Available online at www.sciencedirect.com
SCIENCE DIRECT
Talanta 69 (2006) 1215–1220
Talanta
www.elsevier.com/locate/talanta

Micro flow sensor on a chip for the determination of terbutaline in human serum based on chemiluminescence and a molecularly imprinted polymer

Deyong He, Zhujun Zhang *, Houjiang Zhou, Ying Huang

Institute of Analytical Science, The Key Lab of Analytical Chemistry of Chongqing, Southwest Normal University, Beibei, Chongqing 400715, PR China

Received 4 September 2005; received in revised form 16 December 2005; accepted 17 December 2005
Available online 31 January 2006

Abstract

Based on a molecularly imprinted polymer (MIP) as the recognition element, a novel chemiluminescence (CL) micro flow sensor on a chip for the determination of terbutaline in human serum is described. The MIP was prepared by using terbutaline as the template, methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linking monomer, and acetonitrile as the solvent. The chip was fabricated from two 50 mm × 40 mm × 5 mm transparent poly (methylmethacrylate) (PMMA) slices. The microchannels on the chip etched by CO_2 laser were 200 μm wide and 150 μm deep. The microsensor cell filled with 2 mg MIP for selectively on line adsorbing terbutaline was 10 mm long, 1 mm wide, and 0.5 mm deep. All reagents were controlled by the syringe pump with an accurate timer. The on line adsorbed terbutaline by the MIP can enhance the CL intensity of the reaction of luminol with ferricyanide. The enhanced CL intensity is linear with terbutaline concentration from 8.0 to 100 ng/mL with a detection limit of 4.0 ng/mL (3σ). The micro flow sensor provides for good reproducibility with the relative standard deviation of 3.6% ($n = 7$) for 20 ng/mL terbutaline.

Keywords: Micro flow sensor; Molecularly imprinted polymer; Terbutaline; Chemiluminescence

1. Introduction

Terbutaline (Fig. 1), 1-(3,5-dihydroxyphenyl)-2-(tert-butylamino) ethanol, is a selective β_2-receptor agonist, which widely is used in the treatment of asthma and chronic obstructive pulmonary diseases. Terbutaline has been abused as a growth promoter in animals [1], and its use has been prohibited by the food and agricultural organizations of European Union. Due to the stimulation of the central nervous system and certain anabolic like effects obtained when higher doses of terbutaline are administered [2], the use of terbutaline by athletes was prohibited by the International Olympic Committee (IOC) [3]. Various methods have been developed to determine the presence of terbutaline, including colorimetry [4], spectrophotometry [5], electrophoresis [6–8], and HPLC [9,10]. However, these methods require expensive instruments [6–10] and complex sample pretreatment such as liquid–liquid extraction [11] and immunoaffinity chromatography [12], which greatly limits the applications of these methods in practice.

In recent years, the use of a microsensor on a chip, for its inherent advantages of portability, low reagents consumption and the reduction of analysis time, has received noticeable attention in a variety of applications. These applications include genomic analysis, environmental testing, and clinical applications [13–21]. As far as the above microsensors are concerned, the recognition elements are common cells, antibodies, enzymes, or receptors, which have excellent specificity and reversibility. However, these micorsensors also have the disadvantages of requiring the consumption of expensive biological recognition materials and suffering from poor stability.

Molecular-imprinting polymerization (MIP) is a technique of creating recognition sites for an analyte molecule in a synthetic polymeric substrate. MIP is synthesized by a polymerizing reaction of a mixed solution containing a functional monomer, a cross-linker and a template in a water bath [22]. After the removal of template molecules, the artificially generated recognition sites have their shapes, sizes and functionalities complementary to the analyte, and they are capable of rebinding the

* Corresponding author. Tel.: +86 23 6825 3863; fax: +86 23 6825 3863.
E-mail address: zzj18@hotmail.com (Z. Zhang).

