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Research Article

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Electrocatalytic oxidation of acetaminophen using 1-ethyl-3-methylimidazolium tetrafluoroborate-nickel hexacyanoferrate nanoparticles gel modified electrode

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ABSTRACT

In this work 1-ethyl-3-methylimidazolium tetrafluoroborate, ($EMIMBF_4$) a hydrophilic room temperature ionic liquid (RTIL) was used as a binder to make a new kind of ionic liquid-nickel hexacyanoferrate nanoparticles (NiHCF-NP) gel modified electrode, which was characterized by cyclic voltammetry. The modified electrode showed good electrocatalytic behavior towards the oxidation of acetaminophen with an enhancement of peak current and reduction in overpotential. The modified electrode exhibited good linear relation in the concentration range from 1.5 μM to 1.5 mM for the quantitative analysis of acetaminophen with a limit of detection of 0.51 μM (3σ).

Keywords: Cyclic voltammetry, 1-Ethyl-3-methylimidazolium tetrafluoroborate, Acetaminophen, Room temperature ionic liquid

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INTRODUCTION

RTILs are liquids composed entirely of ions and exist in the liquid state at room temperature. They have several characteristic properties, including intrinsic conductivity, low (or near zero) volatility, high polarity, good chemical and thermal stability, almost negligible vapor pressure, low toxicity and wide electrochemical windows. They are increasingly being used in applications such as green synthesis [1, 2], catalysis [3], and in electrochemical applications such as electrodeposition of metals [4], as electrolytes in lithium batteries, capacitors, fuel cells, photovoltaic, actuators and in electrochemical sensors [5]. RTILs, as promising binders to fabricate carbon ionic liquid electrodes (CILEs), have been used to replace the non conducting pasting liquids in traditional carbon paste electrodes (CPEs) due to their high viscosity [5]. CILE modified with polyoxomolybdate has shown good electrocatalytic activity for the reduction of nitrite [6]. CILE was electropolymerized with Prussian Blue (PB) and a better electrocatalytic reduction of hydrogen peroxide was observed [7]. Ionic nature and limited miscibility will make RTILs as materials for electrochemistry especially for electroanalysis [8].

Prussian Blue has received much attention from the scientific community in the past few years due to its high versatility, especially for its electrocatalytic and electrochromic properties [9-11]. A number of studies concerning the catalytic properties of PB analogues have been reported in the literature, mainly related to the application in chemical sensors and biosensors due to their ability to catalyze the oxidation of biologically, environmentally and pharmaceutically important compounds [12-15]. Recently, the applications of metal hexacyanoferrate nanoparticles have attracted the attention in the area of chemical sensors and biosensors. Among them NiHCF-NP has received a special attention, and its use as a mediator for H_2O_2 detection is reported [16]. Recently we have reported the detection of uric acid using NiHCF-NP-RTIL-Gel modified electrode [17].

Acetaminophen (N-acetyl-p-aminophenol or Paracetamol) is one of the most common drug used in the world and has a very similar structure to aspirin. Acetaminophen acts as a painkiller by inhibiting prostaglandin's synthesis in the central nervous system and relieves fever, other pains like migraine headache, muscular aches, neuralgia, backache, joint pain, rheumatic pain, general pain, toothache, teething pain, cough, and cold. It is suitable for most people, including elderly and young children, because it has very few side effects [18, 19]. Generally acetaminophen does not exhibit any harmful side effects but hypersensitivity or overdose in few cases leads to the formation of some liver and nephrotoxic metabolites [20, 21]. Thus, it is very important to have an analytical technique for the determination of acetaminophen in pharmaceutical preparations.

Numerous methods have been reported for the determination of acetaminophen, such as titrimetry [22], spectrophotometry [23], spectroflurometry [24], HPLC [25-27], HPTLC [28] capillary electrophoresis [29], flow injection analysis [30] and FT-IR spectroscopy [31]. However, these techniques are generally expensive and time-consuming. Hence, there is a great interest in the development of new analytical methods for the determination of acetaminophen in pharmaceuticals without the necessity of a previous separation of the sample components, besides being rapid and low cost.

