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Electrochemical Detection of Norepinephrine Using Sponge-like Co_3O_4 Modified Screen Printed Carbon Electrode

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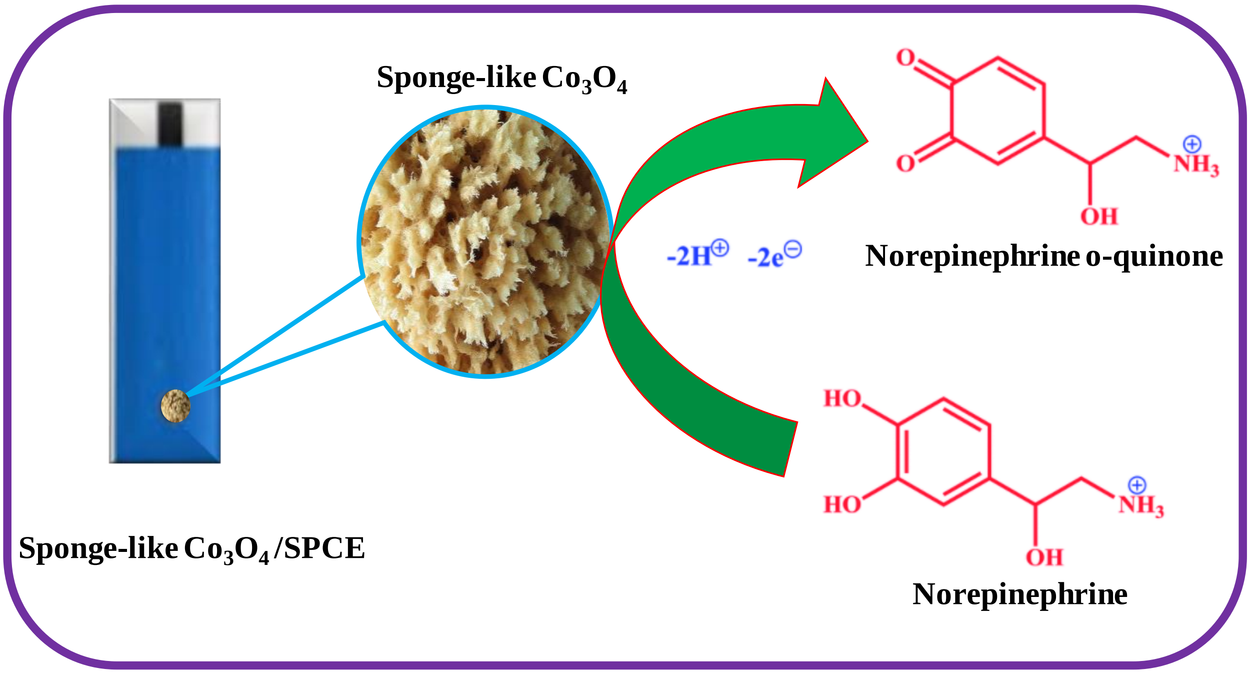
Neural diseases, like Parkinson's and Alzheimer's widely increase portions of degenerative nerve disease, are related to norepinephrine (NE) concentration with proportional correlation. Quantification of NE is difficult as NE coexists with dopamine (DA), ascorbic acid (AA), and uric acid (UA), which interfere with the detection of NE in biological samples. We report the fabrication of sponge-like Co_3O_4 particles modified screen printed carbon electrode the for highly selective and sensitive detection of NE. Compared with recent studies, our newly developed sensor appears to have not only a wide detection range (0.1-1525 μM) but also superior detection limit (75 nM). The Co_3O_4 particles were prepared by simple, very cheaper and reproducible method. The effect of concentration and kinetics of electrochemical detection of NE were studied. Furthermore, the modified electrode was appreciable stability, repeatability and reproducibility. In addition, the practical feasibility of the modified sensor is demonstrated in biological samples.

Keywords: Nanomaterials, metal nanoparticles, electrocatalysis, electrochemical methods, sponge like Co_3O_4, neurodegenerative diseases, norepinephrine.

