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# Nano-molar level determination of isoprenaline in pharmaceutical and clinical samples; a nanostructure electroanalytical strategy

**Sareh Sadat Moshirian-Farahi[a] |Hassan Ali Zamani[a,*] |Mohamadreza Abedi[b]**

[a]*Department of Applied Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran*

[b]*Department of Applied Chemistry, Quchan Branch, Islamic Azad University, Quchan, Iran*

A high sensitive electroanalytical-based sensor for determination of isoprenaline was fabricated by modification of carbon paste electrode (CPE) by NiO-Pt-Pd/CNTs composite as conductive mediator and n-hexyl-3-methylimidazolium hexafluoro phosphate (NHIHP) as conductive binder. The NHIHP/NiO-Pt-Pd/CNTs/CPE was improved the oxidation signal of isoprenaline □3.47 times and reduced oxidation over-potential of drug □180 mV. The pH investigation confirmed that redox behavior of isoprenaline is depended of pH solution with equal value of electron and proton in redox mechanism. The NHIHP/NiO-Pt-Pd/CNTs/CPE was successfully used for determination of isoprenaline in the concertation range 0.003-300 µM with detection limit 0.9 nM by square wave voltammetric method. The standard addition results showed powerful ability of NHIHP/NiO-Pt-Pd/CNTs/CPE as an electroanalytical tool for determination of isoprenaline in the pharmaceutical and clinical samples with recovery data 98.76-105.06%.

***Corresponding Author:**
Hassan Ali Zamani
Email:haszamani@yahoo.com
Tel.: +98 (51) 36630781

**KEYWORDS**
Isoprenaline; NiO-based composite; nanostructure sensor; modified carbon paste electrode; drug sensor.

## Introduction

Isoprenaline is one of the famous catecholamine drugs that prescribed for treatment of heart block and bradycardia. In addition, the treat isoprenaline prescribed for treat asthma and is not suggest for patients with tachyarrhythmias [1]. Although isoprenaline is an active and useful drug in premature infants, a few published papers described this point [2]. The nervousness, visual blurring, headache, nausea and dizziness are the most important side effect associated with overdose of isoprenaline. Therefore, the fast detection and sensitive determination of isoprenaline in biological and clinical sample are very important and many analytical methods were suggested for this goal in recent years [3-6].

The tendency to use electrochemical sensors for the analysis of pharmaceutical compounds such as isoprenaline has grown significantly in recent years due to the high rate of analysis and the lack of use of toxic solvents [7-19]. With the advent of modified sensors and greater variability in the manufacture of electrochemical sensors, this technique has been introduced as a serious competitor for chromatographic methods in drug composition analysis [20-30]. The use of nanomaterial-modified sensors has been a major revolution in the design of sensitive sensors in recent years [31-41]. Nanomaterials with wide range application and new properties can be introduced as

powerful catalysts in different industries such as electrochemical sensor filed [42-58]. The using nanomaterials and especially metal-based nanoparticle can be improve sensitivity of electrochemical sensors and suggest as a new strategy for design of new type of electroanalytical sensors in drug analysis [59-79]. In addition, ionic liquids showed more advantages for modification of electrochemical sensors due to high electrical conductivity and wide electrochemical windows range [80-83]. Recently, the many of scientific reports showed high performance ability of ionic liquid/nanomaterial composite for modification of electrochemical sensor with highly sensitive properties [84-86].

In this research, we fabricated NHIHP/NiO-Pt-Pd/CNTs/CPE as a powerful and highly sensitive electroanalytical sensor for determination of isoprenaline. For this goal, the NiO-Pt-Pd/CNTs composite was synthesized by a simple precipitation method. Due to presence of NiO-Pt-Pd/CNTs nanocomposite and NHIHP as two conductive mediators, the NHIHP/NiO-Pt-Pd/CNTs/CPE showed highly sensitive activity for nano-molar determination of isoprenaline.