0039-9140/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2005.12.043

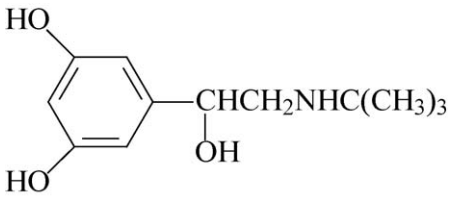

Fig. 1. Molecular structure of terbutaline.

analyte molecules in preference to other closely related structures [23]. MIP is stable and resistant to a wide range of pH, humidity, and temperature [24], so that a sensor modified with a MIP is easily stored and prepared and has a long lifetime [25]. Many sensors based on the MIP have been utilized. These include electrochemical sensing devices [26–28], optical sensors [29–31], the Bulk Acoustic Wave sensor [32], piezoelectric quartz sensor [33], Quartz-crystal-microbalance sensor [34], and surface plasmon resonance sensors [35,36]. The CL-MIP flow through sensor was firstly devised by Lin and Yamada [37] and was developed greatly as it can improve the selectivity of CL method [38–41].

To the best of our knowledge, the studies on synthesizing the MIP of terbutaline have not been reported. Some chemiluminescence systems, such as $KMnO_4$-luminol [42] and $K_3Fe(CN)_6$-rhodamine 6G [43] CL systems, have been proposed for the determination of terbutaline by our group. However, the $KMnO_4$ is a strong oxidizer that can destroy the structure of the MIP, and the sensitivity of the $K_3Fe(CN)_6$-rhodamine 6G CL system is poor.

In this work, a novel micro flow chemiluminescence sensor on a chip using MIP as the recognition element for the determination of terbutaline in human serum is described. All reagents are controlled by the syringe pump with an accurate timer. The precision of the timer is 0.01 s. The terbutaline-MIP is prepared by using terbutaline as the template, methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linking monomer, and acetonitrile as the solvent. The micro flow sensor cell is filled with the MIP to selectively adsorb terbutaline that can react with the mixed solution of ferricyanide and luminol at once and greatly enhance the CL intensity of the reaction of ferricyanide with luminol in $NaHCO_3$–Na_2CO_3 buffer solution. The distinguished possible advantages of this micro flow sensor are shown as following:

First, since it is very difficult to extract the analyte from the cavities of MIP, organic reagents and buffer solution are required as the eluent to extract the analyte, which makes the analysis procedure more complicated. So it is difficult to fabricate reversible recognition elements using MIP. Moreover, it is more difficult to fabricate a reusable micro flow sensor on a chip using the MIP. In this proposed microsensor, because the structure of the analyte can be destroyed through the CL reaction, the reacted analyte can be easily washed off from the MIP by using water as the eluent. The cavities can be released and retain their memory for the target analyte. So the proposed microsensor is reversible and reusable.

Second, the use of organic reagents and buffer solutions as the eluents, which can affect the CL reaction, is avoided. So the analysis procedure is simplified and the sensitivity of the CL reaction is improved. MIP can on-line enrich the analyte for the CL determination. The enrichment effect of the MIP can also improve the sensitivity of the CL reaction.

Third, all the reagents are controlled by the syringe pump with an accurate timer, of which the precision was 0.01 s. Therefore, the micro flow sensor provides for very reproducible measurements. The proposed system can control the reagents more simply and accurately and it can be applied to the complicated flow systems.

The proposed micro flow sensor is very sensitive for the terbutaline with a detection limit of 4 ng/mL and provides for good reproducibility with a relative standard deviation of 3.6% ($n = 7$) for 20 ng/mL terbutaline. A complete analysis can be finished within 6 min, including sampling, purge, and detection.

2. Experimental

2.1. Instrumentation

The microchannels of the microsensor were etched by CO_2 laser (Tian Fang Facility Co., Ltd., Luoyang China). All reagents were controlled by Ts2-60 syringe pump (Longer Precision Pump Co., Ltd., Baoding, China) through PTFE tubing (0.4 mm i.d.). The CL signal was detected and recorded with a BPCL ultra-weak luminescence analyzer (operated at −800 V) (Institute of Biophysics, Academia Sinica, China). All water was produced by Milli-Q water system (Millipore, Bedford, MA, USA). The UV absorbance was performed with the UV-2001 spectrophotometer (Hitachi Ltd., Japan).

