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A Novel Electrochemical Sensor for Determination of Salbutamol Based on Graphene Oxide/Poly (O-nitrobenzoic acid) Modified Glassy Carbon Electrode and its Analytical Application in Pharmaceutical Formulation and Human Urine

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Abstract

Cyclic and differential pulse voltammetric techniques were employed to determine salbutamol at a glassy carbon electrode modified with a graphene oxide and poly(O-nitrobenzoic acid). The modified electrode was characterized by $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ couple and show high catalytic activity towards the oxidation of SAL in PBS (pH 7.6). The effect of scan rate, pH and concentration of SAL were studied at the modified electrode where a radical change in the anodic peak current was observed. The important parameters such as electrode real surface area, electron-transfer number, the surface concentration of the electroactive species, detection and quantification limits were determined and calculated to be 0.485 cm^2, 1, 3.51 × 10^{-8} mol/cm^2, 56 and 188 nM, respectively. The modified electrode was achieved excellent reproducibility and good stability. In addition, there is no interference with the determination of SAL except ascorbic acid and p-nitrophenol. The sensitivity of the modified electrode shows acceptable recoveries in the detection of SAL in pharmaceutical formulations and a human urine sample.

Keywords: Salbutamol; Asthma; Graphene oxide; O-Nitrobenzoic acid; Cyclic voltammetry; Differential pulse voltammetry; Glassy carbon electrode; Pharmaceutical forms and urine sample

Introduction

The shortness of breath and coughing were mostly caused by asthma which means disorder of the respiratory system. And therefore, prevents air to pass into and out of the lungs periodically. One of the most common forms of asthma is an inflammation of the airways known as bronchial asthma. However, in the case of fluid accumulation in the lungs, asthma is referred to as cardiac asthma, which acts as a multiplier of heart failure. Asthma can be treated by relaxing the smooth muscles and thus the bronchial passages are enlarged by using β adrenergic agonists such as salbutamol. First-line drugs in the treatment of airway narrowing, the optimum choice for the treatment of bronchial asthma and chronic obstructive pulmonary disease [1].

Salbutamol (SAL) or albuterol [1-(4-hydroxy-3-hydroxymethyl phenyl)-2-(t-butylamino)ethanol], also known as Ventolin is a type of β adrenergic agonists which a class of drugs primarily used to treat asthma and other forms of allergic diseases [2,3]. In addition to, high doses of SAL illegally are given to animals to raise a preferential muscle in fat ratio, resulting in financial gain for the farmer [4,5]. It is worth noting that the accumulation SAL in animal organs can enter the human body through the food chain leads to headache, muscular pain, muscular tremor, nervousness, fever, vomiting and cardiac palpitation [6-8]. Hence, it became an urgent necessity to determine SAL in different pharmaceutical dosage forms and in clinical chemistry.

In recent years, a different methods of analysis have been reported to determine SAL in pharmaceutical formulations or biological samples, such as spectrophotometry [9-11], high performance liquid chromatography [12-14], gas chromatography [15], capillary electrophoresis [16,17], potentiometry [18,19] and flow injection analysis [20]. Unfortunately, these techniques are expensive, time consuming and relatively complicated even though proven and widely accepted. In other side, Electrochemical methods is sensitive, accurate, simple, rapid, inexpensive and helpful to identify and detection of SAL. The response of analyte on the bare electrode can be affected on the sensitivity of electrochemical determination [21]. So, development of a sensitive modified electrode to detect SAL pharmaceutical samples through electroanalytical technique is significant.

Recently, graphene oxide (GO), an oxidized form of graphene, has become the preferred electrode modification materials for the sensitive detection of organic substances, bioactive molecules, nutrients, food additives, as well as contaminants. This is due to many unusual features of GO such as easily prepared, low cost, wide potential windows, good electrocatalytic activity, high charge transport mobility, high transparency, fairly inert electrochemistry and mechanical strength greater than steel [22-25]. In other hand, graphene exhibits a surface area of 2630 m^2/g, which is much greater than that of graphite (~10 m^2/g) and even that of carbon nanotubes (1315 m^2/g) as reported in literature [26], but it might not provide a significant advantage over existing electrode materials [27].

To this day, fabrication of polymer film on the surface of the electrode by electropolymerization, has received a great attention from scientific research groups, as the polymer film has good stability, reproducibility, high selectivity, strong adherence to the electrode surface, and large service area which means more active sites [28,29]. It is recognized that

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the redox reaction rate increases in the presence of the polymer film on the electrode surface. So, for a first time a glassy carbon electrode (GCE) modified with graphene oxide followed by a polymer film of o-nitrobenzoic acid (GO/poly(O-NBA)/GCE) to determine SAL in its pharmaceutical forms and humin urine.

The electrooxidation of target compounds usually requires high potentials in case of using conventional material electrodes. Also, the achieved detection limits are relatively high as a result of the fouling of electrode surface by the generated from electrochemical reactions. Consequently, this paper aims to describe the preparation a modified electrode of (GO/poly(O-NBA)/GCE) which was expected to enhance the electron transfer kinetics of the modified surface and lowers the oxidation potential of SAL. This electrochemically modified electrode could become a suitable sensor for the electroanalytical detection of SAL in the pharmaceutical formulations and urine samples.

Experimental Part

Chemical reagents and solutions

Salbutamol sulphate, graphite powder (<20 μm) and sulfuric acid (H_2SO_4) were purchased from Sigma-Aldrich. O-nitrobenzoic acid (O-NBA), potassium permanganate ($KMnO_4$), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2) were received from Merck. Sodium hydroxide (NaOH), monopotassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), disodium phosphate (Na_2HPO_4) were obtained from El-Nasr Pharmaceutical Chemicals (Egypt). Sodium nitrate ($NaNO_3$), potassium nitrate (KNO_3), and potassium ferricyanide ($K_3[Fe(CN)_6]$) were purchased from BDH Chemicals Ltd (England). In our experiments all the chemical reagents were of analytical grade unless otherwise stated and were used directly without any further purification.

