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Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 667–670
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Rapid determination of clindamycin in medicine with myoglobin–luminol chemiluminescence system

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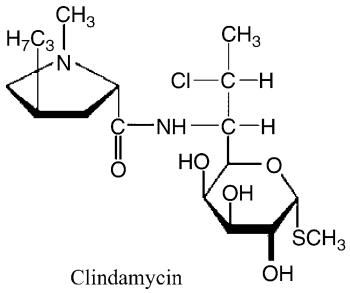
Received 29 October 2005; received in revised form 13 December 2005; accepted 20 December 2005
Available online 24 January 2006

Abstract

A sensitive chemiluminescence method, based on the inhibitory effect of clindamycin on the chemiluminescence reaction between luminol and myoglobin in a flow-injection system, is proposed for the determination of clindamycin. The decrement of chemiluminescence intensity is linear with the logarithm of clindamycin concentration over the range from 0.1 to 70.0 ng ml^{-1} ($r^2 = 0.9995$), with the detection limit of 0.03 ng ml^{-1} (3σ). At a flow rate of 2.0 ml min^{-1}, a complete analytical process could be performed within 0.5 min, including sampling and washing, with a relative standard deviation of less than 3.0% ($n = 5$). The proposed procedure was applied successfully to the determination of clindamycin in capsules without any pretreatment process.

Keywords: Clindamycin; Myoglobin; Luminol; Chemiluminescence

1. Introduction



Clindamycin

Clindamycin [7(*S*)-chloro-7-deoxylincomycin] is a lincosamide antibiotic, which is synthesized from microbially fermented lincomycin by replacing a hydroxyl group at the 7-position of lincomycin by chlorine that significantly increases its activity. The effect of clindamycin, which is primarily bacteriostatic, is exerted by its binding to the 50 S ribosomal subunit and the consequent inhibition of bacterial protein synthesis [1]. It is active against aerobic Gram-positive, anaerobic bacteria and mycoplasmas. Clindamycin is mainly used in the treatment of diseases like staphylococcal skin infections and osteomyelitis, periodontal disease, bacterial prostatitis, toxoplasmosis, and neosporosis [2].

Spectrophotometry [3], capillary electrophoresis [4], HPLC with MS [5,6], UV [7,8], electrochemical [9] and chemiluminescence (CL) [10] detection have been reported for the determination of clindamycin in pharmaceuticals.

Progress in flow-injection (FI) CL analysis has received much attention in pharmaceutical analysis for its high sensitivity, rapidity and simplicity [11–13]. We have currently reported the determination of thiamine [14], risperidone [15] and Vitamin B_{12} [16] with different CL methods. To the best of our knowledge, no CL procedure has been used for the determination of clindamycin. It was previously reported that myoglobin (Mb), which contains a single iron protoporphyrin or heme moiety in the ferric state Mb(Fe^{III}) reacts with luminol yielding CL emission. This was exploited for the determination of myoglobin [17]. It was also found that ligands such as fluoride, cyanide and thiocyanate binding to the iron ion in the heme structure inhibit the CL reaction. This was used for the indirect determination of these ions [17]. In this paper it is described that clindamycin also inhibits the CL reaction between luminol and myoglobin. On the basis of this a simple, sensitive and rapid procedure was developed for the indirect determination of clindamycin. The decrement of CL signal was linear with the logarithm of clindamycin concentration over the range from 0.1

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doi:10.1016/j.jpba.2005.12.027

to 70.0 ng ml^{-1} with R.S.D.s of less than 3% (n = 5). At a flow rate of 2.0 ml min^{-1}, a complete determination of clindamycin, including sampling and washing, could be completed in 0.5 min, offering the sampling efficiency of 120 h^{-1} accordingly.

2. Experimental

2.1. Apparatus

The FI system used in this work is shown schematically in Fig. 1. A peristaltic pump was utilized to deliver all flow streams. PTFE tubing (1.0 mm i.d.) was used as connection material in the flow system. A six-way valve with a loop of 100 μl was employed for sampling. The flow cell was made by coiling 30 cm of colorless glass tube (2.0 mm i.d.) into a spiral disk shape with a diameter of 2.0 cm and placed close to the photomultiplier tube (PMT) (Model IP28, Hamamatsu, Japan). The CL signal produced in the flow cell was detected without wavelength discrimination, and the PMT output was amplified and quantified by a luminosity meter (Model GD-1, Xi'an Remax Electronic Science-Tech. Co. Ltd.) connected to a recorder (Model XWT-206, Shanghai Dahua Instrument and Meter Plant). A UV–vis spectrophotometer (Model Lambda-40P, PE Co. Ltd.) was used and the optical path length was 1.0 cm.

