Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 64 (2004) 156–159
Talanta
www.elsevier.com/locate/talanta

Potassium permanganate–glyoxal chemiluminescence system for flow injection analysis of cephalosporin antibiotics: cefalexin, cefadroxil, and cefazolin sodium in pharmaceutical preparations

Yuanyuan Sun, Yuhai Tang*, Hong Yao, Xiaohui Zheng

Department of Chemistry, College of Science, Xi'an Jiaotong University, Xi'an 710061, PR China

Received 27 October 2003; received in revised form 2 February 2004; accepted 2 February 2004

Available online 2 April 2004

Abstract

A sensitive flow injection chemiluminescence (FL-CL) method for the determination of cephalosporin antibiotics, was developed. The method was based on that cephalosporin antibiotics could enhance the CL reaction of glyoxal and $KMnO_4$ in sulfuric acid. Method development included the optimization of reagent concentrations and flow-rate. Under the optimized conditions, three cephalosporin antibiotics: cefalexin, cefadroxil, and cefazolin sodium, were determined. The detection limits of the method are 10 ng ml^{-1} cefalexin, 2 ng ml^{-1} cefadroxil, and 2 ng ml^{-1} cefazolin sodium. The method was successfully applied to the determination of three cephalosporin antibiotics in pharmaceutical preparations.

Keywords: Chemiluminescence; Flow injection; Cephalosporins; Pharmaceutical preparations

1. Introduction

Cephalosporins, a kind of β-lactam antibiotics with a basic structure of 7-aminocephalosporanic acid, are widely used to treat respiratory tract infection, prostatitis, urinary tract infection, skin, and soft tissues infection that often result from encroachment of sensitive bacteria. Many methods have been reported for the determination of cephalosporins, such as HPLC [1–3], CE [4,5], spectrophotometry [6–9], fluorimetry [3,10], and polarography [11]. There have been only few reports on chemiluminescence (CL) methods for the determination of cephalosporins. Kubo et al. [12] reported a flow injection analysis method for the detection of cephalothin. It was based on the direct chemiluminescence reaction of β-lactam antibiotics with luminol in the presence of hexacyanoferrate(III) and hexacyanoferrate(II) in alkaline solution. Aly et al. [13] developed a flow injection chemiluminescent (FL-CL) method for the determination of cefadroxil monohydrate with a detection limit of 50 ng ml^{-1}. The method is based upon the chemiluminescence reaction of cefadroxil with potassium permanganate in sulphuric acid, sensitized by quinine.

The present paper described a new flow injection CL method for the determination of three cephalosporin antibiotics: cefalexin, cefadroxil, and cefazolin sodium. The method was based upon the enhancing effect of these antibiotics on the CL reaction of glyoxal with potassium permanganate in acid condition. Compared with the previous reported chemiluminescence methods for cephalosporins [12,13], the present method shows lower detection limits and wider calibration ranges. The method was applied to the determination of cefalexin, cefadroxil, and cefazolin sodium in pharmaceutical formulations with satisfactory results.

2. Experimental

2.1. Reagents

Analytical reagent grade chemicals and double distilled water were used to prepare all solutions.

Cefalexin, cefadroxil, and cefazolin sodium samples were purchased from National Institute for the Control of

* Corresponding author. Tel.: +86-29-5275399; fax: +86-29-5275135.
E-mail address: tyh57@mail.xjtu.edu.cn (Y. Tang).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.02.012

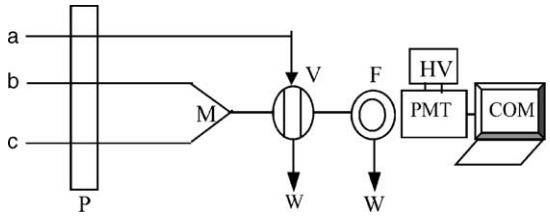

Fig. 1. Schematic diagram of the flow system for determination of cephalosporins. (a): Glyoxal solution; (b): $KMnO_4$ solution; (c): Sample solution; (M): Manifold (P): Peristaltic pump; (V): Six-way injection valve; (F): Flow cell; (PMT): Photomultiplier tube; (HV): High voltage; (COM): Computer; (W): Waste solution.