All solutions were freshly prepared using doubly distilled water at room temperature. A 0.1 M phosphate buffer solution (PBS, pH 7.6) was employed as the supporting electrolyte and prepared by mixing equi-molar (0.1 M) of KH_2PO_4 and K_2HPO_4. 1.0 M of NaOH and HCl solutions were used to adjust the desired pH of the buffer solutions.

Electrochemical equipment and cell

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed using EG&G Princeton applied research potentiostat/galvanostat model 263A (USA) is used [30,31]. The cell (model K0264 micro-cell) was used for all electrochemical experiments contains three electrodes, an Ag/AgCl (saturated KCl) - model K0265-electrode was used as the reference electrode, the counter-model K0266-electrode was of high purity platinum wire and a bare glassy carbon electrode - model G0229 -(2 mm) and (GO/poly(O-NBA)/GCE) were used as the working electrode. Finally, the pH values were adjusted through CyberScan pH 500 Meter (Euteoh-India).

Synthesis of graphene oxide (GO)

1.0 g of commercial natural graphite powder was oxidized by a modified Hummers' method (GO-H2) [32,33]. In an ice-water bath, 3 g of $KMnO_4$ was slowly added to a suspension of the concentrated H_2SO_4 (30 mL), $NaNO_3$ (3 g) and graphite (1.0 g) to keep the temperature of the oxidation reaction between 10 and 25°C. Subsequently, 100 mL of doubly distilled water and 60 mL of 35% H_2O_2 were slowly added to reduce residual permanganate and manganese dioxide. After stirring for 30 min and then centrifuged, the resulting black product was filtered followed by dry in oven at 50°C overnight. Aqueous colloids of GO were prepared from the dried graphite oxide powder by magnetic stirring and heating as reported in literature [34]. Briefly, 0.5 g of graphite oxide was stirred with 50 mL of doubly distilled water at 70°C using magnetic stirring at 400 r.p.m for 12 hours.

Fabrication of the GO/poly(O-NBA)/GCE

A GCE was cleaned sequentially by polishing with alumina powder (3 μm) to a mirror finish surface then rinsing with doubly distilled water and ultrasonically cleaned with water, ethanol for 2 min. Using drop-casting method, a 10 μL aliquot of GO-H_2O suspension (0.5 g/50 mL) was dropped onto a cleaned surface of the GCE, followed by drying at 50°C in an oven. Afterward, the GO/GCE was electropolymerized by immersing in electrolyte solution of 0.1 M Na_2HPO_4 (pH=7.0) containing 5 mM monomer of O-NBA by cyclic the potential between 1.3 and -1.5 V (*vs.* Ag/AgCl) for 10 cycles at a scan rate of 100 mV/s. After electropolymerization, the modified electrode was rinsed thoroughly with doubly distilled water to remove unreacted monomer and dried at room temperature.

Preparation of real samples

5.0 mL of salbutamol sulfate syrup was diluted by dilution factor of 50 with PBS (pH 7.6). Analysis the amount of SAL in each sample was carried out by DPV using the standard addition method. As for the salbovent tablets (2 mg salbutamol sulfate), each tablet was weighed, carefully crushed and dissolved in 55 mL of PBS (pH 7.6).

Urine samples of healthy specimen were analyzed immediately after collection. Two milliliters of fresh samples were diluted to 50 mL with PBS (pH 7.6). The diluted urine samples were spiked with different amount of SAL.

Results and Discussion

Preparation and characterization of the GO/poly(O-NBA)/GCE

There are several papers go directly towards the modification of the physico-chemical properties of the electrodes through the utilization of various kinds of conducting polymers. So, our interest in this work was to further improve the GCE by adding a polymer film of O-NBA on the GO/GCE as shown in Figure 1.

Where the polymer film of O-NBA can be growth by immersing the GO/GC modified electrode in 0.1 M Na_2HPO_4 (pH=7.0) containing 5 mM of O-NBA and cycling the potential between 1.3 and -1.5 V for 10 cycles at scan rate of 100 mV/s as can be seen in Figure 2. In the

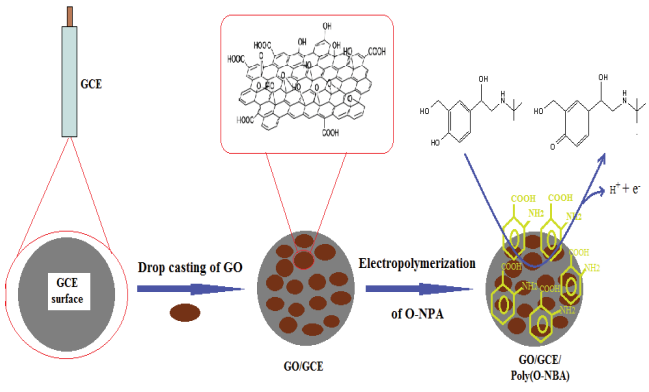

Figure 1: Schematic representation of fabrication of GO/poly(O-NBA)/GCE and the proposed mechanism for oxidation of SAL.

first scan, cathodic peak (1), cathodic peak (2) and anodic peak (3) were observed with peak potentials -0.271 V, -0.837 V and -0.024 V, respectively. Also, the effect of scan rate was studied for O-NBA at GO/GCE as show inset Figure 2, where the peaks currents increase linearly with the increasing in scan rate from 50 to 400 mV/s indicating a diffusion control process.

