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Molecularly imprinted sensor based on *o*-aminophenol for the selective determination of norepinephrine in pharmaceutical and biological samples

Rosy, Himanshu Chasta, Rajendra N. Goyal *

Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247667, India

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ABSTRACT

An electrochemical sensor has been developed for the selective determination of norepinephrine (NE) using the molecularly imprinted technique. The imprinted polymer film at the surface of glassy carbon electrode is prepared by the electropolymerization of *o*-aminophenol in the presence of NE. Imprinted polymer film was characterized by atomic force microscopy (AFM), field emission scanning electron microscopy (FE-SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The imprinted sensor showed a well-defined anodic peak at a potential of ~198 mV in phosphate buffer of pH 7.2 using square wave voltammetry. A linear increase in peak current was found with the increasing concentration of NE in the range from 50×10^{-9} to 10×10^{-6} mol $L^{-1}$and the limit of detection ($3\sigma/b$) was found to be 4.9×10^{-10} mol L^{-1}. The imprinted sensor has been successfully employed to ascertain the content of NE in the commercially available pharmaceutical preparations. The biological applicability of the developed sensor has been delineated by the determination of NE in human plasma and urine samples using the standard addition method. The proposed sensor exhibited high degree of selectivity for NE in comparison to other structurally similar biomolecules present in biological samples, along with long term stability, good reproducibility and excellent capacity of regeneration of molecular recognition sites.

1. Introduction

Norepinephrine (NE) is an important member of catecholamine neurotransmitters exuded by the adrenal medulla in the central nervous system of mammals, and plays crucial physiological roles in the function of the renal, hormonal, cardiovascular, central nervous and reproductive systems [1,2]. High level of catecholamines is interconnected with stress, a fall in blood pressure or blood volume, thyroid hormone deficiency, congestive heart failure, and arrhythmias, whereas, low level of catecholamines is implicated towards idiopathic postural hypotension and depression [3]. Recent reports have suggested that the catecholamines may affect the progression of ovarian cancer cells by modulating the expression of Vascular Endothelial Growth Factor (VEGF) and Matrix Metalloproteinase (MMP). In addition, the expression of VEGF, MMP-2 and MMP-9 as well as VEGF, IL (interleukin)-6 and IL-8 is regulated by NE in nasopharyngeal carcinoma tumor cells and human melanoma tumor cell lines [4,5]. NE also enhances adhesion of human immunodeficiency virus-1-infected leukocytes to cardiac microvascular endothelial cells and also triggers HIV replication *via* protein kinase [6]. NE is an important constituent of drugs which are used for the treatment of various types of disorders such as myocardial infarction, hypertension, bronchial asthma, organic heart disease, diabetes, anxiety and attention-deficit/hyperactivity disorder (ADHD) and is also used in cardiac surgery [7,8]. Also, NE is a stimulant drug which belongs to prohibited list of 2005 chemicals of World Anti-Doping Agency. Due to its significant role in doping, neuroscience, clinical diagnosis and pharmaceutical applications, various analytical techniques have been implemented for NE determination. These techniques include chromatographic [9–14], fluorescent [15], spectrophotometric [16,17], capillary electrophoresis [18] and chemiluminescence methods [19]. Almost, all of these techniques require expensive devices and maintenance along with complicated, tedious and time consuming derivatization, sample preparation and extraction steps. From the last two decades, electrochemical techniques have been used as a highly-sensitive, convenient and effective tool for the analysis of the catecholamine

* Corresponding author. Tel.: +91 1332 285794 (O); +91 1332 285029 (R).
E-mail address: rngcyfcy@iitr.ac.in (R.N. Goyal).

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neurotransmitters and their metabolites *in vivo* or *in vitro* owing to their simplicity, low cost and rapidness as compared to the other routine analytical techniques. Various types of modified electrodes have also been introduced for the analysis of NE including, eriochrome cyanine R film modified glassy carbon electrode [20], MWCNTs–PNDGAChi biocomposite film modified gold electrode [1], ZrO_2 nanoparticles-modified carbon paste electrode [3], 2,2′-[1,2 buthanediylbis (nitriloethylidyne)]-bis-hydroquinone (BH) and TiO_2 nanoparticles modified carbon paste electrode [21], multi-walled carbon nanotubes modified edge plane pyrolytic graphite electrode [22], gold nanoparticles modified ITO electrode [23], and many more [24–27], but the selectivity of analyte on modified electrodes is largely influenced by the high concentration of other metabolites such as uric acid, ascorbic acid *etc*., present in biosamples. Thus, the aim of this study was to prepare a sensor based on the molecularly imprinted polymer (MIP) technique for the selective and sensitive determination of NE in human biological fluids.

