Flow Injection Chemiluminescence Analysis of Some Penicillins by Their Sensitizing Effect on the Potassium Permanganate-Glyoxal Reaction

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A new chemiluminescence method using flow injection is described for the determination of four penicillins, namely: phenoxymethylpenicillin potassium, amoxicillin, ampicillin, and ampicillin sodium. The method is based on sensitizing effect of these drugs on the chemiluminescence reaction of potassium permanganate in sulfuric acid with glyoxal. The different experimental parameters affecting the chemiluminescence intensity were carefully studied and incorporated into the procedure. The method allows the determination of 0.1 – 1.0 µg/ml phenoxymethylpenicillin potassium, 0.1 – 1.0 µg/ml amoxicillin, 0.1 – 1.0 µg/ml ampicillin, and 0.1 – 1.0 µg/ml ampicillin sodium. The method was successfully applied to the determination of four penicillin antibiotics in pharmaceutical preparations.

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Penicillins, a kind of β -lactam antibiotics, are active on sensitive gram-positive and gram-negative organisms. Many analytical procedures have been reported for their determination. The Chinese Pharmacopoeia (2nd ed., 2000) recommends HPLC or titration assays for their dosages.¹ Other reported methods include: spectrophotometry,²⁻⁴ mercurimetric titration,⁵ NMR,⁶ HPLC,⁷⁻¹⁰ and HPLC-APCI-MS.¹¹

There have been a few reports on chemiluminescence (CL) methods for the determination of penicillins. Jingshun *et al.* described a CL method for the determination of penicillin G with a linear range of 0.01 – 0.1 mg/ml and dicloxacillin with a linear range of 0.2 – 1.0 mg/ml.¹² It was based on the CL reaction of penicillins with potassium superoxide dissolved by 18-crown-6-ether. Kubo *et al.* described a flow-injection CL method for the determination of β -lactam antibiotics, which was based on CL directly produced by the reaction of luminol with β -lactam antibiotics in the presence of a catalyst in an alkaline solution. The method allowed the determination of penicillin G in linear range of 0.12 – 6.0 mg/ml with a detection limit of 12 µg/ml.¹³

The present article describes a simple flow injection CL method for the determination of four penicillins: phenoxymethylpenicillin potassium, amoxicillin, ampicillin, and ampicillin sodium. The method is based on an enhancing effect of penicillins on the CL reaction of glyoxal with KMnO_4 in sulfuric acid. The experimental conditions for the reaction were optimized, and the final procedure allowed a successful determination of the studied drugs in pharmaceutical preparations.

Experimental

Reagents and chemicals

All of the reagents were of analytical reagent grade, and

doubly distilled water was used to prepare all solutions. Phenoxymethylpenicillin potassium, amoxicillin, ampicillin, and ampicillin sodium standard samples were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). KMnO_4 was obtained from Xi'an Chemical Reagent Factory (Xi'an, China). Glyoxal was provided by Department of Chemistry of Xi'an Jiaotong University (Xi'an, China).

The 1.0 mg/ml standard solutions of phenoxymethylpenicillin potassium, amoxicillin, ampicillin, and ampicillin sodium were freshly prepared by dissolving 250 mg of each in water and diluting with water to 250 ml. The working solutions were prepared by appropriate dilution of these standard solutions with water before being used. A KMnO_4 solution 1.0×10^{-3} mol/l was prepared by diluting an appropriate 0.1 mol/l stock solution in 1.3 mol/l H_2SO_4 . A glyoxal solution (0.05 mol/l) was prepared from a 1 mol/l stock solution.

Instruments

The flow system used for the determination and CL detection for the studied penicillins is shown schematically in Fig. 1. One peristaltic pump (two channels) was used to drive KMnO_4 in sulfuric acid and glyoxal solutions. Another peristaltic pump was used to deliver the sample solution. Each pump was run at with a constant flow rate of 2.75 ml/min. PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system. The CL signal produced was measured with a photomultiplier tube and recorded with an IBM-compatible computer, which was employed IFFL-D Model flow-injection CL analysis system software (Xi'an Ruike Electronic Equipment Corporation, Xi'an, China).

Procedure for calibration

A series of working solutions of four penicillin antibiotics with different concentrations were prepared by diluting concentrated standard solutions. As shown in Fig. 1, a KMnO_4 solution in sulfuric acid and a glyoxal solution were pumped at

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Fig. 1 Schematic diagram of the flow system for the determination of penicillins. A, sample solution; B, KMnO_4 solution; C, glyoxal solution; V, six-way injection valve; F, flow cell.

