A new sensor for determination of nalbuphine using NiO/functional single walled carbon nanotubes nanocomposite and ionic liquid

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Abstract

Using a NiO/functional single walled carbon nanotubes nanocomposite incorporated with ionic liquid (IL/NiO/CNT/CPE), we prepared a sensitive and selective sensor for determination of nalbuphine (NA). Nalbuphine is a semi-synthetic analgesic drug. Electrochemical investigations showed that the nanocomposite had catalytic effect on oxidation of nalbuphine and enhanced the oxidation current. This modified electrode had linear dynamic range from 0.2 – 500.0 mol L⁻¹ NA with detection limit of 0.07 mol L⁻¹ NA. Selectivity of the sensor has been studied and proposed method has been applied for determination of nalbuphine in biological samples.

Keywords: nalbuphine, NiO, carbon nanotubes, nanocomposite, ionic liquid

Introduction

Nalbuphine, (-)-17-(cyclobutylmethyl)- 4,5 α -epoxymorphinan- 3,6 α ,14-triol hydrochloride, is a semi-synthetic opioid drug derived from the oxymorphone[1,2]. It is an analgesic drug which its analgesic potency is approximately two-thirds that of morphine[2]. Nalbuphine is applied for treatment of moderate to severe pain associated with acute and chronic medical disorders such as cancer, renal or biliary colic, migraine or vascular headaches, and surgical pain. Also, it is used during obstetric analgesia [3]. The bioavailability of oral administrated nalbuphine is lower than intravenous and intramuscular administrated and it is not as effective

for pain relief, mainly because of first-pass metabolism in the gastrointestinal mucosa and/or the liver [2, 3]. In order to study the pharmacokinetics of nalbuphine, high performance liquid chromatography [4, 5], liquid chromatography-tandem mass spectrometry [6] and LC-MS/MS [7] have been used. Sometimes because of the limited availability of other opioids such as methamphetamine, abusers use nalbuphine as an alternative for opioids. Yoo et al. have extracted nalbuphine and its metabolites from drug abusers' urine by solid phase extraction and trimethylsilyl derivatization. Then they have used gas chromatography for measurement of them [8]. Nalbuphine quickly disappears from biological fluids. Kyo In et al. have been applied gas chromatography-mass spectrometric method for determination of accumulated nalbuphine in human hair [9]. El-Didamony et al. have been used oxidation of nalbuphine with Ce(IV) for determination of nalbuphine in pharmaceutical and biological fluids by spectrofluorimetric and spectrophotometric methods [10].

In comparison to above methods, electrochemical methods are simple, fast and inexpensive analytical methods for determination of pharmaceutical samples [11-14]. According to our best knowledge, there are a few electrochemical methods for determination of nalbuphine. Sirajuddin et. al have fabricated spherical gold nanoparticles using tranexamic acid (Tr) as reducing as well as capping agent. Then, they have put Tr-Au nanoparticles between the surface of glassy carbon electrode and nafion and have used this sandwiched layer as sensor for determination of nalbuphine[15].

Nanomaterials have received significant attention for modification of electrodes due to their high surface area and high conductivity which exhibit good electrocatalytic activity [16-18]. The most commonly used electrode active nanomaterials are metal nanoparticles, metal oxides and carbon nanotubes or nanocomposites of them.

In this work, we synthesized NiO/ functional single walled carbon nanotubes nanocomposite (NiO/CNT) and used it for modification of carbon paste electrode. Also, we applied an ionic liquid as a binder with the aim of increasing electrical conductivity of the carbon paste (IL/NiO/CNT/CPE). This modified electrode has been used for determination of nalbuphine (NA) in real samples. The detection limit, linear dynamic range, and sensitivity to NA with the IL/NiO/CNT/CPE are comparable to, and even better than, those previous developed methods. Table 1 presents comparisons of the results obtained from the proposed method and those from methods previously reported.

