Talanta 79 (2009) 995–1000

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Flow injection based microfluidic device with carbon nanotube electrode for rapid salbutamol detection

Chanpen Karuwan, Anurat Wisitsoraat, Thitima Maturos, Disayut Phokharatkul, Assawapong Sappat, Kata Jaruwongrungsee, Tanom Lomas, Adisorn Tuantranont*

Nanoelectronics and MEMS Laboratory, National Electronics and Computer Technology Center, Pathumthani 12120, Thailand

ARTICLE INFO

Article history:
Available online 20 February 2009

Keywords:
Flow injection
Microfluidic device
Carbon nanotube
Salbutamol
In-channel electrochemical detection
Lab-on-a-chip

ABSTRACT

A microfabicated flow injection device has been developed for in-channel electrochemical detection (ECD) of a β-agonist, namely salbutamol. The microfluidic system consists of PDMS (polydimethylsiloxane) microchannel and electrochemical electrodes formed on glass substrate. The carbon nanotube (CNT) on gold layer as working electrode, silver as reference electrode and platinum as auxiliary electrode were deposited on a glass substrate. Silver, platinum, gold and stainless steel catalyst layers were coated by DC-sputtering. CNTs were then grown on the glass substance by thermal chemical vapor deposition (CVD) with gravity effect and water-assisted etching. 100-μm-deep and 500-μm-wide PDMS microchannels fabricated by SU-8 molding and casting were then bonded on glass substrate by oxygen plasma treatment. Flow injection and ECD of salbutamol was performed with the amperometric detection mode for in-channel detection of salbutamol. The influences of flow rate, injection volume, and detection potential on the response of current signal were optimized. Analytical characteristics, such as sensitivity, repeatability and dynamic range have been evaluated. Fast and highly sensitive detection of salbutamol have been achieved. Thus, the proposed combination of the efficient CNT electrode and miniaturized lab-on-a-chip is a powerful platform for β-agonists detection.

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1. Introduction

Microfluidic system is a potential tool for biochemical processing and analysis because of low sample/reagent consumption and high sample throughput [1,2]. Many previous reports have been used glass, silicon and plastic based microfluidic device. Different polymeric materials, including polycarbonate [3], polymethylmethacrylate (PMMA) [4] and polydimethylsiloxane (PDMS) [5] have been commonly employed for the fabrication of plastic based microfluidic devices. PDMS has been one of the most popular materials for chemical microsystems due to its high chemical and mechanical stability [6]. In addition, a variety of PDMS based microfluidic chip for miniaturized analytical system [6,7] have been fabricated by micromolding based on standard photolithography [8,9].

Electrochemical technique offers high performance detection on miniaturizing electrode in miniaturizing system [10]. Therefore, various works have been focused on coupling electrochemical detection (ECD) to microfluidic systems with both end-channel [11,12] and in-channel detection scheme [10,13,14] using different electrode materials, including gold, platinum and carbon nanotube (CNT). Carbon nanotubes (CNTs) is a promising material for electrochemical electrodes due to its high reaction area and excellent electron transfer rate [15]. CNTs have been used as sensors in various applications of electroanalysis [16–21]. However, there have been no reports on direct carbon nanotubes growth on glass substrate for fabrication of microfluidic device with in-channel detection.

[2-(Tert-butylamino)-1-(4-hydroxy-3-hyroxymethyl) phenylethanol] or salbutamol is the most widely used β_2-adrenergic receptor agonist which induces bronchodilation, making it highly useful for curing bronchial asthma, chronic obstructive pulmonary disease (COPD) and other allergic diseases associated with respiratory pathway [22–24]. However, high dose of salbutamol is prohibited in sports because of its abuse as a stimulant and as anabolic agent. Hence, the use of salbutamol is permitted by inhaler only for athletes having asthma or exercise induced asthma. The 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 [25,26]. Thus, devices or instruments for salbutamol detection must be highly sensitive and selective. Presently, complicated and expensive liquid/gas chromatography and spectrophotometric techniques including GC–MS and HPLC with UV-detection have normally been utilized for the determination of salbutamol in athletes [26–28].

* Corresponding author. Tel.: +66 2 564 6900; fax: +66 2 564 6771.
E-mail addresses: adisorn.tuantranont@nectec.or.th, anurat.wisitsoraat@nectec.or.th (A. Tuantranont).

