Microchim Acta (2008) 161: 169–173
DOI 10.1007/s00604-007-0931-8
Printed in The Netherlands

Microchimica Acta

Original Paper

Chemiluminescence determination of atenolol in biological fluids by a europium-sensitized permanganate-sulfite system

Dongdong Li, Jianxiu Du, Jiuru Lu

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an, P.R. China

Received 20 September 2007; Accepted 19 November 2007; Published online 11 February 2008

Abstract. A simple flow injection chemiluminescence (CL) method was developed for the determination of atenolol using Eu^{3+} as the probe. It was found that the weak CL generated by the $KMnO_4$-Na_2SO_3 reaction can be significantly enhanced by the atenolol-Eu^{3+} complex. The experimental conditions were optimized. The CL intensity was linearly related to atenolol concentration in the range from 8.0×10^{-9} to 1.0×10^{-5} g mL^{-1}. The detection limit ($3s_b$) was 3×10^{-9} g mL^{-1} and the relative standard deviation for 1.0×10^{-7} g mL^{-1} atenolol solution was 2.4% ($n = 11$). The method has high sensitivity, wide linear range, inexpensive instrumentation, and has been applied to the determination of atenolol in spiked human urine and plasma samples with recoveries within the range 95.5–104.0%.

Keywords: Atenolol; chemiluminescence; flow injection; europium

Atenolol is one of the most widely used β-blockers in the treatment of various cardiovascular disorders, such as angina pectoris, cardiac arrhythmia and hypertension [1]. β-Blockers were reported to be exceptionally toxic and most of them acted in a narrow therapeutic range [2]. The overdose of atenolol may cause lethargy, disorder of respiratory drive, wheezing, sinus pause, bradycardia, congestive heart failure, hypotension, bronchospasm and hypoglycemia [3]. Since the β-blockers are also misused as doping agents in sports, these drugs have been added to the list of forbidden drugs by the World Anti-doping Agency. The minimum required performance limit for β-blockers is 5×10^{-7} g mL^{-1} [4]. Therefore, the development of sensitive and selective analytical methods for the determination of the β-blockers is of great importance.

Several analytical methods have been reported for the determination of atenolol in human plasma, urine, or pharmaceutical preparations, such as spectrophotometry [5, 6], fluorimetry [7], atomic absorption spectrometry [8], electrochemical method [9–11], liquid chromatography [12–14], capillary electrophoresis [15, 16], and mass spectrometry [17, 18]. CL analysis with the advantages of high sensitivity, wide linear range and simple instrumentation has also exploited for the determination of atenolol [19–21]. Li et al. reported a flow injection CL method for the determination of atenolol based on its inhibitory effect on the luminol-KIO_4-H_2O_2 reaction; the method could determine 5×10^{-7}–1×10^{-4} g mL^{-1} atenolol [19].

Electronic supplementary material: Discussion of the reaction mechanism and additional figures are available online as electronic supplementary material (ESM) at http://springerlink.metapress.com/content/103392/.

Correspondence: Jianxiu Du, Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, P.R. China, e-mail: jxdu@snnu.edu.cn

$Ru(bpy)_3^{2+}$-based electrogenerated CL method was also used for the determination of atenolol, the linear ranges for atenolol were 1×10^{-7}–1×10^{-4} mol L^{-1} and 5×10^{-7}–5×10^{-5} g mL^{-1}, respectively, when coupling with high performance liquid chromatography [20] and capillary electrophoresis [21], respectively.

Depending on the origin of the CL, CL reactions have been classified as direct and indirect or sensitized or energy transfer CL [22]. In indirect CL reaction, the primary excited state molecule is not the final emitter, but transfers its energy to a fluorescent substance, which then produces light emission. The indirect CL reaction is important for improving the sensitivity of the detection and broadening the analytical range of the CL analysis.

Trivalent lanthanide species display high photoluminescence efficiencies (>5% in H_2O), large Stokes' shifts (~300 nm), long excited-state lifetimes (on the order of several hundred microseconds), and narrow emission spectra [23], all of which make them become a useful fluorescent probe [24, 25]. Recently, these characteristic of lanthanide species have been introduced into CL analysis to develop sensitive method for the determination of several fluoroquinolone antibiotics [26, 27]. Reviewing the literature indicated that no CL method was reported for the determination of atenolol with lanthanide species as sensitizer.

