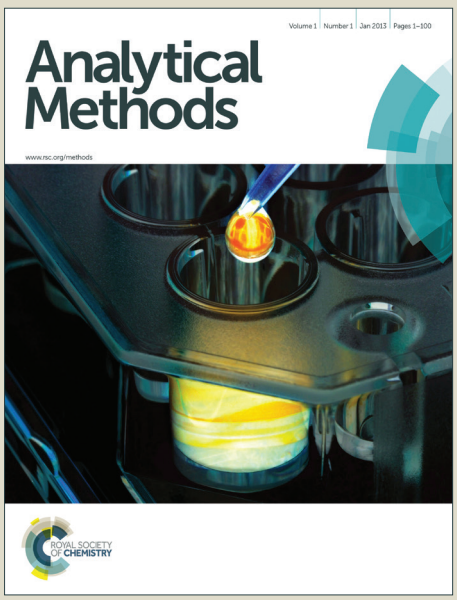
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Design of highly sensitive fluorescence sensor and its application based on inhibiting $NaIO_4$ oxidizing rhodamine 6G

Jia-Ming Liu [a,*], Qitong Huang [b], Pei-Yuan Cai[a], Chang-Qing Lin [b], Li-Hong Zhang [b], Zhi-Yong Zheng [b]

[a] College of Chemistry and Environmental, Minnan Normal University, Zhangzhou, 363000, P.R. China

[b] Department of Food and Biological Engineering, Zhangzhou Institute of Technology, Zhangzhou, 363000, P.R. China

Abstract A novel fluorescence sensor has been designed for determination of terbutaline sulfate (TBS). It was validated by determining selectivity, linearity, accuracy and precision for analysis. And all the experiments presented in this work were based on the inhibition effect on $NaIO_4$ oxidizing rhodamine 6G (Rh 6G), which led to severe enhancing of fluorescence signal. Limit of detection (LOD: 5.8×10^{-12} g mL^{-1}) of this sensor was evaluated and compared with other methods, indicating better sensitivity for TBS determination using this technique. And it has been applied successfully to determine TBS in human serum and urine samples due to the high sensitivity of the sensor. The linear range was from 0.026 to 5.2 ng mL^{-1}, allowing wide determined range of TBS. Meanwhile, the mechanism of this sensor was also discussed.

Keywords Terbutaline sulfate, Rhodamine 6G, Fluorescence sensor

1.Introduction

Up to now, a new analysis method and instruments of research for drugs are facing new challenges [1-2]. TBS is a synthetic β2-adrenoceptor widely used in the treatment of bronchial asthma, chronic bronchitis and emphysema [3-4]. However, the massive-dose or long-term use of TBS would cause tremor, tachycardia, gastrointestinal disturbances and sometimes fatal

* Corresponding author: Tel.:+86 596 2591352.
E-mail address: zzsyliujiaming@163.com (J.-M. Liu).

consequences [5]. Therefore, the detection of trace TBS is important for clinical diagnosis and treatment.

Though many analytical methods, such as capillary electrophoresis with chemiluminescence detection (LOD is 8.2×10^{-9} g mL^{-1})[3], enhanced chemiluminescence method (LOD is 1.0×10^{-8} g mL^{-1}) [4], voltammetric sensor (LOD is 3.3×10^{-9} g mL^{-1})[5], electrochemical voltammetric method (LOD is 9.3×10^{-8} g mL^{-1}) [6], adsorptive stripping voltammetry (LOD is 3.1×10^{-9} g mL^{-1}) [7], chemiluminescence method (LOD is 6.7×10^{-9} g mL^{-1}) [8], chemiluminescence method (CL, LOD is 1.7×10^{-10} g mL^{-1}) [9], spectrophotometric and stability-indicating HPLC (LOD is 2.0×10^{-7} g mL^{-1}) [10], microemulsion high performance liquid chromatography method (LOD is 8.0×10^{-9} g mL^{-1}) [11], column-switching liquid chromatography (LOD is 1.5×10^{-9} g mL^{-1}) [12], high performance thin layer chromatography method (7.1×10^{-6} g mL^{-1}) [13]; 4.7×10^{-6} g mL^{-1} [14]), micro flow sensor (LOD **is** 4.0×10^{-9} g mL^{-1}) [15], HPLC–MS (LOD is 5.0×10^{-10} g mL^{-1}) [16] have been developed for the determination of TBS in tablets and human urine samples. The application of these methods is limited, because, for example, the sensitivity is low and the HPLC apparatus is expensive. So researches on the methods for determination of trace TBS with high sensitivity are of high academic value and practical meaning.