2.2. Reagents

All reagents were of analytical grade, and the water used was purified in a Milli-Q system (Millipore, Bedford, MA, USA). Clindamycin standard solution (1.988 mg ml^{-1}) was obtained from Shaanxi Institute for Drug Control, and the working strength solutions were prepared from the above stock standard solutions as required. Luminol (2.5×10^{-2} mol l^{-1}) was prepared by dissolving 4.40 g luminol (Fluka, Switzerland) in 1 l of 0.1 mol l^{-1} NaOH solution. Horse heart myoglobin (Sigma) was used as received without further purification.

2.3. General procedures

The carrier (pure water) and the solutions (luminol, Mb and clindamycin) were propelled at a flow rate of 2.0 ml min^{-1} on each flow line. The pump was started to wash the whole flow system until a stable baseline was recorded. Then 100 μl mixing solution of clindamycin and Mb was injected into the carrier stream, which was then mixed with the luminol stream. The mixed solution was delivered to the CL flow cell, and the peak height of the CL signal was measured with the luminometer. The PMT voltage was set as −700 V. The concentration of sample could be quantified on the basis of the decrement of CL intensity, $\Delta I = I_o - I_s$, where I_s and I_o were CL signals in the presence and in the absence of clindamycin, respectively.

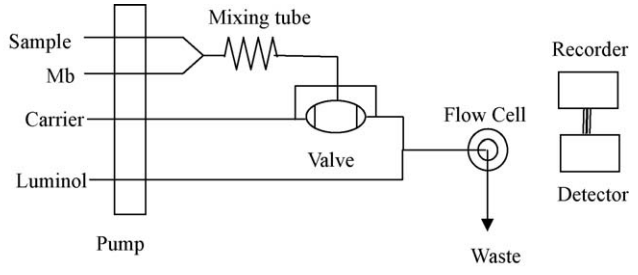

Fig. 1. Schematic diagram of the present FI-CL system for clindamycin.

3. Results and discussion

3.1. CL intensity–time profile

Before the FI method was developed, the kinetic curve was examined by static method. The kinetic profile for CL intensity of luminol–Mb reaction versus time was tested using 5.0×10^{-9} mol l^{-1} Mb and 1.5×10^{-5} mol l^{-1} luminol in 0.05 mol l^{-1} NaOH. It can be seen in Fig. 2 of the static system, the CL signal of luminol–Mb reached a maximum at 3 s after initiating the reaction, and tended to be vanishing in the following 12 s. It was demonstrated that clindamycin inhibited the CL reaction and greatly depressed the CL intensity.

3.2. Effect of Mb, luminol and NaOH concentration

The effect of Mb and luminol concentration on the CL intensity was investigated over the ranges of 5.0×10^{-10} to 5.0×10^{-7} mol l^{-1} and 5.0×10^{-7} to 5.0×10^{-4} mol l^{-1}, respectively. It was found that the CL intensity reached maximum with 5.0×10^{-9} mol l^{-1} Mb and afforded approximately constant CL intensity over 5.0×10^{-9} mol l^{-1}; and the maximum CL intensity could be obtained when using a concentration of 1.5×10^{-5} mol l^{-1} luminol. Therefore, 5.0×10^{-9} mol l^{-1} Mb and 1.5×10^{-5} mol l^{-1} luminol were chosen as the optimum concentration and used in subsequent experiment. Owing to the nature of the luminol reaction, which is more favored under alkaline conditions, NaOH was introduced into the luminol solution to increase the sensitivity of the

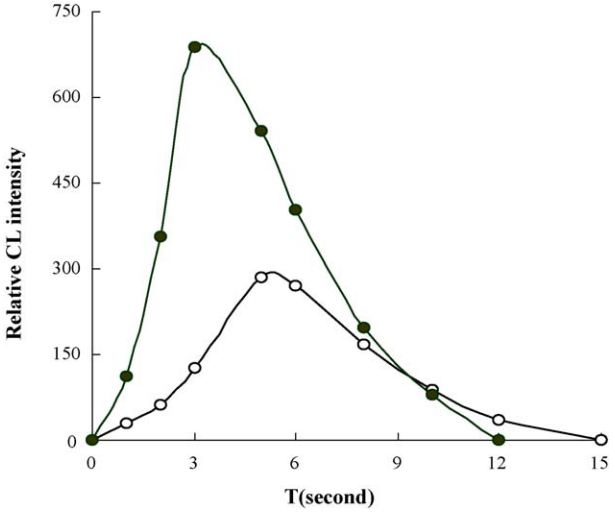

Fig. 2. Kinetic CL intensity–time profile in static system. (●) CL intensity in the absence of clindamycin; (○) CL intensity in the presence of clindamycin (1.0 ng ml^{-1}).

system. A series of NaOH solutions with different concentration (0.005, 0.01, 0.03, 0.05, 0.1 and 0.2 mol l^{-1}, respectively) were tested. The CL intensity versus concentration of NaOH plot reached maximum at about 0.05 mol l^{-1}, and this concentration was employed in subsequent experiments.