Recently, molecularly imprinted polymers (MIPs) have gained interest to the scientists involved in the sensor development. This attention is attributed to the potential advantages of using MIPs in place of natural receptors and enzymes because of their superior stability, affordability and easy preparation in contrast to the enzymes, antibodies, or receptors. Most of these molecules are biomacromolecules, and their sensitive properties, such as instability on exposure to high temperatures, organic solvents and variation in pH conditions, causing hindrance in their practical application [28,29]. Molecularly imprinted polymer receptors (MIPs) combine the robust mechanical and chemical strength, characteristic of cross-linked materials (pyrrole, aniline, *o*-phenylenediamine, *m*-aminophenol, *o*-aminophenol *etc*.) with selectivity for the target analytes [30]. It involves formation of a complex, between a given target (template) molecule and functional monomers dissolved in an appropriate solvent, which is then inserted by polymerization into a growing polymer chain [31]. Subsequent extraction of the template creates a molecular memory into the polymer, which has a complementary shape and size of the imprint species and arraying functional groups [32].

Electropolymerization [33], drop coating [34] and composite membranes techniques [35] have been used for the preparation of MIP films. Among these, the electropolymerization procedure has more advantages over the others in generation of a rigid, uniform, and compact MIP film with good adherence onto an electrode surface of any shape and size [29]. Moreover, the thickness as well as morphology of MIP film is easily controlled by electropolymerization [36]. In recent years, different sensors exploiting the characteristic of MIP had been designed [37,38]. In this paper, the fabrication of a highly selective and sensitive NE sensor is investigated using an *o*-aminophenol MIP as an artificial recognition element, because it exhibits several advantages with respect to other electroactive polymers. *o*-Aminophenol can be *in situ* electropolymerized on different electrode surfaces, and the polymer thickness can be controlled within 10–100 nm due to a self-limiting growth. In addition, the poly(*o*-aminophenol) film can be easily regenerated after use. Another interesting characteristic of poly(*o*-aminophenol) is the presence of an electron-donating OH group next to the imine nitrogen that increases the electron density at the imine sites. Also, OH by itself is also a potential coordinating site [39].

The fabrication of MIP based sensor using electropolymerization of *o*-aminophenol at a glassy carbon electrode was carried out by using cyclic voltammetry. The concentration of NE in human urine and plasma samples, in the presence of high concentration of interfering metabolites, was determined using the standard addition method. NE is used as a template due to its strong electrocatalytic activity [40]. The proposed sensor exhibited large number of recognition sites for NE along with good stability and reproducibility.

2. Experimental

2.1. Reagents and instrumentation

NE, *o*-aminophenol, perchloric acid ($HClO_4$) and $NaClO_4$ were obtained from Sigma Aldrich, USA. NORAD injection (noradrenaline bitartrate, Neon laboratories ltd. Thane, Maharashtra) was purchased from the local market of Roorkee. Phosphate buffer solutions of $\mu = 1$ mol L^{-1} were made according to the method of Christian and Purdy [41]. All other reagents used during the experiment were of analytical grade. Double distilled water was used for the preparation of all solutions.

Electropolymerization and other electrochemical studies were performed using the Bioanalytical system (BAS, West Lafayette, USA) Epsilon EC-USB voltammetric analyzer equipped with a conventional three-electrode single compartment cell. The electrodes used were Ag/AgCl (3 M NaCl) as the reference, a platinum wire as the auxiliary, and a MIP-modified glassy carbon (imprinted sensor) as the working.

Field emission scanning electron microscopy (FE-SEM) images were obtained with Zeiss ultra plus 55. Atomic force microscopy (AFM) was performed using NTEGRA TS-150 with an Illuminator OPTEM VSI 220 instrument. Electrochemical impedance spectroscopy (EIS) was done using a Galvanostat VERSA STAT-3 (PAR, USA).

2.2. Fabrication of imprinted and non-imprinted sensor

The imprinted sensor was prepared by the electropolymerization of *o*-aminophenol on the surface of the glassy carbon electrode. Prior to the electropolymerization, the surface of the glassy carbon was polished with a paste of alumina powder (grade I) and ZnO on polishing cloth to a mirror like finish surface. Polymerization was then carried out in a solution of 0.01 M NE, 0.02 M *o*-aminophenol and 0.1 M $NaClO_4$ for 30 cycles using cyclic voltammetry in the potential range between -0.20 V and $+1.20$ V. The sweep rate during polymerization was 100 mV s^{-1}. After electropolymerization, the imprinted sensor was dipped in 0.5 M H_2SO_4 overnight to release the imprinted molecules [42]. In strongly acidic condition, release of NE from the stereo-cavity of the molecular imprinting membrane is attributed to the destruction of the hydrogen bonds between NE and the molecular imprinting membrane. Consequently, an electrode with a NE imprinted membrane was obtained. Non-imprinted sensor was also fabricated under identical experimental conditions by taking a solution without NE during the electropolymerization.