Fig. 2 Effect of the H_2SO_4 concentration of KMnO_4 on the CL intensity (phenoxymethylpenicillin potassium $1.0 \mu\text{g/ml}$, glyoxal 0.05 mol/l , KMnO_4 $1.0 \times 10^{-3} \text{ mol/l}$).

2.75 ml/min , and mixed with each other in a manifold. The sample solution was also pumped through another pump at 2.75 ml/min and added into the flow cell with the aid of an injection valve with a $80 \mu\text{l}$ sampling loop to combine with a mixed KMnO_4 -glyoxal solution. Calibration graphs were prepared by plotting the CL peak height against the concentration of the penicillins.

Analysis of the studied penicillins in pharmaceuticals

Phenoxymethylpenicillin potassium tablets (250 mg/tablet), amoxicillin capsules (250 mg/capsule), ampicillin capsules (250 mg/capsule), and ampicillin sodium for injection (500 mg/injection) were purchased from a local market. The average weight of each penicillin was calculated, respectively, from the weight of 10 capsules, tablets or injections. Each penicillin was finely powdered and homogenized; a portion of the powder, equivalent to 250 mg of this penicillin, was accurately weighed and dissolved in 250 ml of water as sample solutions. Before analysis, the sample solutions were diluted appropriately. The actual content of each penicillin was calculated from the corresponding regression equation.

Results and Discussion

Effect of different acid concentrations

It was observed that the CL signal of the KMnO_4 -glyoxal system was stronger in an acid solution than in a neutral or basic solution. Four different acids (*i.e.* HCl , HNO_3 , H_3PO_4 and H_2SO_4) of different concentrations, as the media for KMnO_4 , over the range of $0.1 - 2.0 \text{ mol/l}$ were tested. The results showed that the maximum CL intensity was obtained with 1.3 mol/l H_2SO_4 in $1.0 \times 10^{-3} \text{ mol/l}$ KMnO_4 . The effect of H_2SO_4 on the CL reaction is shown in Fig. 2.

Fig. 3 Effect of the flow-rate on the CL intensity. (phenoxymethylpenicillin potassium $1.0 \mu\text{g/ml}$, KMnO_4 $1.0 \times 10^{-3} \text{ mol/l}$, glyoxal 0.05 mol/l).

Effect of the KMnO_4 concentration

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

Effect of the glyoxal concentration

The influence of the glyoxal concentration on the CL intensity was investigated in the range of 0.02 to 0.15 mol/l . It showed that the maximum increase in the relative CL intensity was obtained as the concentration of glyoxal increased up to 0.05 mol/l . Above 0.05 mol/l of the glyoxal concentration, the increase in the relative CL intensity decreased. Therefore, 0.05 mol/l of the glyoxal concentration was selected as being the optimum.

Effect of the reagents flow-rate

The flow rate of reagent solutions was tested in order to obtain a satisfactory CL peak. In this experiment, we controlled all of the solutions (glyoxal, KMnO_4 , and sample solutions) at the same flow rate, and changed the flow rate from 1.1 ml/min to 3.8 ml/min to study the effect of the flow rate on the CL intensity. Figure 3 shows the influence of the flow rate over the range $1.1 - 3.8 \text{ ml/min}$. The results showed that, with $1.0 \mu\text{g/ml}$ phenoxymethylpenicillin potassium as the analyte when the concentration of glyoxal was at 0.05 mol/l , and KMnO_4 was at $1.0 \times 10^{-3} \text{ mol/l}$, the CL intensity continued to increase with increasing flow-rate up to 2.75 ml/min . A greater flow-rate could lower the CL intensity. In this work, $1.0 \times 10^{-3} \text{ mol/l}$ KMnO_4 was selected.

Calibration curves, detection limits and precisions

Under the optimum conditions, four penicillin antibiotics were determined, respectively. The method allowed for the determination of $0.1 - 1.0 \mu\text{g/ml}$ phenoxymethylpenicillin potassium, amoxicillin, ampicillin, and ampicillin sodium with detection limits ($S/N = 3$) of $0.05 \mu\text{g/ml}$ phenoxymethylpenicillin potassium, $0.03 \mu\text{g/ml}$ amoxicillin, $0.03 \mu\text{g/ml}$ ampicillin, and $0.05 \mu\text{g/ml}$ ampicillin sodium.

The relative standard deviations for 11 replicate measurements of phenoxymethylpenicillin potassium, amoxicillin, ampicillin, and ampicillin sodium were 4.4 , 0.8 , 2.1 , and 1.1% when their concentrations were at $0.1 \mu\text{g/ml}$. Table 1 lists the parameters of the calibration curves and the calculated detection limits ($S/N = 3$).