Among various analytical methods, electrochemical methods offer useful alternatives since they are faster, cheaper and safer. However, the redox reaction of acetaminophen at conventional electrodes requires a large overpotential. One of the most promising methods is to modify the electrode surface with suitable catalyst, which can not only improve the redox response of acetaminophen, but also provide a means of extending the dynamic range in analytical determinations [15]. In this work, an $EMIMBF_4$-NiHCF-NP gel composite was prepared using NiHCF-NP mixed with $EMIMBF_4$ as binder instead of non-conductive binder. The gel was coated on the surface of the paraffin wax impregnated graphite electrode (PIGE) which was used for acetaminophen determination. The sensor showed good stability and reproducibility. Electrochemical oxidation of acetaminophen was performed using cyclic voltammetry, hydrodynamic voltammetry and amperometric measurements.

EXPERIMENTAL SECTION

2.1 Apparatus and reagents

An electrochemical workstation (CH Instruments, USA. Model CHI 660 B) was used for all the electrochemical experiments with a traditional three-electrode system composed of a $EMIMBF_4$-NiHCF-NP-Gel modified electrode as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode.

$EMIMBF_4$ (purity ≥98.5%) was obtained from Alfa Aesar, acetaminophen was received from Himedia laboratories (P) Ltd, Mumbai, India and Spectroscopic grade graphite rod (3mm diameter) was used as received from Aldrich. Commercial tablets were purchased from a local pharmaceutical shop. All other chemicals were of analytical grade and double-distilled water was used in all experiments.

2.2 Synthesis of NiHCF-NP

NiHCF-NPs were prepared as reported by dropwise addition of 35 mL of a 10 mM aqueous solution of $NiCl_2$ to a 35 mL solution of 50 mM $K_3Fe(CN)_6$ containing 50 mM KCl under stirring [16]. After complete addition, the liquid was vigorously agitated for 5 min and then immediately subjected to filtration using a 0.4 μm Millipore cellulose filter membrane. The retentate was continuously washed with water and then collected after filtration and dried overnight in vacuum at room temperature to get a powdered substance.

2.3 Construction of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode

The construction of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode has been reported already in our previous study [17]. Briefly, the modified electrode was prepared by a simple coating method. The ratio of $EMIMBF_4$ to NiHCF-NP can influence the performance of the modified electrode and excessive amount of RTIL could cause instability of the modified electrode (give very high background current and high ΔE_p of the redox mediator) and the modified electrode would have poor conductivity if less RTIL is used. To obtain good cyclic voltammetric responses of $EMIMBF_4$-NiHCF-NP-Gel modified electrode, the concentrations and the mass ratios of NiHCF-NP and $EMIMBF_4$ in the mixture were optimized. Typically, about 20 mg of NiHCF-NP mixed with 10 µL of $EMIMBF_4$ was ground in an agate mortar for about 10 min, and a gel was obtained. PIGEs with a diameter of 3 mm were prepared as reported [32] and were used for electrode modification. One end of the electrode was carefully polished on a smooth surface and then with 0.5µm alumina, washed with distilled water and dried in air. Then, this end was coated by mechanically attaching the $EMIMBF_4$-NiHCF-NP-Gel placed on a smooth glass slide. The gel on the electrode surface was spread with a spatula to have a thin gel film on the PIGE surface. For comparison, paraffin oil-NiHCF-NP-Gel modified electrode was also prepared using paraffin oil as binder with NiHCF-NP and following the same procedure.

2.4 Tablet sample preparation

Two tablets of each Calpol and Dolo-650 were individually weighed, ground into powder, and then dispersed in 10 mL of 0.5 M acetic acid in a 100 mL standard flasks. The flasks were shaken vigorously using a cyclomixer and centrifuged to obtain a clear solutions which were filtered through a Whatmann 41 filter paper and finally diluted to the required concentration. The supernatant was adjusted to the experimental pH.