2.3. Voltammetric procedure

Stock solution of NE (1 mM) was prepared by dissolving the required amount of the compound in double distilled water. The desired volume of the stock solution was added to the electrolytic cell containing 2 mL of phosphate buffer solution. Final volume was made 4 mL with the help of double distilled water. The imprinted sensor was then dipped in this solution for 30 min after which it was washed with double distilled water and then voltammograms were recorded under optimized parameters. The optimum conditions for cyclic voltammetry (CV) were initial (E): 0 mV, switching potential 1 (E): 800 mV, switching potential 2 (E): -600 mV, final (E): 0 mV and scan rate (ν): 50 mV s^{-1}. The optimized square wave voltammetric parameters used were initial (E): 0 mV, final (E): 800 mV, square wave amplitude (E_{sw}): 25 mV, potential step (E): 4 mV and square wave frequency (f): 15 Hz. All

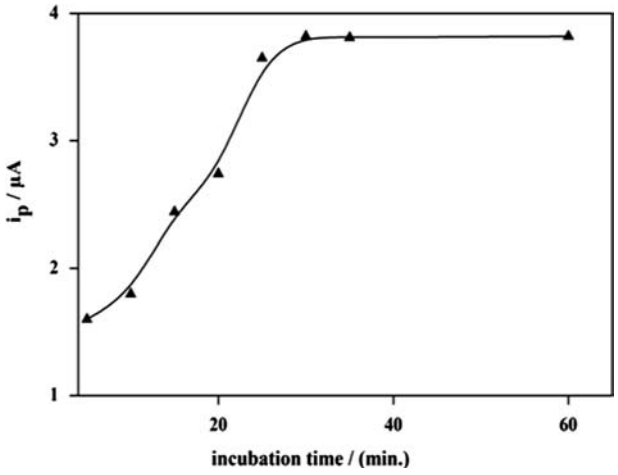


Fig. 1. Effect of incubation time on the peak current response of 1 μM NE observed using the imprinted sensor at pH 7.2.

the potentials reported are with respect to Ag/AgCl electrode at an ambient temperature of 25 ± 2 °C.

3. Results and discussions

3.1. Optimization of incubation time

Incubation time is an important parameter which strongly influences the interaction between imprinted film and analyte molecules and is measured as the function of peak current or current response. The effect of incubation time on binding capacity was evaluated by immersing the proposed sensor into 1 μM concentration of NE from 5 min to 50 min and the current response was measured in phosphate buffer solution of pH 7.2. The current response was found to increase with increasing incubation time, and then reached at a constant value because of occupancy of the molecular recognition sites by NE molecules. The maximum current response was observed at an incubation time of 30 min as shown in Fig. 1. Thus, the incubation time of 30 min was optimized for the subsequent studies.

3.2. Surface morphology

The surface morphologies of imprinted film were investigated by an atomic force microscope (AFM) and a field emission scanning electron microscope (FE-SEM). The AFM and FE-SEM images of imprinted film and non-imprinted film are presented in Fig. 2. The imprinted film exhibited a three dimensional network of porous molecular recognition sites embedded with NE molecules as clearly shown in Fig. 2b and d, and non-imprinted film was relatively flat and compact (Fig. 2a and c). From AFM, the average surface roughness for bare, MIP and nMIP was also calculated. It was observed that polymerization leads to increased roughness of the sensor as the average surface roughness corresponding to bare is 3.5770 nm in comparison to 8.5712 nm for nMIP; average surface roughness for MIP (7.7574 nm) is although little less than nMIP. The differences in these images and the values of average surface roughness are due to the presence of the template molecules that interrupted the growth of the electrochemically synthesized polymeric chain as reported in literature [43]. Moreover, the thickness of the film was found to be ~30 nM. It was thus concluded that the thin film of non-conducting polymer (10–100 nm) is responsible for the fast response time and high selectivity.

3.3. Electrochemical characterization

Cyclic voltammetric and impedance studies were performed for further characterization of imprinted sensor. Typical cyclic voltammograms of NE imprinted sensor before and the after removal of NE molecules and non-imprinted sensor in phosphate buffer solution of pH 7.2 are depicted in Fig. 3. The non-imprinted and imprinted sensors after removal of NE did not exhibit any peak in CV. On the other hand NE imprinted sensor exhibited an oxidation peak (I_a) at 0.240 V. In the reverse sweep two cathodic peaks are observed (I_c; II_c). On further reversal of sweep direction a new oxidation peak II_a was observed. Peaks I_c and II_c formed quasi-reversible couples with peaks I_a and II_a respectively. The observed CV behavior is similar to that reported in literature and represents conversion of NE (I) to 3-hydroxy-2,3-dihydro-1H-indole-5,6-dione (IV). The tentative mechanism for this conversion is presented in Scheme 1 [44]. The presence of peaks in NE imprinted sensor indicates that the oxidation of NE embedded inside the molecular cavities occurs in CV studies. No significant peaks were obtained after the removal of NE molecules from imprinted sensor in 0.5 M H_2SO_4, indicating that NE molecules were effectively removed from the imprinted sensor and the observed cyclic voltammograms were identical to that of the non-imprinted sensor.