1. INTRODUCTION

Norepinephrine (NE) is a very important catecholamine with numerous roles in mammalian central nervous systems and it plays a central role in health and disease [1]. This one kind of endogenous hormone released by specific adrenal medulla, with relinquished as a metabotropic neurotransmitter from nerve in the sympathetic nervous system [2]. The human adrenal medulla releasing about 20 percentage of NE, So the adrenergic neurons are more important for the major NE production [3]. NE has more important for attention, focus, learning, memory and the sleep–wake cycle [4], moreover it is also used as a performance increasing drug in competitive game by a athletes; So prohibited by the world anti-doping agency [5]. This promotes the convert of glycogen to glucose in the liver and helps in converting the fats into fatty acids, resulting in an increment of in energy production in animals [6][7].

Many different classical methods for determination of norepinephrine were developed, including HPLC (high-performance liquid chromatography) [8, 9], GC (gas chromatography) [10], ion chromatography [11], and spectrophotometry [12, 13]. Researcher focused modified electrodes were used for electrochemical analysis of norepinephrine [14, 15]. Also many analytical methods can be used for the determination of NE, electrochemical techniques are unique due to their simplicity, cheap, very easy-handling, rapid response time, portability and low power consumption [16-19]. Unmodified electrodes are poor in selectivity, sensitivity, and encounter fouling issues. Metal and metal oxide NPs has been used to modify electrodes for use as electrocatalysts and biosensors; hence they play an most important role in medical device [20-23]. Co_3O_4 is important nanomaterial many researchers used in catalysis, gas sensors, electrochromic films, battery, catalytic materials and magnetic materials [24-27]. A number of methods such as co-precipitation, hydrothermal synthesis, thermal decomposition and chemical reduction were used for the preparation of Co_3O_4 particles [28-30].



Scheme 1 Schematic Representation for Electrochemical Detection of Norepinephrine using sponge-like Co_3O_4 modified screen printed carbon electrode

Herein, we have prepared sponge-like Co_3O_4 particles to fabricate an electrochemical sensor. The nanomaterial was prepared by a straightforward solution-assisted method using low-cost precursors. We have adopted screen-printed carbon electrodes (SPCE) to prepare working electrode because of its low-cost, easy fabrication, flexibility and reproducibility. The sponge-like Co_3O_4 particles modified SPCE electrode is found to be a suitable electrode material for the detection of NE present in biological samples. The as-synthesized of sponge-like Co_3O_4 particles were characterised by SEM, EDX and EIS analysis.

2. EXPERIMENTAL METHODS

2.1. Apparatus and Chemicals

Cobalt chloride ($CoCl_2$), and norepinephrine, those chemicals were were purchased from Sigma-Aldrich. DD water (Double distilled water) was used for all the electrochemical experiments. 0.05 M phosphate buffer (pH 7.0), prepared from phosphate solution was used as supporting electrolyte. Electrochemical studies were performed in a conventional three-electrode cell using SPCE as a working electrode (area 0.3 cm^2), Ag|AgCl saturated KCl as a reference electrode and Pt wire as a counter electrode. All the electrochemical measurement was carried out using CHI 1205A and CHI 1205B (U.S.A). Surface studies were carried out using (SEM) Hitachi S-3000 H-Scanning Electron Microscope. (EDX) Energy-dispersive X-ray spectra were recorded using Horiba Emax x-act. Electrochemical impedance spectroscopy (EIS) studies were carried out using EIM6ex Zahner (Kronach).

2.2. Preparation of sponge-like Co_3O_4 particles /SPCE

Sodium acetate (3.0 g), $CoCl_2 \cdot 6H_2O$ (1.2 g), and trisodium citrate dihydrate (0.2 g) were dissolved in the mixture of glycerol (30 mL) and distilled water (10 mL) at room temperature [31, 32]. The homogeneous suspension has then transferred into an autoclave. Sodium hydroxide (1.6 g) and $NaH_2PO_2.H_2O$ (3.2 g) dissolved in 20 mL distilled water was slowly brought into the autoclave. After heating at 140 °C for 15 h, the solution was cooled. The resultant product deposited of the autoclave was then rinsed with distilled water, absolute ethanol and finally dried under air at 70 °C for 12 h. The purified Co_3O_4 was dried and redispersed (0.5 mg mL^{-1}) in water and ethanol (1:1; v/v) mixture. Further, 6 μL of sponge-like Co_3O_4 particles was dropped at the SPCE surface and dried at ambient condition. The resulting sponge-like Co_3O_4 particles modified SPCE was used to further detection studies.

