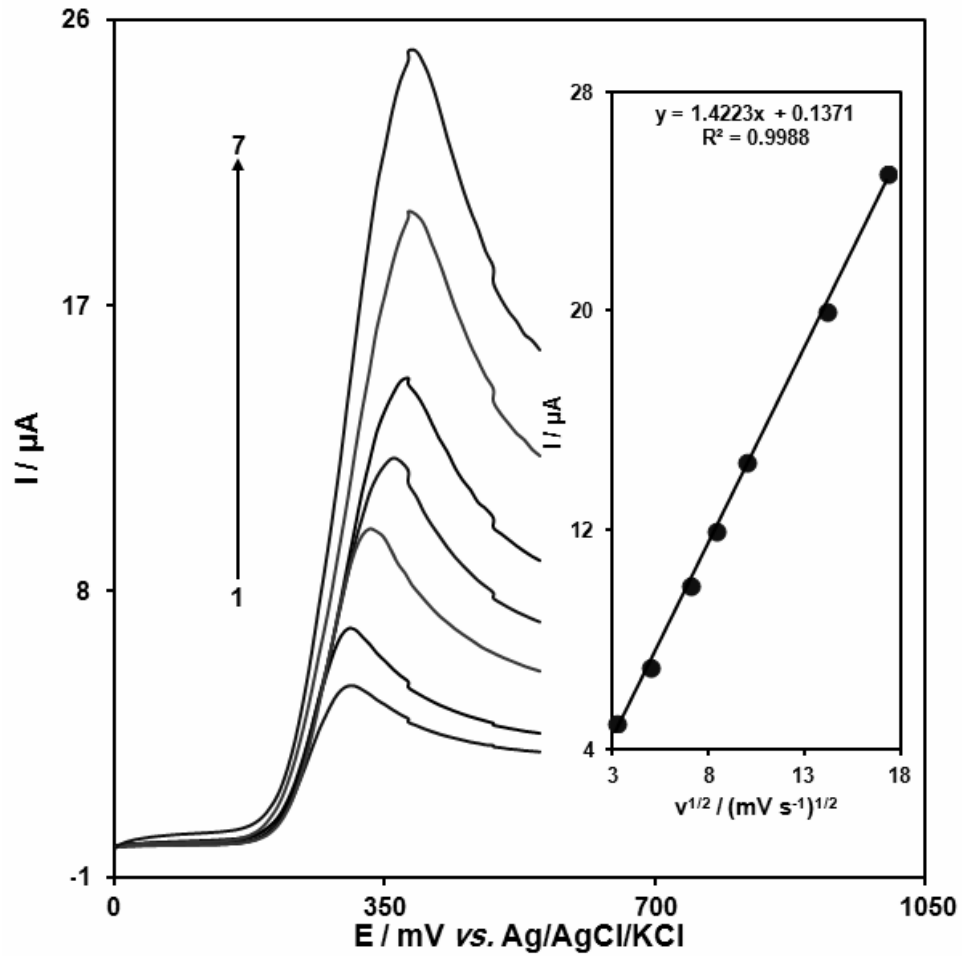

Fig. 4. LSVs of BTOILCPE in 0.1 M PBS (pH 7.0) containing 100.0 µM acetaminophen at various scan rates; numbers 1-7 correspond to 10, 25, 50, 70, 100, 200 and 300 mV s^{-1}, respectively. Inset: variation of anodic peak current *vs*. $v^{1/2}$.