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doi:10.1016/j.talanta.2009.02.015

In this work, a new microfluidic device has been developed using PDMS/glass chip with in-channel amperometric detection based on carbon nanotube electrode. The three-electrode system was patterned across the channel as an in-channel detection using thin-film sputtering and chemical vapor deposition (CVD) technique. The system is applied for determination of salbutamol. The simple, rapid, inexpensive and reliable method will be presented.

2. Experimental

2.1. Chemicals and reagents

All of chemicals used in this work were analytical grade reagent. Standard solutions of salbutamol were purchased from Sigma (USA). Various buffers including citrate (pH 4), phosphate (pH 7) and borate (pH 10) were purchased from Merck (Germany). Tris buffer solution (pH 8.3) was prepared from tris (hexahydroxy) aminomethane (Merck, Germany) and 1.0 M hydrochloric acid (HCl) (Lab Scan, Ireland). The stock solution (0.01 mol l^{-1}) of salbutamol was prepared by dissolving 0.03 g of salbutamol in deionized-distilled water. Glass substrates were purchased from Sperior (Germany). PDMS were purchased from Dow Chemical (USA). Photoresist (SU-8 2100) was purchased from Micro Chem (USA).

2.2. Apparatus

A potentiostat, μ-autolab Type III (Metrohm, Switzerland) was used for all the cyclic voltammetric (CV) and amperometric studies. A single-compartment three electrode system has a carbon nanotube as a working electrode, platinum (Pt) wire as a counter electrode and silver (Ag) wire as a reference electrode. Spin coater (Laurell Technologies Corp., model WS-400A-6NPP) was used for spin coating of photoresist for mold fabrication. The MJB4 mask aligner (SUSS microtec, Germany) was used for the UV-lithography process to obtain photoresist patterns on Si substrate. The oxygen plasma cleaner (Harrick scientific Corp., model PDC-32G) was used for treatment of PDMS and glass surface to obtain good bonding.

2.3. Electrochemical cell for cyclic voltammetry

Cyclic voltammetry is an initial technique, which is used for characterization behavior of an analyte. Fig. 1 shows a home made electrochemical cell with three electrode system consisted of Pt wire auxiliary electrode, Ag wire reference electrode and CNT working electrode. The volume of cell was 1.5 ml. The working electrode was fabricated on ⟨1 0 0⟩ 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 (0.07 cm^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 was deposited by reactive sputtering at a pressure of 3×10^{-3} mbar with 1:5 Ar:O_2 gas mixture while other metallic layers was deposited by pure Ar gas at the same pressure.

The CNT is grown by chemical vapor deposition [19–21]. The CVD process is used because of its low cost and high quality CNT structure [28]. The catalyst layers on substrates were placed upside down along gravitational field on an alumina carrier in a horizontal furnace thermal CVD system. The CNT synthesis was conducted at atmospheric pressure and growth temperature of 700 °C. During CNT growth, acetylene was flown for 1.5 min and hydrogen to acetylene 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 min while acetylene gas was turned off. CNTs growth and water-assisted etching were repeatedly performed for two cycles. The sensing area of CNT electrode was 0.07 cm^2. This electrochemical cell is used for all cyclic voltammetry.

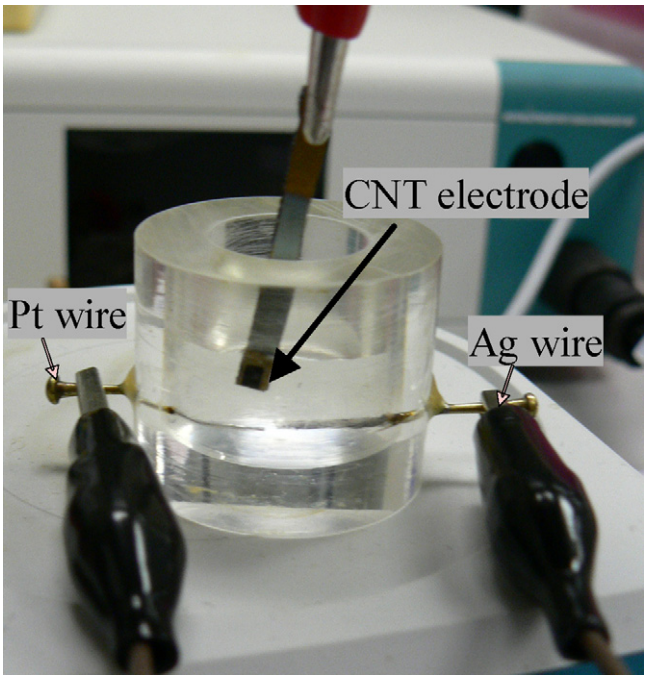

Fig. 1. Electrochemical cell consist of CNTs: working electrode, Pt: auxiliary electrode and Ag: reference electrode.