The adsorption controlled nature of the process was also examined by performing scan rate studies in the range of 10–300 mV s^{-1} using cyclic voltammetry and observing the influence of square wave frequency (f) on peak current (i_p) of 1 μM NE in the frequency range of 5–100 Hz at pH 7.2 using the imprinted sensor. The peak current was found to increase with increasing scan rates and similar behavior was observed on increasing the frequency in Square wave voltammetry. The plot of peak current vs. scan rate and peak current vs. square wave frequency clearly suggested that the electrode process for NE is adsorption controlled at imprinted sensor [23] and the linear relationship between peak current and scan rate can be represented as

$$i_p\ (\mu A) = 0.105\nu\ (\text{mV s}^{-1}) + 1.389$$

with a correlation coefficient of 0.992, where ν is the scan rate and i_p is the peak current and the dependence of peak current on square wave frequency can be expressed by the following relation:

$$i_p(\mu\ A) = 0.0.221f\ (\text{Hz}) + 0.790(\text{correlation coefficient of } 0.995)$$

Adsorption of NE at imprinted sensor was further confirmed by linearity of $i_p/\nu^{1/2}$ vs. log ν and log i_p vs. log ν plots [45,46]. The relation between log i_p vs. log ν can be expressed by the following equation:

$$\log\ i_p = 0.851\ \log\ \nu - 0.590$$

having correlation coefficient of 0.987. The values of peak potential of the oxidation peak I_a were shifted towards more positive potentials with increasing scan rate. The following linear regression equation was observed for peak potential (E_p) vs. log ν plot

$$E_p = 82.957\ (\log\ \nu) + 82.415$$

with a correlation coefficient of 0.984.

Impedance measurements of the imprinted and non-imprinted sensors were recorded in an 1:1 solution of 10 mM $K_3[Fe(CN)_6]$ and 1 M KCl. Fig. 4 represents the electrochemical impedance spectra of bare GCE, imprinted sensor before and after removal of template and non-imprinted sensor, respectively. The charge transfer resistance (R_{CT}) of bare GCE was found to be 763 Ω, which is much lower in comparison to 1685 Ω corresponding to imprinted sensor. High R_{CT} value suggests that polymer layer fabricated on the electrode surface acquired non-conductive properties under given experimental conditions. After immersing