RESULTS AND DISCUSSION

3.1 Electrochemical characterization of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode

Fig.1 shows the cyclic voltammograms of bare PIGE, paraffin-oil-NiHCF-NP-Gel modified electrode and $EMIMBF_4$-NiHCF-NP-Gel modified electrode respectively in 0.1 M $NaNO_3$ at the scan rate of 20 mV/s. No electrochemical response was obtained on the bare PIGE (curve a), indicating no electroactive substance existed on the electrode surface. With the paraffin oil-NiHCF-NP-Gel modified electrode a pair of redox peak was observed. The peak-to-peak separation (ΔE_p) was 0.26 V for this electrode (curve b), which indicates that the electron transfer rate in the NiHCF-NP with non conductive liquid paraffin is slow. However with the $EMIMBF_4$-NiHCF-NP-Gel modified electrode a pair of well-defined redox peaks appeared (curve c) with the enhanced peak currents and the ΔE_p has decreased to a greater extent (0.07 V) [17]. The results indicate that $EMIMBF_4$-NiHCF-NP-Gel modified electrode shows better reversibility than the paraffin oil-NiHCF-NP-Gel modified electrode, suggesting that the presence of $EMIMBF_4$ promotes the electron transfer rate of the modified electrode.

3.2 Electrocatalytic oxidation of acetaminophen by $EMIMBF_4$-NiHCF-NP-Gel modified electrode

The electrocatalytic behavior of the bare and the modified electrodes were evaluated for the oxidation of acetaminophen using cyclic voltammetry. As seen from Fig. 2A, at the bare PIGE (curve a), the oxidation of acetaminophen requires very high potential (curve b) and gives an ill-defined anodic wave involving very slow electrode kinetics. In contrast, oxidation of acetaminophen at the paraffin oil-NiHCF-NP-Gel electrode (Fig. 2B) and $EMIMBF_4$-NiHCF-NP-Gel electrode (Fig. 2C) in 0.1M $NaNO_3$ occurred at much less positive potentials, associated with an increased anodic current. This indicates that these modified electrodes can catalyze the electrocatalytic oxidation of acetaminophen due to the existence of redox active Fe^{2+}/Fe^{3+} system in the NiHCF-NP and the reaction can be as shown in the Scheme 1.

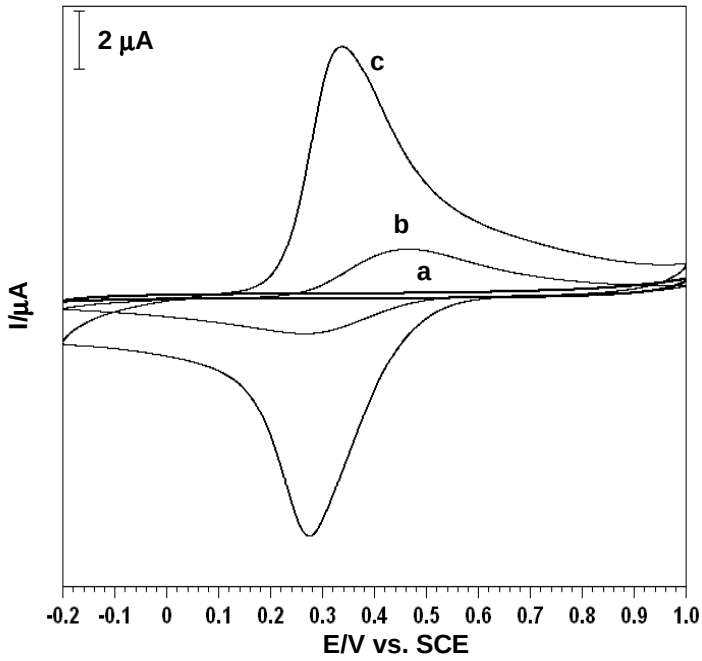
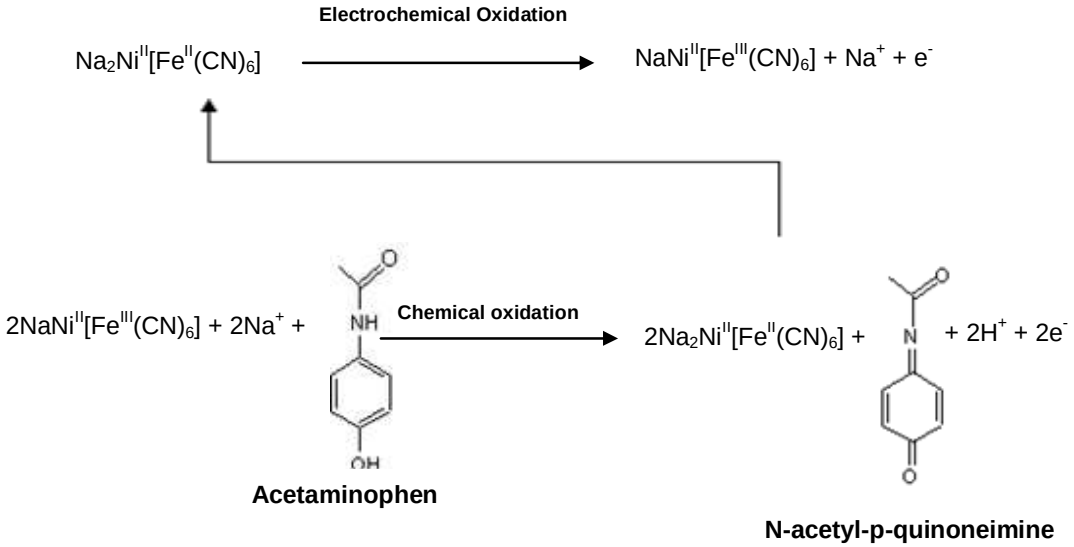


