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RESEARCH ARTICLE

Vertically Aligned Carbon Nanotube Based Electrochemcial Sensor for Salbutamol Detection

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This paper reports electrochemical sensing of salbutamol, a prohibited drug in sports, with a new working electrode based on vertically aligned carbon nanotubes (V-CNT). V-CNTs were synthesized by chemical vapor deposition using acetylene and argon gases at 700 °C on gold coated silicon substrates. A simple electrochemical cell including V-CNTs, silver wire (Ag) and platinum (Pt) wire was designed. Electrochemical characterization by cyclic voltammetry (CV) was carried out with different salbutamol concentrations ranging from 10^{-7} to 10^{-4} mol·l^{-1}. CV curves exhibited irreversible oxidation peak at ~0.7 V. The current response was linear with sensitivity of 0.13 μA/mol·l^{-1} and a minimum detection of 3×10^{-7} mol·l^{-1}. In addition, its pharmaceutical applications were demonstrated. The direct analysis of salbutamol in pharmaceutical products yielded a good analytical feature with wide dynamic working range (0.5 to 100 μM) and matrices' interference was found to be negligible. Thus, V-CNTs electrode is a potential candidate for the electrochemical detection of salbutamol.

Keywords: Vertically Aligned Carbon Nanotube, Electrochemical Sensor, Salbutamol, Cyclic Voltammetry.

1. INTRODUCTION

Carbon nanotubes (CNTs) have been recently studied by analytical chemists as sensors because of its excellent properties including unique electronic band structure, extreme strength, very high thermal conductivity and superconductivity. CNTs have been widely used as electrochemical electrodes in various applications of electroanalysis[1–7] due to their high reaction area and excellent electron transfer rate.[8] CNTs can be grown by chemical vapor deposition (CVD).[5–7,9] CVD is one of the most suitable methods for CNTs growth because of its low cost and low deposition temperature.[10]

Salbutamol or 4-[2-(tert-butylamino)-1-hydroxyethyl]-2(hydroxymethyl) phenol is the most widely used β_2 adrenergic receptor agonists, which induces bronchodilation, making the drug highly useful for curing bronchial asthma and other allergic diseases associated with respiratory pathway.[11–13] However, high dose of salbutamol is prohibited in sports because of its abuse as a stimulant and anabolic agent. Hence, the use of salbutamol is only permitted for athletes having asthma. World Anti-Doping Agency (WADA) has prohibited the oral use of salbutamol and a concentration greater than 1000 ng/ml (3 μM) in urine is considered as an indication of doping.[14,15]

Due to the necessity to detect and monitor β-agonists, several devices and methods have been developed for determination of these compounds, including gas chromatography with mass spectrometric detection (GC-MS)[16,17] and high performance liquid chromatographic (HPLC) method with UV-detection. These methods have normally been utilized for gold-standard determination of salbutamol in athletes.[18–20] Recently, quick detection methods are reported based on electrochemical[21] and MS[22–23] detections or capillary electrophoresis (CE) with MS[24] and amperometric[25] detections.

Among all techniques, electrochemical sensing is a promising alternative for β-agonists sensing due to its fast detection and high sensitivity. Recently, electrochemical detection of β-agonists by various electrodes including glassy carbon, carbon paste, carbon disk, boron doped diamond and fullerene electrodes have been reported.[21,25–30] In this work, CNTs are used as electrodes for salbutamol sensing. A simple electrochemical cell including CNTs as

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working electrode, silver wire as reference electrode and platinum wire as auxiliary electrode is designed for cyclic voltammetric measurement.

2. EXPERIMENTAL DETAILS

2.1. Apparatus

A potentiostat, μ-autolab Type III (Metrohm, Switzerland) was used for all cyclic voltammetric (CV) studies. A single-compartment three electrode system comprised a CNTs working electrode, a platinum wire counter electrode and a silver wire reference electrode. The CNTs electrode was fabricated by CVD technique. The 0.5-mm Pt and 1.0-mm Ag wires were purchased from Aldrich (USA).