In order to determine the ultra-trace TBS, we designed a fluorescence sensor of Rhod.6G-$NaIO_4$**,** which could emit a strong fluorescence signal. The sensitivity of this sensor (LOD: 5.8×10^{-12} g mL^{-1}) was 29.3 times higher than that (LOD: 1.7×10^{-10} g mL^{-1}) of Ref. [9], and to our knowledge, fluorescence sensor for the determination of trace TBS in human serum samples has not been reported with its inhibition effect on the $NaIO_4$ oxidizing Rh 6G. This rapid, accurate, selective and repeatable sensor has been applied to TBS detection in practical samples; showing better application prospects. The research provided a new technique for the TBS detection, which promoted the progress in study of trace drugs analysis.

2 Experimental

2.1 Apparatus and reagents

Phosphorescent measurements were carried out on a Perkin-Elmer LS-55 luminescence spectrometer (Perkin Elmer, USA). The instrument's main parameters are as following: Ex slit:

15.0 nm, Em slit: 15.0 nm; scan speed: 1500 nm min^{-1}. pHS-3B precision acidometer and AE240 electron analytical balance (Mettler Toledo instruments company) were used to introduce solution.

TBS (China pharmaceutical biology preparation testing centre) working solutions: 0.10 μg mL^{-1} TBS stock solution was diluted to 0.10, 1.00, 10.00 ng mL^{-1}; 1.0×10^{-4} mol L^{-1} Rh 6G solution; 0.50 % (W/V) $NaIO_4$ solution; $KHC_8H_4O_4$-HCl buffer solution (pH = 3.50). All the reagents are A.R. grade except that TBS is primary standard regent. Doubly-distilled water (18.2 MΩ) was used throughout.

2.2 Experimental methods

Certain amount of TBS working solution, 1.50 mL Rh 6G, 2.00 mL buffer solution and 1.50 mL $NaIO_4$ were added to a 25–ml colorimetric tube, and the mixture was vortexed thoroughly and incubated at 70 ℃ for 10 min, and then cooled by flowing water for 5 min. At the same time, a reagent blank was prepared. The fluorescence intensity of test solution (F) and reagent blank (F_0) are directly measured at $\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 528/554nm. Then ΔF ($=F-F_0$) was calculated.

3 Results and discussion

3.1 Fluorescence spectra

The fluorescence spectra of Rh 6G-$NaIO_4$-buffer solution-TBS system were scanned by experimental method (Fig. 1). Results show that after heated at 70 ℃ for 10 min, Rh 6G could emit strong and stable fluorescence ($\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 529.1/556.3 nm, F = 721.0). $NaIO_4$ can oxidize Rh 6G to quench the fluorescence signal ($\lambda_{ex}^{max}/\lambda_{em}^{max}$ =528.0/552.4 nm, F = 380.5). TBS can inhibit $NaIO_4$ from oxidizing Rh 6G, which results in the sharp enhancement of the fluorescence signal of Rh 6G ($\lambda_{ex}^{max}/\lambda_{em}^{max}$ = 527.7/554.4 nm, F = 512.3), ΔF = 131.8, $\lambda_{ex}^{max}/\lambda_{em}^{max}$ remained unchanged. So 528/554 nm was chosen as the working wavelength.