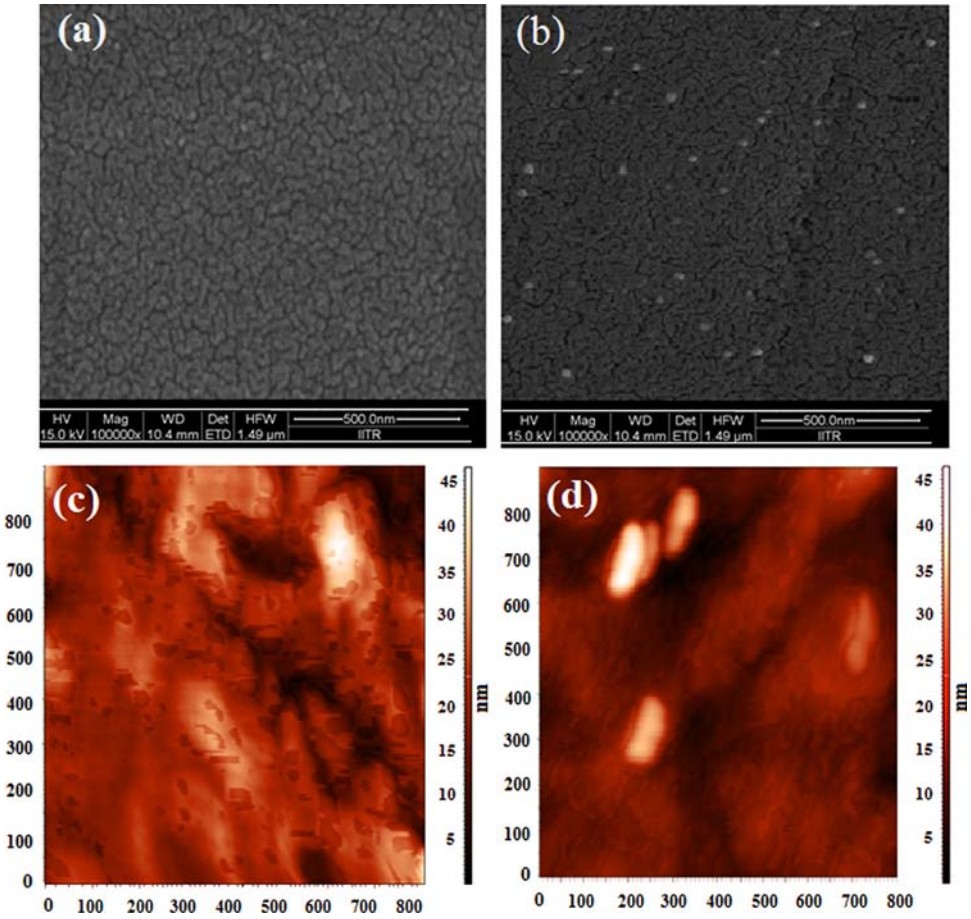


Fig. 2. FE-SEM images of (a) non-imprinted sensor and (b) imprinted sensor and AFM images of (c) non-imprinted sensor and (d) imprinted sensor.

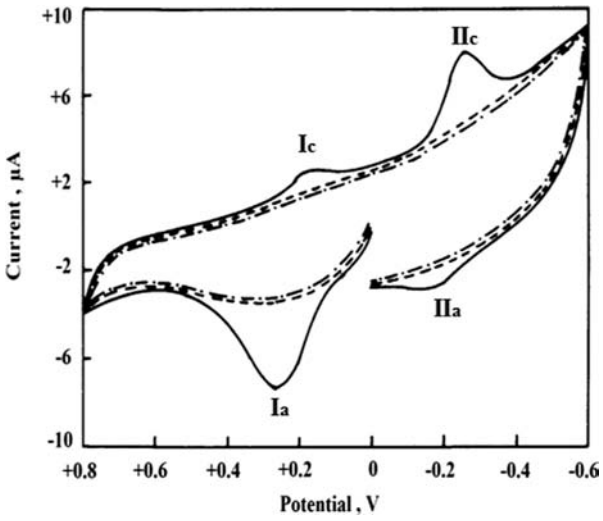


Fig. 3. Cyclic voltammograms of imprinted sensor (a) before removal of NE (—), (b) after removal of NE (-·-·-) and (c) non-imprinted sensor (–·–·–·) at pH 7.2 at 50 mV s^{-1}.

the modified electrode in NE for 30 min, the R_{CT} substantially increased from 1685 Ω to 5402 Ω, which could be attributed to the recognition of NE by the imprinted cavity of *o*-aminophenol membrane on the electrode. Binding of NE results in blocking of the available cavity which is the part of surface not covered with non-conducting polymer and consequently higher R_{CT} value for the redox process of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ is witnessed. On the other hand, the resistance of non-imprinted sensor was found to be much higher (8163 Ω). This high resistance indicates that the polymer membrane does not have available recognition site and probably not a good conductive surface for the electrochemical processes. From these results, it can be concluded that the NE imprinted poly(*o*-aminophenol) membrane has specific recognition sites for NE [42]. Hence, imprinted sensor has been employed for further detailed studies for NE determination.

3.4. Voltammetric studies

3.4.1. Effect of pH

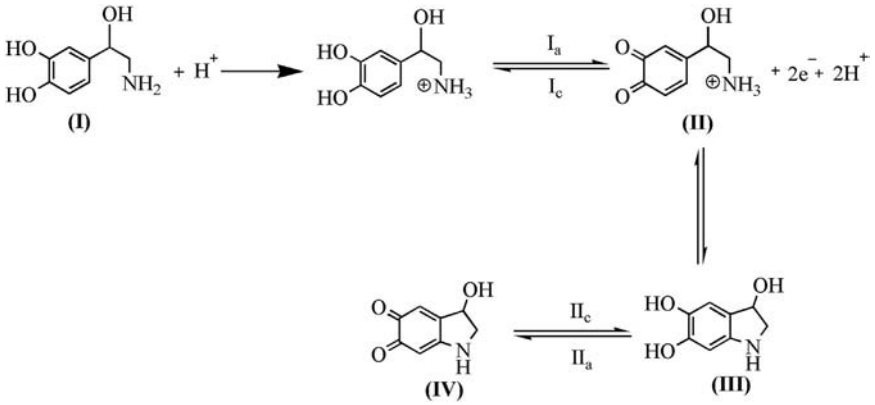
The influence of pH on anodic peak potential of 1 μM NE was determined by using square wave voltammetry at imprinted sensor in the pH range 2.4–10.0 (incubation time: 30 min before each measurement). It was observed that peak potential (E_p) of NE shifted to less positive potentials with increase in pH. The E_p vs. pH plot was linear and dependence of anodic peak potential of NE on the pH of supporting electrolyte can be expressed by the following equation:

$$E_p = [633.750 - 59.273\ \text{pH}]\ \text{mV vs. Ag/AgCl}$$

having correlation coefficient of 0.993. The value of dE_p/dpH ~60 mV/pH clearly indicated that an equal number of electrons and protons were involved in the oxidation of NE as reported in the literature [44].