2.2. Chemicals and Reagents

All chemicals used in this work were analytical grade reagents. Salbutamol was purchased from Sigma (USA). 0.1 mM citric acid (pH 4) was prepared by mixing 10 ml of 2 M citric acid (Merck, Germany) and 4 ml of 2 M sodium hydroxide (Merck, Germany). 0.1 mM Citric acid (pH 6) was made by mixing 10 ml of 2 M citric acid (Merck, Germany) and 6 ml of 2 M sodium hydroxide (Merck, Germany). A 0.1 mM phosphate buffer (pH 7) was prepared by mixing 19.5 ml of 0.2 M sodium phosphate monobasic monohydrate (Merck, Germany) and 30.5 ml of 0.2 M sodium phosphate dibasic (Merck, Germany). A 0.1 M tris buffer (pH 8) was made by mixing 50 ml of 0.2 M tris(hexahydroxy)aminomethane (Merck, Germany) and 29.2 ml of 0.1 M hydrochloric acid (HCl) (Lab Scan, Ireland). 0.1 M Tris buffer (pH 10) was prepared similarly by pH adjustment using 0.1 M hydrochloric acid solutions. The stock solution (0.01 mol l^{-1}) of salbutamol was prepared by dissolving ~30 mg of salbutamol in deionized-distilled water. Finally, salbutamol solutions with different concentrations were prepared by adding the stock solution in these buffers solutions.

2.3. Electrode Fabrication

The structure of CNTs electrode is shown in Figure 1. The working electrode was fabricated on ⟨100⟩ Si substrate. First, SiO_2 (400 nm), Ti (50 nm) and Au (500 nm) were successively sputtered on the substrate. Next, titanium dioxide (300 nm) was sputtered on the gold layer over a defined electrode region, which excludes active sensing (1 mm^2) and electrical contact area. Next, aluminum oxide (10 nm) and stainless steel (SS) catalyst (5 nm) were successively sputtered over the active area through shadow masking for CNT synthesis. The titanium dioxide and aluminum oxide layer were deposited by reactive sputtering at a pressure of 3×10^{-3} mbar with 1:5 Ar:O_2 gas mixture while other metallic layers were deposited by pure Ar gas at the same pressure.

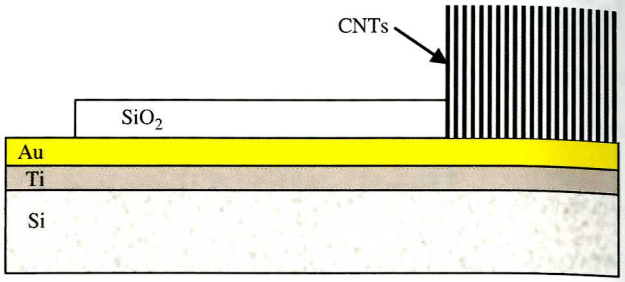

Fig. 1. Schematic diagram of V-CNTs electrode structure.

2.4. V-CNT Synthesis

V-CNTs were then grown by thermal chemical vapor deposition (CVD) with gravity effect and water-assisted etching.[31–32] The catalyst layers on substrates were placed upside down along gravitational field on an alumina carrier in a horizontal furnace thermal CVD system. CNTs synthesis was conducted at atmospheric pressure and growth temperature of 700 °C. During CNTs growth, acetylene was flowed for 1.5 minute and hydrogen to acetylene flow ratio was 4:3:1. In the course of CNT growth, *in-situ* water-assisted etching was employed to remove undesired amorphous carbon formation from random acetylene decomposition. In water etching process, 300 ppm of water vapor was introduced by water bubbling through Ar gas for 3 minutes while acetylene gas was turned off. CNTs growth and water-assisted etching were repeatedly performed for two cycles.

2.5. Electrochemial Cell and Electrochemical Set Up

Cyclic voltammetry is a basic technique, which is widely used for characterization behavior of an analyte. A home made electrochemical cell with three electrode system consisted of a Pt wire auxiliary electrode, an Ag wire reference electrode and a CNTs working electrode. The volume of cell was 1.0 ml.

3. RESULTS AND DISCUSSION

3.1. CNTs Electrode

The surface morphology of the sensing area of CNTs based electrode was examined using scanning electron microscopy (SEM) as shown in Figure 2. It can be seen that the fabricated CNTs are vertically aligned with a height of ~30 μm. Figure 3 shows a 1.0-ml home made electrochemical cell with three electrode system consisted of a Pt wire auxiliary electrode, an Ag wire reference electrode and a CNTs working electrode.