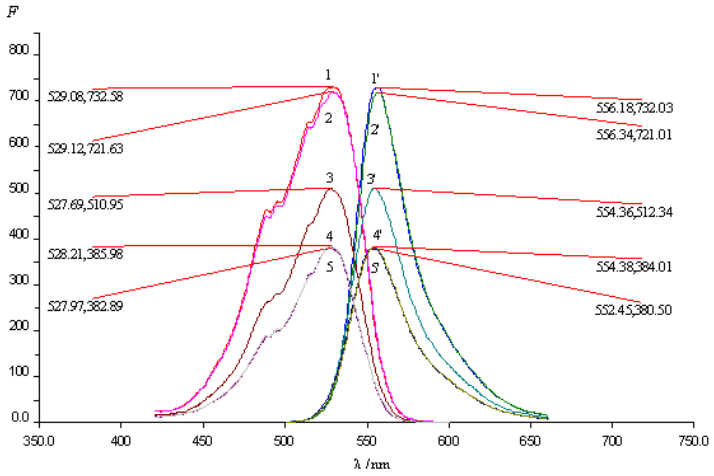


Fig.1 Fluorescence spectra of the Rh 6G-$NaIO_4$-buffer solution-TBS system

1.1' 1.50 mLRhod.6G 2.2' 1.1' + 2.00mL buffer solution

3.3' 5.5' + 130.00 ng TBS 4.4' 5.5' + 0.065 ng TBS

5.5' 2.2' + 1.50mL $NaIO_4$

3.2 Optimum measurement condition

For the system containing 1.30 ng TBS mL^{-1}, the effects of the concentration and dosage of reagent, oxidant, the pH of the solution, reaction temperature and time on the ΔF of the system were investigated in a univariate approach.

3.2.1 Concentration and dosage of reagents

The effects of concentration or dosage on the ΔF of the system were studied (Table **S**1), respectively. Results show that the ΔI_p of the system increased with the increasing concentration and dosage of Rhod.6G, $NaIO_4$ and buffer solution (pH = 3.5). And the ΔF of the system reached the maximum and remained stable when 1.50 mL of 1.0×10^{-4} mol L^{-1} Rhod.6G, 1.50 mL of 0.50% $NaIO_4$ and 2.00 mL of buffer solution were used, and the pH of the system was in the range of 3.50-5.23. The reason might be that the inhibiting ability of TBS to $NaIO_4$ oxidizing Rhod.6G reached the maximum.

3.2.2 Selecting luminescence substrate

The effects of 0.50 mL of 1.0×10^{-4} mol L^{-1} eosin Y (A), acriflavine (B), Rh 6G (C), calcein

(D) and orange yellow G (E) the ΔF were studied (Fig. **S1**), respectively. Results show that when Rh 6G was chosen, the ΔF of the system reached the maximum and remained stable. The reason for the experiment phenomena may be that the quantity yield of Rhod.6G higher than A, B, D and E， which lead to more luminescent molecular for each spot.

3.2.3 Selecting oxidant

The effects of 1.50 mL of 1.00 % (W/V) H_2O_2 (A), KIO_3 (B), $(NH_4)_2S_2O_8$ (C), $NaIO_4$ (D), $KClO_3$ (E) and $KBrO_3$ (F) were studied (Fig. **S2**), respectively. Results show that when $NaIO_4$ was chosen, the ΔF of the system reached the maximum and remained stable. Compared with H_2O_2, KIO_3, $(NH_4)_2S_2O_8$, $KClO_3$ and $KBrO_3$, $NaIO_4$ had the greatest effect on the ΔF of the system due to the strongest oxidability of $NaIO_4$.

3.2.4 Temperature and time for reaction

The effects of reaction time and temperature the ΔF were examined (Fig. **S3-4**), respectively. The ΔF of the system gradually increase with the increasing of time and temperature which might result from increasing of the inhibiting ability of TBS gradually. When the reaction temperature and time were 70 °C and 10 min, respectively, the ΔF of the system reached the maximum, which might be that the inhibiting ability of TBS reached the peak. Henceforth, with the continuous increase of the reaction temperature and time, the ΔF of the system decreased due to the decreasing of inhibiting ability of TBS gradually.

3.2.5 Acidity for reaction

The effects of pH values（3.01, 3.50, 5.23, 11.50 and 12.05）on the ΔF of the system were examined (Fig. **S5**). The ΔF of system increased with the increasing of pH value gradually, and reached the maximum and kept stable in the range of 3.50-5.23, which might be that the inhibiting ability of TBS reached the maximum, while the ΔF of the system decreased due to the decreasing of inhibiting ability of TBS gradually when pH >5.32.