“Here Table 1”

2. Experimental

2.1. Chemicals and apparatus

All chemicals were purchased in the analytical grade. Nalbuphine hydrochloride was purchased from sigma. 1-Butyl-3-methylimidazolium hexafluorophosphate (ionic liquid) was purchased from Sigma-Aldrich. Phosphate buffer (sodium dihydrogen phosphate and disodium monohydrogen phosphate plus sodium hydroxide, 0.1 mol L⁻¹) solutions (PBS) with different pH values were used.

All Voltammetric measurements were carried out using Autolab PGSTAT 12N, potentiostat/galvanostat connected to a three-electrode cell, Metrohm Model 663 VA stand linked with a computer (Pentium IV, 1200 MHz) and with Autolab software. The system was run on a PC using GPES and FRA 4.9 software. For impedance measurements, a frequency range of 0.005 Hz- 100 kHz was employed with the AC voltage amplitude of 10 mV. The 5.0 mmolL⁻¹ K₃[Fe(CN)₆]/K₄[Fe(CN)₆] was used as a redox probe in 0.1molL⁻¹ KCl(0.1molL⁻¹ PBS pH =4.0) at a polarization potential of 0.15V. A conventional

three electrode cell assembly was employed for the experiments in a 50 mL glass cell containing an Ag/AgCl electrode as reference electrode, a platinum wire as counter electrode and IL/NiO/CNT/CPE as working electrode. All of the potentials were measured and reported vs. Ag/AgCl reference electrode. The pH of the solutions was controlled with a Metrohm pH meter (model 827).

2.2. Synthesis of NiO/CNT nanocomposite

In order to synthesis the NiO/CNT nanocomposite, in the first step, 2.0 g commercial functional single walled carbon nanotubes (COOH group) was dispersed in the 100ml of 0.5 molL⁻¹ NaOH solution using ultrasonic bath for 30 min. In the second step, NiO nanoparticles obtained by adding 100 ml of 0.25 molL⁻¹ nickel nitrate solution to previous solution drop by drop and stirring (1,500 rpm) at 30 °C. After completion of the precipitation procedure, the mixture was stirred at room temperature for 2 h. Then, the precipitate was separated from solution using buchner funnel. The precipitate was washed with deionized water and ethanol several times. It was dried at 120 °C for 12 h, and then calcined at 400 °C for 2 h.

2.3. Optimization of sensor

To determine the best composition of the modified electrodes (carbon paste), several modified electrodes were prepared with different percentages of ionic liquid. Then, cyclic voltammograms of 200 mol L⁻¹ NA solution were recorded at the surfaces of theses electrodes. In the presence of 16.0 percent of ionic liquid in the composition of carbon paste, maximum oxidation current was obtained. Similar experiments were conducted to study the effect of nanocomposite percentage. When carbon paste was contained 10.0 percent nanocomposite, the maximum oxidation current was obtained.

2.4. Preparation of the sensor

Modified carbon paste electrode was prepared by hand mixing of 0.8 g graphite powder, 0.2 g NiO/CNT nanocomposite, 0.32 g ionic liquid and 0.68 g viscose paraffin. The mixture was hand mixed in mortar and pestle for 10 min until uniformly wetted paste obtained. The prepared paste was inserted into the glass tube by pushing tube on the mixture. Then a copper wire was pushed to the paste from another side of the tube for electrical connection. When necessary, new surface was obtained by pushing the paste with copper wire and polishing it on a paper. The unmodified carbon paste electrode was prepared in the same way without adding NiO/CNT nanocomposite and/or ionic liquid to the mixture.

3. Results and discussion

3.1. Characterization of synthesized NiO/CNT nanocomposite

To obtain the morphology of NiO/CNT nanocomposite, it was characterized by TEM. As can be seen in Figure 1A, carbon nanotubes have provided surfaces to locate NiO nanoparticles. NiO nanoparticles with nano scale sizes have sat on the carbon nanotubes and have provided rough surfaces that act as active surfaces. Also, Figure 1 B and C show TEM images of CNTs and NiO nanoparticles, respectively. On the other hand, the XRD patterns of the NiO/CNTs showed diffraction peaks absorbed at 2θ values (Fig. 2D). The peaks were observed at the (111), (200), (220), (311) and (222) planes (for FCC structure). These peaks correspond to NiO. On the other hand, it clearly proves the presence of CNTs with a diffraction peak at about 26° .