3.2. Performance of GC and CNTs Electrodes

In order to assess the sensitivity of CNTs electrode, the electrochemical characteristics of CNTs electrode in 1 mM

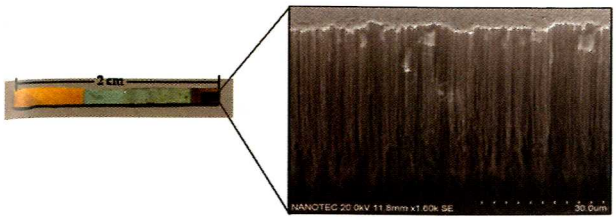

Fig. 2. Photograph of fabricated electrode and SEM micrograph of V-CNTs electrochemical electrode.

salbutamol in citric buffer (pH 6.0) was compare to that of commercial glassy carbon (GC) electrodes as shown in Figure 4. It can be seen that the CNTs electrode exhibits much higher irreversible oxidation peak at ~0.75 V than GC electrode does. Thus, CNTs significantly enhance the electrochemical activity with salbutamol due to its high reaction area and excellent electron transfer rate. The CV curves at 5 mM salbutamol concentration of electrode with no CNTs and with CNTs have been reported by Karuwan et al.[33] Similarly, CNTs electrode exhibits much higher irreversible oxidation peak than gold electrode does. The oxidation peak of salbutamol at ~0.7 V can be explained by oxidative reaction of phenolic hydroxyl group.[34] The mechanism of salbutamol oxidation has been proposed based on the pathway of phenol.[29] The oxidative reaction of salbutamol can be illustrated in Figure 5.[13, 28, 35] In the reaction, sabutamol molecule (A) is oxidized by electrochemical potential and becomes salbutamol free radical (B) giving one electron and one proton. The free radicals are unstable so they will react with each other to form dimers (C).

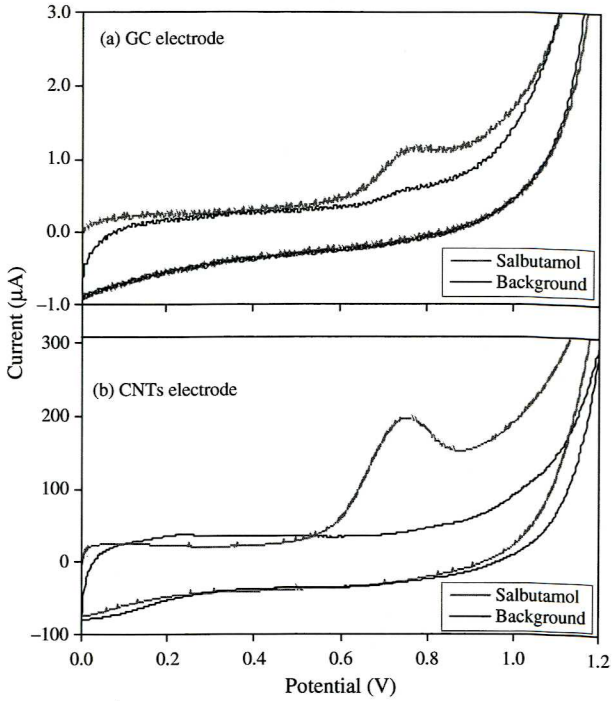

Fig. 4. Cyclic voltammogram of 1 mM salbutamol using GC (a) and CNTs (b) electrode. Scan rate was 100 mVs^{-1}. Buffer solution is citric acid/sodium hydroxide pH 6.0.

3.3. Selection of Electrolyte

In general, solubility and dissociation of an analyte are affected to some extent by pH of its buffer. The electrochemical characteristics of salbutamol were thus studied in buffer solutions with different pHs ranging from 4 to 10.