3.2.6 Stability of the reaction system

The stability of fluorescence was the key to determine trace TBS by inhibiting fluorescence method of TBS to $NaIO_4$ oxidizing Rhod.6G. The stability of the reaction system was studied

under the optimum conditions above (Fig. **S6**). Results show that the ΔF of the system remained almost unchanged among 10-30 min after being cooled by flowing water for 5 min.. It indicated that the system has good repeatability. Moreover, when the standing time was over 30 min the ΔF of the system declined gradually due to the decomposition of TBS.

3.3 Working curve, linear range and detection limit

Under the optimum conditions, the concentration of TBS was linear with ΔF in the range of 0.026—5.2 ng mL $^{-1}$, the working curve and its linear regression equation were ΔF = 3.160 + 24.95C $_{TBS}$ (ng mL^{-1}), correlation coefficients (r) was 0.9994, (n = 6) (Fig. 2). The systems containing 0.026 ng mL^{-1} and 5.2 ng mL^{-1} were measured for 8 times, their RSDs (%) were 2.6 % and 1.5 % respectively, showing good precision. The blank reagent was determined for 11 times, standard deviation (Sb) was 0.050, calculated by 3 Sb /k (3 Sb referred to the quotient between triple of the blank reagent's standard deviation and the slope of the working curve), the LOD was 5.8 ×10^{-12} g mL^{-1}, showing the higher sensitivity than that of CL in Ref. [9]. This was attributed to effect of TBS from $NaIO_4$ oxidizing Rh 6G, which makes the fluorescence signal of Rh 6G enhance sharply.

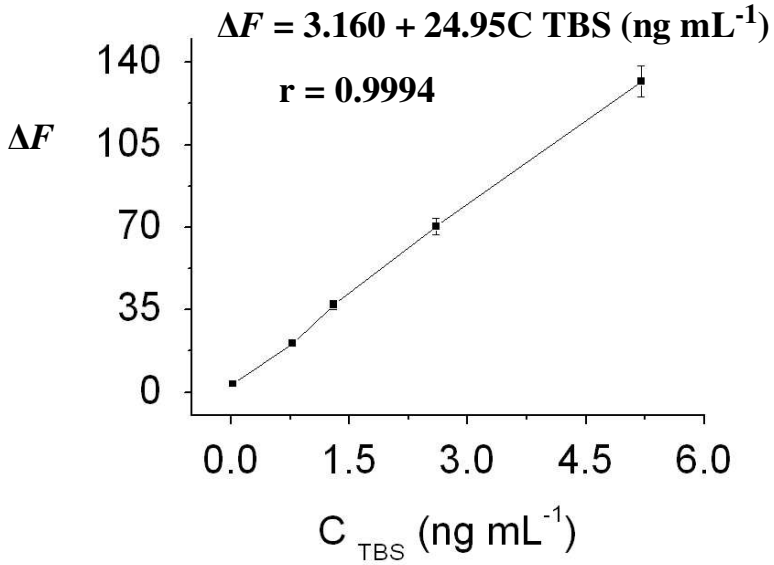


Fig. 2 Working curves of TBS detection with error bars and its the linear regression equation (n = 6, detection conditions: 1.50 mL of 1.0×10^{-4} mol L^{-1} Rhod.6G, 1.50 mL of 0.50% $NaIO_4$ and 2.00 mL of buffer solution at 70 °C for 20 min with pH 3.50)

3.4 Interference Experiment

For the system of 1.30 ng TBS mL^{-1}, the allowed concentration (μg mL^{-1}) of coexistent ions or coexistent materials (Relative error (Er) is ±5 %) are as following: NO_3^-, K^+, Na^+, Ca^{2+}, HCO_3^-, CO_3^{2-}, glucose, galactose, lactose, starch and fructose (130); Zn^{2+}, Al^{3+}, Mg^{2+}. Cl^-, Br^-, PO_4^{3-}, SO_4^{2-}, Fe^{2+}, Fe^{3+} and dextrine (26); vitamine C, citric acid, sucrose, nicotinamide, pantothenic acid and tartaric acid (0.65); glutamate, proline, serine, leucine, glycine, lysine, tryptophan, tyrosine and arginine (0.13); cimaterol, clenbuterol, penbutolol, rackdopamine and salmeterol (0.065), indicating good selectivity of this fluorescence sensor, which might be the result of the high selectivity of the inhibiting reaction.