“Here Figure 1”

3.2. pH effect

According to previous reports, the oxidation peak current of NA is closely related to the pH value of electrolyte solution [15]. Therefore, cyclic voltammograms of NA at surface of IL/NiO/CNT/CPE were obtained in solutions with varying pHs. Figure 2A shows results. As can be seen, at pH 7.0 oxidation peak current has been obtained. So, this value was selected as optimum pH for further experiments. The voltammetric response of NA was obtained in solutions with varying pH from 5.0 to 9.0 (Fig. 2A, inset) at the surface of IL/NiO/CNT/CPE. The results show that the oxidation peak potential shifted to lower potentials with increasing the pH which indicates that protons are directly involved in the oxidation of NA (Figure 2B). The obtained slope value is very close to the anticipated Nernstian value suggesting that the number of electron transfer equals to the proton number involved in the electrode reaction [19].

“Here Figure 2”

3.3. Electrochemical studies

In order to investigate the effect of modifiers, cyclic voltammograms of 200 $\mu\text{mol L}^{-1}$ NA solution (pH=7.0) were recorded at bare carbon paste electrode (CPE), modified CPE with NiO/CNT nanocomposite (NiO/CNT/CPE), with ionic liquid (IL/CPE) and with both of them (IL/NiO/CNT/CPE). As can be seen in figure 3, oxidation peak current at IL/NiO/CNT/CPE has been observed at lower potential (430 mV) with higher peak current (5.8 A) compared with other electrodes. In consequence, IL/NiO/CNT/CPE had high redox activity peak indicated the catalytic ability of IL/NiO/CNT/CPE to NA oxidation. In addition, results clearly demonstrate that the presence of ionic liquid in the carbon paste caused decreasing oxidation peak potential and enhancing peak current. Catalytic ability of IL/NiO/CNT/CPE may be due to excellent characteristics of NiO/CNT

nanocomposite such as good electrical conductivity, high surface area and high chemical stability together with the high electrical conductivity of ionic liquid [20]. As we know, nanocomposite have a high ratio of surface area and so can be used as conductive compounds in sensors filed. On the other hand, carbon nanotubes can show metallic or semiconducting behavior and also they have high surface area. So, carbon nanotubes based nanocomposites have a good electrical conductivity according to previous publications in electrochemical sensors [20-24]. Figure 3 inset shows the current densities derived from cyclic voltammograms at the surface of different electrodes confirm catalytic ability of IL/NiO/CNT/CPE. In addition the active surface area of carbon paste electrode and modified electrodes were obtained by cyclic voltammetry using $K_3Fe(CN)_6$ as a probe at different scan rates [25]. The results obtained were as follows: 0.09, 0.16, 0.17 and 0.22 cm^2 for CPE, NiO/CNT/CPE, IL/CPE and IL/NiO/CNT/CPE, respectively.

“Here Figure 3”

To study the effect of potential scan rate (ν) on the oxidation current, cyclic voltammograms of 300 $\mu mol L^{-1}$ NA (pH=7.0) were recorded at IL/NiO/CNT/CPE with different scan rates (Figure 4). The results showed that there was a linear relationship between the peaks current and the square root of scan rate according to regression equation of:

$$I_{pa} = 0.5899 \nu^{1/2} + 1.4608 \quad (R^2 = 0.994, I_{pa} \text{ in } A \text{ and } \nu \text{ in } mVs^{-1})$$

This linear relation indicated that the electrode process is controlled under the diffusion step.