3.4.2. Effect of concentration

To investigate the influence of various NE concentrations on peak current, square wave voltammograms were recorded in the phosphate buffer solution of pH 7.2 at the imprinted sensor. It was



Scheme 1. Representation of the tentative redox mechanism of NE at imprinted sensor.

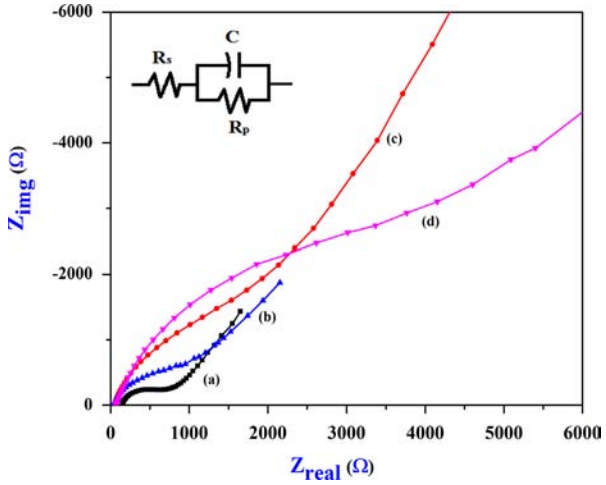


Fig. 4. Observed impedance of (a) bare GCE, (b) imprinted sensor without NE, (c) imprinted sensor with NE and (d) non-imprinted sensor. Inset shows the equivalent circuit model.

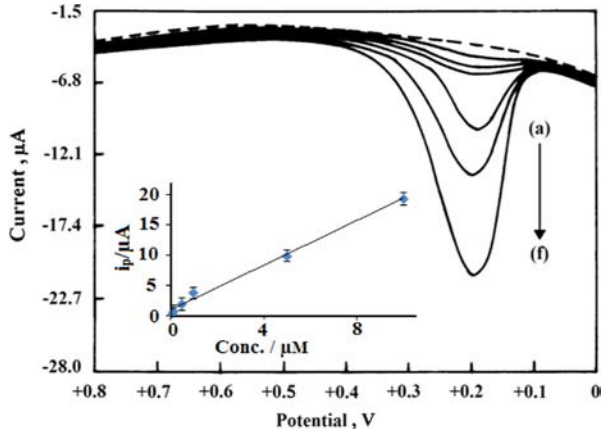


Fig. 5. Observed square wave voltammograms for (i) blank phosphate buffer solution (…..) and (ii) increasing concentration of NE [curves were recorded at (a)=0.05; (b)=0.1; (c)=0.5; (d)=1; (e)=5; (f)=10 μM concentration (incubation time: 30 min)] using imprinted sensor in phosphate buffer solution of pH 7.2. Inset: the observed calibration plot.

observed that the oxidation peak current systematically increased with increasing concentration of NE in the range 0.05–10 μM as presented in Fig. 5. The plot between peak current (i_p) vs. concentration (C) showed a good linearity as depicted in the inset of Fig. 5 and the dependence of peak current (after subtracting background current of phosphate buffer solution) on concentration can be expressed as

$$i_p\ (\mu A) = 1.859\ C\ (\mu M) + 0.906$$

with a correlation coefficient of 0.994 for NE. The detection limit at imprinted sensor was evaluated by using the relation $3\sigma/b$, where σ is the standard deviation of blank solution (phosphate buffer solution of pH 7.2) and b is the slope of the calibration curve and was found to be 4.9×10^{-10} M. The sensitivity of NE determination was calculated as 1.859 μA μM^{-1}. A comparison of detection limit and linear range of concentration for NE with a recently published work on NE determination is tabulated in Table 1.

3.4.3. Selectivity

To examine the selectivity of the imprinted sensor towards NE, influence of some analogs and potential interfering substances such as uric acid, ascorbic acid and serotonin was evaluated. For this purpose square wave voltammetric response of a solution having a mixture of NE and 10–100 folds amount of aforementioned interfering substances was recorded at pH 7.2 using imprinted sensor. The experimental results show that no significant changes in peak current response of NE were observed for these interfering substances upto 100 fold amounts. It was found that the oxidation peak current of NE varied in the range 93–100% as shown in Fig. 6. The interference of epinephrine (EP) and dopamine (DA) was also investigated. It is observed that 250×10^{-9} mol L^{-1} EP and 350×10^{-9} mol L^{-1} DA do not affect the determination of 50×10^{-9} mol L^{-1} NE; however, at higher concentrations of EP and DA, the peak current of NE increased significantly. These observed results can be explained by the presence of suitable molecular complementary cavities (Fig. 7) and unique binding, which resulted from hydrogen bonds as well as weak interactions between the imprinted sites and NE molecules. In addition, the adsorption of NE molecules into the film and the selective binding within the film attributed to the porosity of imprinted sensor. These results indicate that the imprinted sensor is highly selective and can be applied for the quantitative determination of NE in biological fluids and pharmaceutical formulations.