3.5 Analysis of samples

An asthmatic orally took one TBS tablet (total: 40 μg TBS), after 24 hours, 20.00 mL of human serum and 1.00 mL of urine were obtained, respectively. 10.00 mL of 0.050 mol L^{-1} Tris-HCl was added to human serum, mixed homogeneously for 10 min. After centrifugalization, the supernatant was deserted. Thereafter, 20.00 mL buffer solution was added and mixed homogeneously for 10 min again and stored at 4 ℃ before use. After that, the adsorbent Sepharose 4B was added, and supernatant was deserted after centrifugalizating. 20.00 mL Tris-HCl was added again and shaken in an oscillator (300 r min^{-1}) for 20 min. After centrifugalization (200 r min^{-1}) for 5 min, supernatant was obtained for use. The human urine was dealt according to the method described in Ref. [17], the test solution was obtained for use. The content of TBS in test solution was determined according to the experimental method, and a standard addition recovery experiment was also conducted. The results were compared with CL [9].and are listed in Table 2.

Table 2 Analytical results of TBS in samples

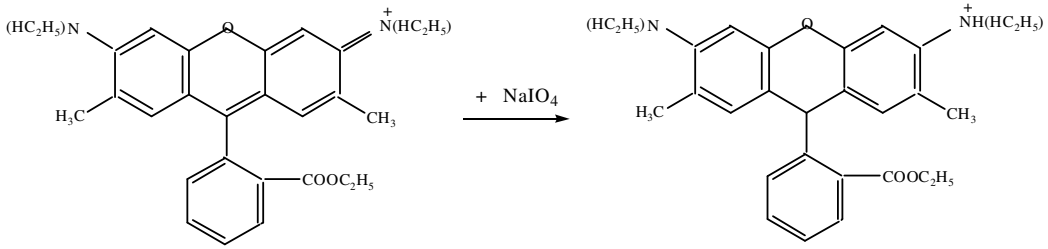
Sample	Found (ng mL^{-1})	RSD (%)	Added (ng mL^{-1})	Obtained (ng mL^{-1})	Recovery (%)	Found (CL) (ng mL^{-1})	E_r (%) (CL)

Serum A	0.0601	2.2	0.0020	0.00200	100	0.0609	– 1.3
Serum B	0.0609	3.6	0.0020	0.00202	101	0.0613	– 0.65
Serum C	0.0598	2.1	0.0020	0.00199	99.5	0.0605	– 1.2
Serum D	0.0612	2.3	0.0020	0.00204	102	0.0607	+ 0.82
Serum E	0.0603	3.4	0.0020	0.00203	102	0.0608	– 0.82
Serum F	0.0607	3.7	0.0020	0.00198	99.0	0.0614	– 1.1
Serum G	0.0602	2.5	0.0020	0.00201	100.5	0.0615	– 2.1
Serum H	0.0608	3.8	0.0020	0.00206	103	0.0601	+ 1.2
Serum I	0.0604	3.9	0.0020	0.00197	98.5	0.0609	– 0.82
Serum J	0.0606	3.0	0.0020	0.00205	103	0.0613	– 1.1
Average	0.0605	3.0	0.0020	0.00202	100.8	0.0610	
Urine A	16.5	2.0	2.0	1.94	97.0	16.7	– 1.2
Urine B	17.3	1.4	2.0	2.05	102	17.8	– 2.8
Urine C	16.6	1.8	2.0	1.98	99.0	17.1	– 2.9
Urine D	17.2	2.1	2.0	2.00	100.0	16.6	+ 3.6
Urine E	16.9	1.9	2.0	2.01	100.5	17.3	– 2.3
Urine F	17.1	1.5	2.0	2.03	102	17.7	– 3.4
Urine G	16.4	1.7	2.0	2.02	101	16.9	– 3.0
Urine H	17.4	1.2	2.0	2.06	103	16.8	+ 3.6
Urine I	16.7	1.6	2.0	1.97	98.5	17.2.	– 2.9
Urine J	17.1	1.3	2.0	2.07	104	17.5	– 2.3
Average	16.92	1.6	2.0	1.95	97.5	17.2	

Seen from the table 2, this method not only can measure the TBS content in human serum, can also be used to analyze residual trace TBS in human urine. The results of this method were in accordance with those of CL. The recovery was 97.0-104 (%), and the RSD was 1.2-3.9 (%), which shows this sensor has high accuracy, high sensitivity and precision. Moreover, the content of TBS in human serum was 0.0605 ng mL^{-1} by the oral dose of 40 μg every day, while the residual TBS in human urine was 16.92 ng mL^{-1}, indicating that 0.040% (16.92 ng /40000 ng) TBS discharged from the urine, and it could predict that the patients had no not life-threatening.