Fig. 1 Cyclic voltammograms of the (a) bare PIGE, (b) paraffin oil-NiHCF-NP-Gel modified electrode, (c) $EMIMBF_4$-NiHCF-NP-Gel modified electrode and in 0.1M $NaNO_3$ at a scan rate of 20 mV/s.



Scheme 1 Mechanism for electrocatalytic oxidation of acetaminophen at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode.

Nevertheless, the anodic oxidation current for acetaminophen at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode is significantly larger than that at the paraffin oil-NiHCF-NP-Gel modified electrode due to the presence of $EMIMBF_4$ with NiHCF-NP. The oxidation of acetaminophen at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode surface occurs at a potential of 300 mV less positive than at the paraffin oil-NiHCF-NP-Gel modified electrode. The results suggested that $EMIMBF_4$-NiHCF-NP-Gel modified electrode showed good electrocatalytic activity to the acetaminophen oxidation with decrease in overpotential and with an increase in peak current. The presence of

$EMIMBF_4$ in the modified electrode facilitates a suitable charge-transfer bridge to the electron transfer rate and thus exhibits excellent electrocatalytic ability. The catalytic oxidation current for acetaminophen oxidation showed linearity in the concentration range of 1.53×10^{-6} to 1.50×10^{-3} M. The linear regression equation is expressed as I_{pa} = 0.0897 c + 0.5433, with correlation coefficient R = 0.9945 (Fig. 2D), where I_{pa} is the oxidation peak current (μA) and c is the concentration of acetaminophen (μM). The detection limit for acetaminophen determination was found to be 5.1 x 10^{-7} M.