2.2. Reagents

All the reagents were of analytical grade except for 2-2′ azobisisobutyronitrile (AIBN) and Milli-Q water was used for the preparation of solutions. Methacrylic acid and AIBN were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Ethylene glycol dimethacrylate was kindly provided by the Institute of Analytical Science of Shaan Xi Normal University (Xi'an, China). Before use, EGDMA and MAA were distilled.

A stock solution of terbutaline (1×10^{-4} g/mL) was prepared by dissolving 10 mg terbutaline (Institute of Pharmaceutical and Biomaterial Authentication of China) in 100 mL water and was stored in a refrigerator (277 K). Working standards were prepared daily from the stock solution by appropriate dilution. Luminol solution (1.0×10^{-2} mol/L) was prepared by dissolving 1.772 g luminol (Shaan Xi Normal University, China, >96%) in 1000 ml of 0.5 mol/L NaOH solution. A stock solution of potassium ferricyanide (1×10^{-3} mol/L) was prepared by dissolving 33 mg $K_3Fe(CN)_6$ (Chongqing Chemical Reagent Company, China) in 100 mL water. $NaHCO_3$ solution (0.01 mol/L) was prepared by dissolving 84 mg $NaHCO_3$ (Chongqing Chemical Reagent Company, China) in 100 mL water.

2.3. Preparation of molecularly imprinted polymer

MIP was prepared by using terbutaline as the target molecule. Four millimoles of terbutaline, 16 mmol of MAA and 80 mmol EGDMA were dissolved in 20 mL acetonitrile, and then mixed in an ultrasonic water bath for 1 h in a glass vial. Then, 2 mmol AIBN was added, and the solution was purged with nitrogen gas for 15 min and polymerized at 338 K for 24 h. Non-imprinted polymer (NIP) was prepared in the absence of template.

A portion of polymer particles as collected above was crushed in a mortar. Then the powder was wet sieved through a 280-mesh sieve. The polymer particles larger than 50 μm were ground again until the whole material passed through the sieve.

The binding characteristic for terbutaline was evaluated in comparison to the CL intensity when MIP and NIP was used as recognition elements, respectively. The results showed that terbutaline was selectively adsorbed on the MIP and greatly sensitized the CL reaction. Under the same condition, the CL intensity would still keep the blank signal without any increase because of non-special recognition to terbutaline. The binding capacity of MIP was performed by the UV absorbance change at 276 nm. The result showed that the maximum binding capacity of MIP was 1.094 mg/g for terbutaline.

2.4. Fabrication of the device on chip

The homemade micro flow sensor on chip (Fig. 2) was fabricated by two transparent poly(methylmethacrylate) (PMMA) substrates. The dimension of the channels was shown in Fig. 2. The micro flow channels etched by CO_2 laser were 200 μm wide and 150 μm deep except that the micro flow sensor cell was 10 mm long, 1 mm wide, and 0.5 mm deep.

The etched PMMA was selected as the base plate and a non-etched PMMA was selected as the top plate. Firstly, the micro cell was equipped with a small amount of glass wool at both ends. Secondly, 2 mg of the above-prepared MIP particles were packed into the micro cell carefully and were leveled off manually. Thirdly, the top plate was placed on the base plate and bonded at a pressure of 1.0–1.5 MPa at 353 K for 20 min, then cooled to room temperature whilst maintaining a pressure of 1.0–1.5 MPa. Its bottom except for the cell was masked by the black tape. Thus, only the light from the CL reaction in micro flow sensor cell could be detected by photomultiplier (PMT).

2.5. Procedures

As shown in Fig. 2, four syringes, each with a volume of 1 mL, were filled with luminol, $K_3Fe(CN)_3$, sample (terbutaline) solution and H_2O, respectively, and were fixed on the syringe pumps. The whole analysis includes five steps, namely, sampling, two cleaning procedures and two detecting procedures. Steps 2, 3, 4, and 5 form a circular procedure before next sample was injected and detected. A complete analysis could be finished within 6 min.

Step 1 P_2 was firstly started to wash the whole microchannels at rate of 20 μL/min. Then P_2, P_3 were shut off and P_1 was started to deliver luminol and $K_3Fe(CN)_6$ into the microsensor cell at the rate of 10 μL/min for 1.5 min. The obtained CL signal was used as the blank signal.