3. RESULTS AND DISCUSSION

3.1 Characterization of sponge-like Co_3O_4 particles

The Co_3O_4 (**Fig. 1A**) clearly reveals the sponge -like surface from SEM image. The EDX spectrum of sponge-like Co_3O_4 particles (**Fig. 1B**) verified the presence of expected elements, oxygen (O) and cobalt (Co) with weight percentage of 32 and 68. **Fig. 2A** displays the corresponding EDX image of the sponge-like Co_3O_4 particles, which clearly showed signals for Co and O atoms.

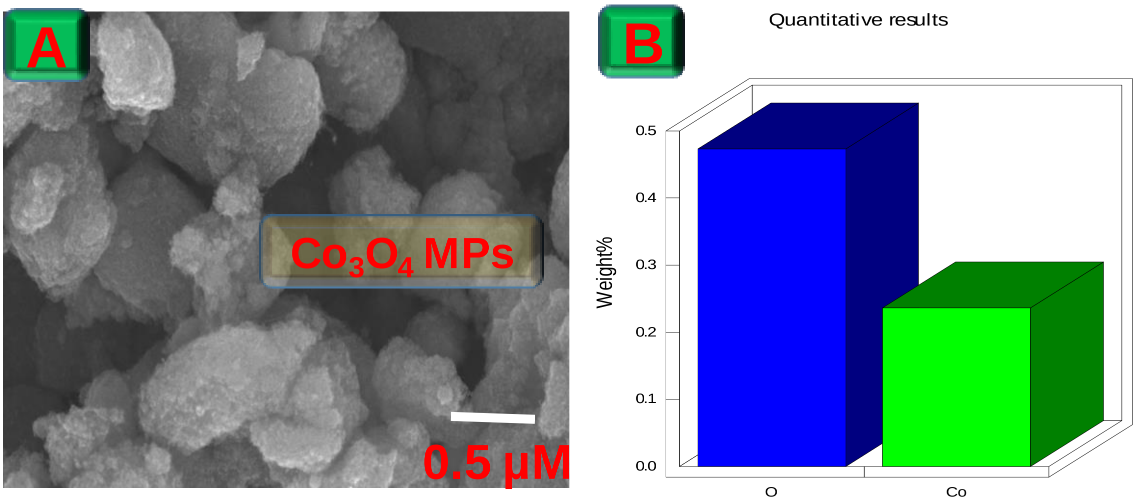


Figure 1. A) SEM image of sponge-like Co_3O_4 Particles, B) Elemental Weight Percentage from EDX spectrum.

Fig. 2B shows obtained EIS (a) at unmodified SPCE only, and (b) sponge-like Co_3O_4 /SPCE in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. The charge transfers resistance (R_{ct}) values obtained at unmodified SPCE and sponge-like Co_3O_4 modified SPCE are 201 and 60 Ω, respectively. The sponge-like Co_3O_4 /SPCE has higher electrical conductivity compare to other electrode. So high conductivity and low resistance of the materials is useful for the developments of sensitive and sensitive sensors [33, 34].