## Experimental

### *Materials and instruments*

Isoprenaline hydrochloride, 98% was purchased from Across Company. The stock solution of isoprenaline hydrochloride was prepared by dissolving 0.0247 g isoprenaline hydrochloride powder into 100 mL distillated water. Graphite powder, diethyl ether and paraffin oil were purchased from Merck Co. and used for preparation of carbon paste electrode. Nickel nitrate hexahydrate, platinum(II) chloride, palladium(II) acetylacetonate, sodium hydroxide and single wall carbon nanotubes were purchased from Sigma-Aldrich Co. and used for synthesis of NiO-Pt-Pd/CNTs nanocomposite. Phosphoric acid was purchased from Across Co. and used for preparation of phosphate buffer solution (PBS 0.1 M).

Electrochemical instrument (The Ivium-Vertex and Potentiostat/Galvanostat) was used for voltametric investigation. Ag/AgCl/KCl and Pt wire were purchased from Azar Electrode CO. and used as reference and counter electrodes, respectively.

### *Synthesis of NiO-Pt-Pd/CNTs nanocomposite*

The 8.0 mg platinum(II) chloride + palladium(II) acetylacetonate was dissolved in 100 mL solution containing nickel nitrate hexahydrate (0.5 M). The solution was stirred for 30 min at 35 °C. In next step, 200 mL sodium hydroxide 1.0 M was added to solution.The precipitate was washed seven times and then filtered. The filtered precipitate was dried at 2 °C for 2 h and then calcined in the furnace at 2 °C.

### *Preparation of NHIHP/NiO-Pt-Pd/CNTs/CPE*

The NHIHP/NiO-Pt-Pd/CNTs/CPE was ready by mixing 0.95 gr graphite powder + 0.05 g Pt- NiO-Pt-Pd/CNTs + 0.95 gr graphite powder into mortar and pestle in the presence of diethyl ether as solvent for better mixing. After the evaporation of diethyl ether in the room temperature, 1 drops of NHIHP and 14 drops of paraffin oil were added to the dried powders as binders hand-mixed for 3.0 h. A homogeneous paste was obtained and then filled into a glass tube and it was attached to an electrical contact (copper wire).

### *Preparation of real sample*

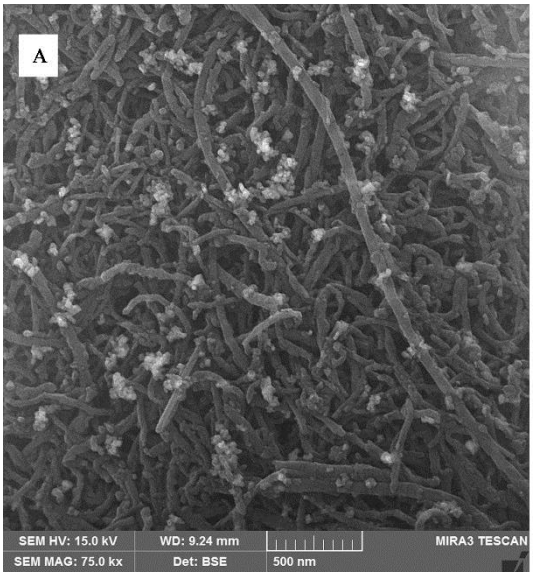
Isoprenaline injection was purchased from local pharmacy and then mix with PBS (pH=7.0). The injection solution of isoprenaline with specified concentration was used for testing of NHIHP/NiO-Pt-Pd/CNTs/CPE ability for real sample using standard addition method. In addition, the

pharmaceutical serum was dilute 5-times with PBS and used for real sample analysis.

## Results and discussion

*Characterization of NiO-Pt-Pd/CNTs nanocomposite*

The NiO-Pt-Pd/CNTs nanocomposite was characterized by FESEM and EDS methods. The results in Figure 1A confirm spherical NiO-Pt-Pd deposit as surface of single wall carbon nanotubes. In addition, the analysis results relative to EDS investigation are presence in figure 2B and presence of C, Ni, O, Pt and Pt confirm purity of synthesized NiO-Pt-Pd/CNTs nanocomposite.