3.4.4. Stability and reproducibility of imprinted sensor

To examine the reproducibility of imprinted sensor, five repetitive square wave voltammetric measurements were recorded at pH 7.2 by incubating the imprinted sensor for 30 min in 1 μM solution of NE. The observed results showed a relative standard deviation (R.S.D.) of 1.21% which indicated the reproducibility of the imprinted sensor. Interday precision was also evaluated by measuring the response of peak current at the imprinted sensor for eight consecutive days for the 1 μM concentration of NE and the R.S.D. value was found to be 2.03%. To assure the reproducibility of the results further, five different imprinted sensors were

Table 1
A comparison of electrochemical response of the imprinted sensor with previously reported electrochemical sensors for the determination of norepinephrine.

Sr. no.	Electrochemical sensor	Linear range of concentration (M)	Detection limit (M)	Ref. no.
1	ECR/GCE	2×10^{-6} to 50×10^{-6}	15×10^{-7}	[20]
2	ZONMCPE	1×10^{-7} to 2×10^{-3}	8.95×10^{-8}	[3]
3	C-Ni/GCE	2×10^{-7} to 8×10^{-5}	6×10^{-8}	[42]
4	BHTME	4×10^{-6} to 11×10^{-4}	50×10^{-8}	[21]
5	MC-CNPEs	8×10^{-8} to 70×10^{-5}	43×10^{-9}	[26]
6	DDP-CNPE	1×10^{-7} to 38×10^{-6}	79×10^{-9}	[7]
7	MWCNTs/CILE	3×10^{-7} to 45×10^{-5}	9×10^{-8}	[25]
8	PANI/TPA	8×10^{-8} to 2×10^{-3}	5×10^{-8}	[27]
9	Poly(cresol red)/GCE	3×10^{-6} to 3×10^{-5}	15×10^{-7}	[24]
10	Imprinted sensor	5×10^{-8} to 10×10^{-6}	4.9×10^{-10}	Proposed method

ECR – eriochrome cyanine R; ZONMCPE – ZrO_2 nanoparticles-modified carbon paste electrode; BHTME – 2,2′-[1,2 buthanediylbis(nitriloethylidyne)]-bis-hydroquinone TiO_2 nanoparticles modified carbon paste electrode; MC-CNPE – molybdenum (VI) complex carbon nanotube paste electrode; DDP-CNPE – 3,4-dihydroxybenzaldehyde-2,4-dinitrophenylhydrazone carbon nanotube paste electrode; MWCNTs/CILE – multi-wall carbon nanotubes modified carbon ionic liquid electrode; PANI/TPA – nanostructured polyaniline doped with tungstophosphoric acid.

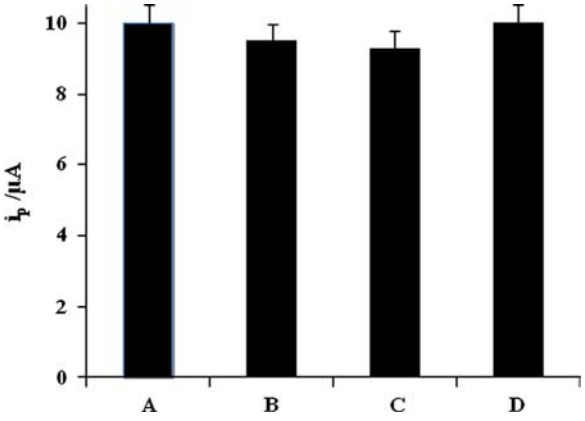


Fig. 6. Observed selectivity of imprinted sensor for (A) 5 μM NE, (B) 5+300 μM ascorbic acid, (C) 5+300 μM serotonin, and (D) 5+500 μM uric acid. (Incubation time: 30 min.)

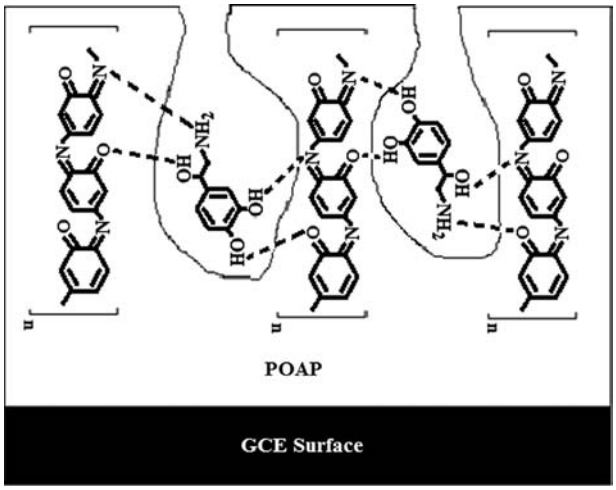


Fig. 7. Representation of the tentative mechanism of electrochemically synthesized imprinted sensor containing the template NE molecules entrapped in the imprinted site.

prepared by the same procedure as mentioned in Section 2. The R.S.D. value of 1.57% for 1 μM concentration of NE was observed at these sensors, which confirmed the excellent reproducibility of imprinted sensor.