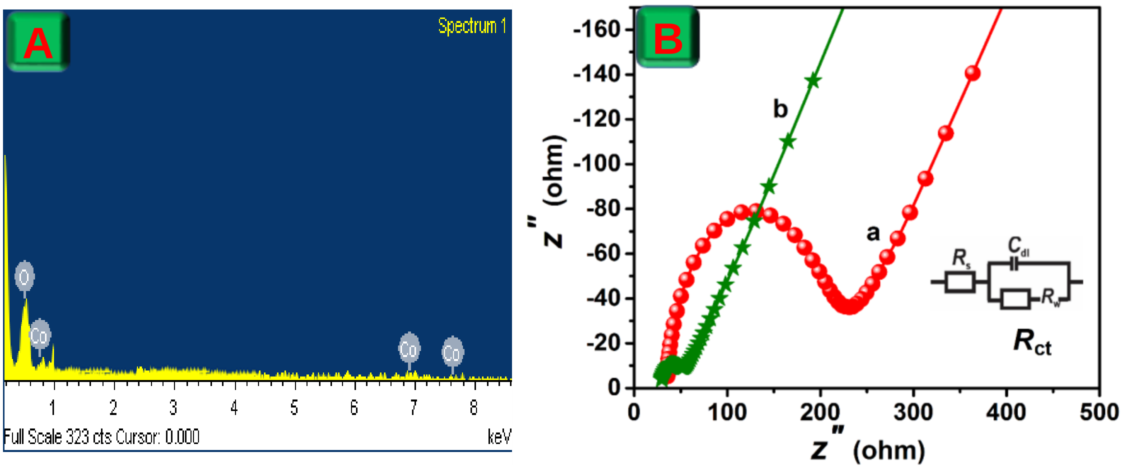


Figure 2. A) EDX spectrum of sponge-like Co_3O_4 particles, B) EIS curves of unmodified SPCE (a), sponge-like Co_3O_4 particles/SPCE (b) in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$ solutiuon.

3.2 Detection of NE at sponge-like Co_3O_4 / SPCE

The cyclic voltammogram (CVs) (Fig. 3A shows) obtained at (a) unmodified SPCE, (b) sponge-like Co_3O_4 particles/SPCE in phosphate buffer solution (pH 7.0) containing 50 µM NE at the scan rate of 50 mV s^{-1}. The unmodified SPCE shows poor electrocatalytic ability of oxidise NE but modified material of sponge-like Co_3O_4 particles/SPCE has shown better electrocatalytic efficiency to NE. Moreover, sponge-like Co_3O_4 particles/SPCE was excellent electrocatalytic ability to oxidise NE at lower over-potential (0.24 V) with a sharp peak and enhanced peak current [14, 35]. The voltammetric results revealed that the sponge-like Co_3O_4 particles/SPCE has the good catalytic ability over unmodified electrode. Because the Co_3O_4 particles owns the high surface areas, high conductivity and more catalytic sites (Fig.1A) and these all characteristics of the Co_3O_4 particles reason the obtained more electrocatalytic ability of this material [36, 37]. Fig. 3B shows the CV curves obtained at sponge-like Co_3O_4 particles/SPCE in phosphate buffer solution (pH 7) containing change the concentration of NE. The oxidation peak current (i) linearly increases as the concentration of NE, and plot to the concentration of NE and the response current exhibited excellent linearity (Fig. 3C).