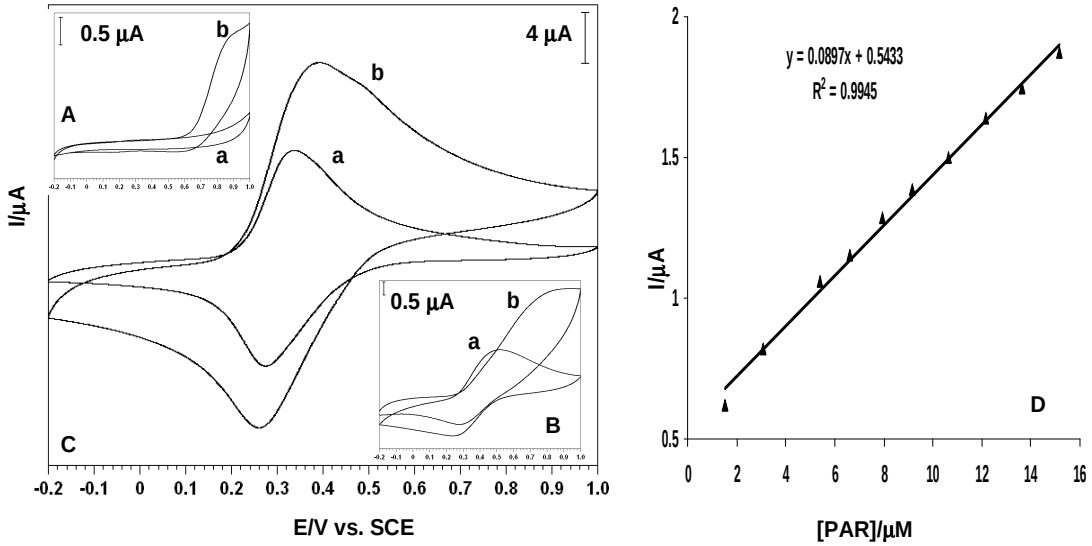


Fig. 2 Cyclic voltammograms obtained for bare PIGE (A), paraffin oil-NiHCF-NP-Gel modified electrode (B), $EMIMBF_4$-NiHCF-NP-Gel modified electrode (C) in 0.1 M $NaNO_3$ in the absence (a) and presence of 1.9 x 10^{-4} M acetaminophen (b). Scan rate 20 mV/s. (D) Plot for catalytic current vs. different concentrations of acetaminophen.

3.3 Effect of pH and scan rate

The influence of pH on the oxidation of acetaminophen at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode was investigated in the pH range from 2-9. The effect of pH on the oxidation peak current is shown in Fig.3A. The current increases from pH 2.0 and was maximum at pH 7.0, and then a decrease in current at higher pHs was observed which is due to the hydroxylation of the mediator [15]. Hence pH 7.0 was chosen as the optimum pH for the determination of acetaminophen. The pH of the supporting electrolyte was maintained using phosphate buffer for subsequent experiments of electrocatalytic oxidation of acetaminophen.

The effect of scan rate on the oxidation of acetaminophen was investigated in the range from 10 to 300 mV/s. The peak current increased with a positive shift in the peak potential when the scan rate was increased. The relationship between the peak current and the square root of the scan rate is given in Fig. 3B. The linear curve indicated that the electrochemical reaction is predominantly diffusion controlled in the confined thin film. The linear regression equation was i_{pa} = 4.2904x - 7.2953 $\nu^{1/2}$ (i_{pa}: μA, ν: mV/s), i_{pc} = 3.8512x - 5.2224 $\nu^{1/2}$ (i_{pc}: μA, ν: mV/s), with correlation coefficients of 0.9957 and 0.9993 respectively. According to Randles equation: i_{pa} = 2.69 x $10^5 n^{3/2} A D^{1/2} \nu^{1/2} c$, the diffusion coefficient D of acetaminophen was calculated and found to be 3.69 x 10^{-7} $cm^2 s^{-1}$.

3.4 Amperometric determination of acetaminophen at $EMIMBF_4$-NiHCF-NP-Gel modified electrode

To obtain optimum conditions for amperometric determination of acetaminophen in flow systems, the hydrodynamic behavior of 1.40 x 10^{-4} M acetaminophen at the bare electrode, paraffin oil-NiHCF-NP-Gel modified electrode and $EMIMBF_4$-NiHCF-NP-Gel modified electrode was investigated in the potential range of -0.2 to 1 V (Fig. not shown). As expected, a poor response was observed at the bare electrode and paraffin oil-NiHCF-NP-Gel modified electrode, whereas on the $EMIMBF_4$-NiHCF-NP-Gel modified electrode acetaminophen oxidation occurred at approximately 0.45 V and the current reached a limiting value in the potential range of 0.5 to 1.0 V. This behavior illustrates that the oxidation of acetaminophen is greatly enhanced at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode due to electrocatalysis which is effectively assisted by the highly conductive $EMIMBF_4$. Hence,

a potential of 0.5 V was selected as the working potential for amperometric determination of acetaminophen using $EMIMBF_4$-NiHCF-NP-Gel modified electrode under hydrodynamic conditions.

