Scientific paper

Electrocatalytic Oxidation of Isoproterenol and its Voltammetric Determination in Pharmaceuticals and Urine Samples Using a Poly(1-methylpyrrole)-DNA Modified Electrode

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Abstract

Determination of isoproterenol (ISP) was carried out using a DNA incorporated poly(1-methylpyrrole) modified glassy carbon electrode (GCE). The poly(1-methylpyrrole)-DNA/GCE showed an excellent electrocatalytic effect on the oxidation of ISP. The poly(1-methypyrrole)-DNA/GCE also accelerated the rate of electron transfer reaction of ISP. Compared with a bare GCE, the poly(1-methylpyrrole)-DNA/GCE exhibits a distinct shift of the oxidation potential of ISP in the cathodic direction and a marked enhancement of the current response. A linear calibration plot was obtained covering the concentration range from 2.0×10^{-6} to 6.0×10^{-5} M with a detection limit of 1.60×10^{-7} M by cyclic voltammetry. The electrode system has also successfully resolved the overlapping anodic peak of ISP and uric acid (UA) into two well-defined voltammetric peaks in cyclic voltammetry at 0.416 V and 0.552 V for ISP and UA, respectively. The poly(1-methypyrrole)-DNA/GCE has successfully been utilised for the determination of ISP in pharmaceutical preparations. The validity of the proposed method was also assured by the recovery of ISP and UA in urine samples.

Keywords: DNA, 1-methylpyrrole, isoproterenol, uric acid, modified electrodes

1. Introduction

Isoproterenol (ISP) is widely used for the treatment of primary pulmonary hypertension and allergic emergencies, status asthmaticus, bronchial asthma, venticular bradycardia, cardiac arrest, glaucoma.[1] It is also used to bronchitis, cardiac chock and heart attack. Nevertheless, the excess of the drug may cause heart failure and arrhythmias.[2] A number of flow injection procedures have been appeared in the literature for the determination of ISP.[3–8] Nevado et al. has reported a flow injection spectrophometric procedure for the determination of ISP based on the reaction of ISP with metaperiodate.[3] Also, a flow injection spectrophotometric procedure has been developed for the determination of ISP based on the formation of a coloured complex between ISP and Fe(II) in aminoacetic-carbonate buffer at pH 8.3 and measuring the absorbance at 530 nm.[4] Two flow injection procedures based on the inhibition of the intensity of chemiluminescence (CL) from the luminol–hypochlorite system have also been reported.[5,6] Also, a flow injection procedure based on imidazole catalyzing the decomposition of catecholamines and generating hydrogen peroxide has been described.[7] Another flow injection spectrophotometric determination of ISP based on the oxidation reaction of ISP with immobilized polyphenol oxidase has also been reported.[8] However, these methods are quite complicated and have several disadvantages such as the need of the long waiting times, high costs, requirements for sample preparation and low sensitivity and selectivity. Electrochemical methods have been useful for the determination of electroactive species in pharmaceuticals and body fluids due to their simplicity and low cost.[9–11] However, in electrochemical detection of ISP, voltammetric methods may suffer from low sensitivity and selectivity that leads to an inactive overpotential due to the irreversibility of its voltammetric behaviour of catecholamines.[12] However, compared to other techniques, modified electrodes provide certain advantages such

as long term stability, sensitivity and homogeneity in electrochemical deposition.[13–17]

In this paper, a novel electrode system was fabricated for the determination of ISP using a poly(1-methylpyrrole)-DNA/GCE. It is shown that the presence of DNA into the polymer film causes a remarkable increase in the anodic peak current of ISP. The poly(1-methylpyrrole)-DNA/GCE has successfully been applied for determination of ISP in pharmaceuticals and body fluids.

2. Experimental

2. 1. Chemicals

Isoproterenol (ISP), 1- methylpyrrole, and uric acid (UA) were purchased from Fluka (Germany). DNA obtained from Sigma (Germany) was used as received. Solutions of 1-methylpyrrole and DNA were prepared in 0.2 M KCl at pH 7.4. All other reagents were of analytical grade or equivalent, and obtained from Merck or Fluka. Solutions of ISP and UA were prepared in 0.1 M phosphate buffer solution (PBS) at pH 4.0. Aqueous solutions were prepared with doubly distilled water. Oxygen-free nitrogen was bubbled through the cell prior to each experiment. All experiments were carried out at *ca.* 25 °C.