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

where D and C_b are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I *vs*. $t^{-1/2}$ were employed with the best fits for different concentrations of acetaminophen (Fig. 5A). The slopes of the resulting straight lines were then plotted *vs*. acetaminophen concentration (Fig. 5B). From the resulting slope and Cottrell equation the mean value of the D was found to be 1.3×10^{-6} cm^2 s^{-1} which is about one-order smaller than the values reported on ILCPE (2.3×10^{-5} cm^2 s^{-1}) [13] and CPE (7.51×10^{-5} cm^2 s^{-1}) [14] and comparable to the value on GCE (4.97×10^{-6} cm^2 s^{-1}) [15]

Calibration Plot and Limit of Detection

The peak current of acetaminophen oxidation at the surface of the modified electrode can be used for determination of acetaminophen in solution. Therefore, differential pulse voltammetry (DPV) experiments were performed for different concentrations of acetaminophen. The oxidation peak currents of acetaminophen at the surface of the modified electrode were proportional to the concentration of the acetaminophen within the ranges 1.0-600.0 µM (Fig. 6). The detection limit (3σ) of

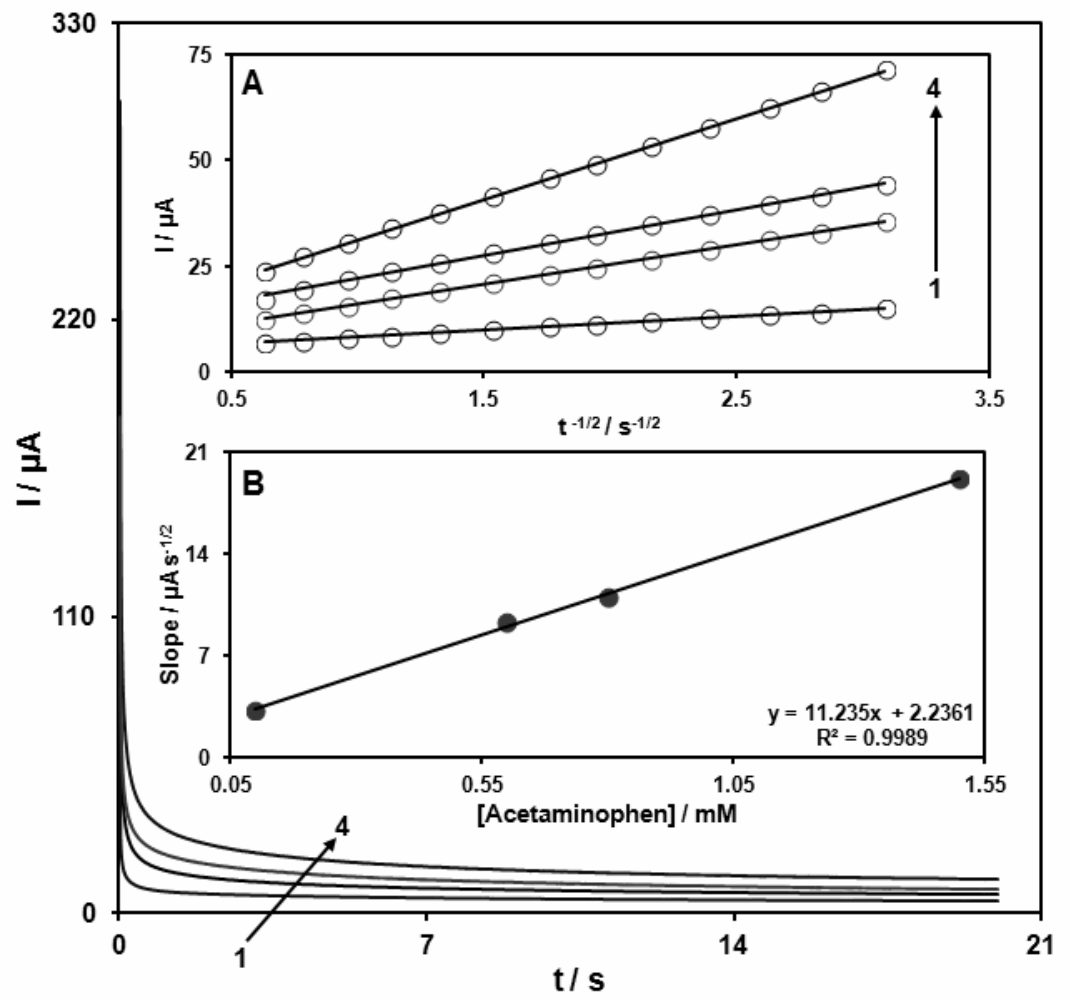

Fig. 5. Chronoamperograms obtained at BTOILCPE in 0.1 M PBS (pH 7.0) for different concentrations of acetaminophen. The numbers 1-4 correspond to 0.1, 0.6, 0.8 and 1.5 mM of acetaminophen. Insets: (A) Plots of I *vs*. $t^{-1/2}$ obtained from chronoamperograms 1-4. (B) Plot of the slope of the straight lines against acetaminophen concentration.