The GO/poly(O-NBA)/GCE was characterized by cyclic voltammetry of 5.0 mM $K_3[Fe(CN)_6]$ in electrolyte solution of 0.1 M KNO_3 at bare GCE and the GO/poly(O-NBA)/GCE at scan rate 100 mV/s as illustrated in Figure 3. The cyclic voltammogram (Figure 3, curve a) exhibits a quasireversible redox couple with a peak separation (ΔEpeak) of 465 mV with small redox peak currents at bare GCE. On the other hand, A large peak current for $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ couple was observed (Figure 3, curve b) when GO/poly(O-NBA)/GCE used as working electrode and ΔEpeak was obviously decreased to 151 mV. This is due to the better electron transfer through GO and poly(O-NBA) as the redox mediators between the analyte and the GCE which indicates the high electrocatalytic activity of the GO/poly(O-NBA)/GCE [35].

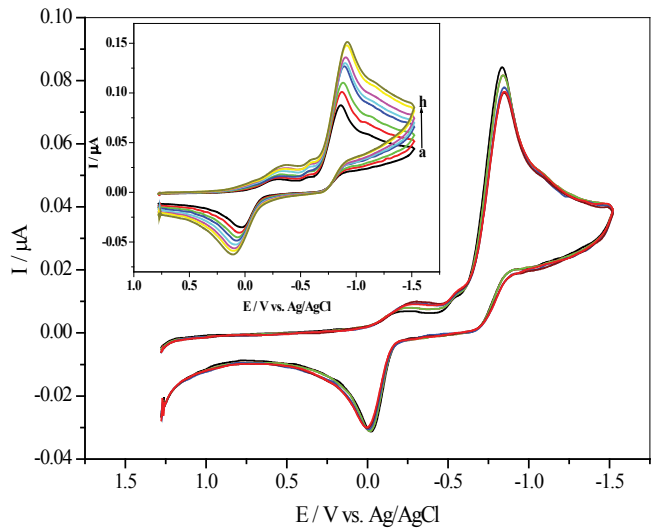

Figure 2: CVs of 0.1 M Na_2HPO_4 (pH = 7.0) containing 5 mM of O-NBA at GO/GCE in scan rate of 100 mV/s for 10 cycles. Inset: effect of scan rate (a-h: 50, 100, 150, 200, 250, 300, 350 and 400 mV/s, respectively).

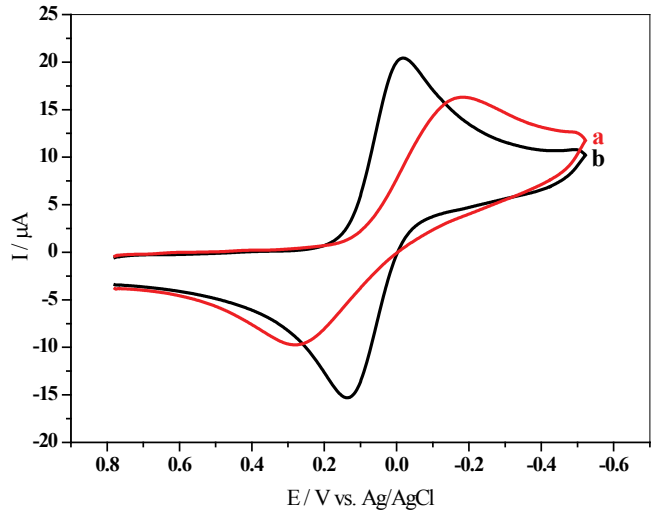

Figure 3: CVs of (a) bare GCE and (b) the GO/poly(O-NBA)/GCE in 0.1 M KNO_3 containing 5.0 mM $K_3[Fe(CN)_6]$ at scan rate 100 mV/s.

The active surface area of the modified electrode must be determined because it refers to electrode reaction rate. So, the cyclic voltammetry of 5.0 mM potassium ferricyanide as the redox probe was recorded. Figure 4 shows the CVs of 0.1 M KNO_3 contains 5.0 mM $K_3[Fe(CN)_6]$ at the GO/poly(O-NBA)/GCE at different scan rate (50-1000 mV/s). Its clear that, each peak current increased with increase of scan rate. In addition to, a straight line obtained by plotting Ip against square root of the scan rate. by compensation of the slope in the following Randles-Sevcik equation [36-38]:

$$Ip = 0.446\ nFAC(nFDv/RT)1/2$$

were, n is the number of electrons (for this system n=1), Ip is the peak current, F is the Faraday constant (C/mol), C is the concentration of the electroactive species (mM), ν is the scan rate (mV/s), R is the universal gas constant (J/mol.K), T is the temperature in Kelvin, A is the electroactive surface area of the modified electrode (cm^2) and D is the diffusion coefficient (cm^2/s). With known the precise value of D which is 5.6×10^{-6} cm^2/s [39], the value of A was estimated as 0.485 cm^2. The electroactive surface area of the GO/poly(O-NBA)/GCE is high compared to the A of bare GCE (0.126 cm^2), which provided an effective evidence for the superior conductivity of GO/poly(O-NBA) films as expected.

Electrochemical behaviour of SAL

The electrochemical behaviour of SAL at unmodified and GO/poly(O-NBA) modified glassy carbon electrodes was investigated using cyclic voltammetry. Figure 5 displays the cyclic voltammograms of 0.1 M PBS (pH=7.6) in: (curve a) GO/poly(O-NBA)/GCE in the absence of SAL, (curve b) unmodified GCE with 0.3 mM of SAL, (curve c) GO/GCE with 0.3 mM of SAL and (curve d) GO/poly(O-NBA)/GCE with 0.3 mM of SAL at scan rate 100 mV/s. its observed that, there is no any peaks in anodic or cathodic scan for the GO/poly(O-NBA)/GCE in PBS containing no SAL (curve a). In other side, SAL was found to give an