“Here Figure 4”

In order to calculate transfer coefficient (α), Tafel plots were developed for IL/NiO/CNT/CPE using the data derived from the raising part of the current–

voltage curves with two scan rates (Figure 5). The slope of the Tafel plot is equal to $2.3RT/n(1-\alpha)F$. Using this data gives average of $\alpha=0.43$ (with assuming $n = 1$).

“Here Figure 5”

Chronoamperometric measurements of NA at IL/NiO/CNT/CPE were carried out for 200 and 300 mol L⁻¹ NA (pH=7.0) by setting the working electrode potential at 550 mV vs. Ag/AgCl/KCl_{sat}. The plots of I_{pa} versus $t^{-1/2}$ were obtained for each solution. From the slopes of the resulting straight lines mean value of the diffusion coefficient (D) was found to be $8.72 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [26].

Electrochemical impedance spectroscopy (EIS) was investigated in the presence of K₃[Fe(CN)₆]/K₄[Fe(CN)₆] as a redox probe at the surface of electrodes. Figure 6 shows Nyquist plots of the imaginary impedance (Z_{im}) vs. the real impedance (Z_{re}) of the EIS obtained at the IL/NiO/CNT/CPE (curve d), IL/CPE (curve c), NiO/CNT/CPE (curve b) and CPE (curve a). In Nyquist plots, the electron transfer resistance (R_{ct}) can be estimated by the radius of the semicircle part of curve. The curve corresponding to CPE had big semicircle part. For NiO/CNT/CPE, IL/CPE and IL/NiO/CNT/CPE the R_{ct} has decreased dramatically, indicating that NiO/CNT and IL can act as effective electron conduction pathway between the electrode and electrolyte. This effect causes increasing electronic conductivity of electrode by addition of them to the CPE.

“Here Figure 6”

3.4. Analytical features

Calibration curve of method was obtained using square wave voltammetry at the IL/NiO/CNT/CPE in solutions with different concentrations of NA. There was a linear relationship between oxidation current (I_{pa}) and concentration of NA in the range of 0.2 – 500.0 mol L⁻¹ NA according to the equation: $I_{pa}(\text{A}) = 0.0247 C_{NA}(\text{mol L}^{-1}) + 0.8466$ (Figure 7). The lower detection limit of the method was

determined using the equation $C_m = 3S_b/m$, where S_b is the standard deviation of the blank response (μA) and m is the slope of the calibration curve. Using the data from calibration curve and measurements of blank, the lower detection limit was obtained to be $0.07 \mu mol L^{-1}$ NA.

3.5. Interference and real sample analysis study

To investigate the influence of foreign species on the determination of $20.0 \mu mol L^{-1}$ NA at IL/NiO/CNT/CPE, square wave voltammograms of solutions contain NA were compared with solutions contain NA accompanying by another species. If the difference between oxidation currents was more than $\pm 5\%$, the species was considered as interference. The results showed that 1000 fold of glucose, fructose, lactose, sucrose, alanine, phenyl alanine, 750 fold histidine, glycine, L-threonine, L-isoleucine, Na^+ , Al^{3+} , F^- , K^+ , Cl^- , Br^- , Ca^{2+} , CO^{2+} and Li^+ , 500 fold tryptophan, ascorbic acid (after addition of $1 mmol L^{-1}$ ascorbic oxidize), 200 fold urea and saturated starch solution did not effect on NA oxidation current.

The concentration of NA in urine (from healthy volunteers), serum and water was determined with the aim of study the application of proposed method in real samples. The concentrations of NA in spiked samples were determined by standard addition method. The results are given in Table 2, confirm that the modified electrode retained its efficiency for the determination of NA in real samples.

“Here Table 2”

4. Conclusion

In summary, application of modified carbon paste electrode by NiO/carbon nano tubes nanocomposite and ionic liquid caused catalytic effect for oxidation of nalbuphine. This feature was applied for determination of nalbuphine in real

samples as a sensor. Also, the modified electrode displays high selectivity in the SWV measurement of NA.