The aim of this work is to develop a sensitive CL method for the β-blocker atenolol using Eu^{3+} as the sensitizer. It was found that the weak CL signal from $KMnO_4$-Na_2SO_3 reaction could be greatly enhanced in the presence of Eu^{3+} and atenolol. The experimental conditions were optimized and a new flow injection CL method was proposed for the determination of atenolol. The proposed method was sensitive, selective, and applied to the determination of atenolol in spiked human urine and plasma samples with satisfactory results.

Experimental

Chemicals

Atenolol standard (No. 100117-199903) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Eu_2O_3 was purchased from General Research Institute for Nonferrous Metals of China (Beijing, China). Other reagents were purchased from Xi'an Chemicals (Xi'an China). All of chemicals were of analytical grade; redistilled water was used throughout.

Atenolol stock solution (2.00×10^{-4} g mL^{-1}) was prepared by dissolving 0.0200 g atenolol in 100 mL of water. This solution was stored in a refrigerator and protected from light. Atenolol working solutions were prepared by the dilution of the stock solution with water when used. Eu^{3+} solution (2×10^{-2} mol L^{-1}) was prepared by dissolving 0.352 g Eu_2O_3 with a small amount of concentrated HCl, evaporating the solution to near dryness on a water bath, and then diluting to 100 mL with water. $KMnO_4$ solution (5×10^{-2} mol L^{-1}) was prepared in water, stored in a brown bottle, and used after two weeks. More dilute solutions of $KMnO_4$ were prepared by appropriate dilution with water before use. Na_2SO_3 solution (2×10^{-4} mol L^{-1}) was freshly prepared by dissolving an appropriate amount of Na_2SO_3 in water.

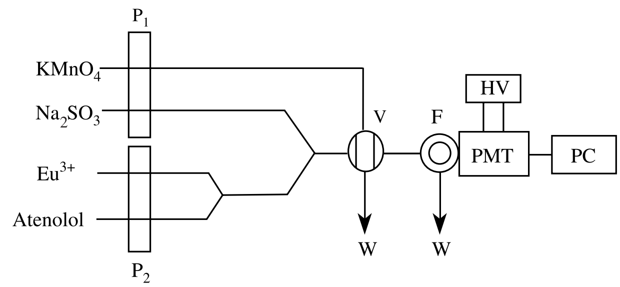

Fig. 1. Schematic diagram of CL flow system. P_1, P_2 peristaltic pump; *V* six-way value; *F* flow cell; *PMT* photomultiplier tube; *HV* high voltage; *PC* personal computer; *W* waste

Apparatus

Figure 1 shows the schematic diagram of the FI-CL system used. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. Peristaltic pumps were used to deliver reagent solutions and sample solution, each channel at a flow rate of 2.0 mL min^{-1}. Injection was operated by means of a six-way valve equipped up with a 50 μL sample loop. The distance between the injection valve and the flow cell was about 20 cm. The CL signal produced in the flow cell (1 mm × 25 cm spiral colorless glass tubing in 3.5 turns) was detected with a CR105 photomultiplier tube (Beijing Hamamatsu Photo Techniques Inc.). CL data acquisition and treatment were performed using IFFL-D type of flow injection CL data processing system (Xi'an Remex Eletronic Science-Tech Co. Ltd.).

Procedure

As shown in Fig. 1, atenolol solution was firstly mixed with 8×10^{-3} mol L^{-1} Eu^{3+} solution, followed with 2×10^{-4} mol L^{-1} Na_2SO_3 solution. Finally 50 μL solution of 5×10^{-4} mol L^{-1} $KMnO_4$ was injected into above merged stream by means of the six-way valve for producing CL. The concentration of atenolol was quantified by the enhanced CL intensity ΔI, $\Delta I = Is - I_0$, where *Is* is the CL signal of sample, and I_0 is the blank signal.

Results and discussion

In the preliminary experiments, several CL systems with $KMnO_4$ as the oxidant were investigated using flow injection mode. These systems were of $KMnO_4$-Na_2SO_3, $KMnO_4$-$Na_2S_2O_3$, $KMnO_4$-HCOOH, $KMnO_4$-HCHO, and $KMnO_4$-H_2O_2 system. The experiments showed atenolol-Eu^{3+} complex can enhance the CL signal from the reactions of $KMnO_4$-Na_2SO_3 and $KMnO_4$-$Na_2S_2O_3$, while no enhancement was observed for other CL systems. Finally, $KMnO_4$-Na_2SO_3 system was selected to determine atenolol because it gave larger enhancement and better precision than those of $KMnO_4$-$Na_2S_2O_3$ system.

Effect of manifold and flow rate

The different mixing procedure of reagents and the different injection mode were designed to obtain the maximum sensitivity. The manifold shown in Fig. 1 had the highest sensitivity and better reproducibility.