2. 2. Apparatus

Electrochemical experiments were performed using an EcoChemie Autolab PGSTAT 12 potentiostat/galvanostat (Utrect, The Netherlands) with the electrochemical software package 4.9 or an Epsilon potentiostat (Bioanalytical Systems, Lafayette, USA) with the electrochemical software 1.6.70_XP. A three-electrode system was used: a 3 mm sized glassy carbon electrode (GCE) as working electrode [(Bioanalytical Systems, Lafayette, USA)], a Pt wire counter electrode and an Ag/AgCl reference electrode.

2. 3. Preparation of Modified Glassy Carbon Electrodes

Prior to electrochemical modification, the bare GCE was polished with 0.05 μm alumina slurry on a polishing pad. Then it was rinsed with water, and sonicated with 1 + 1 HNO_3 and acetone, and water for 10 min, respectively. After being cleaned, the electrode was activated by 5 cyclic sweepings from –0.6 to +0.8 V in PBS at pH 7.2. Then, the electrode was immersed in a solution of 5 mM DNA and 10 mM 1- methylpyrrole dissolved in 0.2 M KCl at pH 7.4 and was conditioned by cyclic sweepings from –1.5 to +2.5 V for 10 scans (Fig. 1). Afterwards, the modified electrode was electroactivated by cyclic voltammetry from –0.6 to +0.8 V at 100 mV/s in 0.1 M PBS at pH 4.0.

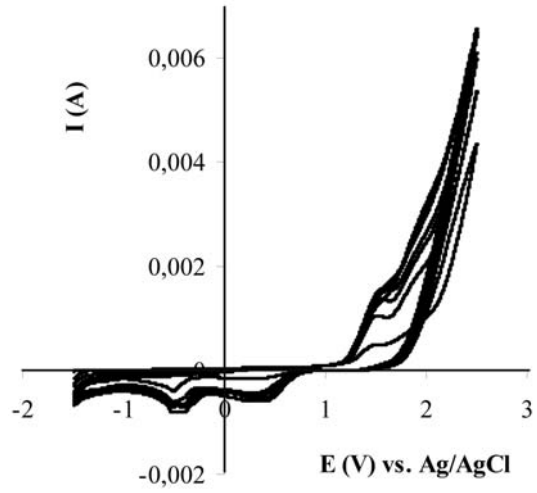


Fig. 1. Cyclic voltammograms of the mixture of 0.01 M 1-methylpyrrole and 5 mM DNA in 0.2 M KCl at pH 7.4. Scan rate: 100 mV/s.

3. Results and Discussion

3. 1. Voltammetric Behaviour of Isoproterenol (ISP) at Poly(1-methylpyrrole)-DNA/GCE

Fig. 2 exhibits the cyclic voltammograms of ISP at bare GCE, poly(1-methylpyrrole)/GCE and poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0. At bare GCE, ISP shows an oxidation peak at 0.750 V and a corresponding reduction peak at 0.083 V. The separation in peak potential (ΔEp) is about 667 mV. This indicates that the electrochemical process at bare GCE is very slow. At the poly(1-methylpyrrole)/GCE, a well-defined redox wave of ISP was obtained. In addition, poly(1-methylpyr-