2.4. Fabrication of the miniaturized microfluidic system

The microfluidic chip was designed to have two microchannel inlets and one microchannel outlet. For two microchannel inlets, one is used for buffer carrier stream and the other is used for injection of analyte. The microchannel is 100 μm deep and 500 μm wide. For the fabrication of PDMS chip, the microfluidic channels have been fabricated using a standard photolithography technique (as shown in Fig. 2a). SU-8 photoresist was spin on Si substrate using spin coater and then soft baked to remove solvent in the layer. The

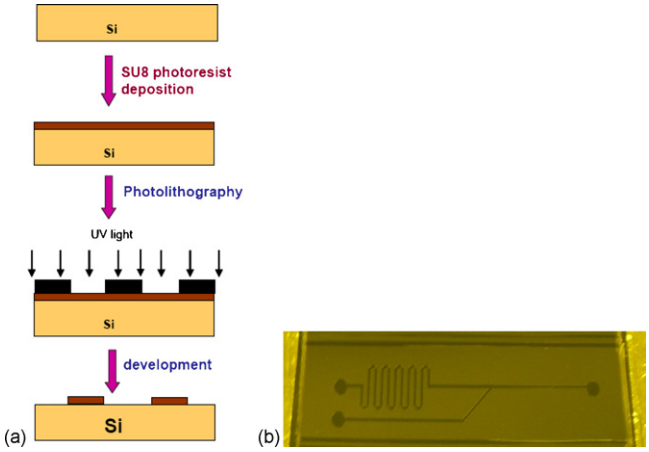

Fig. 2. (a) Fabrication process and (b) photograph of SU-8 mold.

UV-lithography was performed to obtain photoresist patterns on Si substrate using MJB4 mask aligner and then post-baked in order to selectively cross-link the exposed portion of photoresist. Finally, the photoresist was then developed and cleaned with deionized water and isopropyl alcohol. Typical photograph of SU-8 mold is shown in Fig. 2b.

The detection part was designed to be three electrode system, which consists of CNTs, Pt and Ag electrode layers on glass substrate. The three electrodes were fabricated opposite to the outlet of separation channel. Cr/Pt, Cr/Ag and Cr/Au/Stainless steel were patterned on glass substrate by sputtering through electroplated microshadow mask. 200 μm wide and 300 nm thick platinum auxiliary electrode and silver reference electrodes were achieved. To obtain the working electrode, CNTs were grown on Cr/Au/stainless layers by CVD technique as previously described in Section 2.3 but in this case glass substrate is used and CNTs were grown at a lower temperature of 600 °C. The PDMS microfluidic part containing microfluidic channel and glass substrate with three electrodes were bonded using oxygen plasma treatment.

2.5. Microfluidic procedure

Before to use, the channel was treated with deionized water for 10 min. The running buffer is 50 mM tris buffer (pH 8.3), prepared by dissolving the required amount of tris-(hydroxymethyl)aminomethane, then adjusted pH with 1.0 M HCl and added deionized water as required to complete the solution. The solutions were filtered through 0.2 μm cellulose acetate filter to prevent clogging. The electropherograms were recorded with time while applying a detection potential at +0.6 V versus Ag reference electrode. Sample injections were performed after stabilization of baseline buffer signal. The running buffer was delivered into the channel using a syringe pump at a flow rate of 40 μl/min. The analytes were also injected into the channel by propelling of syringe pump.

2.6. Sample preparation

Pharmaceutical products containing salbutamol including Asmasal and Ventolin syrup, and Ventolin tablet were purchased from drug stores. These syrup samples were diluted in 50 mM tris buffer pH 8.3 before analysis (1/40 dilution for Asmasal and Ventolin syrup). Ventolin tablet was ground and filtered through 0.2 μm cellulose acetate membrane, followed by suitably dilution before analysis.