3.3. Effect of flow rate and the length of mixing tubing

The effect of the mixing tube length on CL intensity was tested from 5.0 to 20.0 cm. It was observed that the CL intensity was much stronger using 15.0 cm mixing tube than that of other mixing tube in the presence of 1.0 ng ml^{-1} clindamycin. Thus, 15.0 cm mixing tube was selected. The influence of flow rate on determination was examined by investigating the signal-to-noise ratio (S/N) under different flow rate. Flow rate of 2.0 ml min^{-1} offering highest S/N ratio was then chosen as suitable condition considering analytical precision.

3.4. Analytical performance for determination of clindamycin

Under the optimal conditions, a series of standard solutions of clindamycin were tested by the CL system illustrated in Fig. 1. The decrement of CL intensity was proportional to the logarithm of clindamycin concentration over the range from 0.1 to 70.0 ng ml^{-1} with the detection limit of 0.03 ng ml^{-1} (3σ). The regression equation for clindamycin is $\Delta I_{CL} = 5.8133 \ln C_{clindamycin} - 26.14$, $r^2 = 0.9995$. At the flow rate of 2.0 ml min^{-1}, a typical analysis including sampling and washing, could be completed in 0.5 min giving a throughput of 120 h^{-1} with R.S.D.s of less than 3.0% ($n = 5$).

3.5. Interference studies

The interference of foreign species was tested by analyzing a standard solution of clindamycin (1.0 ng ml^{-1}) into which increasing amounts of potential interfering substances were added. The tolerable relative concentration of foreign species with respect to 1.0 ng ml^{-1} clindamycin for interference at 5.0% level were over 10000 for Cl^-, NO_3^-, Ac^-, I^-, SO_4^{2-}, PO_4^{3-}, BrO_3^-, Na^+, amylum, glucose, borate, malic acid, maltose and ethanol, and 5000 for NH_4^+, Mg^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, oxalate, methanol, tartrate, sucrose, citrate, and salicylic acid, and 1000 for glutin, urea and dextrin, and 100 for uric acid, and 10 for Cu^{2+}, Zn^{2+}, Ni^{2+}, Cr^{3+}, Fe^{2+}/Fe^{3+}, respectively. Common excipients such as agar and cellulose in capsules caused no interference for the determination of clindamycin.

4. Application

4.1. Determination of clindamycin in capsules

The proposed method was applied to the determination of clindamycin in capsules (Chongqing Kerui Pharmaceutical Co. Ltd.) purchased from the local market. Ten capsules were weighed and ground to a fine powder using a pestle and mortar. The powder was dissolved in water, and the resulting solution was filtered through an ordinary filter paper and diluted to the mark in a 100 ml brown calibrated flask. Suitable aliquots from this solution were taken for the determination of clindamycin so that the concentration of clindamycin was in the working range of its determination without pretreatment. For the procedure described, the samples were determined by the standard addition method into which a known quantity of clindamycin was added. The data in Table 1 show that the results obtained by the proposed method agree well with the results obtained by UV spectrophotometry. The recoveries range from 92.3% to 106.5%.

Table 1
Content of clindamycin (mg $capsule^{-1}$)

By the proposed method	By UV 200.1 nm
147.1	148.2
152.1	151.8
146.8	149.3
155.8	152.6
157.0	153.5
150.9	151.2
155.4	150.7
152.7	148.8

Label claim: 150.

5. Possible mechanism of the CL reaction

The possible mechanism of clindamycin inhibiting luminol–Mb CL reaction was investigated by the static CL and UV methods. Based on the fact that the absorption spectrum of $Mb(Fe^{III})$-clindamycin is the same as that of $Mb(Fe^{II})$, with the characteristic λ_{max} at 415 nm, it is suggested that Mb in the ferric state $Mb(Fe^{III})$ with the characteristic λ_{max} at 409 nm, can be reduced by clindamycin to form $Mb(Fe^{II})$. The CL reaction is $Mb(Fe^{III}) \rightarrow Mb(Fe^{II})$ + aminophthalate. However, when clindamycin is present, it reacts with $Mb(Fe^{III})$, decreasing its concentration and hence, less intensity is emitted, i.e. two competitive reactions takes place with $Mb(Fe^{III})$. As shown in Fig. 2, the CL intensity in the presence of 1.0 ng ml^{-1} clindamycin was half of that in the luminol–Mb CL reaction; its time period of CL process was also extended from 12 to 15 s and the CL signal reaches its maximum from 3 to 5 s.

6. Conclusions

Compared with other methods for the assay of clindamycin, the proposed method offers advantages of simplicity of apparatus, less reagent consumption, higher sensitivities and higher sample throughput.

Acknowledgments

The authors gratefully acknowledge the financial support from Shaanxi Province Nature Science Foundation and Ministry of Education, China, Grant No. 2003B15 and No. 04JK145.

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