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References

- [1] Y. C. Yoo, H. S. Chung, I. S. Kim, W. T. Jin, M. K. Kim, Determination of nalbuphine in drug abusers' urine, *J. Anal. Toxicol.* 19 (1995) 120–123.
- [2] A. R. Aitkenhead, E. S. Lin, K. J. Achola, The pharmacokinetics of oral and intravenous nalbuphine in healthy volunteers, *Br. J. clin. Pharmac.* 25 (1988) 264–268.
- [3] S.J. Enna, D. B. Bylund, *xPharm: The Comprehensive Pharmacology Reference*, Elsevier, 2008.
- [4] L. M. Dube, N. Beaudoin, determination of nalbuphine by high-performance liquid chromatography with electrochemical detection: application to clinical samples from postoperative patients, *J. Chromatogr. B*, 427 (1988) 113-120.
- [5] F. Bressolle, S. Khier, A. Rochette, J. M. Kinowski, C. Dadure, X. Capdevila, Population pharmacokinetics of nalbuphine after surgery in children, *Br. J. Anaesth* 106 (2011) 558–565.
- [6] D. L. Keller, D. S. Guzman, J. M. Klauer, B. KuKanich, S. A. Barker, J. R. Fernández, J. R. Paul-Murphy, Pharmacokinetics of nalbuphine hydrochloride after intravenous and intramuscular administration to hispaniolan amazon parrots (*amazona ventralis*), *Am. J. Vet. Res.* 72 (2011) 741–745.
- [7] L. J. Caia, J. Zhanga, X. M. Wanga, R. H. Zhua, J. Yanga, Q. Z. Zhanga and W. X. Penga, Validated LC-MS/MS assay for the quantitative determination of nalbuphine in human plasma and its application to a pharmacokinetic study, *Biomed. Chromatogr.* 25 (2011) 1308–1314.
- [8] Y. C. Yoo, H. S. Chung, I. S. Kim, W. T. Jin and M. K. Kim, Determination of nalbuphine in drug abusers' urine, *J. Anal. Toxicol.* 19 (1995) 120–123.

- [9] J. Y. Kim, M. Kyo In, K. J. Paeng, B. C. Chung, Simultaneous determination of nalbuphine and opiates in human hair by gas chromatography-mass spectrometry, *Chromatographia* 59 (2004) 219–226.
- [10] A. M. El-Didamony, I. I. Ali, New spectrofluorimetric and spectrophotometric methods for the determination of the analgesic drug nalbuphine in pharmaceutical and biological fluids, *Luminescence* 28 (2012) 734–741.
- [11] A. Taherkhani, T. Jamali, H. Hadadzadeh, H. Karimi-Maleh, H. Beitollahi, M. Taghavi, F. Karimi, ZnO nanoparticle-modified ionic liquid-carbon paste electrode for voltammetric determination of folic acid in food and pharmaceutical samples, *Ionics* 20 (2014) 421–429.
- [12] H. Beitollah, M. Goodarzian, M. A. Khalilzadeh, H. Karimi-Maleh, Marjan Hassanzadeh, M. Tajbakhsh, Electrochemical behaviors and determination of carbidopa on carbon nanotubes ionic liquid paste electrode, *J. Mol. Liq.* 173 (2012) 137–143.
- [13] R. Sadeghi, H. Karimi-Maleh, A. Bahari, M. Taghavi, A novel biosensor based on ZnO nanoparticle/1,3- dipropylimidazolium bromide ionic liquid-modified carbon paste electrode for square-wave voltammetric determination of epinephrine, *Phys. Chem. Liq.* 51 (2013) 704–714.
- [14] T. Jamali, H. Karimi-Maleh, M. A. Khalilzadeh, A novel nanosensor based on Pt:Conanoalloy ionic liquid carbon paste electrode for voltammetric determination of vitamin B9 in food samples, *LWT-Food Sci. Technol.* 57 (2014) 679–685.
- [15] T. Shaikh, A. Nafady, F. N. Talpur, Sirajuddin, M. H. Agheem, M. R. Shah, S. T. H. Sherazi, R. A. Soomro, S. Siddiqui, Tranexamic acid derived gold nanoparticles modified glassy carbon electrode as sensitive sensor for determination of nalbuphine, *Sens. Actuators B* 211 (2015) 359–369.