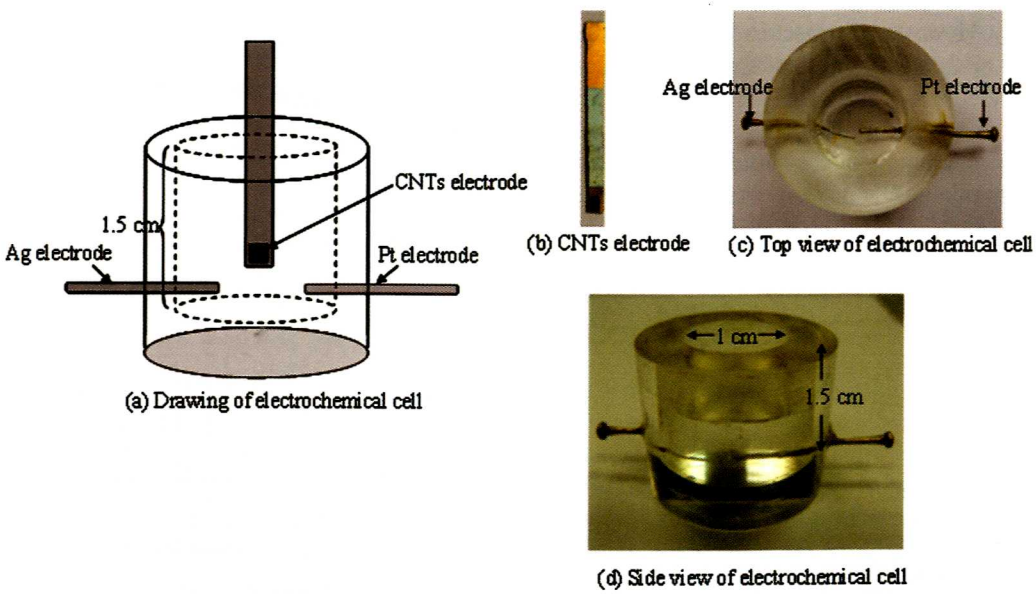

Fig. 3. Home-made electrochemical cell with three electrode system consisted of platinum wire auxialary electrode, Ag wire reference electrode and CNTs working electrode. The electrochemical cell was made from acrylic piece with 1.0 cm diameter and 1.5 cm height. The voltage window used for CV experiments is from 0 to +1.0 V.

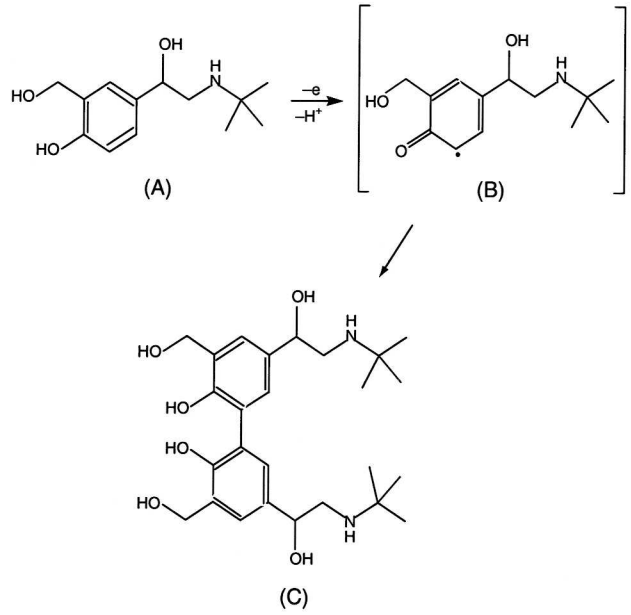

Fig. 5. Oxidation reaction of salbutamol on V-CNTs electrode.

It was found that the salbutamol current response was weakly depending on pH and pH value of 6 was a locally optimal condition that gave high electrochemical response. Thus, this buffer was selected for further studies.

3.4. Scan Rate Dependence Study

CV responses were then taken with different scan rates as illustrated in Figure 6. The oxidation peak amplitude is found to increase linearly with the square root of scan rate, indicating that the current is limited by semi-infinite diffusion of salbutamol on CNTs electrode. It should be noted that the effect of scan rate on the background is not shown for clarity. However, the inset in Figure 6 has been properly plotted with background subtraction at different scan rates.