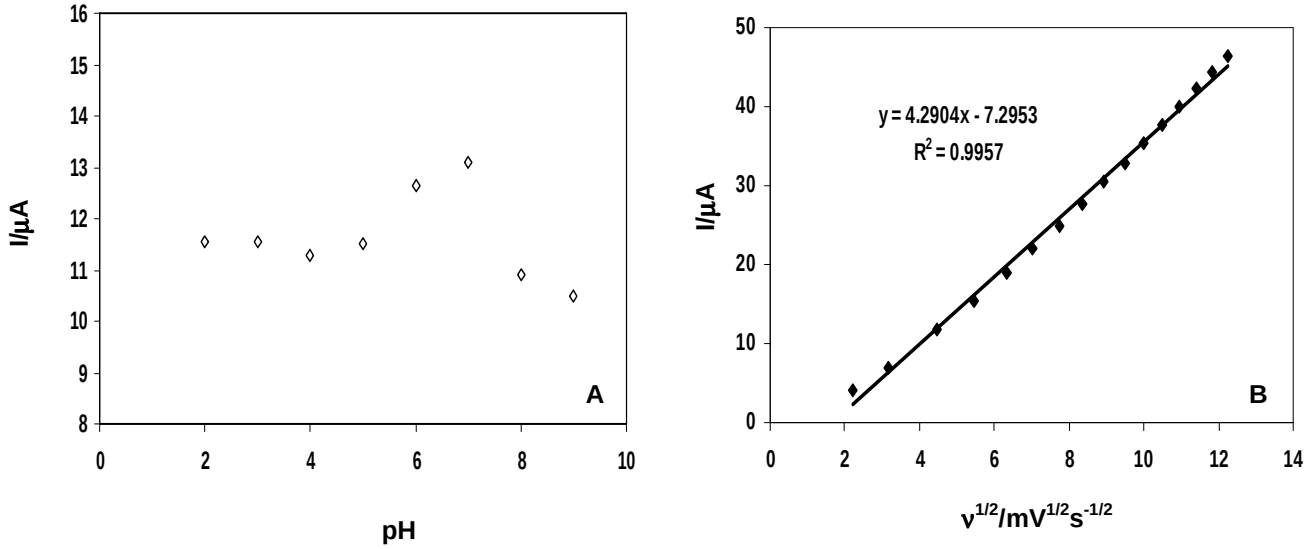


Fig. 3 (A) Effect of pH on the catalytic peak current for the electrocatalytic oxidation of 8.26 x 10^{-5} M acetaminophen at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode. Scan rate 20 mV/s. (B) Plot of catalytic current with the square root of the scan rate for the electrocatalytic oxidation of 8.26 x 10^{-5} M acetaminophen at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode.