acetaminophen was found to be 4.6×10^{-7} M. These values are comparable with the values reported by other research groups for electro- oxidation of acetaminophen at the surface of chemically modified electrodes (see Table 1).

The Repeatability and Stability of the BTOILCPE

The long term stability of the BTOILCPE was tested over a 3-weeks period. The CVs were recorded using the modified electrode that was stored in atmosphere at room temperature for a given time, the peak potentials for acetaminophen oxidation were unchanged and the current signals showed less than 2.6% decrease relative to the initial response. The antifouling properties of the modified electrode toward acetaminophen oxidation and its oxidation products were investigated by recording the CVs of the modified electrode before and after use in the presence of acetaminophen. CVs were recorded in the presence of acetaminophen after having cycled the potential 10 times at a scan rate of 50 mV s^{-1}. The peak potential was unchanged and the current decreased by less than 2.3%. Therefore, at

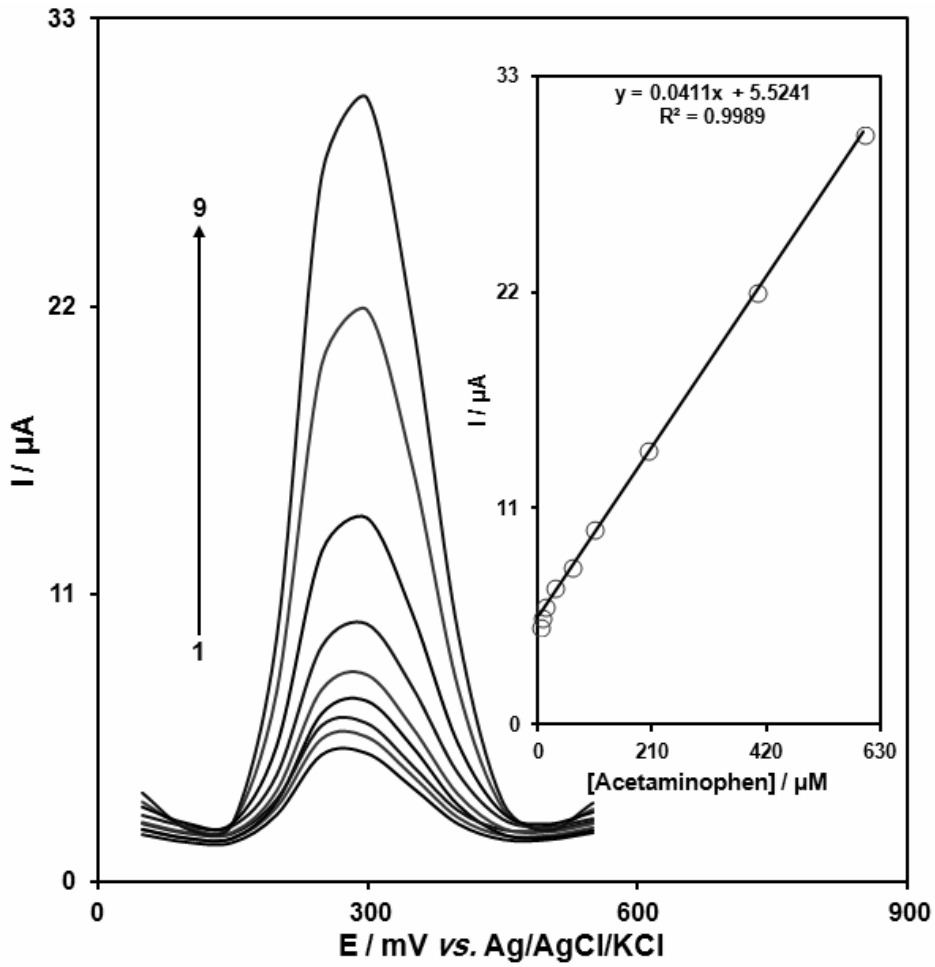

Fig. 6. DPVs of BTOILCPE in 0.1 M (pH 7.0) containing different concentrations of acetaminophen. Numbers 1-9 correspond to 1.0, 5.0, 10.0, 30.0, 60.0, 100.0, 200.0, 400.0 and 600.0 µM of acetaminophen. Inset: The plot of the acetaminophen peak currents as a function of acetaminophen concentration in the range of 1.0-600.0 µM.

the surface of the BTOILCPE, not only does the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decrease.

Interference Study

In continuous to evaluate the selectivity of the proposed sensor in the determination of acetaminophen, the influence of various foreign species on the determination of 40.0 µM acetaminophen was investigated. The determination of each solution was repeated three times and the average current values were obtained. Tolerance limit was taken as the maximum concentration of foreign substances that caused an approximate relative error of ±5%. The results demonstrated that glucose, fructose, sucrose, lactose, methanol, ethanol, F^-, SO_4^{2-}, K^+, Cl^-, SCN^-, Br^-, Mg^{2+}, Na^+, Li^+; L-theronine, L-isoleucin, hystidine, alanine, phenylalanine, glycine, methionine, L-cysteine, tryptophan, ascorbic acid and uric acid had no influence on the selectivity.