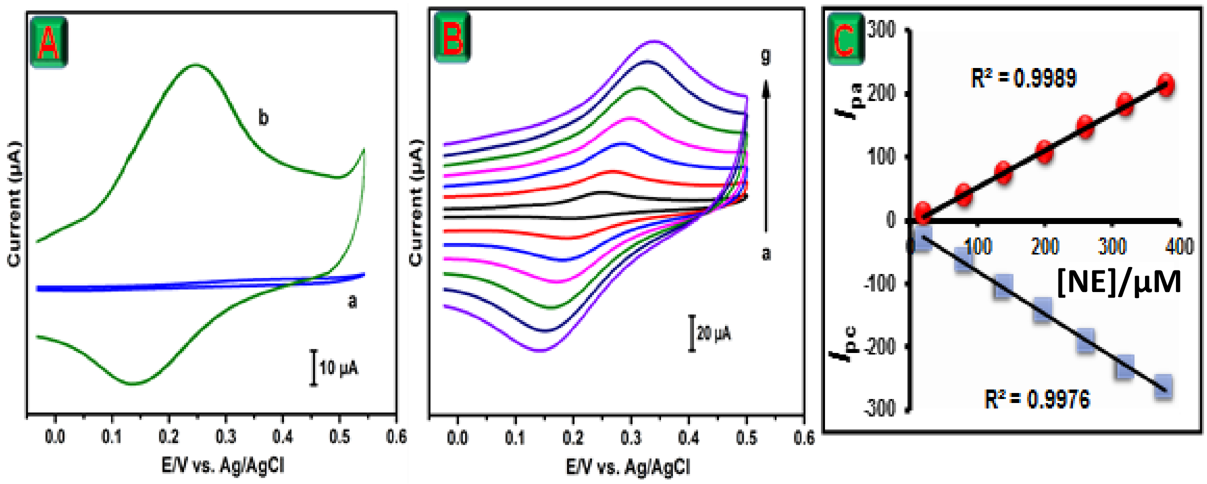


Figure 3. (A) Cyclic voltammogram shown at (a) bare SPCE, (b) sponge-like Co_3O_4 particles/SPCE in 0.05M phosphate buffer (pH 7) containing 50 µM NE at the scan rate of 50 mV s^{-1}. (B) Cyclic voltammogram obtained at Co_3O_4 /SPCE with different concentration of NE (50 to 350 µM) using 0.05M PBS (pH 7.0). (C) Plot of Peak Current vs. [NE]

3.3 Different scan rate study

Influence of different scan rate towards the electrocatalytic reaction of NE at sponge-like Co_3O_4 particles/SPCE was studied (**Fig. 4A**). The anodic peak of NE increased linearly as the scan rate increases, (0.05 –0.5 Vs^{-1}). The plot between anodic peak current and scan rate shown linearity, and oxidation process is a surface controlled diffusion process studied (**Fig. 4B**).

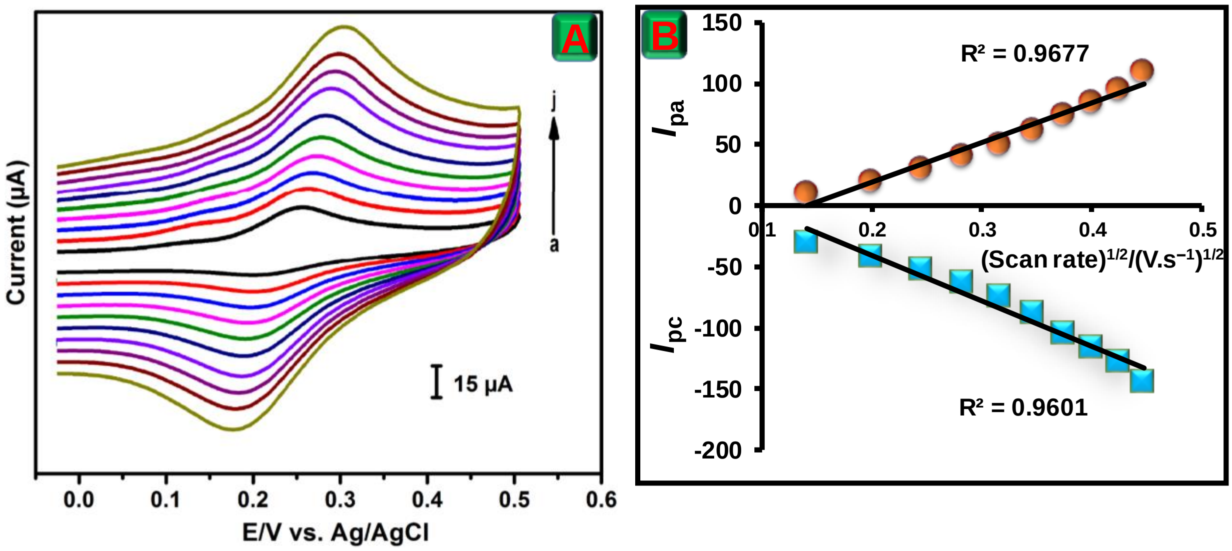


Figure 4. (A) Effect of the kinetics study on the cyclic voltammogram of 50 µM NE at the sponge-like Co_3O_4 particles/SPCE at 0.01 to 100 V s^{-1} and (B) Plot for (scan rate)$^{1/2}$, V.S^{-1} vs. peak current, µA.