3.6 Sensing mechanism

Rh 6G can emit fluorescence (Fig.1, Curve 2.2'). However, the fluorescence signal of Rh 6G quenched intensively in the Rh 6G-$NaIO_4$-buffer solution system (Fig. 1, Curve 3.3'), maybe Rh 6G was oxidized by $NaIO_4$ to non-fluorescence complex, and the oxidation reaction (Scheme 1) [18] can be expressed as follows:

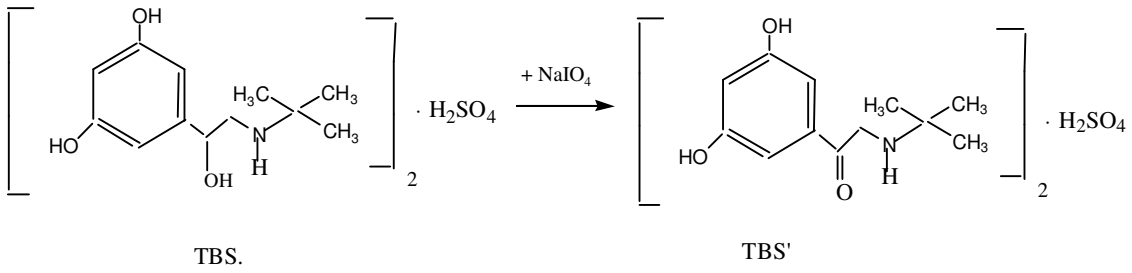


Rhod.6G Rhod.6G '

Scheme 1 Reaction between Rhod.6G and $NaIO_4$

The main IR characteristic absorption peaks of Rhod.6G (υ-NH_2 3420 cm^{-1}, υ-CH_3 1466 cm^{-1}, υ C-O-C 1230 cm^{-1}, υC_6H_5- 1505 cm^{-1}, υ-C=N- 2203 cm^{-1}, υ-benzoquinone base 1675 cm^{-1}), and Rhod.6G' (υ-NH_2 3423 cm^{-1}, υ-CH_3 1467 cm^{-1}, υ C-O-C 1231 cm^{-1}, υC_6H_5- 1507 cm^{-1}, υ C=C 1633 cm^{-1}, υ C-NH 1164 cm^{-1}) can be seen, υ-C=N- absorption peaks at 2203 cm^{-1} and 1675 cm^{-1} of Rhod.6G disappeared, but C-CH and C-NH absorption peaks at 780 $cm^{-1}$and 1164 cm^{-1} of Rhod.6G' appeared. This experiment corroborated the fact that Rhod.6G could be oxidized to Rhod.6G' by $NaIO_4$.

The oxidization-reduction reaction expression took place between TBS and $NaIO_4$ when TBS was added. The reaction (Scheme 2) can be expressed as follows:



TBS. TBS'

Scheme 2 Reaction between TBS and $NaIO_4$

The above reaction inhibited $NaIO_4$ from oxidizing Rh 6G, which caused the fluorescence signal of Rh 6G to enhance sharply, and ΔF of the system was linear correlation to the content of TBS, hereby fluorescence sensor was suitable for the determination of TBS based on the inhibition effect on $NaIO_4$ oxidizing Rh 6G.

The main IR characteristic absorption peaks of TBS (υ-OH 3612 cm^{-1}, υC_6H_5- 1511 cm^{-1}, υC=C 1640 cm^{-1}, υ-CH_3 1470 cm^{-1}, υ N-H 1623 cm^{-1}, υ-OH 1060 cm^{-1}), and TBS' (υ-OH 3277 cm^{-1}, υC_6H_5- 1514 cm^{-1}, υC=C 1645 cm^{-1}, υ-CH_3 1473 cm^{-1}, υ N-H 1626 cm^{-1}, υ C=O 1725 cm^{-1}) can be seen, υ-OH absorption peaks at 1060 cm^{-1} of TBS disappeared, but C=O absorption peaks at 1725 cm^{-1} of TBS' appeared. This experiment corroborated the fact that TBS could be oxidized to TBS' by $NaIO_4$.