The stability of the imprinted sensor was characterized by measuring the voltammetric current response of constant concentration of 1 μM of NE after storing it over a period of 10 days. The sensor was used each day and stored at 4 °C. No significant decrease in current response was observed during first 5 days and over the next 5 days the current response decreased about 2.63% of its initial value. These results indicated the adequate stability of imprinted sensor for NE determination.

Table 2
Determination of NE in norepinephrine containing injection (NORAD).

Sample	Stated content (mg/mL)	Detected content (mg/mL)[a]	Error (%)
1	2	1.97	−1.50
2	2	1.92	−4.00
3	2	1.99	−0.50
4	2	1.98	−1.00

[a] The RSD value for determination was less than 2.5% for $n=3$.

4. Analytical applicability of imprinted sensor

4.1. Determination of NE content in norepinephrine injection

In order to evaluate the pharmaceutical relevance of the imprinted sensor, commercially available norepinephrine injection (NORAD, noradrenaline bitartrate) was analyzed. For this purpose, 100 μL of injection was diluted using 5 ml distilled water without any pretreatment. It was further diluted with double distilled water so that the concentration of NE reached in the working range. To ascertain the concentration of NE in different samples prepared from NORAD, the peak current response at pH 7.2 was measured by incubating the imprinted sensor for 30 min in samples. The results are represented in Table 2 and show that the observed value of concentration is close to the labeled value suggesting the analytical utility of the imprinted sensor for NE determination.

4.2. Biological sample analysis using imprinted sensor

Biological applicability of the imprinted sensor was investigated by measuring the NE concentration in human blood plasma as well as in urine samples using the standard addition method. Urine and plasma samples of healthy volunteer were used for this analysis. At first, samples were prepared by centrifuging the blood plasma for 10 min at a speed of 1000 rpm. The obtained supernatant of blood plasma was diluted 10 times, and urine was also diluted 10 times with phosphate buffer solution of pH 7.2 to minimize the matrix complexity. Samples were spiked with known concentration of standard NE ranging from 1 to 10 μM, and their square wave voltammograms were recorded using the

Table 3
Recovery results obtained for NE in human urine and plasma samples at the imprinted sensor (incubation time: 30 min).

Spiked (μM)	Urine		Plasma	
	Detected (μM)	Recovery (%)[a]	Detected (μM)	Recovery (%)[a]
Sample 1				
1.00	1.03	103.0	1.01	101.0
5.00	4.98	99.6	4.93	98.6
10.00	10.22	102.2	9.98	99.8
Sample 2				
1.00	0.98	98.0	0.97	97.0
5.00	5.01	100.2	4.87	97.4
10.00	9.87	98.7	10.02	100.2
Sample 3				
1.00	1.02	102.0	0.99	99.0
5.00	5.06	101.2	5.09	101.8
10.00	9.94	99.4	9.78	97.8

[a] The R.S.D. value was less than ± 3.1% for plasma and was less than ± 2.2% for urine for $n=3$.

imprinted sensor. A well-defined peak for the oxidation of NE was observed at the peak potential of ~198 mV, no other peaks were found in entire measurements. These measurements further confirmed the selectivity of imprinted sensor for NE determination. The results obtained for NE determination in urine and blood plasma samples are listed in Table 3. The urine and blood plasma recoveries were obtained in ranges of 98–103% and 97–101.8%, respectively.

The recovery data showed acceptable range, which indicated that the proposed method can be utilized successfully for the detection of NE in human biological fluids with adequate accuracy and reliability.

5. Conclusions

In the present work, an extremely selective electrochemical sensor was successfully employed for the monitoring of norepinephrine content present in pharmaceuticals formulation and human blood as well as urine samples using the standard addition method. The proposed sensor was based on the molecular imprinted method in which *o*-aminophenol was electropolymerized in the presence of NE at glassy carbon surface. A linear relation at the imprinted sensor was observed in between current response and NE concentration with a detection limit of 4.9×10^{-10} mol L^{-1}. It has been found that the detection limit of the imprinted sensor is better than several papers reported in recent years. In addition, the sensor is selective as no interference from uric acid and ascorbic acid, commonly present in biological fluids, is observed. Thus, it is concluded that the proposed molecularly imprinted sensor can be used as a recognition tool for the selective determination of NE with good reproducibility, long term stability and adequate accuracy in the presence of analogs molecules, which are generally present in biological fluids.

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