Step 2 P_1, P_3 were shut off and P_2 was started to clean the whole microsensor cell at the rate of 20 μL/min for 1 min to purge the remaining luminol and $K_3Fe(CN)_6$.

Step 3 P_1, P_2 were shut off and P_3 was started to syringe the sample into the microsensor cell at the rate of 6 μL/min for 2 min to on line adsorb terbutaline by the MIP.

Step 4 P_1, P_3 were shut off and P_2 was started to inject water into the micorsensor cell at the rate of 20 μL/min for 1 min to clean the non-specific adsorbed terbutaline on the surface of MIP.

Step 5 P_2, P_3 were shut off and P_1 was started to inject the CL reagents, luminol and $K_3Fe(CN)_6$ solution, into the microsensor cell at the rate of 10 μL/min for 1.5 min, monitoring the change of the CL intensity. The concentration of terbutaline was quantified by the relative CL intensity.

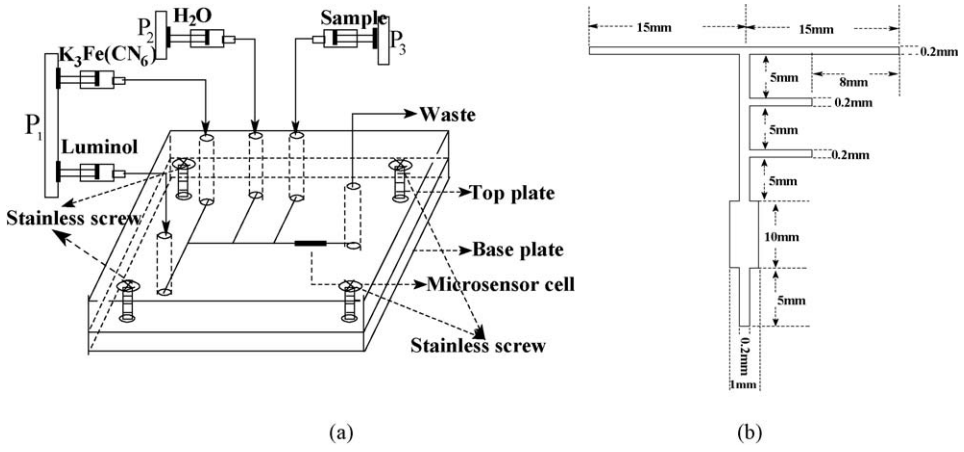

Fig. 2. (a) Schematic diagram of micro flow sensor on chip for determination of terbutaline (P_1, P_2, P_3: Ts2-60 syringe pump). (b) Schematic diagram of dimension of the microchannel.

3. Results and discussion

3.1. Conditions optimization of the CL reaction

In the previous experiment, it was found that terbutaline could greatly enhance the CL emission of luminol–ferricyanide system in basic medium. Before MIP was introduced into the CL system and the system was conducted on the sensor chip, as the present work, the optimal conditions for the determination of terbutaline were investigated in a flow-injection manner firstly.

It is well known that most CL reactions of luminol must be in an alkaline medium. For the studied CL system, optimum reaction media was tested at first with the concentration of luminol being 1×10^{-4} mol/L, ferricyanide being 5×10^{-5} mol/L and terbutaline being 40 ng/mL. The result shows that the strongest relative CL intensity was obtained in basic Na_2CO_3–$NaHCO_3$ buffer solution. And the optimum pH of Na_2CO_3–$NaHCO_3$ buffer solution for the system was found to be 10.4.

The effect of the concentration of luminol on the CL intensity was investigated in the range of 1×10^{-5} to 5×10^{-4} mol/L. It was found that the CL intensity increased with the increase of luminol concentration. For the consideration of good signal to noise ratio (S/N) and relative low baseline, 8×10^{-5} mol/L luminol was selected as the optimum concentration for the system.

The response of the CL system was also greatly affected by $K_3Fe(CN)_6$, the oxidant of the CL reaction. The influence of its concentration on the CL system was examined in the range of 1×10^{-5} to 2×10^{-4} mol/L. For a comprehensive consideration, 5×10^{-5} mol/L $K_3Fe(CN)_6$ was selected for subsequent research.