4 Conclusion

A ultra-sensitive sensor for the determination of trace TBS was designed based on the inhibition effect on $NaIO_4$ oxidizing Rh 6G. This method was suitable for the residue analysis of trace TBS in human body for its convenience and rapidness, providing new detection technique for maintaining human health and also driving the research progress of drugs detection technique and sensor.

Acknowledgements

This work was supported by Fujian Province Natural Science Foundation (Grant No. 2010J01053,

2009J1017 and 2008J0313), Fujian Province Education Committee (A10277, JA08252, JB08262 and JB09278), Fujian provincial bureau of quality and technical supervison (FJQI 2011006) and Scientific Research Program of Zhangzhou Institute of Technology Foundation (ZZY1215, ZZY 1007 and ZZY1009). At the same time, we are very grateful to precious advices raised by the anonymous reviewers.

Reference

[1] J.A. Ribeiro, F. Silva, C.M. Pereira, *Anal Chem*, 2013, **85**, 1582-1590.

[2] Q. Huang, S. Hu, H. Zhang, J. Chen, Y. He, F. Li, W. Wen, J. Ni, X. Bao, Y. Lin, *Analyst*, 2013, **138**, 5417-5423.

[3] S. Li, J Wang, S. Zhao, *J Chromatogr B*, 2009, **877**,155-158.

[4] L. Han, Y. Zhang, J. Kang, J. Tang, Y. Zhang, *J Pharmaceut Biomed*, 2012, **58**, 141-145.

[5] Y. Li, Z. Ye, J. Zhou, J. Liu, Ge Song, K. Zhang, B. Ye, *J Electroanal Chem (Switz)*, 2012, **687**, 51-57.

[6] W. Xu, R. Lei, W. Cao, C. Guo, X. Zhang, S. Wang, *J Anal Sci (Methods & Instrum)*, 2013, **3**, 75-79.

[7] A. M. Beltagi, H. S. El-Desoky, M. M. Ghoneim, *Chem Pharm Bull*, 2007,**55**, 1018-1023

[8] Yi Lv, Z. Zhang, Y. Hu, D. He, S. He, *J Pharm Biomed Anal*, 2003, **32**, 555-561.

[9] Z. Wang, Z. Zhang, Z. Fu, X. Zhang, *Anal Bioanal Chem*, 2004, **378**, 834-840.

[10] H.A. Hashem, M.S. Elmasry, W.E. Hassan, *J AOAC Int*, 2012, **95**, 1412-1417.

[11] M.S. Althanyan, K.H. Assi , B.J. Clark, J. Hanaee, *J Pharm Biomed Anal*, 2011, **55**, 397-402.

[12] Y. Zhang, Z. R. Zhang, *J Chromatogr B*, 2004, **805**, 211-214.

[13] Md. Faiyazuddin, A. Rauf, N. Ahmad, S. Ahmad, Z. Iqbal, S. Talegaonkar, A. Bhatnagar, R.K. Khar, F.J. Ahmad, *Saudi Pharm J*, 2011,**19**, 185–191.

[14] Md. Faiyazuddin, S. Ahmad, Z. Iqbal, S. Talegaonkar, F.J. Ahmad, A. Bhatnagar, R.K. Khar, *Anal Sci*, 2010, **26**, 467-472.

[15] D. He, Z. Zhang, H. Zhou, Y. Huang, *Talanta*, 2006, **69** , 1215-1220.

[16]Y. Yamini, C.T. Reimann, A. Vatanara, J.Å. Jönsson, *J Chromatogr A*, 2006, **1124**, 57-67

[17] K.H. Peng, X.Q. Tang, S.X. Xiang. Chinese Journal of Health Laboratory Technology, 2006,16, 307-308

[18] J.M. Liu, F. Gao, W. Y. Gao, L. Q. Zeng, X. M. Huang, Z. M. Li, X. C. Huang, W. N. Lin, F.

M. Wang, C. L. Nie. J Fluoresc (2008) 18:573–579