The effect of flow rate on the CL reaction was tested in the range from 1.2 to 2.8 mL min^{-1}. The enhanced CL signal continued to increase with increase in flow rate. Finally, a flow rate of 2.0 mL min^{-1} was employed by considering the sensitivity, reagents consumption and reproducibility.

Effect of $KMnO_4$ concentration

The effect of $KMnO_4$ concentration on the CL reaction was examined in the range from 1×10^{-5} to 3×10^{-3} mol L^{-1}. The maximum enhanced CL signal was observed when $KMnO_4$ concentration was 5×10^{-4} mol L^{-1}. Higher or lower concentration of $KMnO_4$ caused a decrease in the enhanced CL signal. Therefore, 5×10^{-4} mol L^{-1} $KMnO_4$ was selected.

Effect of Na_2SO_3 concentration

The effect of 5×10^{-5}–1×10^{-3} mol L^{-1} Na_2SO_3 on the CL reaction was examined. The enhanced CL signal rapidly increased with increasing Na_2SO_3 concentration up to 1×10^{-4} mol L^{-1}, and then varied slightly. Finally, 2×10^{-4} mol L^{-1} Na_2SO_3 was used.

Effect of Eu^{3+} concentration

The influence of Eu^{3+} concentration on the CL reaction was examined up to 1×10^{-2} mol L^{-1}. No obvious enhancement on the CL signal was observed in the absence of Eu^{3+}. The enhanced CL signal increased rapidly with increasing Eu^{3+} concentration from 1×10^{-4} to 8×10^{-3} mol L^{-1}. When the concentration of Eu^{3+} was higher than 8×10^{-3} mol L^{-1}, the blank increased rapidly and the enhancement on the CL signal became slowly. Therefore, 8×10^{-3} mol L^{-1} Eu^{3+} was selected.

Effect of the pH of $KMnO_4$ solution

$KMnO_4$ can react with some substances to generate CL in acidic, neutral, or alkaline medium [28–31]. The effect of the pH of $KMnO_4$ solution on the CL reaction was examined in the range from pH 2 to pH 10. It was observed that the enhanced CL signal had no obvious change when the pH of $KMnO_4$ solution was in the range of pH 2–10. Therefore, $KMnO_4$ aqueous solution was used.

Analytical parameters

Under the optimum experimental conditions, the enhanced CL intensity (ΔI) was proportional to the concentration of atenolol (C) in the range from 8.0×10^{-9} to 1.0×10^{-5} g mL^{-1}. The linear regression equation was $\Delta I = 9.67 + 11.5\ C$ (C: 10^{-8} g mL^{-1}) with a correlation coefficient was 0.9983. The relative standard deviations ($n = 3$) for the slope and intercept were 2.2 and 2.9%, respectively. The intra-day ($n = 11$) and inter-day ($n = 3$) precision for 1.0×10^{-7} g mL^{-1} atenolol solution was 2.4 and 3.8%, respectively. The detection limit (blank plus three times its standard deviation) was 3×10^{-9} g mL^{-1} atenolol. The determination of atenolol could be completed in 1 min, including sampling and injection, giving a sample throughput of 60 h^{-1}.

Interference

The effect of some common inorganic ions and organic compounds was investigated on the CL determination of 1.0×10^{-7} g mL^{-1} atenolol. A foreign species was considered not to interfere if it caused a relative error less than 5% in the enhanced CL signal. The tolerable ratios were 1000-fold glucose, starch, lactose, K^+, Na^+, Ca^{2+}, Ni^{2+}, Mg^{2+}, Cu^{2+}, NO_3^-; 500-fold metoprolol tartrate, Zn^{2+}; 100-fold dextrin, oxalate, propranolol hydrochloride, Al^{3+}, Cr^{3+}; 10-fold Fe^{2+}, Fe^{3+}, ascorbic acid, and uric acid.