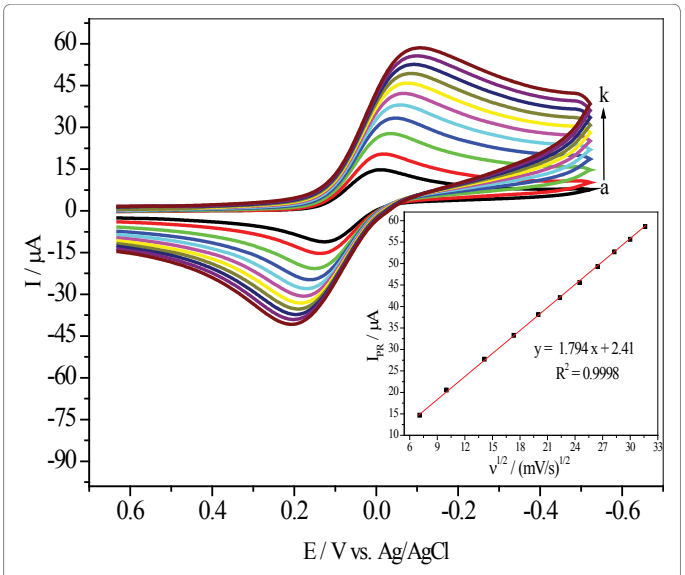

Figure 4: CVs of 0.1 M KNO_3 containing 5.0 mM $K_3[Fe(CN)_6]$ at the GO/poly(O-NBA)/GCE at different scan rate (a-k: 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mV/s, respectively).

oxidative peak at 0.59, 0.585 and 0.53 V (vs. Ag/AgCl) at an unmodified GCE (curve b), a GO/GCE (curve c) and a GO/poly(O-NBA)/GCE (curve d) respectively. Knowing that, SAL undergo an irreversible behaviour due to no cathodic peak was observed. Its clear that, the addition of GO to the bare GCE leads only to increase the anodic peak current value of SAL. While in case of using the GO/poly(O-NBA)/GC modified electrode, a relatively sharp oxidative peak with a two-fold peak current at 60 mV lower potential indicated the high catalytic activity of the modifier towards the oxidation of SAL. Which means the modified electrode could be attributed to an increased surface area [40].

Effect of scan rate

The electron-transfer number (n) and the surface concentration of the electroactive species (Γ) can be determined by investigate the effect of scan rate. Figure 6 shows the cyclic voltammograms at different scan rates (20-500 mV/s) for the electrocatalytic oxidation of 0.3 mM SAL in 0.1 M PBS (pH=7.6) at the GO/poly(O-NBA)/GCE. From this figure, the anodic peak current of SAL was observed to increase with scan rate due to heterogeneous kinetics. Also, the oxidation reaction of SAL at GO/poly(O NBA)/GCE is irreversible due to the oxidation peak potential of SAL shifted in the positive direction [41].

A linear dependence (IP=0.016 ν+2.267, R^2=0.9967) of peak current on scan rate from 100 to 500 mV/s (Figure 6) indicated that the oxidation reaction kinetics of SAL at GO/poly(O-NBA) modified GCE is predominantly adsorption-controlled process [41,42]. The electron-transfer number (n) and the surface concentration of the electroactive species (Γ) could be deduced according to Laviron theory [43,44] using the following equation:

$I_p=nFQν/4RT=n^2F^2νAΓ/4RT$ here, A is the surface area, Q is the peak area (known quantity) of certain scan rate, Γ is the surface concentration of the electroactive species, other symbols have their usual meaning and the values of n and Γ were found to be 1 and 3.51 × 10^{-8} mol/cm^2 respectively.

Effect of pH

The change in the value of pH plays important role in the mechanics of the oxidation and reduction reactions of most compounds. Consequently, the effect of pH on the electrochemical oxidation of SAL at GO/poly(O-NBA)/GCE was studied. Cyclic voltammetry was carried out to examine the effects of pH (PBS, pH 3.33 to 11.61) on voltammetric determination of 0.3 mM SAL at 100 mV/s. The results were illustrated in Figure 7. Initially the anodic peak current increases gradually with increasing pH and reaches a maximum at 7.6 and then the peak current decreases indicating 7.6 of pH is the optimum value which is in agreement with reported papers [45,46]. But there is a slightly increase in the alkaline medium as shown inset Figure 7. Moreover, the oxidative peak potential markedly shifts to more negative value with increasing in pH value indicating the participation of protons during oxidation of SAL [42].

For further analyzing the data from Figure 7, a good linear relationship (Figure 8) was estimated between the oxidative peak potential (EP) and pH (over a pH range between 6.71 and 11.61) with a linear regression equation of EP=1.03-0.0615 pH (R^2=0.9994). The obtained slope value -61.5 mV/pH was very close to the theoretical value of -59 mV, demonstrating that the number of the electrons taking part in the oxidation of SAL is equal with that of protons. That is, one electron and one proton are producing from the oxidation reaction of SAL at GO/poly(O-NBA)/GCE. Hence, a reaction oxidation mechanism of SAL was proposed as shown in Figure 1.

Effect of concentration of SAL

The DPV was employed to investigate the effect of concentration of SAL due to its ability to discriminate faradic current from non-faradic current [43]. DP voltammograms of various concentrations of SAL (64.23-626.2 μM) in 0.1 M PBS (pH=7.6) at the GO/poly(O-NBA)/GCE was presented in Figure 9 where: pulse high 25 × 10^{-3} V, pulse width 50 × 10^{-3} s, step time 0.1 s and scan rate 20 mV/s. The peak current of SAL clearly increases with increasing in its concentration