B

| Element | Wt% |
|---|---|
| C | 40.6 |
| Ni | 29.2 |
| O | 18.1 |
| Pt | 5.8 |
| Pd | 6.3 |

**FIGURE 1** A) FESEM and B) EDS analysis data for synthesized NiO-Pt-Pd/CNTs nanocomposite

*Electrochemical investigation*

Isoprenaline is a catecholamine drug and its electrochemical behavior is expected to be influenced by pH of aqueous solutions. Therefore, this factor was optimized by recording cyclic voltammograms of 600 µM isoprenaline in the pH range 4.0-8.0 (Figure 2 inset). The maximum oxidation current was observe at pH=7.0 and this value was selected as optimum condition. The linear relation with equation of $E_{pa}$=0.0592 pH + 0.9167 ($R^2$=0.9902) was observe between oxidation potential of isoprenaline and pH of solution in the presence of drug that confirm equal value of electron and proton in redox behavior of isoprenaline (Figure 2).

The cyclic voltammograms of 600 µM isoprenaline was recorded at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE (Figure 3 curve a), NHIHP/CPE (Figure 3 curve b), NiO-Pt-Pd/CNTs/CPE (Figure 3 curve c) and NHIHP/NiO-Pt-Pd/CNTs/CPE (Figure 3 curve d) in the solution with pH=7.0. With moving of CPE to NHIHP/NiO-Pt-Pd/CNTs/CPE, the oxidation current of isoprenaline was increased from 12.8 µA to 43.8 µA and oxidation potential was reduce from 680 mV to 500 mV.

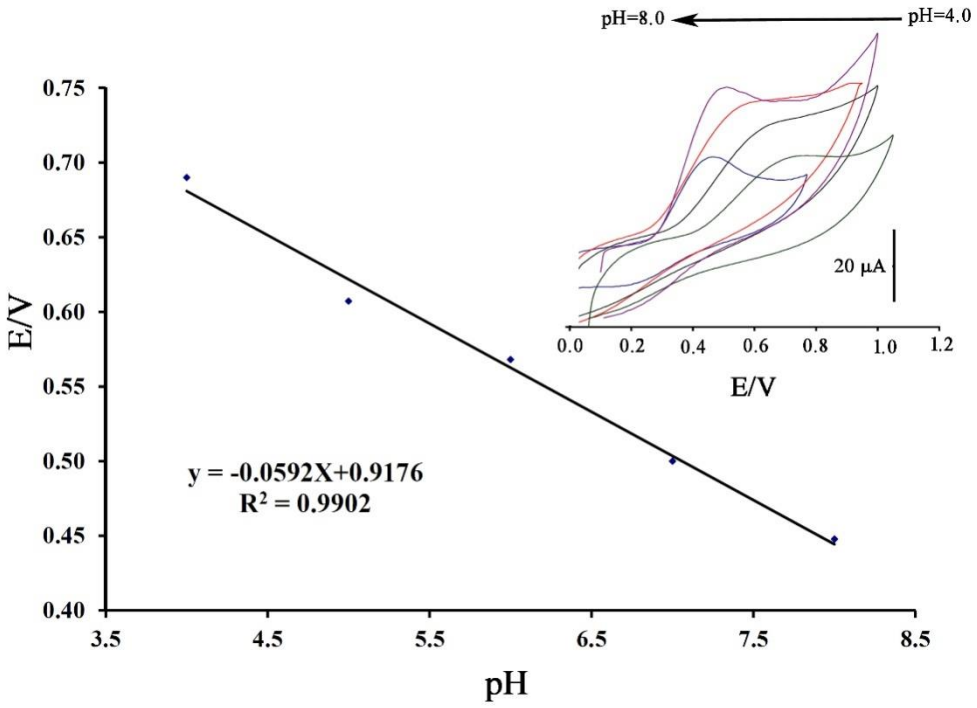



**FIGURE 2** Diagram of $E_P$ *vs.* pH for electro-oxidation of 600 µM isoprenaline at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE. Insert) Cyclic voltammogram relative to electro-oxidation of 600 µM isoprenaline at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE in the pH range 4.0-8.0.