Application

Determination of atenolol in spiked urine samples

In generally, β-blockers are extensively metabolized with less than 5% of the oral dose being excreted unchanged in the urine [32], the expected concentration of the β-blockers excreted in the urine after oral administration is normally in the range of 1.0–5.0 µM

Table 1. Results for the determination of atenolol in spiked urine samples

Samples	Added (10^{-8} g mL^{-1})	Found (10^{-8} g mL^{-1})	Recovery (%)	RSD (%, $n = 5$)
1	0.00	0.00		
	4.00	3.98	99.6	2.4
	40.0	41.3	103.0	2.2
	200	202.1	101.0	2.8
2	0.00	0.00		
	4.00	4.02	100.6	2.1
	40.0	41.5	104.0	2.7
	200	202.5	101.3	2.7
3	0.00	0.00		
	4.00	4.15	103.7	2.5
	40.0	39.7	99.7	2.5
	200	205.1	102.5	2.8

[20]. Therefore, the proposed method was applied to the determination of atenolol in urine samples. Urine samples were obtained from three healthy volunteers. Into 2.5 mL of urine sample, a known amount of atenolol standard solution was added and then diluted to 50 mL with water. The atenolol content in urine sample was determined by the proposed method. The results are summarized in Table 1. The recoveries of urine samples were in the range 99.6–104.0%.

Determination of atenolol in spiked plasma samples

The antihypertensive effects of atenolol means that a single dose of 50 or 100 mg needs to be administered daily, which gives plasma peak concentrations of 200–300 and 500–600 ng mL^{-1}, respectively, obtained between 2 and 4 h after the intake of formulation [33]. Plasma samples were obtained from the blood blank of Shaanxi province. A known amount of atenolol standard solution and 0.2 mL of plasma samples were transferred into a centrifuge tube and mixed. Then 2.0 mL 0.1 mol L^{-1} $Ba(OH)_2$ and 1.8 mL 0.1 mol L^{-1} $ZnSO_4$ were added to remove protein and reducing substances [34, 35]. The resultant solution was diluted to 6 mL with water and centrifuged at 3000 rpm for 10 min. One milliliter supernatant solution was transferred into a volumetric flask, diluted to 25 mL with water, and determined by the proposed method. The results are given in Table 2. The recoveries of serum samples were in the range 95.5–102.6%.

Table 2. Results for the determination of atenolol in spiked plasma samples

Samples	Added (10^{-8} g mL^{-1})	Found (10^{-8} g mL^{-1})	Recovery (%)	RSD (%, $n = 5$)
1	0.00	0.00		
	5.00	5.08	101.7	2.7
	60.0	60.7	101.1	2.9
	400	405.5	101.4	2.1
2	0.00	0.00		
	5.00	4.75	95.0	3.1
	60.0	57.7	96.1	3.2
	400	410.6	102.6	2.2
3	0.00	0.00		
	5.00	4.83	96.7	2.6
	60.0	57.2	96.4	2.0
	400	409.1	102.3	2.8

References

1. You Q D (2004) Medicinal chemistry. Chemical Industry Press, Beijing, p 262
2. Siren H, Saarinen M, Hainari S, Riekkola M L (1993) Screening of β-blockers in human serum by ion-pair chromatography and their identification as methyl or acetyl derivatives by gas chromatography-mass spectrometry. J Chromatogr B 632: 215
3. Snook C P, Sigvaldason K, Kristinsson J (2000) Severe atenolol and diltiazem overdose. J Toxicol Clin Toxicol 38: 661
4. http://www.wada-ama.org/en/dynamic.ch2?pagecategory.id=372.2006
5. Al-Ghannam S M (2006) A simple spectrophotometric method for the determination of β-blockers in dosage forms. J Pharm Biomed Anal 40: 151
6. Khan I U, Yaqoob N, Ahmad M (2005) Determination of atenolol in pure substance and pharmaceutical preparations applying first order derivative spectrophotometry. Chem Anal (Warsaw) 50: 951
7. Murillo Pulgarin J A, Alanon Molina A, Fernandez Lopez P (1998) Simultaneous determination of atenolol, propranolol, dipyridamole and amiloride by means of non-linear variable-angle synchronous fluorescence spectrometry. Anal Chim Acta 370: 9
8. El Ries M A (1995) Indirect atomic absorption spectrometric (AAS) determination of atenolol. Anal Lett 28: 1629
9. Goyal R N, Singh S P (2006) Voltammetric determination of atenolol at C_{60}-modified glassy carbon electrodes. Talanta 69: 932
10. Shamsipur M, Jalali F, Haghgoo S (2005) Preparation of an atenolol ion-selective electrode and its application to pharmaceutical analysis. Anal Lett 38: 401
11. Nikolelis D P, Petropoulou S S E, Mitrokotsa M V (2002) A minisensor for the rapid screening of atenolol in pharmaceutical preparations based on surface-stabilized bilayer lipid membranes with incorporated DNA. Bioelectrochemistry 58: 107
12. Ceresole R, Moyano M A, Pizzorno M T, Segall A I (2006) Validated reversed-phase HPLC method for the determination of atenolol in the presence of its major degradation product. J Liq Chromatogr Related Technol 29: 3009
13. Cheng F C, Chen Y T, Kuo J S, Chen S H, Chang L C (1996) A micro liquid chromatographic assay for the determination of plasma-unbound atenolol. J Pharm Biomed Anal 14: 1169
14. Simmons B R, Stewart J T (1995) HPLC determination of atenolol in human serum on underivatized silica using solid phase extraction. Anal Lett 28: 2017
15. Arias R, Jimenez R M, Alonso R M, Telez M, Arrieta I, Flores P, Ortiz-Lastra E (2001) Determination of the β-blocker aten-