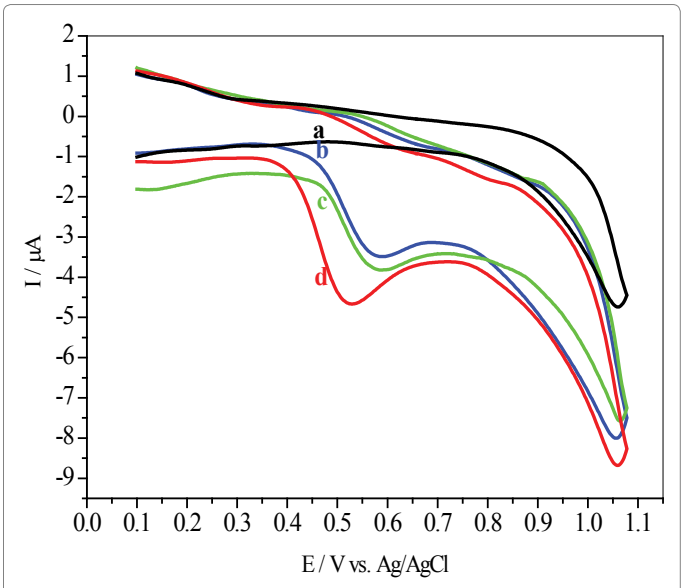

Figure 5: CVs of 0.1 M PBS (pH=7.6) in: (a) GO/poly(O-NBA)/GCE in the absence of SAL, (b) unmodified GCE with 0.3 mM of SAL, (c) GO/GCE with 0.3 mM of SAL and (d) GO/poly(O-NBA)/GCE with 0.3 mM of SAL at scan rate 100 mV/s.

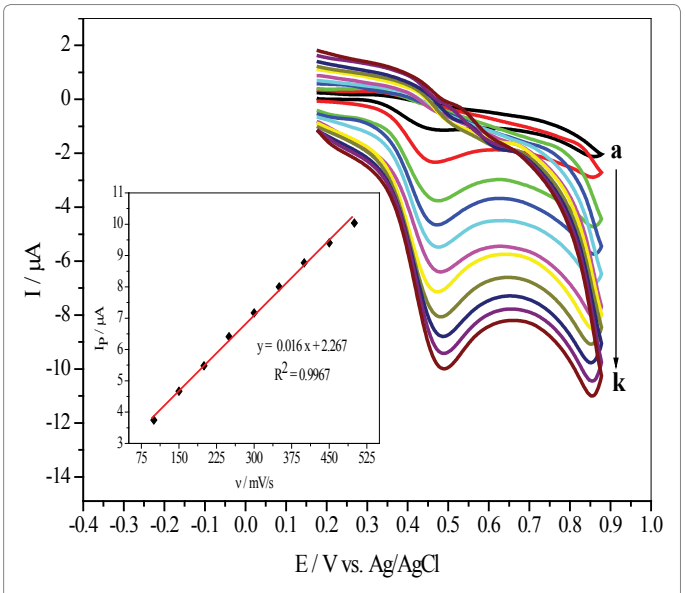

Figure 6: CVs of 0.3 mM of SAL in 0.1 M PBS (pH=7.6) at the GO/poly(O-NBA)/GCE with different scan rate: (a) 20, (b) 50, (c) 100, (d) 150, (e) 200, (f) 250, (g) 300, (h) 350, (i) 400, (j) 450, (k) 500. Inset: plot of peak current vs. scan rates (v) from 100 to 500 mV/s.

and there is gradually shifted in peak potential towards positive values. Moreover, inset Figure 9 shows the plot of anodic peak current against the concentration of SAL in the range 79.52 to 626.2 µM-calibration curve-with linear equation IP=8.53×10^{-4} C+0.496 (R^2=0.9971). The detection (LOD) and quantification (LOQ) limits were calculated from the following two equations:

LOD=3 s/m and LOQ=10 s/m

where, s and m are standard deviation of the intercept and the slope of calibration curve, respectively. The values of DL and QL were calculated to be 56 and 188 nM, respectively. It is known that the detection limit was the lowest concentration sensed by an electrode and being able to give the measurable signal that can be recorded by the technique. Table 1 shows the comparison of various modified electrodes for SAL detection, where the clear superiorities of the present work are the detection sensitivity and wide linear range of concentration.

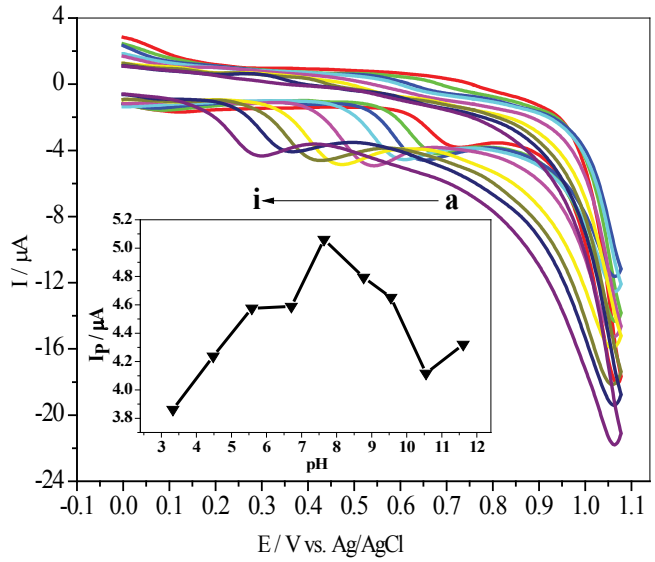

Figure 7: CVs of 0.3 mM SAL at GO/poly(O-NBA)/GCE in PBS of various pH values (a-i: 3.33, 4.48, 5.58, 6.71, 7.64, 8.77, 9.55, 10.55 and 11.61, respectively) at 100 mV/s. Inset: Plot of I_P *vs.* pH

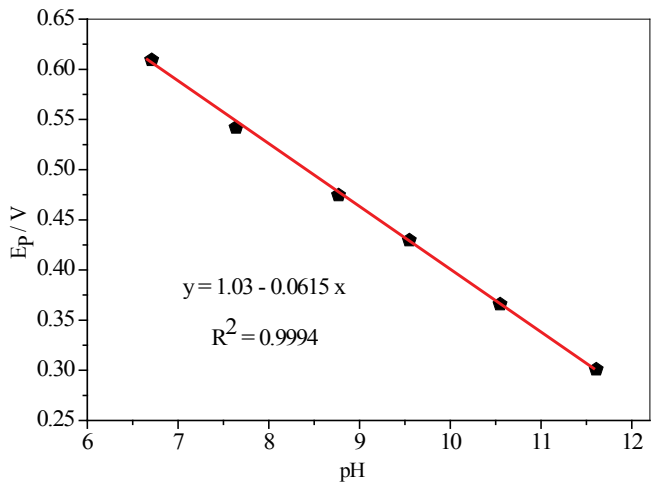

Figure 8: Plot of E_P versus pH, other conditions were the same as figure 7.