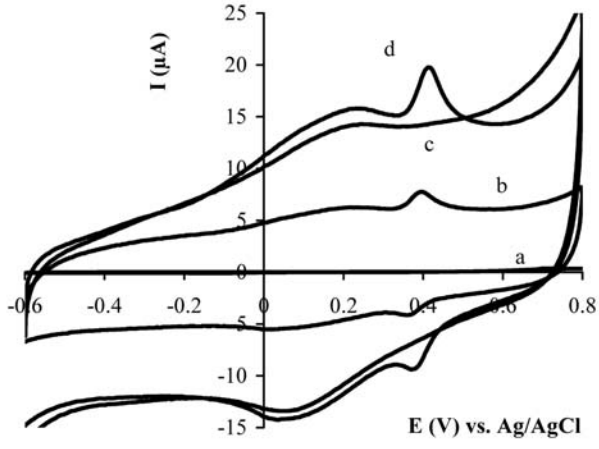


Fig. 2. Cyclic voltammograms of 5.0×10^{-5} M ISP at (a) bare GCE, (b) poly(1-methylpyrrole)/GCE and (d) poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0. (c) a CV of poly(1-methylpyrrole)-DNA/GCE. Equilibrium time: 5 s, scan rate: 50 mV/s.

role)-DNA/GCE exhibited an intensive increase in the anodic peak current for ISP and showed an excellent electrocatalytic effect on the oxidation of ISP. Furthermore, the poly(1-methylpyrrole)-DNA/GCE also accelerated the electron transfer rate for ISP. The oxidation of ISP at poly(1-methylpyrrole)-DNA/GCE occurs at 0.416 V and a corresponding reduction peak at 0.381 V. The separation in peak potential (ΔEp) is about 35 mV. This reveals that ISP undergoes only a reversible two-electron oxidation to form isoproterenolquinone at pH 4.0. The other oxidation/reduction peaks clearly correspond to the modified surface as shown in Fig. 2(c). The proposed ISP reaction at pH 4.0 is given in Scheme 1.

Compared with both bare GCE and poly(1-methylpyrrole)/GCE, the electrochemical response of ISP has greatly been increased on the poly(1-methylpyrrole)-DNA/GCE. Intensive increase in peak current is observed owing to the improvement in the reversibility of electron transfer process and the larger real area of the polymer film.[18–20] This suggests an efficient electrocatalytic oxidation reaction toward ISP at the poly(1-methylpyrrole)-DNA/GCE.

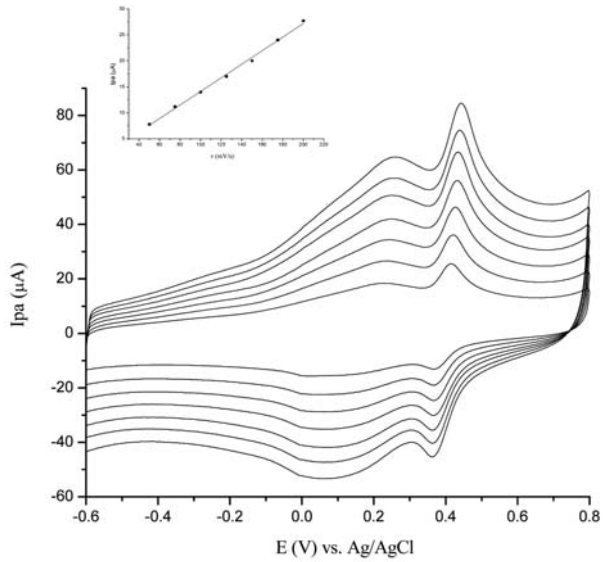
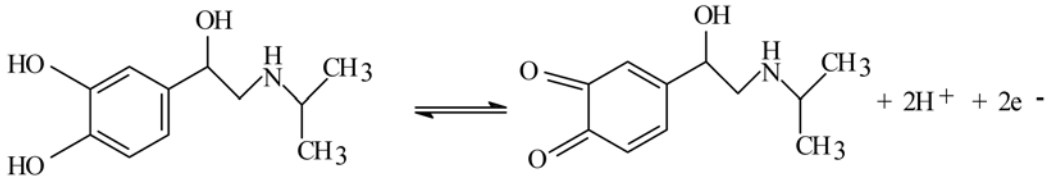


Fig. 3. Cyclic voltammograms of 6.0×10^{-5} M ISP in 0.1 M PBS at pH 4.0. Scan rates increasing from 50 to 200 mV/s. Inset: A plot of Ipa vs scan rate. Equilibrium time: 5 s.



Scheme 1. Proposed ISP Reaction at Poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0.