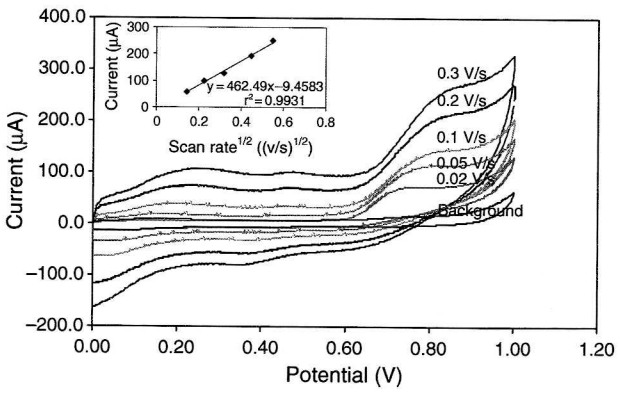

Fig. 6. Cyclic voltammograms obtained at various potential scan rates of 1 mM standard solutions of salbutamol in citric acid/sodium hydroxide pH 6.0. The inset picture shows the relationship between square root of the scan rate and current response. (Working electrode: V-CNTs, Reference electrode: silver wire, Auxiliary electrode: Pt.)

3.5. Concentration Study

The CV measurements were made with different concentrations as illustrated in Figure 7(a). The amperometric response of oxidation peak as a function of concentration is shown in the inset of Figure 7(b). It is observed that peak current of salbutamol varies linearly with concentration in the range 0.5–100 μM. At low concentration (less than 0.5 μM), peak current of salbutamol also varies linearly but with a higher slope value. On the other hand, the response current begins to level off at higher salbutamol concentrations (200–400 μM).

3.6. Stability of CNTs Electrode

The stability of CNTs electrode was checked by recording successive cyclic voltammograms. After 20 cycles, no change was observed in the voltammetric profiles of CNTs electrode. Even in the presence of salbutamol, the electrode remained stable after 20 successive cycles with relative standard deviation (RSD) of 8.9%. Furthermore, no significant change in the response was observed for more than two months after the electrode was stored at room temperature. Moreover, reproducibility of CNTs electrode was determined from seven sensors fabricated in the same batch and RSD of 14.7% was obtained. Therefore, CNTs electrodes have satisfactory repeatability and reproducibility.

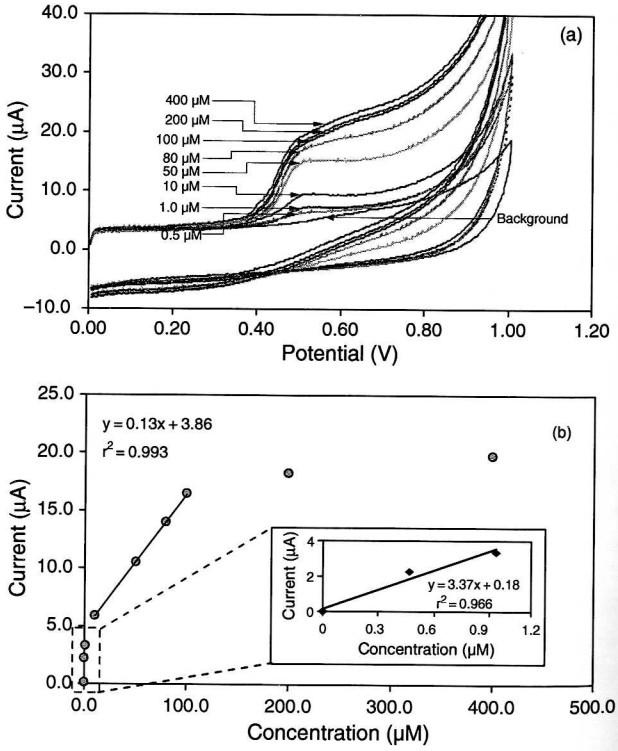

Fig. 7. Cyclic voltammograms of salbutamol solutions with different concentrations in citric acid/sodium hydroxide (pH 6.0) (a). Calibration curve of oxidation peak as a function of concentration (b). (Working electrode: V-CNTs, Reference electrode: silver wire, Auxiliary electrode: Pt.)

Table I. Comparison of analytical performance of CNTs electrode and other data reported in literature.