- [16] H.Bagheri, H.Karimi-Maleh, F.Karimi, S.Mallakpour, M.Keyvanfard, Square wave voltammetric determination of captopril in liquid phase using N-(4-hydroxyphenyl)-3,5-nitrobenzamide modified ZnO/CNT carbon paste electrode as a novel electrochemical sensor, *J. Mol. Liq.* 198 (2014) 193–199
- [17] R. Moradi, S. A. Sebt, H. Karimi-Maleh, RoyaSadeghi, F. Karimi, A. Baharie, H. Arabif, Synthesis and application of FePt/CNTs nanocomposite as a sensor and novel amide ligand as a mediator for simultaneous determination of glutathione, nicotinamide adenine dinucleotide and tryptophan, *Phys.Chem. Chem. Phys.* 15 (2013) 5888–5897.
- [18] H. Karimi-Maleh, P. Biparva, M. Hatami, A novel modified carbon paste electrode based on NiO/CNTs nanocomposite and(9,10-dihydro-9,10-ethanoanthracene-11, 12-dicarboximido)-4-ethylbenzene-1,2-diol as a mediator for simultaneous determination of cysteamine, nicotinamide adenine dinucleotide and folic acid, *Biosens. Bioelectron.* 48 (2013) 270–275.
- [19] E.Afsharmanesh, H.Karimi-Maleh, A. Pahlavan, J.Vahedi, Electrochemical behavior of morphine at ZnO/CNT nanocomposite room temperature ionic liquid modified carbon paste electrode and its determination in real samples, *J. Mol. Liq.* 181 (2013) 8–13
- [20] M. Najafi, M. A. Khalilzadeh, H. Karimi-Maleh, A new strategy for determination of bisphenol A in the presence of Sudan I using a ZnO/CNTs/ionic liquid paste electrode in food samples, *Food Chem.* 158 (2014) 125–131
- [21] H. Karimi-Maleh, F. Tahernejad-Javazmi, N. Atar, M.L. Yola, V.K. Gupta, A.A. Ensafi, A novel DNA biosensor based on a pencil graphite electrode modified with polypyrrole/functionalized multiwalled carbon nanotubes for determination of 6-mercaptopurine anticancer drug, *Ind. Eng. Chem. Res.* 54 (2015) 3634–3639.

- [22] H.Karimi-Maleh, F.Tahernejad-Javazmi, V.K. Gupta, H. Ahmar, M.H.Asadi, A novel biosensor for liquid phase determination of glutathione and amoxicillin in biological and pharmaceutical samples using a ZnO/CNTs nanocomposite/catechol derivative modified electrode. *J. Mol. Liq.* 196 (2014) 258–263
- [23] H.Karimi-Maleh, F.Tahernejad-Javazmi, A.A. Ensafi, R. Moradi, S. Mallakpour, H. Beitollahi, A high sensitive biosensor based on FePt/CNTs nanocomposite/N-(4-hydroxyphenyl)-3,5-dinitrobenzamide modified carbon paste electrode for simultaneous determination of glutathione and piroxicam, *Biosens.Bioelect.* 60 (2014) 1–7
- [24] M.R. Shahmiri, A. Bahari, H. Karimi-Maleh, R. Hosseinzadeh, N. Mirnia, Ethynylferrocene–NiO/MWCNT nanocomposite modified carbon paste electrode as a novel voltammetric sensor for simultaneous determination of glutathione and acetaminophen, *Sens. Actuators B* 177 (2013) 70–77
- [25] M. Fouladgar, S. Mohammadzadeh, Determination of methimazole on a multiwall carbon nanotubes titanium dioxide nanoparticles paste electrode, *Anal. Lett.*, 47 (2014) 763–777.
- [26] M. Fouladgar, H. Karimi-Maleh, R. Hosseinzadeh, Novel nanostructured electrochemical sensor for voltammetric determination of N-acetylcysteine in the presence of high concentrations of tryptophan, *Ionics* 19 (2013) 665–672.