3. 2. The Effect of Scan Rate on the Oxidation of Isoproterenol (ISP)

To investigate the electrochemical process of ISP at the modified electrode, the effect of scan rate on the electrochemical response of ISP at poly(1-methylpyrrole)-DNA/GCE using cyclic voltammetry in 0.1 M PBS at pH 4.0 were carried out (Fig. 3). The anodic peak current (Ipa) was proportional to the scan rate (v) over the range of 50–200 mV/s. No shifts in the oxidation peak potential of ISP were observed with increasing scan rate. The linear regression equation was Ipa (μA) = 0.178571 + 0.124286v (mV). The results indicated that the electrochemical oxidation of ISP at poly(1-methylpyrrole)-DNA/GCE is a surface-controlled process.

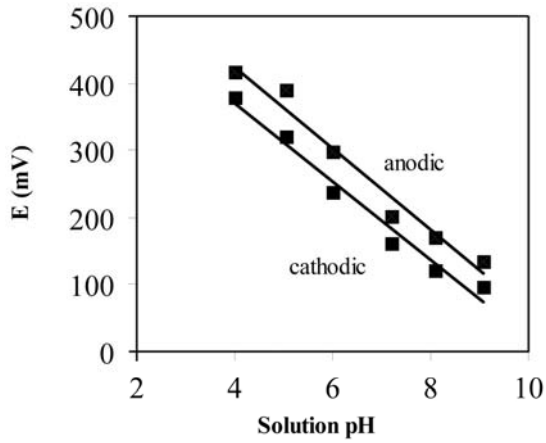


Fig. 4. A plot of oxidation peak potential of ISP vs. solution pH.

3. 3. The Effect of Solution pH on the Oxidation Potential of Isoproterenol (ISP)

In addition, the effect of the pH value of the PBS buffer solution on peak potential of ISP at poly(1-methylpyrrole)-DNA/GCE was also investigated. The peak potential of ISP shifted in the negative direction with increasing pH. This shows that the redox couple of ISP includes proton transfer in the electrochemical processes. The slope of the plot of the peak potential *vs*. pH value of the solution was *ca*. 62.0 mV/pH for anodic and 60.5 for cathodic peaks (Fig. 4). This indicated that the proportion of the electron and proton involved in the reactions is 1:1.

The number of electrons was calculated according to the Nernst equation Since equal numbers of electrons and protons should be involved in the electrode reaction, the number of hydrogen ions involved in the whole electrode reaction is 2.

3. 4. Calibration Equation for the Determination of Isoproterenol (ISP)

Determination of the concentration of ISP at poly(1-methylpyrrole)-DNA/GCE was performed in 0.1 mol L^{-1} PBS at pH 4.0. Cyclic voltammograms of various concentrations of ISP at poly(1-methylpyrrole)-DNA/GCE are given in Fig. 5. The anodic peak currents were plotted against the bulk concentration of ISP after the background subtraction. The response of anodic peak currents of ISP at poly(1-methylpyrrole)-DNA/GCE was linear with the concentration of ISP in the range of $2.0 \times 10^{-6} \sim 6.0 \times 10^{-5}$ M. The linear regression equation was Ipa (μA) = 1.25761 + 0.14725 C (μM) with a correlation coefficient of 0.9967. The detection limit was 1.60×10^{-7} M (S/N = 3). The detection limit obtained using the proposed method is compared well with literature value of 6.25×10^{-5} M observed by flow injection spectrophotmetric determination ISP.[8]