Pharmaceutical and Biological Products (Beijing, China). $KMnO_4$ was obtained from Xi'an Chemical Reagent Factory (Xi'an, China). Glyoxal was provided by Chemistry Department of Xi'an Jiaotong University. Dosage forms containing cefalexin, cefadroxil, and cefazolin sodium were purchased from local markets.

The 1×10^{-3} mol l^{-1} $KMnO_4$ working solution was prepared by diluting appropriate 0.1 mol l^{-1} stock solution in water. The 0.05 mol l^{-1} working solution was prepared by diluting appropriate 1 mol l^{-1} glyoxal solution in water.

The 1.0 mg ml^{-1} standard solutions of cefalexin, cefadroxil, and cefazolin sodium were daily prepared by dissolving 0.2500 g of each in water and diluting with water to 250 ml. The standard solutions were stored in the refrigerator and protected from light. The testing solutions were prepared by appropriate dilution of these standard solutions with water before used.

2.2. Instruments

Fig. 1 shows the schematic diagram of the flow injection chemiluminescence system. One peristaltic pump was used to deliver the cephalosporins and $KMnO_4$ solutions. Another peristaltic pump was used to pump glyoxal solutions. All components were connected with PTFE tubing (0.8 mm i.d.) in the flow system. Reagent solutions were injected into the flow system by a six-way injection valve. A photomultiplier tube was used to detect the CL. The CL signal was recorded with IBM-compatible computer, which was employed an IFFL-D model flow-injection CL analysis system software (Xi'an Ruike Electronic Equipment Corporation, Xi'an, China).

2.3. Procedure

2.3.1. Procedure for calibration

A series of working solutions of three cephalosporin antibiotics with different concentrations were prepared by diluting respective concentrated standard solutions. $KMnO_4$ solution in sulfuric acid was mixed with the cephalosporins solution in a manifold prior to reaching the six-way injection valve. Glyoxal solution was injected into the flow cell by the six-way injection valve to combine with the mixed $KMnO_4$–cephalosporins solution. The calibration graphs were prepared by plotting the CL peak height against the concentration of the cephalosporins.

2.3.2. Procedure for pharmaceutical preparations

Cefalexin capsules (250 mg per capsule), cefadroxil tablets (250 mg per capsule) and cefazolin sodium for injection (500 mg per bottle), were purchased from local markets. Each sample stock solution was prepared by dissolving a quantity of the mixed content, equivalent to 250 mg of this cephalosporin, from 10 capsules, tablets or bottles with water. Before analysis, the stock solutions were diluted appropriately to ensure the concentration of each within the linear range.

3. Results and discussion

3.1. Effect of different acid concentrations

It was observed that the CL signal of $KMnO_4$–glyoxal system was stronger in acid solution than in neutral or basic solution. Four different acids (i.e. HCl, HNO_3, H_3PO_4, and H_2SO_4) of different concentrations, as the mediums for $KMnO_4$, over the range of 0.1–2 mol l^{-1} were tested. The results showed maximum CL intensity was obtained with 1.0 mol l^{-1} H_2SO_4. The effect of H_2SO_4 on the CL reaction is shown in Fig. 2.

3.2. Effect of $KMnO_4$ concentration

The effect of 1×10^{-4}–1×10^{-2} mol l^{-1} $KMnO_4$ on the CL intensity was examined. The CL intensity continued to increase with increasing $KMnO_4$ concentration up to 1.0×10^{-3} mol l^{-1}. The experimental results showed that 1×10^{-3} mol l^{-1} could give rise to the larger CL response and lower background signal. Larger concentration of $KMnO_4$ could lower the CL intensity. Thus, 1×10^{-3} mol l^{-1} $KMnO_4$ was used in the work.

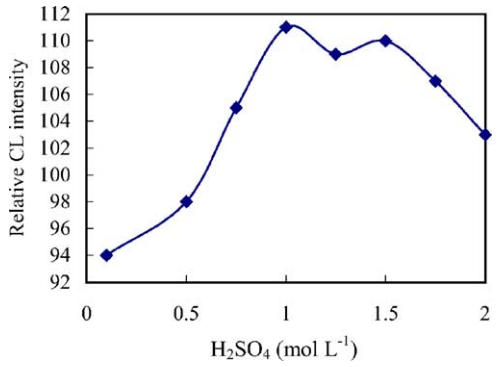

Fig. 2. Effect of H_2SO_4 concentration of $KMnO_4$ on the CL intensity. Conditions: cefalexin, 0.1 μg ml^{-1}; glyoxal, 0.05 mol l^{-1}; $KMnO_4$, 1×10^{-3} mol l^{-1}.