Table 1. Comparison of the features of proposed method with some other methods

Method	Modifier/ Reagent/ Technique	Dynamic range (mol L ⁻¹)	Detection limit (mol L ⁻¹)	Ref.
HPLC	liquid-liquid extraction / Electrochemical detection	0.008-0.100	8.39×10 ⁻⁴	4
Gas Chromatography	solid phase extraction	0.28-27.9	0.28	8
Spectrophotometry	Ce(IV)	4.48-35.81	0.89	10
Voltammetry	Glassy carbon / tranexamic acid capped Au nanoparticles	0.14 -3.5	0.037	15
Voltammetry	Carbon paste/ NiO/functional single walled carbon nanotubes nanocomposite	0.2 – 500.0	0.07	this wor k

Table 2. Determination of nalbuphine in real samples (n=4)

Sample	Added (mol L ⁻¹)	Expected (mol L ⁻¹)	Founded (mol L ⁻¹)	Recovery %
Urine	—	—	<Limit of detection	
	10.0	10.0	10.38±0.45	103.8
	20.0	20.0	20.57±0.63	102.8
Water	—	—	<Limit of detection	
	5.0	5.0	4.87±0.38	97.4
Serum	—	—	<Limit of detection	
	30.0	30.0	30.84±0.95	102.8

±Shows the standard deviation

Legend of Figures:

Figure 1. A) TEM image of synthesized NiO/CNT nanocomposite. B) TEM image of CNT. C) TEM image of synthesized NiO/NPs. D) XRD patterns of as-synthesized NiO/CNT nanocomposite.

Figure 2. A) Current - pH curve for oxidation of $200.0 \mu\text{mol L}^{-1}$ NA at IL/NiO/CNT/CPE (scan rate = 100 mV s^{-1}). Inset, Effect of pH on cyclic voltammograms of NA. B) Plot of potential, E, vs. pH for the electro-oxidation of $200.0 \mu\text{mol L}^{-1}$ NA at surface of IL/NiO/CNT/CPE.

Figure 3. Cyclic voltammograms of (a) IL/NiO/CNT/CPE, (b) IL/CPE, (c) NiO/CNT/CPE and (d) CPE in the presence of $200 \mu\text{mol L}^{-1}$ NA at pH 7.0. inset: The current densities derived from cyclic voltammograms responses at same electrodes

Figure 4. Plot of I_{pa} vs. $v^{1/2}$ for the oxidation of $300.0 \mu\text{mol L}^{-1}$ NA at IL/NiO/CNT/CPE. Inset: cyclic voltammograms of NA at IL/NiO/CNT/CPE with different scan rates (a) 5, (b) 10, (c) 20, (d) 40, (e) 100, (f) 200, (g) 300 and (h) 450 mV s^{-1} in pH 7.0.

Figure 5. Tafel plots for IL/NiO/CNT/CPE in $300.0 \mu\text{mol L}^{-1}$ NA (pH 7.0) with scan rate of 5 and 10 mV s^{-1} .

Figure 6. Nyquist plots of (a) CPE (b), NiO/CNT/CPE (c), IL/CPE (c), and (d) IL/NiO/CNT/CPE in the presence of $5.0 \text{ mmol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ in $0.1 \text{ mol L}^{-1} \text{ KCl}$ (0.1 mol L^{-1} PBS pH = 4.0), frequency range: 0.005 Hz- 100 kHz; E_{dc} : +0.15 V vs. Ag/AgCl; E_{ac} : 10 mV.

Figure 7. Calibration curve for determination of NA. Inset shows Square wave voltammograms in the buffer solution (pH=7.0) containing different concentrations of NA in the range of 0.2 to $500.0 \mu\text{mol L}^{-1}$ NA.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7