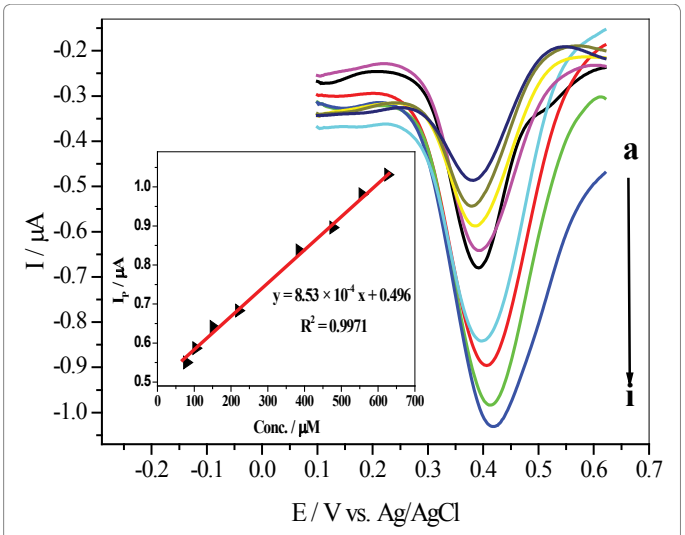

Figure 9: DPVs of GO/poly(O-NBA)/GCME in 0.1 M PBS (pH = 7.6) containing different concentrations of SAL (a-i: 64.23, 79.52, 104.4, 151.8, 220.6, 385.4, 477.1, 556.6 and 626.2 µM, respectively) at pulse high 25 x 10^{-3} V, pulse width 50 x 10^{-3} s, step time 0.1 s and scan rate 20 mV/s. Inset: calibration curve.

Electrode	Technique	Linear range	LOD	Reference
AuNPs/L-cys/MWNTs-NF/GCE	LSV	0.09 -7.0	50	[21]
poly(AHNSA)/GCE	DPV	0.20 - 8.0	68	[42]
MWNT-DHP/GCE	SWV	0.80 - 10	200	[43]
AB-DHP[a]/GCE	LSV	0.40 -7.5, 7.50 -100	100	[46]
Poly taurine/ZrO2/GCE	LSV	5.00 - 220	50	[47]
Nano-Au modified ITO	SWV	0.21-8.36	31	[48]
SMWCNT[b]-NF	DPV	0.10-0.30	100	[49]
CPT-BDDE	SWV	17.3-347	5060	[50]
GO/poly(O-NBA)/GCE	DPV	64.23-626.2	56	Present work

[a]AB-DHP: acetylene black-dihexadecyl hydrogen phosphate and [b]SMWCNT: mixture of SWCNT and MWCNT (1:1).

Table 1: Comparison of different electrochemical sensors for the determination of SAL.

Sample	Spiked (mM)	Found (mM)	Accuracy (% RE)	Recovery (%)
Salbutamol	0.150	0.152	± 1.3	101.3
Syrup	0.200	0.19	± 5.0	95.00
	0.450	0.434	± 3.5	96.44
Salbovent	0.150	0.157	± 4.6	104.6
Tablet	0.200	0.21	± 5.0	105.0
	0.450	0.441	± 2.0	98.00
	0.150	0.146	± 2.6	97.33
Urine	0.200	0.195	± 2.5	97.50
	0.450	0.429	± 4.6	95.33

Table 2: Determination of SAL in real samples.

Reproducibility, stability and interference

The performance of the modified electrodes can be evaluated by checking reproducibility, stability and selectivity. The reproducibility of the results was investigated by taking repetitive cyclic voltammogram of 6 parallel determinations of SAL (89.55 µM) in PBS (pH=7.6) at GO/poly(O-NBA)/GCE and the relative standard deviation (RSD) was 6.2%. The obtained value of RSD indicates the excellent reproducibility

of the modified electrode. In addition, this modified electrode showed a good stability, where only 4.5% decrease of the anodic peak current for SAL was observed after the GO/poly(O-NBA)/GC electrode was stored for ten days at 5.0°C.

The selectivity of the modified electrode was examined for 89.55 μM SAL in PBS (pH=7.6) with the maximum tolerable concentrations of some foreign species and concomitant compounds. Accordingly, 1000-fold concentration of common ions such as Na^+, K^+, Mg^{2+}, Zn^{2+}, Cl^-, NO^{3-}, CO_3^{2-} and SO_4^{2-} don't interference with determination of SAL. Beside, 100-fold of alanine, cysteine, glucose, lactose and 50-fold of maleic, tartaric and citric acids do not influence significantly the analytical results. But 50-fold concentration of ascorbic acid and p-nitrophenol showed a severe influence on the determination of SAL.

Analysis of real samples

In order to evaluate the applicability of the GO/poly(O-NBA)/GCE using DPV, the recoveries of SAL were determined in salbutamol syrup, salbovent tablets and urine samples containing different concentrations of

SAL by the standard addition method. The obtained results were summarized and presented in Table 2. Accordingly, the results of SAL recoveries (95.0-105. 0%) are satisfactory and proved that this modified electrode can readily be applied.