3. Results and discussion

3.1. Cyclic voltammetry

In order to assess the sensitivity of CNTs electrode, the electrochemical characteristics of salbutamol was compare to bare-gold electrodes. It was observed that the CNT electrode exhibits much higher irreversible oxidation peak at ~0.7 V than bare-gold electrode does as shown in Fig. 3. Thus, CNTs significantly enhance the electrochemical activity with salbutamol due to its high reaction area and excellent electron transfer rate. The oxidaiton process of salbutamol can be attributed to the oxidation of the phenolic hydroxy group, which can be observed with $1H^+$ and 1e in irreverible reaction on CNT electrode. The oxidation reaction of salbutamol has been illustrated in previous work [24,29,30].

The effect of pH on sensitivity to 1 mM salbutamol solution was evaluated in order to select an optimum pH value. The cyclic voltammograms were obtained at different pHs of 4.0, 7.0, 8.3 and 10.0. It can be seen that the highest current response from cyclic voltammogram (Fig. 4) was obtained at pH 8.3. It is likely that

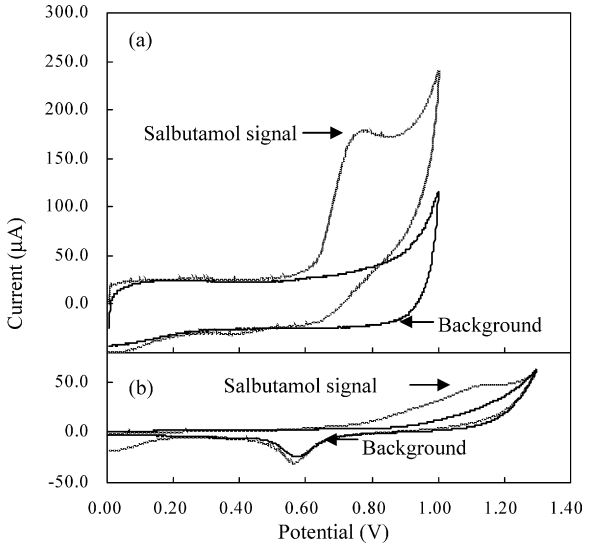

Fig. 3. The oxidation of 5 mM salbutamol on CNT (a) and gold (b) electrode. Scan rate was 100 mV s^{-1}. Buffer solution was 50 mM tris buffer pH 8.3.

salbutamol can be optimally dissociated in the buffer solution at this pH because this pH value is close to dissociation constant (pK_a) of salbutamol (~9.0) [31,32]. Therefore, the buffer pH 8.3 was selected as the optimal pH in all subsequent amperometric-microflow experiments.

3.2. PDMS/glass chip system

Fig. 5 shows the developed microfluidic device for analysis of salbutamol. The microfluidic channels were 100 μm deep with 500 μm wide. Syringe pump (P1) was used for propelling the run-

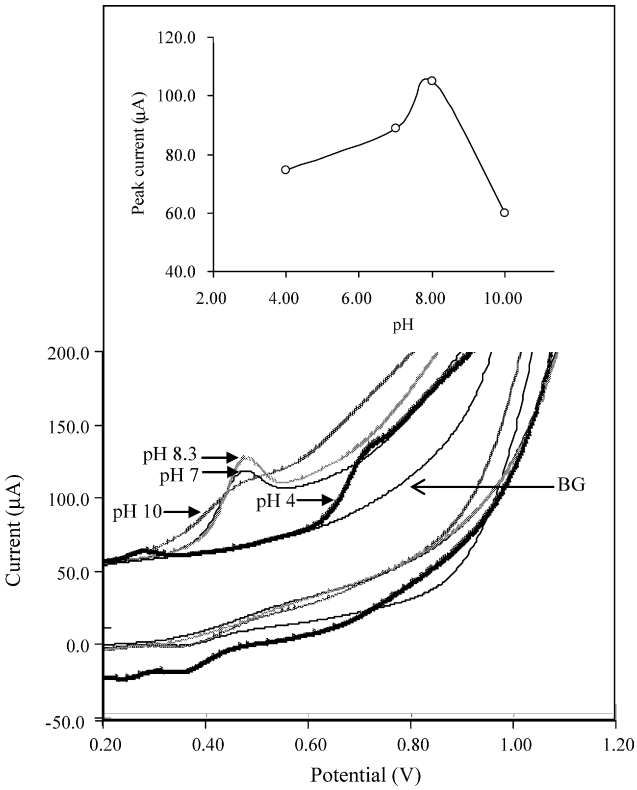

Fig. 4. Effect of pH buffer on 1 mM standard solutions of salbutamol at various pH of CNT based electrode.