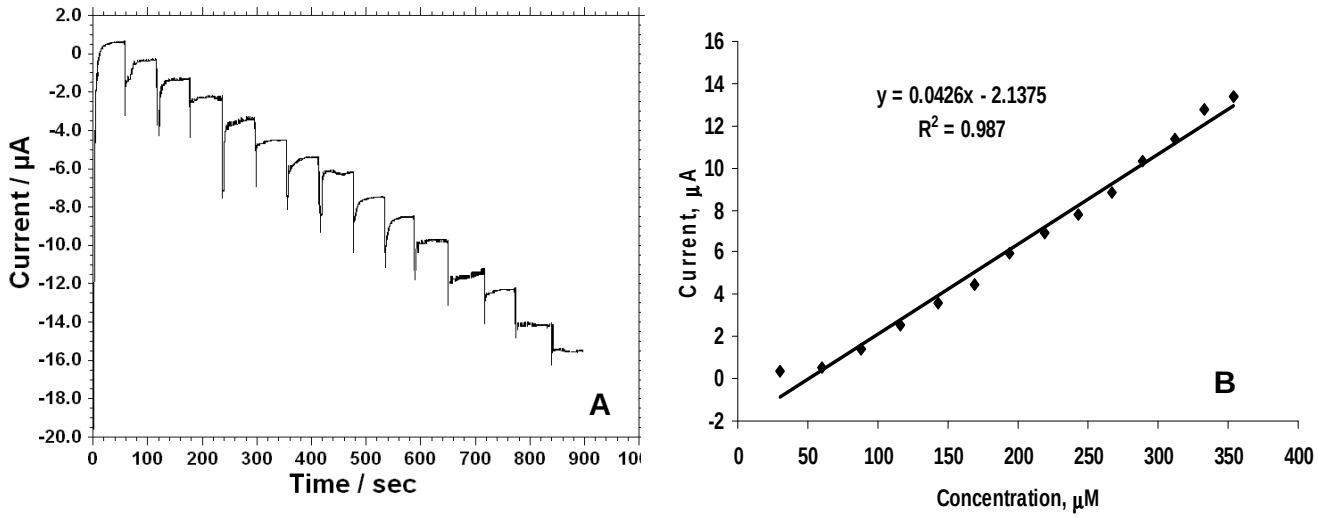


Fig. 4 (A) Amperometric *i-t* curve for the determination of acetaminophen using $EMIMBF_4$-NiHCF-NP-Gel modified electrode in 0.05M PBS (pH.7). Each addition increases the concentration of 2 mM acetaminophen at regular interval of 60 s. E_{app} 0.5 V. (B) Plot for catalytic current vs. different concentrations of acetaminophen.

Fig.4A shows the amperometric response curve of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode to acetaminophen determination. The amperometric response of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode to acetaminophen oxidation was recorded with successive addition of acetaminophen to a continuously stirring background electrolyte at the fixed potential. At 0.5 V, the oxidation current increased to reach a stable plateau within 5 s with each addition of acetaminophen into the buffer solution. The experimental results revealed that the

amperometric current response is linear with the concentration of acetaminophen and the linear equation could be expressed as I (μA) = 2.53 + 0.0437$C_{acetaminophen}$ (μM), with a correlation coefficient of 0.9932 (Fig. 4B). The limit of detection for acetaminophen is 5.1 x 10^{-7} M. High sensitivity can be attributed to the synergistic augmentation of $EMIMBF_4$ and NiHCF-NP-Gel composite towards acetaminophen oxidation. Such a good response of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode for oxidation of acetaminophen under dynamic conditions justifies its viable application in flow systems.

3.5 Interference study

In order to evaluate the specificity of the modified electrode in the determination of acetaminophen, some possible interfering substances were added to 5.0 x 10^{-6} M acetaminophen

solution and the anodic current was measured. There was no substantial change in the peak current response in the presence of 100-fold concentration of K^+, NH_4^+, Ba^{2+}, Zn^{2+}, Mg^{2+}, Cu^{2+}, Fe^{3+}, glucose, *p*-aminophenol and L-cysteine. Ascorbic acid and urea do not affect the oxidation current of acetaminophen up to ten-fold-excess; caffeine does not interfere even up to 50-fold excess. Thus, on this basis the selectivity of the modified electrode is found to be satisfactory.