This improvement is relative to presence of NHIHP and NiO-Pt-Pd/CNTs at surface of CPE as conductive binders.

The cyclic voltammograms of 550 µM isoprenaline was recorded at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE was recording in the scan rate range 10-100 mV/s (Figure 4).

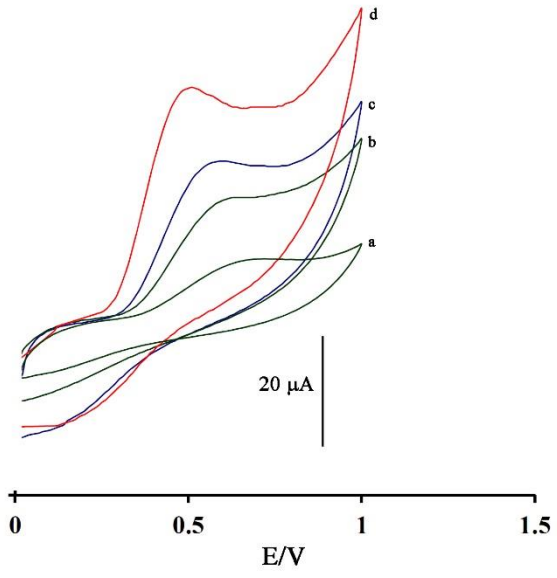



**FIGURE 3** Cyclic voltammogram of 600 µM isoprenaline at surface of a) CPE, b) NiO-Pt-Pd/CNTs/CPE, c) NHIHP/CPE and d) NHIHP/NiO-Pt-Pd/CNTs/CPE

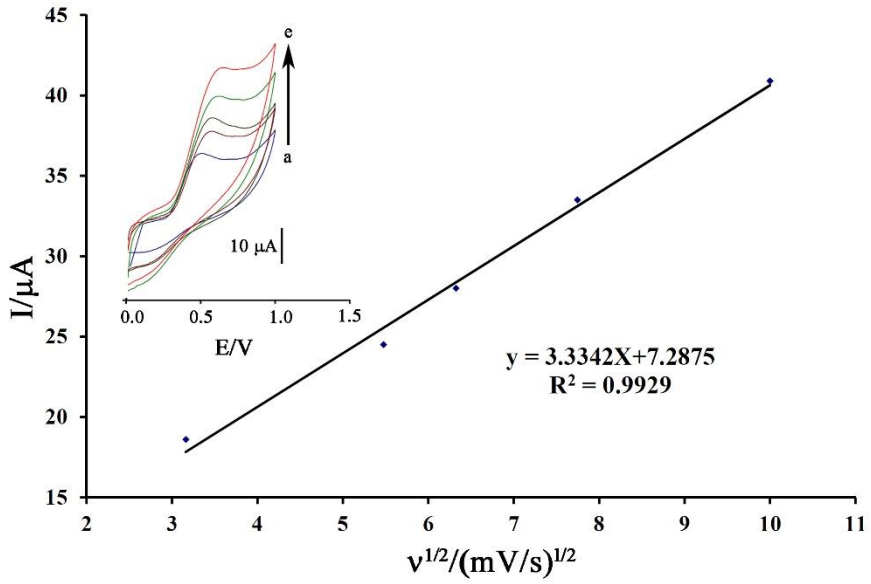



**FIGURE 4** Cyclic voltammogram of 550 μM isoprenaline at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE in the scan rate a) 10, b) 30, c) 40, d) 60 and e) 100 mV/s.