3.4 Determination of NE

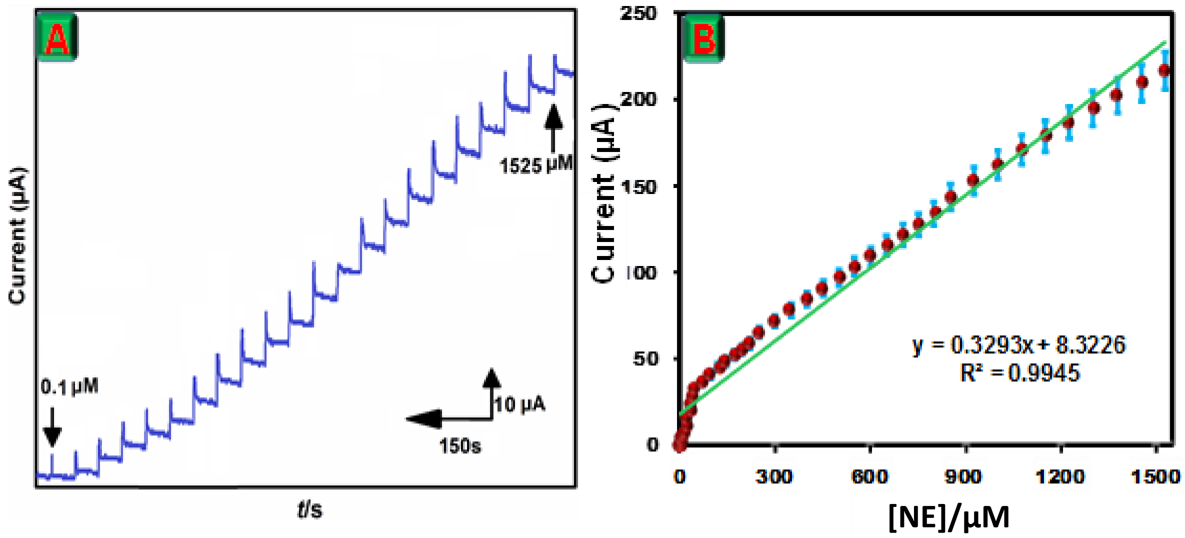


Figure 5. (A) Amperometric i–t response of sponge-like Co_3O_4 particles modified electrode upon each addition of 0.1 µM, 0.3 µM, 1 µM, 10 µM, 30 µM, 50 µM and 75 µM NE into the continuously stirred PBS (pH 7) at the rotation speed of 1200 rpm. Eapp = +0.24 V. (B) Plot of [NE], µM vs. peak current, µA.

Fig. 5A displays the amperometric response of sponge-like Co_3O_4 particles/SPCE towards the sequential addition of NE into phosphate buffer solution (pH 7). The given potential was + 0.24 V and speed of rotation electrode was 1200 RPM, and each addition, a good response current is obtained and its increased 96.8% of steady-state current at 7s of NE addition. Thus, the sponge-like Co_3O_4

particles/SPCE film delivered prominent and sensitive response to NE. The concentration dependent linear plot showed excellent linearity with a slope of 0.3293 µA µM^{-1} (**Fig. 5B**). The working concentration range was found to be linear from 0.1 to 1525 µM with a sensitivity of 2.485 µAµM^{-1} cm^{-2}. The LOD (limit of detection) were calculated as 75 nM and the LOD were calculated using the formula, (LOD= 3 s_b/S)where s_b is the standard deviation of five blank measurements and S is the sensitivity. The following parameters are sensor important parameters such as sensitivity, LOD, and linear range of NE. Table 2. Shown previously reported modified electrodes with present modified electrode work Table 2 [20].

3.4 Interference study, Stability, Repeatability and Reproducibility

Interference study of the sponge-like Co_3O_4 modified electrode to detect NE in presence of interferents has been provd. The electrochemcial signal of the electrode into 200 µM of NE (fig. 6). As shown in the study, sponge-like Co_3O_4 particles/SPCE film modified electrode delivered excellent amperometric response to NE, but very low responses to all the other interfernt analytes added. Then verified the storage stability of the sponge-like Co_3O_4 material modified electrode, its electrocatalytic response to NE was noted day for a week. And then during the one week of storage period, the current slightly decreased, but not more because about 97.4 % of the peak current was retained, and the good storage stability of the modified Co_3O_4 particles/SPCE. The electrode exhibited good repeatability in RSD of 3.22%, for repetitive stuies carried out using the same Co_3O_4 modified electrode and it exhibited reproducibility with RSD of 2.11%.