Conclusion

The present work clearly represents a GCE modified with GO and poly(O-NBA) to determine SAL in PBS using CV and DPV. The peak current of SAL was increased with increase in scan rate and concentration. The effect of pH at the modified electrode shows the high peak current of SAL oxidation at pH 7.6. An excellent sensitivity and good selectivity were achieved by the GO/poly(O-NBA)/GCE towards SAL. The high concentration of some common foreign compounds didn't interference in the detection of SAL at the GO/poly(O-NBA)/GCE. While high concentrations of ascorbic acid and p-nitrophenol showed a severe influence on the determination of SAL. The modified electrode showed good stability, unmatched efficiency and high precision in estimation of SAL. So successfully used in the determination of SAL in pharmaceutical formulations and urine sample.

References

1. Barisione G, Baroffio M, Crimi E, Brusasco V (2010) Beta Adrenergic Agonists. Pharmaceuticals 3: 1016-1044.
2. Yilmaz N, Ozkan SA, Uslu B, Senturk Z, Biryol I (1998) Voltammetric determination of salbutamol based on electrochemical oxidation at platinum and glassy carbon electrodes. Tr J Chem 22: 175-182.
3. Crescenzi C, Bayoudh S, Cormack PAG, Klein T, Ensing K (2001) Determination of Clenbuterol in Bovine Liver by Combining Matrix Solid-Phase Dispersion and Molecularly Imprinted Solid-Phase Extraction Followed by Liquid Chromatography/Electrospray Ion Trap Multiple-Stage Mass Spectrometry. Anal Chem 73: 2171-2177.
4. Shishani E, Chai SC, Jamokha S, Aznar G, Hoffman MK (2003) Determination of ractopamine in animal tissues by liquid chromatography-fluorescence and liquid chromatography/tandem mass spectrometry. Chim Acta 483: 137-145.
5. Du X-D, Wu Y-L, Yang H-J, Yang T (2012) Simultaneous determination of 10 β 2-agonists in swine urine using liquid multi-walled carbon nanotubes as a reversed dispersive solid phase extraction sorbent. J Chromatogr A 1260: 25-32.
6. Doerge DR, Churchwell MI, Holder CL, Rowe L, Bajic S (1996) Detection and confirmation of B-agonists in bovine retina using LC/APCI-MS. Anal Chem 68: 1918-1923.
7. Mitchell GA, Dunnavan G (1998) Illegal use of β-adrenergic agonists in the United States. J Anim Sci 76: 208-211.
8. Rajkumar M, Li YS, Chen SM (2013) Electrochemical detection of toxic ractopamine and salbutamol in pig meat and human urine samples by using poly taurine/zirconia nanoparticles modified electrodes. Colloids Surf B 110: 242-247.
9. Dol I, Knochen M (2004) Flow-injection spectrophotometric determination of salbutamol with 4-aminoantipyrine. Talanta 64: 1233-1236.
10. Dave HN, Mashru RC, Thakkar AR (2007) Simultaneous determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. Analytica Chimica Acta 597: 113-120.
11. Basavaiah K, Somashekar B, Ramakrishna V (2007) Rapid titrimetric and spectrophotometric methods for salbutamol sulphate in pharmaceuticals using N-bromosuccinimide. Acta Pharm 57: 87-98.
12. Bernal JL, del-Nozal MJ, Velasco H, Toribio L (1996) HPLC versus SFC for the determination of salbutamol sulphate and its impurities in pharmaceuticals. J Liq Chromatogr Rel Technol 19: 1579-1589.
13. Alejandraa H, Carlosa F, Marcelaa P, Vivianaa D, Saraa P (2004) Validation of a chiral HPLC assay for (R)-sabutamol sulfate. J Pharm Biomed Anal 34: 45-51.
14. Singh RA, Kumar, Kumar D, Agarwal AK (2005) Simultaneous estimation of cetirizine hydrochloride and salbutamol sulphate in pharmaceutical dosage forms by reverse phase high performance liquid chromatography. Pharma Rev 3: 144-146.
15. Reguerio JAQ, Perez B, Casademant G (1993) Determination of clenbuterol and salbutamol in urine by capillary gas chromatography with capillary columns of 100 μm. J Chromatogr A 655: 73-76.
16. Sirichaia S, Khanatharana P (2008) Rapid analysis of clenbuterol, salbutamol, procaterol, and fenoterol in pharmaceuticals and human urine by capillary electrophoresis. Talanta 76: 1194-1198.
17. Chen C, Li H, Fan Y (2011) Determination of salbutamol sulfate in medicaments by capillary electrophoresis with contactless conductivity detection. Chin J Chromatogr 29: 137-140.
18. Sun XX, Sun LZ, Aboul-Enein HY (2000) Internal Solid Contact Electrode for the Determination of Salbutamol Sulfate in Pharmaceutical Formulation. Electroanalysis 12: 853-857.
19. Ganjali MR, Shirvani-Arani S, Nourozi P, Salimzadeh D, FaalRastegar M (2004) A Novel Sulfate Polymeric Membrane Sensor Based on a New Bis□ Pyrylium Derivative. Electroanalysis 16: 1009-1013.
20. Karuwan C, Wisitsoraat A, Maturos T, Phokharatkul D, Sappat A, et al. (2009) Flow Injection Based Microfluidic Device with Carbon Nanotube Electrode for Rapid Salbutamol Detection. Talanta 79: 995-1000.
21. Li Y, Ye Z, Luo P, Lia Y, Ye B (2014) A sensitive voltammetric sensor for salbutamol based on MWNTs composite nano-Au film modified electrode. Anal Methods 6: 1928-1935.
22. Tang L, Wang Y, Li Y, Feng H, Lu J, et al. (2009) Preparation, structure, and electrochemical properties of reduced graphene sheet films. Advanced Functional Materials 19: 2782-2789.
23. Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, et al. (2004) Electric field effect in atomically thin carbon films. Science 306: 666-669.
24. Li X, Zhang G, Bai X, Sun X, Wang X (2008) Highly conducting graphene sheets and Langmuir–Blodgett films. Nature Nanotechnol 3: 538-542.
25. Lee C, Wei X, Kysar JW, Hone J (2008) Measurement of the elastic properties and intrinsic strength of monolayer graphene. Science 321: 385-388.
26. Pumera M, Smid B, Veltruska KJ (2009) Influence of nitric acid treatment of carbon nanotubes on their physico-chemical properties. Nanosci Nanotechnol 9: 2671-2676.
27. Goh MS, Pumera M (2010) Single-, few-, and multilayer graphene not exhibiting significant advantages over graphite microparticles in electroanalysis. Anal Chem 82: 8367-8370.
28. Gilbert O, Chandra U, Kumara SBE, Panduranga CM, Nagaraj C, et al. (2008) Poly (Alanine) Modified Carbon Paste Electrode for Simultaneous Detection of Dopamine and Ascorbic Acid. J Electrochem Sci 3: 1186-1195.