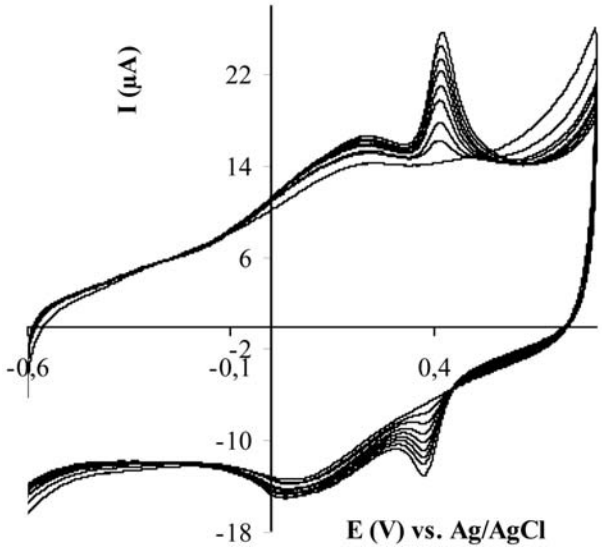


Fig. 5. Cyclic voltammograms of various concentrations of ISP at poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0. ISP concentrations: (a) 2.0×10^{-6} M (b) 1.0×10^{-5} M (c) 2.0×10^{-5} M (d) 3.5×10^{-5} M (e) 4.5×10^{-5} M (f) 5.0×10^{-5} M (g) 6.0×10^{-5} M. Equilibrium time: 5 s, scan rate: 50 mV/s.

3. 5. Detection of Isoproterenol (ISP) in the Presence of Uric Acid (UA)

UA coexisted in samples, can be easily oxidized at a potential rather close to that of ISP using a conventional electrode resulting in electrochemical response of ISP being overlapped by that of UA always interfere with the measurement of catecholamines.[21,22] In this work, it was found that this problem could be eliminated using the poly(1-methylpyrrole)-DNA/GCE. The cyclic voltammograms of the mixture of ISP and UA at bare GCE and the poly(1-methylpyrrole)-DNA/GCE are given in Fig. 6. At bare GCE, a single broad voltammetric peak at 0.560 V was appeared for the mixture of ISP and UA. Compared to the bare GCE, two well-defined anodic peaks were obtained at poly(1-methylpyrrole)-DNA/GCE. These well-separated two anodic peaks were occurred at 0.416 V and 0.552 V in CV correspond to the oxidation of ISP and UA

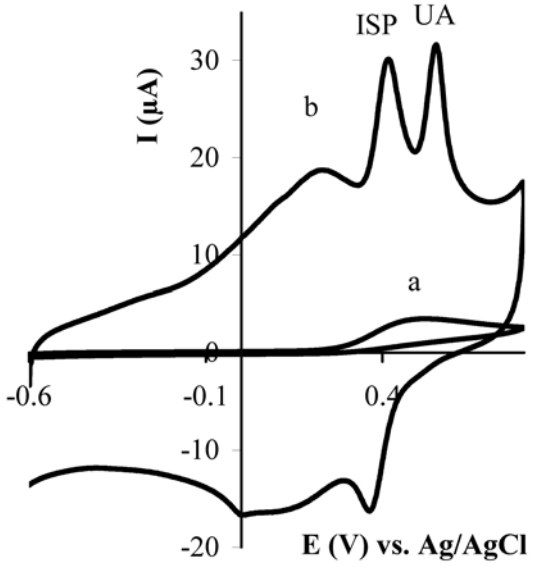


Fig. 6. Cyclic voltammograms of the mixture of 1.0×10^{-4} M ISP and 1.75×10^{-4} M UA at (a) bare GCE and (b) poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0. Equilibrium time: 5 s, scan rate: 50 mV/s.

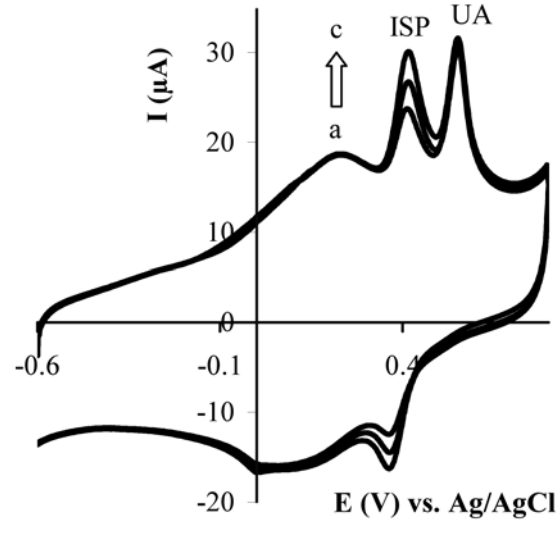


Fig. 7. Cyclic voltammograms of the increasing concentrations of ISP in the presence of 1.75×10^{-4} M UA at poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0. ISP concentrations: (a) 5.0×10^{-5} M (b) 7.5×10^{-5} M (c) 1.0×10^{-4} M. Equilibrium time: 5 s, scan rate: 50 mV/s.