The linear relation between oxidation current of isoprenaline and $\nu^{1/2}$ at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE confirm a diffusion process [87-96] in this study.

Chronoamperometric signals of isoprenaline were recorded at a surface of NHIHP/NiO-Pt-Pd/CNTs/CPE (Figure 5 A). For this goal, we used step potential at 400 mV. The Plot of I versus $t^{-1/2}$ were recorded for 100, 200 and 300 μM isoprenaline (Figure 6B). The value of diffusion coefficient (D) was obtained ~ $2.14 \times 10^{-6}$ $cm^2/s$ by the Cottrell equation.

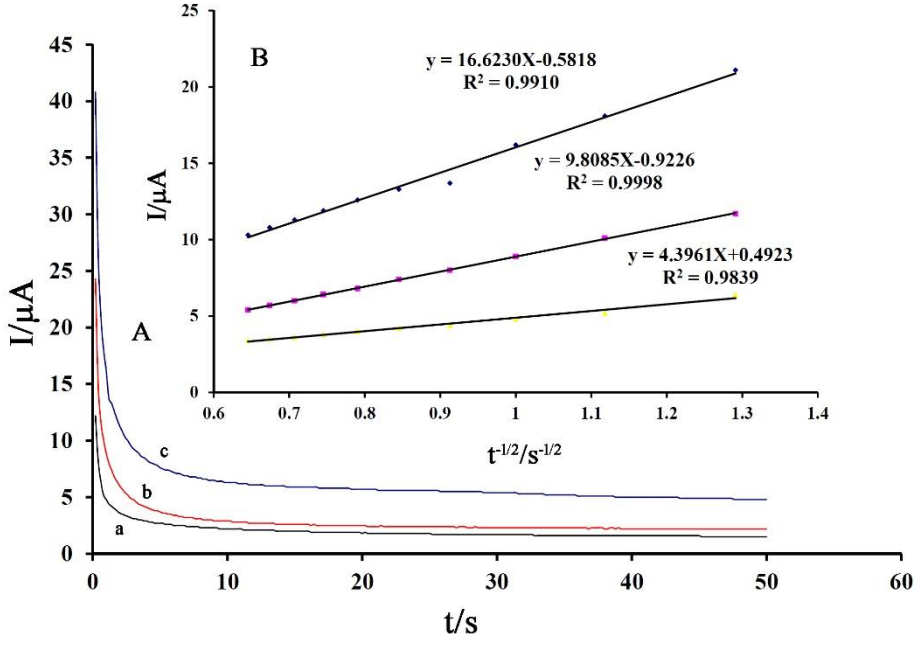



**FIGURE 5** A) Chronoamperograms (a) 100.0, (b) 200.0 and (c) 300.0 μM isoprenaline at the NHIHP/NiO-Pt-Pd/CNTs/CPE. B) Cottrell plots obtained from chronoamperometry

The square wave voltammograms of isoprenaline (in the different concentration range) was recorded at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE (Figure 7 inset). The results show a linear dynamic range 0.003-300 µM ($R^2$=0.9918) and detection limit 0.9 nM that confirm powerful ability of NHIHP/NiO-Pt-Pd/CNTs/CPE as an electroanalytical sensor in determination trace level of isoprenaline.