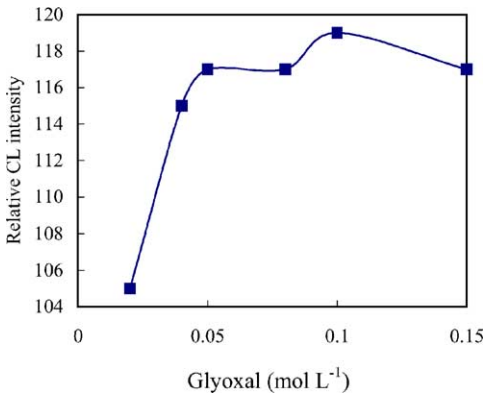

Fig. 3. Effect of glyoxal concentration on the CL intensity. Conditions: cefalexin, 0.1 μg ml^{-1}; $KMnO_4$, 1×10^{-3} mol l^{-1}.

3.3. Effect of glyoxal concentration

The effect of 0.02–0.15 mol l^{-1} glyoxal on the CL intensity was examined. The CL intensity continued to increase with increasing glyoxal concentration up to 0.05 mol l^{-1}. The experimental results (Fig. 3) showed the CL intensity continued to increase with increasing glyoxal concentration up to 0.05 mol l^{-1}. When the glyoxal concentration is beyond 0.05 mol l^{-1}, the intensity of the CL signal is trending stable. In this work, 0.05 mol l^{-1} glyoxal was selected.

3.4. Calibration curves, detection limits, and precisions

The method allowed the determination of 0.01–1 μg ml^{-1} cefalexin, 0.01–1 μg ml^{-1} cefadroxil, and 0.1–5 μg ml^{-1} cefazolin sodium. The relative standard deviations for 11 replicate measurements of cefalexin, cefadroxil, and cefazolin sodium were 1.1, 1.3, and 1.5%, respectively, when their concentrations were at 0.1 μg ml^{-1}. Table 1 lists the parameters of the calibration curves and the calculated detection limits ($S/N = 3$).

3.5. Selectivity

In order to assess the selectivity of the proposed method, the effect of some common inorganic ions and organic compounds was studied by preparing solutions containing 0.1 μg ml^{-1} of cefalexin. It was considered not to interfere if a foreign material caused a relative error of less than ±5% during the determination of 0.1 μg ml^{-1} cefalexin. The results showed that no interference had been found when including up to a 1000-fold Na^+, K^+, Mg^{2+}, Ba^{2+}, Ca^{2+}, SO_4^{2-}, NO_3^-, PO_4^{3-}, glucose, starch, lactose, citric acid, 500-fold carbowax, 100-fold Zn^{2+}, five-fold Al^{3+}, and two-fold CO_3^{2-}.

3.6. Application

Following the procedure detailed in Section 2, the proposed method was applied to the determination of cefalexin, cefadroxil and cefazolin in pharmaceutical formulations. The results were listed in Table 2 and agreed well with those obtained by pharmacopoeia method [14].

3.7. Possible reaction mechanism

It was reported that $KMnO_4$ could react with some reductants in the presence of formaldehyde to produce $^1O_2$ $^1O_2$($^1\Delta_g$ $^1\Delta_g$), a complex oxygen molecule of single state, which could transform into $^3O_2$ ($^3\Sigma_g$), a triplet state oxygen. During the transformation, it could produce CL and the formaldehyde could accelerate oxidation reaction rate [15]. Thus it was assumed that the cephalosporins could also react with $KMnO_4$ to produce CL and the CL reaction could be accelerated by glyoxal.