References	Methods	Linear dynamic range (μM)	Correlation coefficient (r^2)	Detection limit (μM)
Our work	V-CNTs electrode with cyclic voltametry	0.5 and 100	0.993	0.3
Yilmaz et al. [34]	PT electrode with cyclic voltametry	100–1000	0.9996	80
Yilmaz et al. [34]	GC electrode with cyclic voltametry	20–1000	0.9997	10
Karuwan et al. [28]	BDD electrode with amperometric detection	0.5–100	0.999	0.1
Zhou et al. [25]	Carbon-disk electrode with CE-amperometric detection	0.3 to 50	0.998	0.2
Quintino et al. [26]	GC electrode with batch injection analysis	0.8 to 200	0.9995	0.25
Goyal et al. [13]	Au nanoparticles on ITO electrode with differential pulse voltametry	0.2 to 8.4	—	0.3

Pt electrode: Platinum electrode; GC electrode: Glassy carbon electrode; BDD electrode: Boron doped diamond electrode; CE: Capillary electrophoresis; Au: Gold electrode; ITO: Indium thin oxide.

It should be noted that repeatability and reproducibility were measured at 100 μM sabutamol concentration.

3.7. Analytical Features

The analytical performance of CNTs electrode for salbutamol detection is summarized in Table I. Linear concentration dependence or dynamic range is observed between 0.5 and 100 μM. The regression equation is given by $y = 0.13x + 3.86$ ($r^2 = 0.993$), where y and x are the height of peak current (μA) and salbutamol concentration (μM), respectively. The slope of the equation is corresponding to linear sensitivity of 0.13 μA/μM. It should be noted that the calibration line does not go to zero due to nonlinearity of the sensor in the low concentration region. The detection limit (3S/N) is as low as 0.3 μM. The noise value (N) is measured by taken peak–peak amplitude of sinusoidal noise in the base line region of CV curve.

The analytical performance of CNTs electrode with cyclic voltametry is compared to other reports in literature as listed in Table I. It is evident that CNTs electrode is significantly better than GC and Pt electrodes based on cyclic voltametry detection.[34] Moreover, the result is comparable to electrochemical detection of salbutamol by boron-doped diamond (BDD) electrode using amperometric detection,[28] carbon-disk electrode with CE-amperometric detection,[25] GC electrode with batch injection analysis[26] and gold nanoparticles modified indium tin oxide electrode using Osteryoung square wave voltammetry.[13] For a given electrode, it is well known that amperometric detection, batch injection analysis and Osteryoung square wave voltammetry generally yield lower detection limit than cyclic voltammetry. This implies that the CNTs based electrode should offer better salbutamol detection due to its larger surface area compared to gold nanoparticles, BDD, and GC. In addition, the CNTs based electrode exhibits wider dynamic range than the reported gold nanoparticle based sensors, which is only between 0.2 and 6 μM.

Table II. Comparison of the labeled values of salbutamol in pharmaceutical products and the analyzed results by HPLC-UV and CV using V-CNTs electrode.

Sample name	Content of salbutamol (mg/L)		
	Label	HPLC-UV	V-CNTs
1. Ventolin syrup	400	401 ± 3	406 ± 5
2. Asmasal syrup	400	403 ± 2	413 ± 3

3.8. Performance for Pharmaceutical Applications

Salbutamol content in syrup samples were analyzed by the developed electrochemical system. The measured results were compared to the labeled values from their manufacturer and results from high performance liquid chromatography with ultraviolet detection (HPLC-UV) as shown in Table II. HPLC-UV measurement was conducted by the manufacturer (IDS Manufacturing Ltd., Thailand). According to pair t-test,[36] the results for Ventolin and Asmasal are not significantly different at 95% confidence ($t_{stat} = 3.0$, $t_{critical} = 12.70$). It is clear that our results are in good agreement with those from HPLC-UV. Moreover, recovery studies made for the samples show that the matrices have small influence on electrochemical oxidation of salbutamol (115–121% recovery). Thus, the samples can be directly analyzed by our method with low matrices' interference. However, it was found that interference occurred in other pharmaceutical products such as Ventolin tablets. Thus, the electrode doesn't possess general anti-interference capability. Sample pretreatment including separation is needed to overcome the interference problem in general cases especially urine samples, which contain very complex matrices.

4. CONCLUSIONS

This work presents the utilization of vertically align carbon nanotube (V-CNT) for electrochemical detection of salbutamol. Cyclic votammetry (CV) gives a well-defined irreversible oxidation peak of this compound. The method provides good analytical features with wide dynamic working range (0.5 to 100 μM) compared to another report on gold nanoparticle based sensor. In addition, the detection limit (3S/N) is as low as 0.3 μM. Furthermore, this method is suitable for direct analysis of salbutamol in pharmaceutical product with low matrices' interference.

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