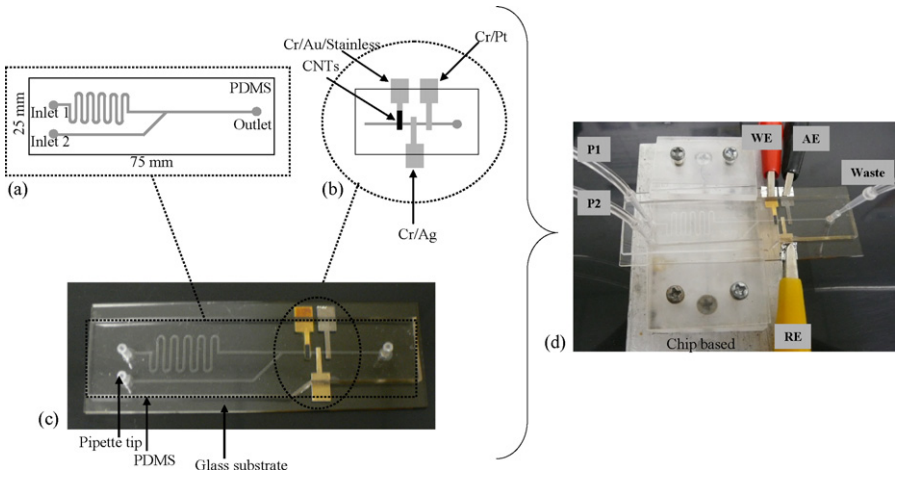

Fig. 5. Microchip and in-channel amperometric detector.

ning buffer through inlet microchannel, Inlet 1. Inlet microchannel, Inlet 2, was used for injection of analyte control by the second syringe pump (P2). Micropipette tips were cut for connection between reservoirs (Fig. 5c) and the micro-tubing (Fig. 5d). The developed system was used for all amperometric experiments.

3.3. Flow injection optimization

The detection potential strongly affects the sensitivity of current signal of the analyte. To obtain the optimal detection potential, the hydrodynamic voltammogram was determined. Hydrodynamic voltammetry were studied from injection of 15 μl of 1 mM standard salbutamol solution into the micro-flow system with varying detection potential from 0.3 to 0.7 V as shown in Fig. 6a. It can be noticed that working potential does not considerably influence the salbutamol signal. It is possible that it is characteristic of CNT electrode which is deposited at low temperature (600 °C) on glass substrate. It was observed that CV from the CNT electrode on glass chip is relatively board with small peak so the current signal is only weakly depending on potential in the oxidation region. To obtain the optimum detection potential, the S/B ratio [33,34], which is salbutamol signal divided by background current, was calculated from the result in Fig. 6a. Fig. 6b is shown the maximum S/B ratio at 0.6 V. Therefore, this potential was used for all micro-flow experiment in amperometric detection.

The effect of volume on the analytical performance of CNTs electrode was investigated over the 1–20 μl. Fig. 7a shows the current response and throughput versus volume of 1 mM standard salbutamol with fixed flow rate at 80 μl/min. It can be seen that as the injection volume increased, throughput decreased while current signal increased as shown in Fig. 7a. Thus, injection volume of 15 μl was chosen for acceptable sensitivity and throughputs (60 samples/h).

In order to achieve the satisfactory sensitivity and sample throughput, the effect of flow rate was optimized using injection of 15 μl of standard salbutamol solution. Fig. 7b shows that the sensitivity decreased with increasing flow rate from 20 to 120 μl/min. Conversely, the increasing flow rate increased sample throughput. To balance between sensitivity and sample throughputs, the flow rate of 40 μl/min was selected.

3.4. Microfluidic system with amperometric detection

Previous studies have shown that salbutamol can be adsorbed on glassy carbon [24], boron-doped diamond [29], gold [35] electrodes. In order to test the fouling or adsorption of the compound on CNTs electrode, the replicate injections of standard salbutamol of 0.5 mM have been evaluated in term of percent of relative standard deviation (%R.S.D.). The developed system gave an acceptable repeatability with R.S.D. of 7.80% ($n = 20$) so fouling was not occurred on the CNT electrode.