Table 1 Calibration curves of penicillins

Species	Con. range ^a /μg ml ⁻¹	Calibration curve ^b	R ^{2c}	Detection limit ^d /μg ml ⁻¹	RSD, % ^e
Phenoxymethylpenicillin potassium	0.1 – 1	y = 5.14 + 38.6x	0.998	0.05	4.4
Amoxicillin	0.1 – 1	y = 12.28 + 1.49 × 10 ² x	0.998	0.03	0.8
Ampicillin	0.1 – 1	y = 18.61 + 25.80x	0.999	0.03	2.1
Ampicillin sodium	0.1 – 1	y = 0.13 + 2.17 × 10 ² x	0.993	0.05	1.1

a. Concentration range of the studied penicillins. b. Calibration curve; y is the CL intensity and x is the concentration of penicillins in 1 μg/ml. c. Correlation coefficients. d. Detection limit; calculated as three-times the standard (n = 3) of the peak intensity. e. Relative standard deviations for 11 replicate measurements of penicillins; their concentrations were at 0.1 μg/ml.

Table 2 Determination results of penicillins in pharmaceuticals

Preparation	Claimed/mg	Proposed method		Official method ^a	
		Found/mg ^b	SD	Found/mg ^b	SD
Phenoxymethylpenicillin potassium tablets	250	243.0	0.9	252.8	0.3
Amoxicillin capsules	250	237.8	0.2	252.5	0.2
Ampicillin capsules	250	228.5	0.2	247.3	0.5
Ampicillin sodium for injection	500	517.0	0.3	500.6	0.3

a. Ref. 1. b. Mean value of five measurements.

Interference study and application

In order to assess the selectivity of the proposed method, the interference of some common inorganic ions and organic compounds on the CL reaction was examined by preparing solutions containing 1.0 μg/ml of phenoxymethylpenicillin potassium. The tolerance of foreign species was taken as the largest concentration, yielding an error of less than ± 5% in CL the signal of phenoxymethylpenicillin potassium. No interference was found when including up to a 1000-fold Na⁺, K⁺, Mg²⁺, Ba²⁺, Ca²⁺, SO₄²⁻, NO₃⁻, glucose, starch, 100-fold Zn²⁺, Al³⁺, 5-fold CO₃²⁻, 2-fold Cl⁻.

The proposed method was successfully applied to the determination of phenoxymethylpenicillin potassium, amoxicillin, ampicillin, and ampicillin sodium in pharmaceutical formulations. The results are listed in Table 2, and agree well with those obtained by pharmacopoeia methods.¹

Discussion on a possible reaction mechanism

It was reported that KMnO₄ could react with some compounds (pyrogallol, ethamsylate and methotrexate) in the presence of formaldehyde or formic acid to produce ¹O₂(¹Δ_g), a complex oxygen molecule having a single state, which could transform into ³O₂(³Σ_g), a triplet state oxygen. During the transformation, it could produce CL and formaldehyde or formic acid, which could accelerate the oxidation reaction rate.^{14–16} It was also reported that the possible emitter of the hypochlorite-hydrogen peroxide CL systems was concerned with the singlet excited oxygen species.¹⁷ Thus, it is reasonable to assume that the singlet excited molecular oxygen species should also be the possible emitter in the present system. The formation of the singlet excited molecular oxygen may due to the oxidation of the β-lactam structure with the acidic potassium permanganate, and glyoxal could accelerate the CL reaction. In order to support this hypothesis, the CL spectrum of the system was detected with a modified RF-540 fluorescence spectrometer. The spectrum showed a wavelength maximum at 630 nm. This wavelength maximum was shown to be similar to the spectrum attained for the above-mentioned CL systems.

In addition, Pang *et al.* studied the fluorescence spectrum of

some penicillin antibiotics (benzathine benzylpenicillin, ampicillin, and carbenicillin) and their degradation products in the presence of Mn(II).¹⁸ The results showed that the fluorescent intensities of the studied penicillin antibiotics in solutions was not detectable, but the fluorescent intensities of their degradation products could be detected. The maximum emission wavelengths of the spectrum of the degenerated products were over the range of 436 – 420 nm. Obviously, since the maximum emission lengths of the spectrum for the degradation products did not agree with that of the CL reaction spectrum in our experiments, it does not support that the possible emitter was the reaction products of the penicillin antibiotics with acidic permanganate.

Based on the above discussions, the possible reaction mechanism was suggested to be as follows:

Conclusion

Based on the chemiluminescence reaction of penicillins with KMnO₄ in sulfuric acid and glyoxal, a new flow injection CL method was developed for the determination of these penicillins. The method was simple, rapid and sensitive, and was applied to the analysis of these penicillins in pharmaceutical preparations and compared with pharmacopoeia methods.

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