Consequently, all these factors influencing the response of the CL system on the chip system were further investigated and similar results were obtained. Therefore, these conditions were selected as optimum for the micro flow sensor chip system.

3.2. Effect of flow rate on the micro flow sensor chip

The flow rate is an important factor influencing the response of the system, including the relative CL intensity, sampling frequency and lifetime of the micro flow sensor. When the CL system was transferred onto the microchip, it also led to some new problems of the micro channels leakproofness, the stability and the reproducibility of the device. Because of the effect of the resistance of mass transfer, the backpressure would be generated in chip. If the flow rate was too high, the backpressure would become too high to make it difficult to transfer the reagent, what's more it could make the chip leak. However, if the flow rate was too low, the analysis time would increase. The effect of the flow rate on the chip system was therefore investigated carefully. The results showed that the excellent stability, reproducibility and good CL response can be achieved when the flow rate of luminol and $K_3Fe(CN)_6$ solution lines was fixed at the range of 5–20 μL/min.

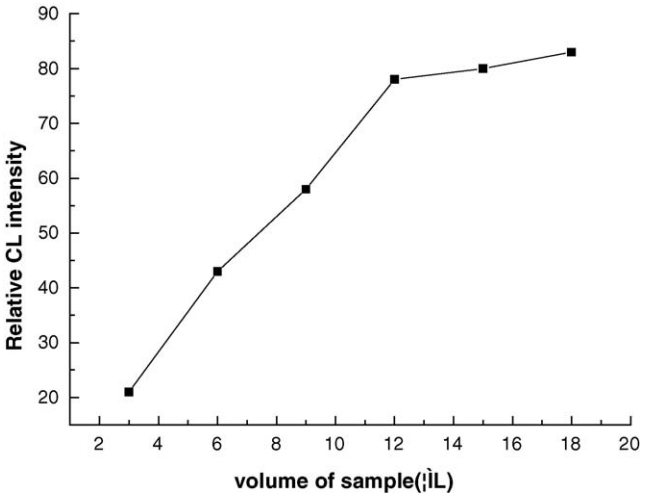

Fig. 3. The effect of volume of sample on CL intensity (flow rate: 6 μL/min; sample: 40 ng/mL).

3.3. Effect of sampling

The flow rate of sampling is important for controlling the content of adsorbed terbutaline with the MIP. If the flow rate was too high, the terbutaline could not be adsorbed enough by MIP. If the flow rate was too low, the analysis time could increase. The results showed that the optimal flow rate was 6 μL/min. The effect of the volume of terbutaline on CL intensity was studied with the sample flow rate fixed at 6 μL/min, as shown in Fig. 3. The CL peak intensity was the greatest when the volume of sample syringed into the microsensor cell was increased to 12 μL. This was probably because the MIP became saturated with adsorbed terbutaline at this point. More terbutaline syringed into the cell could not increase the CL intensity but increased the cleaning time. Therefore, the syringe time of the sample for subsequent work was selected as 2 min.

3.4. Effect of volume of CL reagents

The CL reagents, including luminol and ferricyanide, were syringed into the micorsensor cell together by the pump. The flow rate of the CL reagents was fixed to 10 μL/min and the effect of the volume of CL reagents on CL intensity was studied. It showed that the relative CL intensity and the blank signal both increase with increasing the volume of the CL reagents. The relative CL intensity reached the greatest when the volume of the CL reagents increased to 15 μL, as shown in Fig. 4. This was probably because terbutaline had reacted with the mixed CL reagent completely. More CL reagents syringed into the cell could not increase the CL intensity but increased the cleaning time. So the syringe time of CL reagents was selected as 1.5 min.