Table 1
Calibration curves of the studied cephalosporins

Cephalosporins	Condition range (μg ml^{-1})	Regression equation $Y = a + b\,X$[a]	Regression coefficint	Detection limit (ng ml^{-1})
Cefalexin	0.01 ∼ 0.1	$Y = 0.061 + 0.010X$	0.9994	10
	0.1 ∼ 1	$Y = -39.776 + 0.141X$	0.9942	
Cefadroxil	0.01 ∼ 0.1	$Y = -0.437 + 0.034X$	0.9983	2
	0.1 ∼ 1	$Y = -5.7030 + 0.189X$	0.9984	
Cefazolin sodium	0.1 ∼ 1	$Y = -4.524 + 0.098X$	0.9970	2
	1 ∼ 5	$Y = -738.770 + 1.990X$	0.9973	

[a] $Y = a + b\,X$ where Y is the concentration in 0.1 μg ml^{-1} and X is the CL intensity.

Table 2
Determination results of cephalosporins in samples

Preparation	Proposed method[a]		Pharmacopoeia method[a]	
	Content (%)	R.S.D. (%)	Content (%)	R.S.D. (%)
Cefalexin capsules (No.: C020402)	101.4	1.7	101.1	1.0
Cefadroxil tablets (No.: 020404)	93.8	1.3	100.2	2.0
Cefazolin sodium for injection (No.: B02082916)	105.5	2.2	97.4	0.7

[a] Average of five measurements.

Based on the above discussions, the possible reaction mechanism was suggested as following

$$MnO_4^- + H^+ + \text{glyoxal} + \text{cephalosporins} \rightarrow {}^1O_2({}^1\Delta_g) + H_2O + \text{Mn(II–IV)} + \text{products}$$
$$2\,{}^1O_2({}^1\Delta_g) \rightarrow {}^1O_2{}^1O_2({}^1\Delta_g{}^1\Delta_g)$$
$${}^1O_2{}^1O_2({}^1\Delta_g{}^1\Delta_g) \rightarrow {}^3O_2({}^3\Sigma_g) + h\nu$$

4. Conclusion

Based on the chemiluminescence reaction of the studied cephalosporins with $KMnO_4$ in sulfuric acid, sensitized by glyoxal, a new flow injection CL method was developed for the determination of these cephalosporins. The method was simple, rapid and sensitive, and was applied to the analysis of these cephalosporins in pharmaceutical preparations and compared with pharmacopoeia method.

Acknowledgements

This work was financially supported by Xi'an Jiaotong University.

References

[1] V.F. Samanidou, E.A. Hapeshi, I.N. Papadoyannis, J. Chromatogr. B 788 (2003) 147.
[2] L.K. Sørensen, L.K. Snor, J. Chromatogr. A 882 (2000) 145.
[3] M. Kai, H. Kinoshita, M. Morizono, Talanta 60 (2003) 325.
[4] Y. Mrestani, R.H.H. Neubert, J. Pharm. Biomed. Anal. 24 (2001) 637.
[5] A. Gáspár, M. Andrási, S. Kardos, J. Chromatogr. B 775 (2002) 239.
[6] I.F.A-L. Momani, J. Pharm. Biomed. Anal. 25 (2001) 751.
[7] B. Morelli, J. Pharm. Biomed. Anal. 32 (2003) 257.
[8] V. Ródenas, M.S. García, C. Sánchez-Pedreño, M.I. Albero, J. Pharm. Biomed. Anal. 15 (1997) 1687.
[9] M.M. Ayad, A.A. Shalaby, H.E. Abdellatef, H.M. Elsaid, J. Pharm. Biomed. Anal. 18 (1999) 975.
[10] X.W. He, B. Tang, H.X. Shen, Chem. J. Chin. Univ. 16 (1995) 26.
[11] A.G. Fogg, M.J. Martin, Analyst 106 (1981) 1213.
[12] H. Kubo, M. Saitoh, S. Murase, T. Inomata, Y. Yoshimura, H. Nakazawa, Anal. Chim. Acta 389 (1999) 89.
[13] F.A. Aly, N.A. Alarfaffj, A.A. Alwarthan, Talanta 47 (1998) 471.
[14] I.F.A-L. Momani, The Pharmacopoeia of People's Republic of China, Editorial Committee of the Pharmacopoeia of PR China, second ed., Chemical Industry Press, Beijing, China, 2000, pp. 190, 192, 194.
[15] Y.H. He, J.R. Lu, Chin. J. Anal. Chem. 30 (2002) 598.