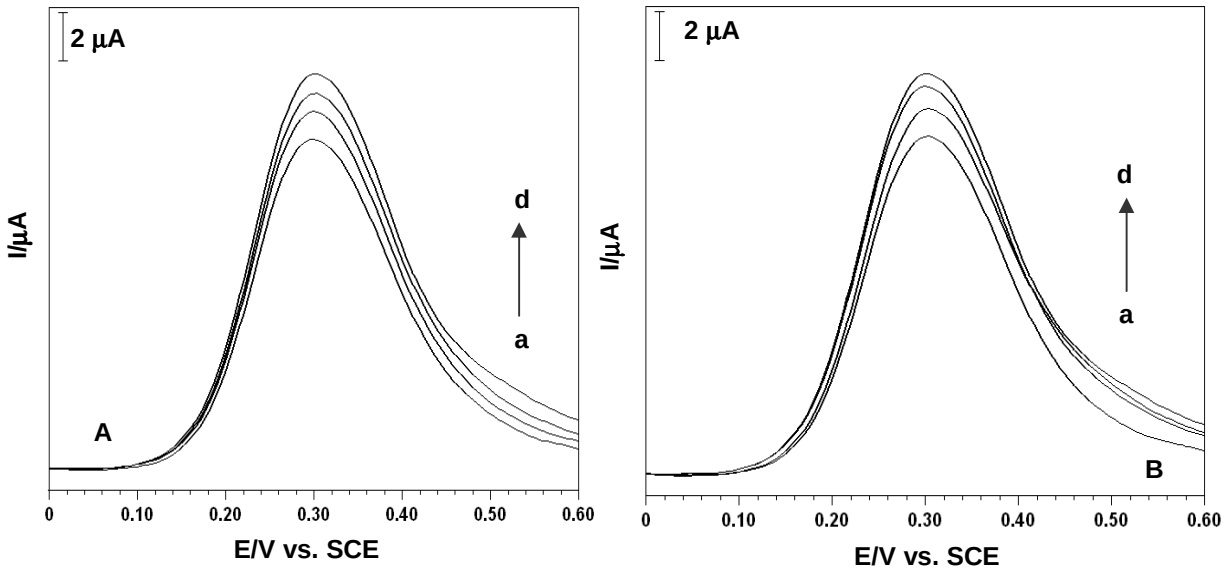


Fig. 5 (A) Differential pulse voltammogram of solution containing (a) 0, (b) 1.5 x 10^{-5}, (c) 3.0 x 10^{-5}, (d) 4.5 x 10^{-5} M. acetaminophen extracted from commercial tablets at the modified electrode. (B) Differential pulse voltammogram of acetaminophen at (a) 0, (b) 1.5 x 10^{-5}, (c) 3.0 x 10^{-5}, (d) 4.5 x 10^{-5} M in 0.1 M $NaNO_3$ modified electrode (pH 7.0).

3.6 Analytical application of the proposed sensor in real sample analysis

The developed method was applied to the analysis of two different commercial tablets. Fig. 5A and B shows DPV curves for different concentrations of acetaminophen at the $EMIMBF_4$-NiHCF-NP-Gel modified electrode under the optimum conditions. The relative standard deviation (R.S.D.) of five replicates of various concentration of acetaminophen was less than 3.5%. The proposed method is simple and rapid and it can thus be recommended for the analysis acetaminophen in pharmaceutical preparations. The results obtained with commercial sample analysis are shown in Table 1 and the values obtained are an average of five replicate measurements. From Table 1, it is seen that the recoveries ranged from 95.3 to be 100.2 %, which indicates that the method is reliable for the determination of acetaminophen.

Table 1 Recovery data observed for commercial samples at different concentrations

Sample	Amount of acetaminophen (μM) Added	Detected[a]	% Recovery	R.S.D. (%)
Calpol	15	15.1	100.6	1.4
	30	29.8	99.3	1.9
	45	44.3	98.4	1.1
Dolo-650	15	14.7	98.6	1.3
	30	28.6	95.3	1.5
	45	44.0	97.7	1.2

[a] *Five replicate measurements were made on the sample*

3.7 Stability and antifouling properties of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode

Repetitive cycling of the modified electrode was done to determine the stability of the $EMIMBF_4$-NiHCF-NP-Gel modified electrode in 0.1 M $NaNO_3$ solution. The results indicated that even after 100 continuous cycles at 20 mV/s, the peak current was found to decrease only less than 3%. The $EMIMBF_4$-NiHCF-NP-Gel modified electrode retained its initial activity for more than 2 months when stored in air-tight container at 4°C. The long term stability of $EMIMBF_4$-NiHCF-NP-Gel electrode towards acetaminophen oxidation was investigated by cyclic voltammetry and amperometric techniques. Also, the current–time profiles related to the catalytic oxidation of 7.6 x 10^{-5} M acetaminophen for a period of 8 h at $EMIMBF_4$-NiHCF-NP-Gel electrode were recorded under stirring conditions (Fig. not shown). All the results have indicated that the modified electrode exhibited a highly stable response with long time usage.

CONCLUSION

In summary, NiHCF-NP was immobilized on PIGE with an ionic liquid, $EMIMBF_4$ which was used as binder to fabricate the $EMIMBF_4$-NiHCF-NP-Gel electrode. The modified electrode was characterized by cyclic voltammetry. The $EMIMBF_4$-NiHCF-NP-Gel electrode showed excellent electrocatalytic activity for the oxidation of acetaminophen. The conductivity of the $EMIMBF_4$-NiHCF-NP-Gel electrode was greatly enhanced by the synergistic effect of the electronic conductivity of NiHCF-NP associated with ionic conductivity of $EMIMBF_4$. The superiority of the $EMIMBF_4$-NiHCF-NP-Gel electrode in terms of reduction in overpotential and higher sensitivity for acetaminophen oxidation than paraffin-oil-NiHCF-NP-Gel electrode indicates that $EMIMBF_4$ can facilitate the electron transfer during redox process. The proposed electrode has the advantages of easy preparation, high stability and sensitivity, and can be used to the determination of acetaminophen in commercially available tablets.

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