3.5. Effect of cleaning time

It was necessary to offer enough cleaning time (Step 2) to completely remove residues of CL reaction in the MIP to make the new binding sites for the next determination and obtain

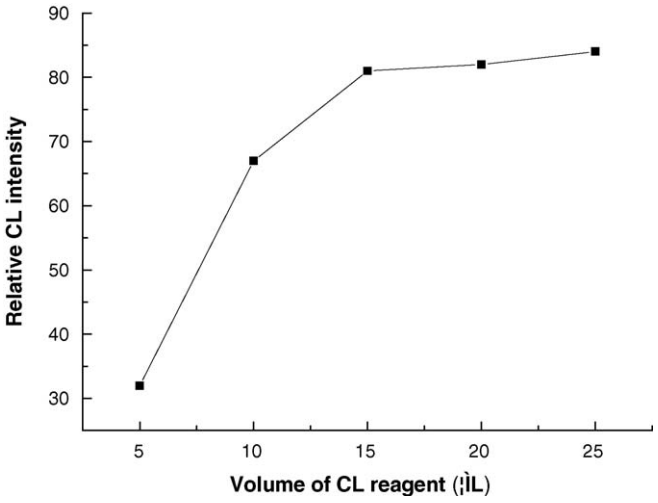

Fig. 4. The effect of the volume of CL reagent on CL intensity (flow rate: 10 μL/min; sample: 40 ng/mL).

good repeatability. The cleaning time was investigated from 0 to 2 min.Optimal results showed that 1 min was enough to renew the micro flow sensor and obtain a good repeatability when the flow velocity was fixed at 20 μL/min.

The cleaning time in the Step 4 was also critical parameter for the selective detection of adsorbed terbutaline. The washing time was studied in the range between 0 and 2 min, when the flow rate of water was fixed at 20 μL/min. The results showed that when the washing time was 1 min, the possible substances residue except adsorbed terbutaline could be completely removed. So the optimal time was 1 min for the following experiments.

3.6. The lifetime of the micro flow sensor

The lifetime of micro flow sensor was evaluated by comparing the CL intensity of the same terbutaline concentration in different times. The result showed that the microsensor could be used more than 100 times in one month. Then the CL intensity began to decrease. The possible reason was that the shrinkage of MIP destroyed some binding sites and resulted in the decrease of binding capacity for MIP used in water solution. However, the micro flow sensor was easy to prepare.

3.7. Selectivity of the microsensor

Under the optimized conditions, the interference of some foreign species normally existed in human serum was investigated by analyzing a standard solution of 2.0×10^{-8} g/mL terbutaline. The tolerable limit of interference substances was taken as relative standard deviation not higher than 5%. At the same time, the interference of these species to terbutaline under the same conditions without MIP was also carried out. The result (Table 1) showed that these substances in human serum in the normal concentration range did not interfere with the determination of terbutaline. Orciprenaline, clenbuterol, and salbutamol have the similar structure with terbutaline. These materials can also occupy some binding sites. Clenbuterol had no effect to CL reaction. Orciprenaline and salbutamol could be sensitized by the CL reaction, but the relative CL intensity was lower than terbutaline. So the similar structure Orciprenaline, clenbuterol, and salbutamol had no interference for the determination of terbutaline. Other substances existing in blood such as anion and cation in the normal level did not interfere with the terbutaline analysis either. Therefore, the microsensor provided for good selectivity and could be used in the complicated sample analysis.

Table 1
The tolerable ratios for some interfering substances in human serum

Interference substances	Without MIP	Micro flow sensor
Ascorbic acid	0.1	500
Uric acid	0.05	500
Glucose	100	1000
Carnine	5	1000
Lactate	50	1000
Vitamin B_1, B_2, B_6	1	1000
Clenbuterol	200	500
Salbutamol	10	200
Orciprenaline	5	50

3.8. Response of the microsensor

Under the optimum conditions described in the on-chip analysis, the CL response was linearly related to the concentration of terbutaline in the range of 8.0–100 ng/mL ($\Delta I = 2.258C - 5.586$, $R^2 = 0.9976$; C: concentration of terbutaline(ng/mL), ΔI: the relative CL intensity) with a detection limit of 4 ng/mL (3σ). At a concentration higher than 100 ng/mL, the CL intensity still increased but it increased slowly and deviated from the calibration curve. The proposed method had good reproducibility with the relative standard deviation 3.6% ($n = 7$) for 20 ng/mL of terbutaline (Fig. 5).