respectively. Fig. 7 represents the CV recordings at various concentrations of ISP where concentration of UA was kept constant. In the presence of UA, the anodic peak current of ISP increased linearly with the increase in its concentration. Fig. 8 also shows the cyclic voltammograms at various concentrations of UA where concentrations of ISP was kept constant.

In the presence of UA, the anodic peak current of ISP increased linearly with the increase in its concentration. These clearly show that UA does not interfere with detection of ISP. Overall facility of the poly(1-methylpyrrole)-DNA/GCE for simultaneous determination of ISP and UA was demonstrated by simultaneously changing the concentration of ISP and UA (Fig. 10). The anodic peak responses of both ISP and UA increase linearly with its concentration indicating that the voltammetric responses of ISP and UA are independent of each other. It is remarkable that poly(1-methylpyrrole)-DNA/GCE enables the determination of ISP in the presence of UA.

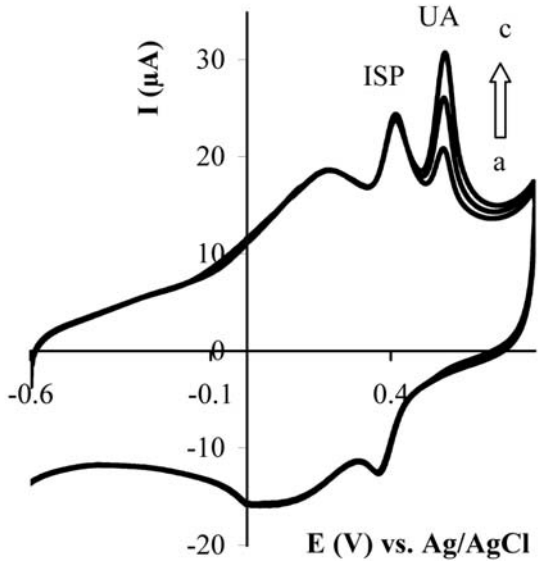


Fig. 8. Cyclic voltammograms of the increasing concentrations of UA in the presence of 5.0×10^{-5} M ISP at poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0. UA concentrations: (a) 7.5×10^{-5} M (b) 1.0×10^{-4} M (c) 1.75×10^{-4} M. Equilibrium time: 5 s, scan rate: 50 mV/s.

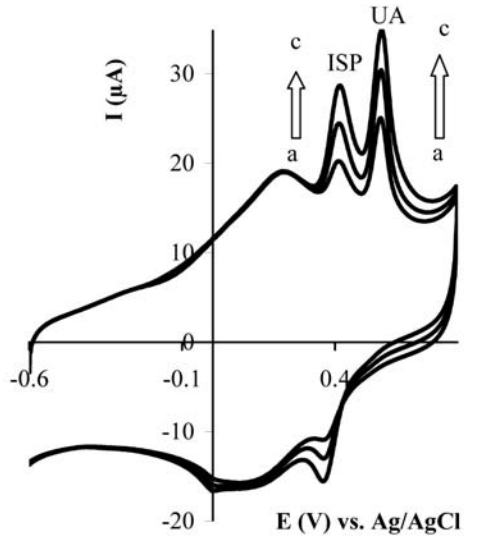


Fig. 9. Cyclic voltammograms of the mixture of the increasing concentrations of ISP and UA at poly(1-methylpyrrole)-DNA/GCE in 0.1 M PBS at pH 4.0. ISP concentrations: (a) 2.0×10^{-5} M (b) 5.0×10^{-5} M (c) 7.5×10^{-5} M. UA concentrations: (a) 1.25×10^{-4} M (b) 1.75×10^{-4} M (c) 2.0×10^{-4} M. Equilibrium time: 5 s, scan rate: 50 mV/s.