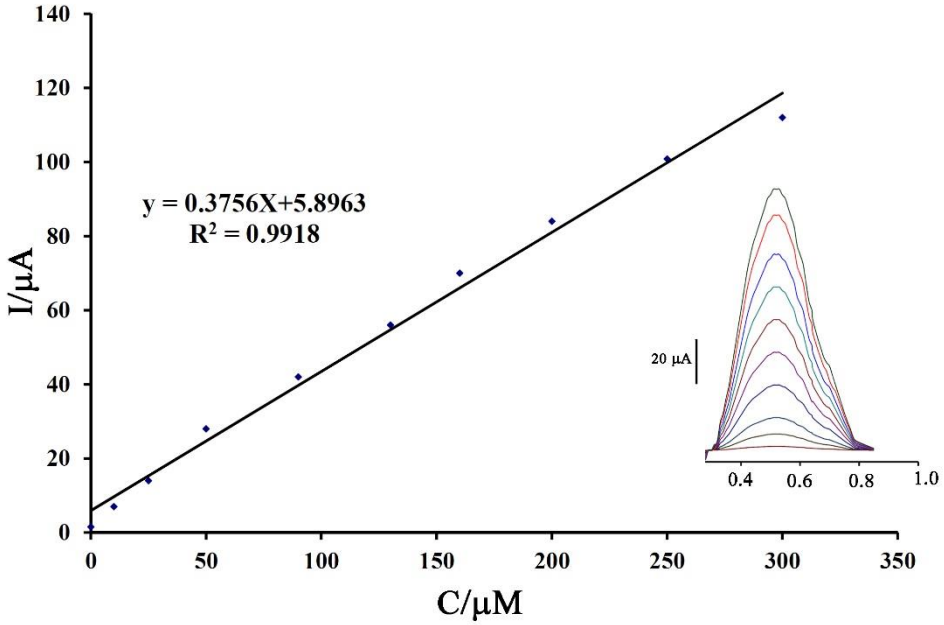



**FIGURE 7** Relation between oxidation current of isoprenaline and its concentration in the linear dynamic range 0.003-300 µM at surface of NHIHP/NiO-Pt-Pd/CNTs/CPE. Insert) Relative square wave voltammograms

The stability of NHIHP/NiO-Pt-Pd/CNTs/CPE was evaluated over a period of 30 days in the presence 600 µM isoprenaline. The results showed 92.12% of initial signal of isoprenaline was remain after 30 days that confirm good stability of NHIHP/NiO-Pt-Pd/CNTs/CPE.

The selectivity of NHIHP/NiO-Pt-Pd/CNTs/CPE for determination of 20.0 µM isoprenaline was investigated using square wave voltammetric method with acceptable error 5% in current. The results showed that 500-fold of glucose, methanol, 800-fold of $Mg^{2+}$, $F^-$ and $K^+$ and 400-fold of valine, methionine, vitamin $B_9$ and tryptophan and 20-fold of dopamine and vitamin $B_2$ did not affect the selectivity.

The capability of NHIHP/NiO-Pt-Pd/CNTs/CPE for determination of isoprenaline in injection, and pharmaceutical serum samples (as real samples) was checked. The obtained data are presence in table 1 and recovery data 98.76-105.06% confirm powerful ability of NHIHP/NiO-Pt-Pd/CNTs/CPE as a new and powerful electroanalytical sensor in determination of isoprenaline.

**TABLE 1** Determination of isoprenaline in real samples (n = 5).

| Samples | Added (µM) | Expected (µM) | Founded (µM) | Recovery % |
|---|---|---|---|---|
| Injection samples | --- | --- | 2.11±0.22 | --- |
| | 10.00 | 11.96±0.67 | 10.16±0.23 | 98.76 |
| Pharmaceutical serum | --- | <LOD | <LOD | --- |
| | 15.00 | 15.00 | 15.76±0.92 | 105.06 |

## Conclusion

The NiO-Pt-Pd/CNTs nanocomposite was synthesis by a simple and fast precipitation method and characterized by FESEM and EDS methods. In continuous the synthesized nanocomposite was used for fabrication of NHIHP/NiO-Pt-Pd/CNTs/CPE. The NHIHP/NiO-Pt-Pd/CNTs/CPE was used as a new electroanalytical sensor for determination of isoprenaline. The NHIHP/NiO-Pt-Pd/CNTs/CPE was successfully used for determination of isoprenaline in the concertation range 0.003-300 µM with detection limit 0.9 nM in real sample with recovery data 98.76-105.06%.

## Orcid:

*Hassan Ali Zamani: https://orcid.org/0000-0002-3156-2781*

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