3.9. Applications of the microsensor

The human serum samples were obtained from local hospital. 500 μL of the serum was added to the ultrafiltration tube and centrifuged at 4000 rpm for 30 min. The filtrate was direct

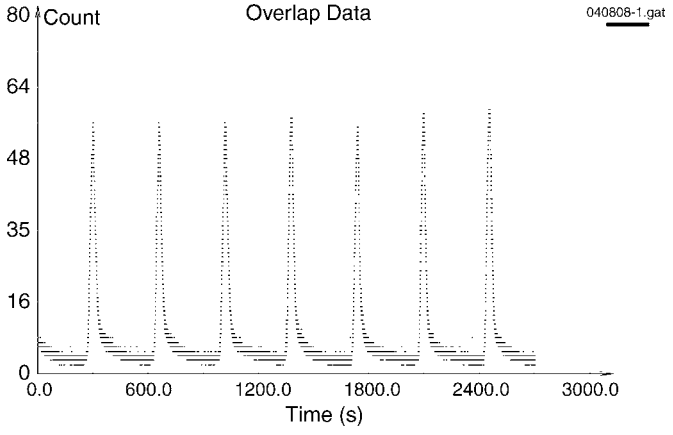

Fig. 5. Typical recording of the microsensor response to terbutaline standard (terbutaline: 20 ng/mL, R.S.D.: 3.6%).

Table 2
The result of determination of terbutaline in human serum

Sample	Amount found (ng/mL)[a] ± S.D.	Added (ng/mL)	Found (ng/mL)	Recovery (%)
Serum 1	13.4 ± 0.52	20.0	19.6	98
Serum 2	23.9 ± 0.92	20.0	21.1	106
Serum 3	47 ± 1.12	40.0	43.3	108

[a] Average of three results.

transferred into the syringe and then used for terbutaline analysis. At the same time, the standard addition tests were taken. The results were given in Table 2.

4. Conclusion

The described micro flow sensor combined with the MIP has important advantages. These include improved selectivity and sensitivity without the requirement of additional organic eluent to wash off the adsorbed terbutaline, simpler and more accurate control of the reagents, and the ability to apply to complicated flow systems. This proposed microsensor has been used successfully to determine the presence of terbutaline in human serum. The proposed microsensor is very sensitive and can be used repeatedly. It can realize integer miniaturization easily.

Acknowledgements

This study was supported by a grant from the Ministry of Science and Technology of the People's Republic of China (2003BA310A05).