In order to obtain a calibration curve, the varied concentrations of standard salbutamol from 5.0 to 1000 μM were injected into the system for evaluation of the correlation coefficient. Fig. 8 shows the effect of concentration on current signal under different concentration from 5 to 1000 μM with three replicate injections.

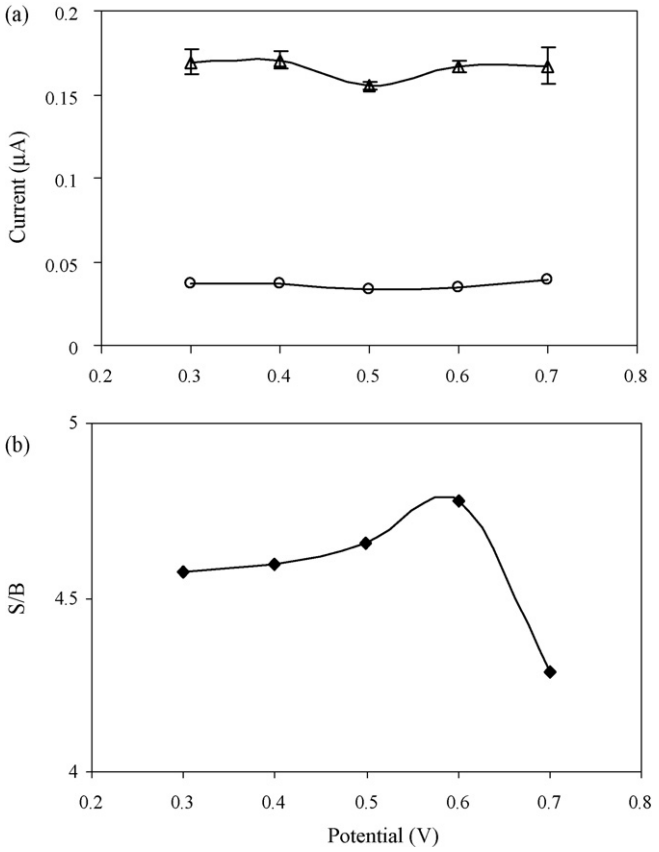

Fig. 6. Results obtained by microfluidic devices with amperomeric detector (Fig. 5d): (a) hydrodynamic signal of the carrier, 50 mM tris buffer or the background current and peak current from injections ($n = 3$) of 1 mM of salbutamol into the carrier stream and (b) plot of the signal-to-background ratio (S/B) and the applied potential.

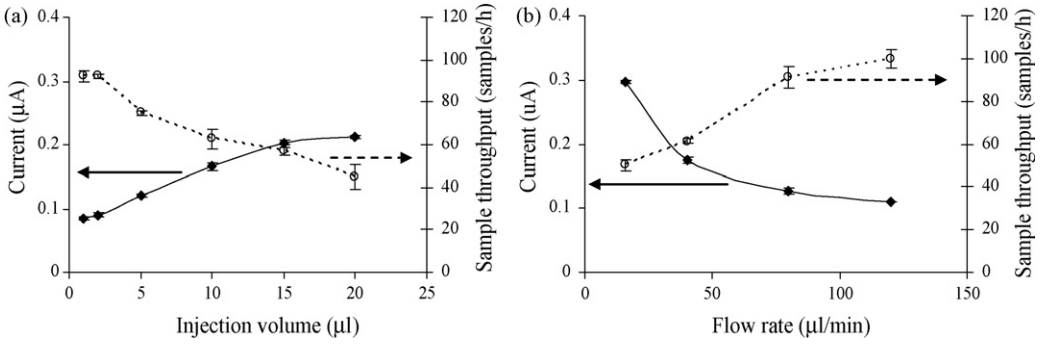

Fig. 7. Effect of injection volume (a) and flow rate (b) on current signal (μA) and sample throughput (samples/h).

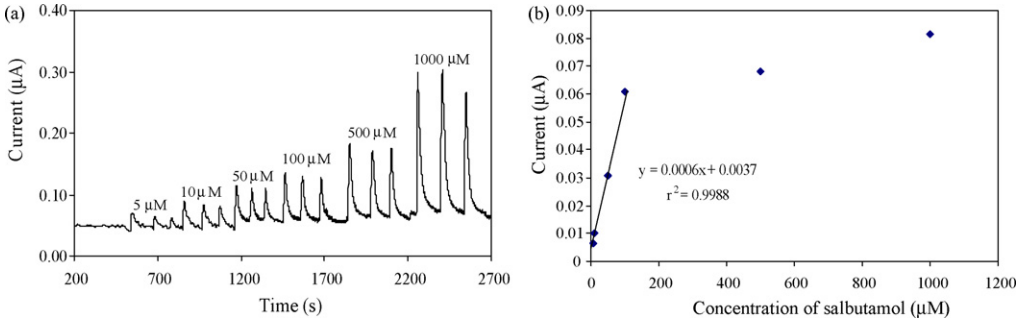

Fig. 8. Effect of concentration of standard salbutamol on current signal (a) and resulting calibration plot (b).