3. 5. Repeatability and Stability of DNA doped Poly(1-methylpyrrole)/GCE

The relative standard deviation (RSD) of 6 successive scans was 2.45% for 5.0×10^{-5} M ISP. This indicated that the reproducibility of the poly(1-methylpyrrole)-DNA/GCE was excellent. However, the modified electrode should be well treated to maintain its repeatability. It was found that leaving the modified electrode in acetone over the night and then performing 20 cycles of scanning in 0.1 M PBS in the potential range 0.0~0.8 V could regenerate a clean background CV curve for the next experiments. A clean modified electrode should be kept in 0.1 M PBS. Also, the current response decreased only by 5% over a week for storage in 0.1 M PBS.

3. 6. Analytical Applications

The proposed method was used for the determination of ISP in pharmaceuticals. ISP samples were diluted with 0.1 M PBS. The injections were analysed by the standard addition method. The results are given in Table 1. The average recovery of 98.5% with an RSD of 2.65% was obtained employing the proposed method for the five different determinations of ISP injections. The data obtained at poly(1-methylpyrrole)-DNA/GCE are in close agreement with the claimed values. The data are also in good agreement with certified values obtained by the United States Pharmacopoeia using UV spectrophotometric method with an average recovery of 104.5% with an RSD of 4.7%.[23] However, the data obtained by the proposed method are also comparable with previously reported flow injection spectrophotometric method with an average recovery of 107.0% with an RSD of 2.8%.[24] The validity of the proposed method was also assured by the recovery of ISP and UA in urine samples (Table 2). The mean recoveries in urine samples were 98.4% with an RSD of 2.74% for ISP and 98.0% with an RSD of 2.58% for UA. The superiority of proposed method is mainly owing to its simplicity, high stability and long usage life. These results indicated that the proposed method could be easily used for the determination of ISP in pharmaceuticals and UA in urine samples.

Table 1. Analysis of isoproterenol injections (n = 5)

	Content (×10 mg/ml)	Found (×10 mg/ml)	Recovery (%)	RSD (%)
ISP	2.0	1.97 ± 0.05	98.5	2.65

Mean ± standard deviation

Table 2. Voltammetric analysis of urine samples (n = 6)

	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
ISP	50.0	49.2 ± 1.35	98.4	2.74
UA	25.0	24.5 ± 0.63	98.0	2.58

Mean ± standard deviation

4. Conclusions

This study has indicated that poly(1-methylpyrrole)-DNA/GCE can be used for the electrocatalytic oxidation of ISP and its determination. The results showed that the presence of DNA in the polymer film provide an improved electrochemical behaviour and a marked enhancement of the anodic current response for ISP. It has also indicated that poly(1-methylpyrrole)-DNA/GCE enables the determination of ISP in the presence of UA. The proposed method could easily be applied for determination of ISP in pharmaceutical preparations and urine samples. The poly(1-methylpyrrole)-DNA/GCE has a good sensitivity and repeatability. The proposed method is accurate, sensitive and easy.

5. Acknowledgements

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Povzetek

Z uporabo elektrode iz steklastega ogljika (GCE), ki smo jo modificirali z DNA z vgrajenim poli(1-metilpirolom) (poli(1-metilpirol)-DNA/GCE) smo določali izoproterenol (ISP). Ugotovili smo, da je tako pripravljen poli(1-metilpirol)-DNA/GCE senzor pri ciklični voltametriji kaže linearno odvisnost od koncentracije ISP v območju med 2.0×10^{-6} in 1.0×10^{-4} M z limito detekcije 1.60×10^{-7} M. S pomočjo poli(1-metilpirol)-DNA/GCE elektrode smo uspeli prekrivajoč anodni vrh za ISP in urinsko kislino (UA) ločiti v dva dobro ločena vrhova, kar kaže na njeno potencialno možnost uporabe za določanje ISP v farmacevtskih preparatih ter pri analizi vzorcev urina.