References

[1] R. Ventura, L. Damasceno, M. Farre, J. Cardoso, J. Segura, Anal. Chim. Acta 418 (2000) 79.
[2] L. Martineau, M.A. Horan, N.J. Rothwell, R.A. Little, Clin. Sci. 8 (1992) 615.
[3] International Olympic Committee, Prohibited classes of substances and prohibited methods, in: IOC Medical Code and Explanatory Document, IOC, Lausanne, Switzerland, 1995.
[4] S. Tanabe, K. Kawanabe, Chem. Pharm. Bull. 37 (1989) 3131.
[5] S. Agatonovic-Kustrin, R. Alany, Anal. Chim. Acta 449 (2001) 157.
[6] A.M. Stalcup, K.H. Gahm, S.R. Gratz, R.M.C. Sutton, Anal. Chem. 70 (1998) 144.
[7] S.R. Gratz, A.M. Stalcup, Anal. Chem. 70 (1998) 5166.
[8] M. Roig, R. Berges, R. Ventura, K.D. Fitch, A.R. Morton, J. Segura, J. Chromatogr. B 768 (2002) 315.
[9] H.K. Kyeong, J.K. Hyun, K. Jeong-Hwan, D.S. Sang, J. Chromatogr. B 751 (2001) 69.
[10] Y. Zhang, Z.R. Zhang, J. Chromatogr. B 805 (2004) 211.
[11] A. Polettini, M.C. Ricossa, A. Groppi, M. Montagna, J. Chromatogr. 564 (1991) 529.
[12] G.V. Vyncht, P. Gaspar, E. Depauw, G. Maghuin-Rogister, J. Chromatogr. A 683 (1994) 67.
[13] D.C. Duffy, H.L. Gillis, J. Lin, Anal. Chem. 71 (1999) 4669.
[14] E. Eteshola, D. Leckband, Sens. Actuators B 72 (2001) 129.
[15] K. Hayashi, Y. Iwasak, R. Kurita, K. Sunagawa, O. Niwa, Electrochem. Commun. 5 (2003) 1037.
[16] A. Yamaguchi, P. Jin, H. Tsuchiyama, T. Masuda, K. Sun, S. Matsuo, H. Misawa, Anal. Chim. Acta 468 (2002) 143.
[17] H. Tanli, K. Maehana, T. kamidate, Anal. Chem. 76 (2004) 6693.
[18] R. Davidsson, F. Genin, M. Bengtsson, T. Laurell, J. Emneus, Lab on A Chip 4 (2004) 481.
[19] S. Kwakye, A. Baeumner, Anal. Bioanal. Chem. 376 (2003) 1062.
[20] M. Pumera, Talanta 66 (2005) 1048.
[21] H.L. Lee, S.C. Chen, Talanta 64 (2004) 750.
[22] A. Blomgren, C. Berggren, A. Holmerg, F. Larsson, B. Sellergren, K. Ensing, J. Chromatogr. A 975 (2002) 157.
[23] K. Mosbach, Trends Biochem. Sci. 19 (1994) 9.
[24] D. Kriz, O. Ramstrom, K. Mosbach, Anal. Chem. 69 (1997) 345.
[25] R. suede, T. Srichana, C. Sangpagai, C. Tunthana, P. Vanichapichat, Anal. Chim. Acta 504 (2004) 89.
[26] S. Kroger, A.P.F. Turner, K. Mosbach, K. Haupt, Anal. Chem. 71 (1999) 3698.
[27] T.L. Panasyuk, V.M. Mirsky, S.A. Piletsky, O.S. Wolfbeis, Anal. Chem. 71 (1999) 4069.
[28] H.H. Weetall, K.R. Rogers, Talanta 62 (2004) 329.
[29] A.J. Tong, H. Dong, L.D. Li, Anal. Chim. Acta 466 (2002) 31.
[30] B. Wandelt, P. Turkewitsch, S. Wysocki, G.D. Darling, Polymer 43 (2002) 2777.
[31] Y.C. Chen, J.J. Brazier, M.D. Yan, P.R. Bargo, S.A. Prahl, Sens. Actuators B 102 (2004) 107.
[32] Y.G. Tan, Z.L. Zhou, P. Wang, L.H. Nie, S.Z. Yao, Talanta 55 (2001) 337.
[33] B.S. Ebarvia, C.A. Binag, F. Sevilla, Anal. Bioanal. Chem. 378 (2004) 1331.
[34] T.Y. Lin, C.H. Hu, T.C. Chou, Biosens. Bioelectron. 20 (2004) 75.
[35] M. Lotierzo, O.Y.F. Henry, S. Piletsky, I. Tothill, D. Cullen, M. Kania, B. Hock, A.P.F. Turner, Biosens. Bioelectron. 20 (2004) 145.
[36] A. Kugimiya, T. Takeuchi, Biosens. Bioelectron. 16 (2001) 1059.
[37] J.M. Lin, M. Yamada, Anal. Chem. 72 (2000) 1148.
[38] J.M. Lin, M. Yamada, Analyst 126 (2001) 810.
[39] H.J. Zhou, Z.J. Zhang, D.Y. He, Y.F. Hu, Anal. Chim. Acta 523 (2004) 237.
[40] F. Nie, J.R. Lu, W.F. Niu, Anal. Chim. Acta 545 (2005) 129.
[41] F. Nie, J.R. Lu, Y.H. He, J.X. Du, Talanta 66 (2005) 728.
[42] Z.P. Wang, Z.J. Zhang, Z.F. Fu, X. Zhang, Anal. Bioanal. Chem. 378 (2004) 834.
[43] Y. Lv, Z.J. Zhang, Y.F. Hu, D.Y. He, S.H. He, J. Pharm. Biomed. Anal. 32 (2003) 555.