29. Harisha KV, Kumara Swamy BE, Jayadevappa H, Vishwanath CC (2015) Voltammetric Determination of Folic acid in presence of Dopamine and Ascorbic Acid at Poly (Alanine) Modified Carbon Paste Electrode. Anal Bioanal Electrochem 7: 454-465.

30. Abou-Krisha MM, Assaf FH, Toghan AA (2007) Electrodeposition of Zn–Ni alloys from sulfate bath. J Solid State Electrochem 11: 244-252.

31. Rageh HM, Abou-Krisha MM, Abo-bakr AM, Abd-Elsabour M (2015) Electrochemical Behavior and the Detection Limit of Ascorbic Acid on a Pt Modified Electrode. Int J Electrochem Sci 10: 4105-4115.

32. Hummers WS, Offeman RE (1958) Preparation of graphitic oxide [J]. J American Chemical Society 80: 1339-1339.

33. Muzyka R, Kwoka M, Smedowski L, Diez N, Gryglewicz G (2017) Oxidation of graphite by different modified Hummers methods, New Carbon Mater 32: 15-20.

34. Zhang TY, Zhang D (2011) Aqueous colloids of graphene oxide nanosheets by exfoliation of graphite oxide without ultrasonication, Bull Mater Sci 34: 25-28.

35. Mousavi MF, Amiri M, Noori A, Khoshfetrat SM (2017) A Prostate Specific Antigen Immunosensor Based on Biotinylated-Antibody/Cyclodextrin Inclusion Complex: Fabrication and Electrochemical Studies. Electroanalysis 29: 2818-2831.

36. Randles JEB (1948) A cathode ray polarograph. Part II. The current-voltage curves. Trans Faraday Soc 44: 327-338.

37. Sevcik A (1948) Oscillographic polarography with periodical triangular voltage. Collect Czech Chem Commun 13: 349-377.

38. Garcia-Miranda Ferrari A, Foster CW, Kelly PJ, Brownson DAC, Banks CE (2018) Determination of the Electrochemical Area of Screen-Printed Electrochemical Sensing Platforms. Biosensors 8: 53-62.

39. Lyons MEG, Keeley GP (2006) The Redox Behaviour of Randomly Dispersed Single Walled Carbon Nanotubes both in the Absence and in the Presence of Adsorbed Glucose Oxidase. Sensors 6: 1791-1826.

40. Amare M, Admassie S (2012) Polymer modified glassy carbon electrode for the electrochemical determination of caffeine in coffee. Talanta 93: 122-128.

41. Amare M, Menkir G (2017) Differential pulse voltammetric determination of salbutamol sulfate in syrup pharmaceutical formulation using poly(4-amino-3-hydroxynap hthalene sulfonic acid) modified glassy carbon electrode. Heliyon 3: e00417.

42. Wei Y, Zhang Q, Shao C, Li C, Zhang L, et al. (2010) Voltammetric determination of salbutamol on a glassy carbon electrode coated with a nanomaterial thin film. J Anal Chem 65: 398-403.

43. Laviron E (1979) General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. J Electroanal Chem Interf Electrochem 101: 19-28.

44. Laviron E (1979) The use of linear potential sweep voltammetry and of a.c. voltammetry for the study of the surface electrochemical reaction of strongly adsorbed systems and of redox modified electrodes. J Electroanal Chem 100: 263-270.

45. Goyal RN, Oyama M, Singha SP (2007) Fast determination of salbutamol, abused by athletes for doping, in pharmaceuticals and human biological fluids by square wave voltammetry. J Electroanal Chem 611: 140-148.

46. Yang X, Wang F, Hu S (2010) Enhanced oxidation of salbutamol sulphate at acetylene black-dihexadecyl hydrogen phosphate composite film electrode and its analytical applications. ACAIJ 9: 86-92.

47. Rajkumar M, Li YS, Chen SM (2013) Electrochemical detection of toxic ractopamine and salbutamol in pig meat and human urine samples by using poly taurine/zirconia nanoparticles modified electrodes. Colloids Surf B 110: 242.

48. Goyal RN, Oyama M, Singh SP (2007) Fast determination of salbutamol, abused by athletes for doping, in pharmaceuticals and human biological fluids by square wave voltammetry. J Electroanal Chem 611: 140.

49. Kuo-Chiang Lin, Chuen-Pon Hong, Shen-Ming Chen (2013) Simultaneous determination for toxic ractopamine and salbutamol in pork sample using hybrid carbon nanotubes. Sens. Actuators B 177: 428.

50. Pinar PT, Ali HS, Abdullah AA, Yardim Y, Senturk Z (2018) Electroanalytical determination of salbutamol in pharmaceutical formulations using cathodically pretreated boron-doped diamond electrode, Marmara Pharm J 22: 460-468.