olol in plasma by capillary zone electrophoresis. J Chromatogr A 916: 297
16. Maguregui M I, Jimenez R M, Alonso R M (1998) Simultaneous determination of the β-blocker atenolol and several complementary antihypertensive agents in pharmaceutical formulations and urine by capillary zone electrophoresis. J Chromatogr Sci 36: 516–522
17. Dong L L, Huang J X (2006) Determination of atenolol in human plasma by pseudo reversed phase liquid chromatography-tandem mass spectrometry. Chromatographia 64: 583
18. Angier M K, Lewis R J, Chaturvedi A K, Canfield D V (2005) Gas chromatographic-mass spectrometric differentiation of atenolol, metoprolol, propranolol, and an interfering metabolite product of metoprolol. J Anal Toxicol 29: 517
19. Li X X, Qi H L (2004) Flow-injection chemiluminescence determination of atenolol. J Northwest Univ (Natural Science Edition) 34: 687
20. Park Y J, Lee D W, Lee W Y (2002) Determination of β-blockers in pharmaceutical preparations and human urine by high-performance liquid chromatography with tris(2,2-bipyridyl)ruthenium(II) electrogenerated chemiluminescence detection. Anal Chim Acta 471: 51
21. Huang J S, Sun J Y, Zhou X G, You T Y (2007) Determination of atenolol by capillary electrophoresis electrogenerated chemiluminescence. Anal Sci 23: 183
22. Robards K, Worsfold P J (1992) Analytical applications of liquid-phase chemiluminescence. Anal Chim Acta 266: 147
23. Crosby G A, Whan R E, Allire R M (1961) Intramolecular energy transfer in rare earth chelates: role of the triplet state. J Chem Phys 34: 743
24. Zhu X J, Wang X L, Jiang C Q (2005) Spectrofluorimetric determination of heparin using a tetracycline-europium probe. Anal Biochem 341: 299
25. Oliva M P L A, Olsina R A, Masi A N (2005) Sensitive detection of salbutamol using europium-enhanced fluorescence with trioctylphosphine oxide (TOPO) as coligand. Analyst 130: 1312
26. Wang X, Zhao H C, Hua L H, Jin L P, Zhang Z L (2001) Europium sensitized chemiluminescense determination of rufloxacin. Anal Chim Acta 445: 169
27. Ocana J A, Baragan F J, Callejin M, De la Rosa F F (2004) Application of lanthanide-sensitised chemiluminescence to the determination of levofloxacin, moxifloxacin and trovafloxacin in tablets. Microchim Acta 144: 207
28. Abbott R W, Townshend A, Gill R (1986) Determination of morphine. by flow-injection analysis with chemiluminescence detection. Analyst 111: 635
29. Hinason B J, Barnett N W (2001) Analytical applications of acidic potassium permanganate as a chemiluminescence reagent. Anal Chim Acta 445: 1
30. Li Y H, Lu J R (2006) Flow injection chemiluminescence determination of naproxen based on $KMnO_4$-Na_2SO_3 reaction in neutral aqueous medium. Anal Chim Acta 577: 107
31. Marino D F, Ingle J D (1981) Determination of humic acid by chemiluminescence. Anal Chim Acta 124: 23
32. Ceniceros C, Maguregui M I, Jimenez R M, Alonso R M (1998) Quantitative determination of the β-blocker labetalol in pharmaceuticals and human urine by high-performance liquid chromatography with amperometric detection. J Chromatogr B 705: 97
33. Brent Miller R (1991) A validated high-performance liquid chromatographic method for the determination of atenolol in whole blood. J Pharm Biomed Anal 9: 849
34. Bostick D T, Hercules D M (1975) Quantitative determination of blood glucose using enzyme induced chemiluminescence of luminol. Anal Chem 47: 447
35. Malavolti N L, Piloso D, Nieman T A (1985) Determination of cholesterol with a microporous membrane chemiluminescence cell with cholesterol oxidase in solution. Anal Chim Acta 170: 199