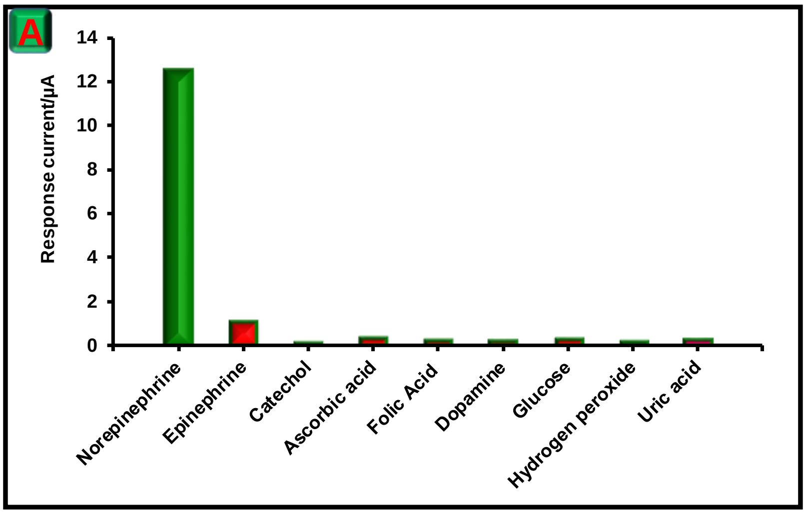


Figure 6. Selectivity study: Plot of Response Current vs. NE and other interfering agents

3.5 Practical applicability

Iinvestigated the practical applicability of newly prepared sponge-like Co_3O_4 particles modified SPCE towards the detection of NE in spiked biological samples. 2 mL of the urine sample was added with 100 mL phosphate buffer solution (pH 7) and known concentration of NE was spiked into the solution. Same method followed to spiked human serum sample also prepared. Amperometric i-t was done the spiked solutions by following the experimental conditions optimised for lab samples. For human urine sample, the sponge-like Co_3O_4 modified electrode sensitive signals. The added, found and recovery value was calculated and shown as Table. 1**.** Consequently, the newly prepared Co_3O_4 modified electrode is proved to have excellent practical ability

Table 1. Determination of NE in real samples using sponge-like Co_3O_4 particles/SPCE

Real Samples	[NE]			
	Added (nM)	Found (nM)	Recovery (%)	*RSD (%)
Human serum	200	194.5	97.25	3.44
	500	493.7	98.74	3.15
Urine sample	200	195.5	97.75	3.12
	500	491.9	98.38	3.18

* RSD (Relative Standard Deviation) of three individual measurements

Table 2. Comparison of electroanalytical parameters obtained at sponge-like Co_3O_4 particles/SPCE modified electrode towards NE with previous reports

Electrode	Linear range/ µM	Detection limit/µM	Ref.
ZrO_2 modified CPE	0.1 – 200	0.85	[14]
Carbon-coated nickel magnetic nanoparticles	0.2 – 500	0.65	[38]
Calix[4]arene crown-4 ether	0.5 – 9.7 and 9.7 – 230	0.25	[39]
Gold nanoparticles-doped DNA composite electrode	0.5 – 80	0.05	[40]
Antimony Doped Tin Oxide-silica composite	0.9 – 150	0.33	[41]
polymer-coated PdNPs	0.5 –80	0.1	[42]
$FeMoO_4$ nanorods	0.5 – 200	0.037	[43]
sponge-like Co_3O_4 particles/SPCE	0.1-1525	0.075	This work

4. CONCLUSION

The electrochemical detection of NE was developed using sponge-like Co_3O_4 particles/SPCE. The successful formation of the Co_3O_4 particles was verified by SEM, EIS, EDX, and electrochemical studies. Those studies proved that the material has the good electrocatalytic property towards the oxidation of NE. The materials displayed less over potential with excellent peak current for the oxidation of NE in PBS. The amperometric determination showed a long linear range of 0.1 to 1525 µM and sensitivity of 2.485 $\mu A \mu M^{-1}$ cm^{-2} with the low detection limit is 75 nM. In addition, the developed sensor has shown good stability, repeatability and reproducibility.

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