Real Sample Analysis

In order to evaluate the analytical applicability of the

Table 1. The Performance of some Reported Modified Electrodes for Determination of Acetaminophen

Electrode	Modifier	Linear range (μM)	LOD (μM)	Ref.
Carbon paste	Ionic liquid-ZnO nanoparticle	0.1-550.0	0.07	[13]
Nitrocellulose membrane	Single-walled carbon nanotube and nafion	50.0-300.0	15.0	[16]
Glassy carbon	Polyethylenimine-functionalized multi-walled carbon nanotubes	0.0999-6.95	0.0558	[17]
Glassy carbon	Nickel and copper oxides-decorated graphene composite	4.0-400.0.0	1.33	[18]
Carbon paste	$BaTiO_3$ nanoparticle and ionic liquid	1.0 to 600.0	0.46	This work

Table 2. The Application of BTOILCPE for Determination of Acetaminophen in Acetaminophen Tablet and Urine Samples (n = 5). All Concentrations are in μM

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
	0	10.0	-	3.1
Acetaminophen tablet	2.5	12.3	98.4	1.8
	5.0	15.2	101.3	2.9
	7.5	17.4	99.4	2.4
	10.0	20.7	103.5	2.6
	0	-	-	-
Urine	5.0	5.1	102.0	1.9
	10.0	9.7	97.0	3.3
	15.0	14.9	99.3	2.8
	20.0	20.3	101.5	2.3

proposed method, it was also applied to the determination of acetaminophen in acetaminophen tablet and urine samples. The results for determination of acetaminophen in real samples using DPV are given in Table 2. Satisfactory recoveries of the experimental results were found for acetaminophen. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

CONCLUSIONS

The BTOILCPE was developed as a high sensitive voltammetric sensor for the rapid determination of acetaminophen in pharmaceutical and biological samples. The BTOILCPE showed a good improvement to the electrode process of acetaminophen compared to the bare CPE. Under the optimum conditions in voltammetric analysis, the oxidation peak current was proportional to the acetaminophen concentration in the range of 1.0-600.0 μM with the detection limit of 0.46 μM. Synergistic effects in the enhanced current response were observed when both $BaTiO_3$ nanoparticles and ionic liquid were employed. Finally, the BTOILCPE was successfully used for the determination of acetaminophen in real samples such as urine and pharmaceutical samples.

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