Table 1
Comparison between the labeled and analyzed value of salbutamol in asthma drugs.

Samples	Salbutamol content	
	Labeled value	Analyzed value
Asmasal syrup	400 mg/l	380 ± 5.1 mg/ml
Ventolin syrup	400 mg/l	375 ± 7.2 mg/ml
Ventolin tablet	2 mg/tablet	1.1 ± 0.1 mg/tablet

Linear concentration dependence was observed between 5 and 100 μM. The regression equation is given by $y = 0.0006x + 0.0037$ ($r^2 = 0.998$), 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 0.0006 μA/μM. The detection limit (3S/N) is as low as 1.0 μM, an order of magnitude below WADA's urine doping specification.

The developed microfluidic device (Fig. 5d) was tested for determination of asthma drugs, which contain salbutamol. Salbutamol contents in three asthma drugs analyzed by the microfluidic device were compared to the labeled values from their manufacturers as shown in Table 1. It can be seen that the analyzed results by the developed method for Asmasal and Ventolin syrub are not significantly different from the labeled values, which are presumably correct since they are provided by their manufacturers. Therefore, the salbutamol content in these syrup samples can be correctly directly analyzed by this method because other components in the syrup, which is surrounding matrices, do not cause interference to electrochemical measurement. In contrast, the analyzed value and labeled value are clearly different for Ventolin tablet. The incorrect analysis results for Ventolin tablet should be due to interference from other components in the tablet, therefore sample pretreatment or separation technique [36,37] should be used to separate salbutamol from other components before electrochemical analysis.

4. Conclusions

In conclusion, the in-channel amperometric microfluidic device has successfully been developed for salbutamol detection. The device demonstrated the first utilization of carbon nanotube electrode on glass based microfluidic ship for detection of salbutamol. The results show that the salbutamol can be effectively detected from some samples by the proposed method. The electrocatalytic activity of salbutamol on CNTs is good when compared with bare-gold electrode. Moreover, the CNTs electrode exhibits a good stability in flowing system and good reproducibility for amperometric detection.

References

[1] H. Becker, L.E. Locascio, Talanta 56 (2002) 267–287.
[2] D.R. Rwyes, D. Iossifidis, P.A. Auroux, A. Manz, Anal. Chem. 74 (2002) 2623–2636.
[3] Y. Liu, D. Ganser, A. Schneider, R. Liu, P. Grdzinski, N. Kroutchinina, Anal. Chem. 73 (2001) 4196–4201.
[4] S.M. Ford, B. Kar, S. McWhorter, J. Davies, S.A. Soper, M. Klopf, G. Calderon, V.J. Saile, Microcolumn 10 (1998) 413–422.
[5] J.C. McDonald, D.C. Duffy, J.R. Anderson, D.T. Chiu, H. Wu, O.J.A. Schueller, G.M. Whitesides, Electrophoresis 21 (2000) 27–40.
[6] M.J. Schoning, M. Jacobs, A. Muck, D.-T. Knobbe, J. Wang, M. Chatrathi, S. Spillmamm, Sens. Actuators B 108 (2005) 688–694.
[7] O. Yassine, P. Morin, O. Dispagne, L. Renaud, L. Denoroy, P. Kleimann, K. Faure, J.-L. Rocca, N. Ouaini, R. Ferrigno, Anal. Chim. Acta 609 (2008) 215–222.
[8] G.S. Virdi, R.K. Chutani, P.K. Rao, S. Kumar, Sens. Actuators B 128 (2008) 422–426.
[9] Micro Chem, Nano SU-8 2000, Negative Tone Photoresis Formulations, pp. 2035–2100.
[10] K.-W. Lin, Y.-K. Huang, H.-L. Su, Y.-Z. Hsieh, Anal. Chim. Acta 619 (2008) 115–121.
[11] M. Castano-Alvarez, M.T. Fernansez-Abedul, A. Costa-Garcia, J. Chromotogr. A 1109 (2006) 291–299.
[12] D.F. Pozo-Ayuso, M. Castano-Alvarez, A. Fernansez-la-Villa, M. Garcia-Granda, M.T. Fernandez-Abedul, A. Costa-Garcia, J. Rodriguez-Garcia, J. Chromotogr. A 1180 (2008) 193–202.
[13] K.-T. Liao, C.-M. Chen, H.-J. Huang, C.-H. Lina, J. Chromotogr. A 1165 (2007) 213–218.

[14] N. Dossi, R. Toniolo, A. Pizzariwllo, S. Susmel, F. Perennes, G. Bontempelli, J. Electroanal. Chem. 601 (2007) 1–7.
[15] S. Shahrokhian, H.R. Zare-Mehrjardi, Electrochim. Acta 52 (2007) 6310–6317.
[16] S. Roy, H. Vedala, W. Choi, Nanotechnology 17 (2006) S14–S18.
[17] J.-E. Huang, X. Hong, H.-L. Li, Carbon 41 (2003) 2731–2736.
[18] A. Wisitsoraat, A. Tuantranont, E. Comini, G. Sberveglieri, W. Wlodarski, IEEE Sensors 2007 Conference, 28–31 October, 2007, IEE Sensors, 2007, pp. 550–553.
[19] Y. Wanna, N. Srisukhumbowornchai, A. Tuantranont, A. Wisitsoraat, N. Thavaungkul, P. Singjai, J. Nanosci. Nanotechnol. 6 (2006) 3893–3896.
[20] A. Wisitsoraat, A. Tuantranont, C. Thanachayanont, V. Patthanasettakul, P. Singjai, J. Electroceram. 17 (2006) 45–49.
[21] S. Chaisitsak, J. Nukeaw, A. Tuantranont, Diamond Relat. Mater. 16 (2007) 1958–1966.
[22] J.E.F. Reynolds (Ed.), Martindale: The Extra Pharmacopoeia, 30th ed., The Pharmaceutical Press, London, 1993.
[23] M.-H. Spyridaki, P. Kiousi, A. Vonaparti, P. Valavani, V. Zonaras, M. Zahariou, E. Sianos, G. Tsoupras, C. Georgakopoulos, Anal. Chim. Acta 573 (2006) 242–249.
[24] R.N. Goyal, M. Oyama, S.P. Singh, J. Electroanal. Chem. 611 (2007) 140–148.
[25] http://www.wada-ama.org.
[26] A. Pichon, N. Venisse, E. Krupka, M.-C. Perault-Pochat, A. Denjean, Int. J. Sports Med. 27 (2006) 187–192.
[27] M.C. Dumasia, E. Houghton, J. Chromotogr. A 564 (1991) 503–513.
[28] M.S. Dresselhaus, G. Dresselhaus, P. Avouris, Carbon Nanotubes: Synthesis, Structure, Properties, and Applications, Springer, 2001.
[29] C. Karuwan, T. Mantim, P. Chaisuwan, P. Wilairat, K. Grudpan, P. Jittangprasert, Y. Einaga, O. Chailapakul, L. Suntornsuk, O. Anurukvorakun, D. Nacapricha, Sensors 6 (2006) 1837–1850.
[30] S. Griese, D.K. Kampouris, R.O. Kadara, C.E. Banks, Electrochim. Acta 53 (2008) 5885–5890.
[31] V.B. Sutariya, R.C. Mashru, M.G. Sankalia, J.M. Sankalia, ARS Pharm. 46 (2005) 337–352.
[32] D.J. Smith, USDA, ARS Biosciences Research Laboratory, Fargo, ND 58105.
[33] O. Chailapakul, M. Amatatongchai, P. Wilairat, K. Grudpan, D. Nacapricha, Talanta 64 (2004) 1253–1258.
[34] W. Siangproh, N. Wangfuengkanagul, O. Chailapakul, Anal. Chim. Acta 499 (2003) 183–189.
[35] M.S.M. Quintino, L. Anges, Talanta 62 (2004) 231–236.
[36] O. Anurukvorakun, W. Suntornsuk, L. Suntornsuk, J. Chromotogr. A 1134 (2006) 326–332.
[37] S. Sirichai, P. Khanatharana, Talanta 76 (2008) 1194–1198.