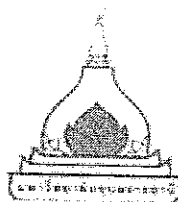


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**CHALLENGES IN CHEMISTRY
FOR SUSTAINABLE DEVELOPMENT**

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Analytical Chemistry

Flow injection chemiluminescence for the determination of cephalixin, cefaclor and cefadroxil using acidic potassium permanganate and formaldehyde system

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Abstract: Flow injection chemiluminescence (FICL) system was designed, fabricated and investigated for the determination of three cephalosporin antibiotics (cephalexin, cefaclor and cefadroxil). The method was based on the chemiluminescence (CL) emitting reaction between each cephalosporin and potassium permanganate (KMnO₄) in an acid medium, which was greatly enhanced by formaldehyde. The optimum conditions for CL reaction were studied in detail using flow injection (FI) system. Under the optimum conditions, calibration graphs over the ranges of 0.1-8.0 µg ml⁻¹ for cephalexin and cefaclor, and 0.1-10.0 µg ml⁻¹ for cefadroxil were obtained. The limits of detection (3σ) for cephalexin and cefaclor were 0.05 µg ml⁻¹, and for cefadroxil was 0.02 µg ml⁻¹. The relative standard deviation (R.S.D.) were less than 2% (n=11) with the percentage recovery of 99.4 ± 1.7%, 99.0 ± 1.5% and 99.2 ± 1.4% for cephalexin, cefaclor and cefadroxil, respectively. The method was successfully applied to the determination of cephalexin, cefaclor and cefadroxil in pharmaceutical formulations with no evidence of interference from common excipients. The results compared favourably with those obtained by the official methods, and were in agreement with the declared compounds. The method can be used for quality control assay of the studied cephalosporins.

Introduction

Cephalexin, cefaclor and cefadroxil are some of the first-generation cephalosporin antibiotics characterized by a broad antibacterial spectrum and a resistance to beta-lactamase-producing organisms in addition to its antimicrobial activity [1]. The structures of them are illustrated in Figure 1.

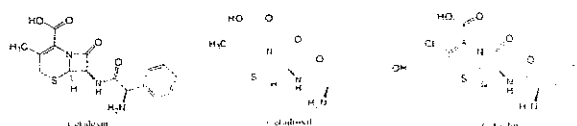


Figure 1 Chemical structures of the investigated drugs

Many methods have been reported for the determination of cephalosporins, such as HPLC [2-4], CE [5], spectrophotometry [6-8], spectrofluorometry [9,10], and polarography [11].

Chemiluminescence (CL) is the emission of radiation, usually in the visible or near-infrared region, as a result of chemical reaction. One of the reaction products is formed in an electronic excited state, and

emits the radiation on falling to the ground state. CL is becoming a powerful analytical tool with widespread application in various fields owing to its high sensitivity, wide dynamic range and simple instrumentation [12-15]. The advantages of flow injection analysis (FIA) has been one of the reasons for the increasing number of applications of CL. The use of FICL as a simple means for pharmaceuticals detection and has been applied to many drugs, such as morphine [16], buprenorphine hydrochloride [17], lorazepam [18], tetracycline [19] and amoxicillin [20]. Since then, a few of such reactions have been reported mainly in cephalosporin analysis [21-24].

The present paper described a simple FICL method for the determination of cephalexin, cefaclor, and cefadroxil. The method was based upon the enhancing effects of these antibiotics on the CL reaction of formaldehyde with potassium permanganate in acid condition which shows lower detections limits and wider calibration ranges. The method was applied to the determination of cephalexin, cefaclor, and cefadroxil in pharmaceutical preparations with satisfactory results.

Materials and Methods

Instruments : The FICL manifold was designed and fabricated as shown in Figure. 2. The reaction reagents were pumped to the three-line manifold by a peristaltic pump (Gilson, Miniplus 2) at a total flow rate of 6.0 ml min⁻¹ through poly(tetrafluoroethylene) (PTFE) flow tubes (Tygon, 0.8-mm i.d. resistant to acid). The water carrier stream, and the reagent streams (4.0 M formaldehyde solution in H₂SO₄ and KMnO₄ oxidant) both at flow rate of 2.0 ml min⁻¹. A 100 µl sample or standard of cephalosporin was injected into water carrier stream. Acidic 0.50 mM KMnO₄ was used as the oxidant and 5%v/v formaldehyde solution in 4.0 M H₂SO₄ as enhancing reagent.

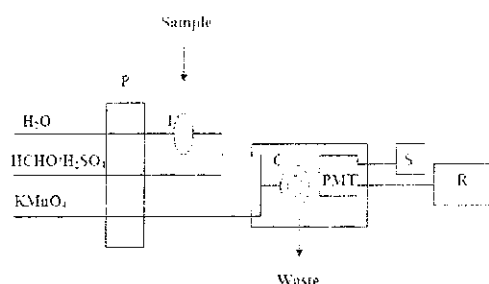


Figure 2 FI-CL manifold designed for determination of cephalixin, cefaclor, and cefadroxil. (P) peristaltic pump; (I) injection valve; (C) flat spiral-coiled glass tube flow cell; (PMT) photomultiplier tube; (S) photomultiplier source power supply; (R) chart recorder.

A home-made detector was used which comprised three components: a T-piece where reagent and carrier streams come together, a flat spiral flow cell and a photomultiplier tube (PMT) was operated at 0.55 kV, provided by a stable high voltage power supply; detector components were placed inside a closed black box to avoid light input. A chart recorder recorded the signal and the peak height was measured for quantification.

Reagents: All of solutions and chemicals were prepared from analytical reagent grade materials using distilled, deionised water. Cephalixin, cefaclor and cefadroxil standards were purchased from Sigma, Poole, Dorset, UK.

A stock standard solution containing $1000 \cdot \text{g} \cdot \text{ml}^{-1}$ of each cephalosporins were prepared by dissolving 100 mg of each drug in 100 ml of water.

Potassium permanganate (KMnO_4) and sulfuric acid (H_2SO_4) were obtained from Fisher Chemicals (Loughborough, UK). The stock standard solution of KMnO_4 was prepared daily and protected from light using amber glass bottles.

A solution of 5%v/v formaldehyde was prepared by dissolving formaldehyde (Fisher Scientific Chemicals, UK) in 4.0 M H_2SO_4 .

Pharmaceutical products containing the investigated drugs were purchased from commercial sources in Phitsanulok and Chiang Mai Province, Thailand.

Optimization studies: The optimization of experimental conditions was carried out by means of a univariate method, using cefadroxil as a model molecule. The optimized FI-CL system for the determination of cefadroxil is represented in Figure 2, which is consisting of three channels FI manifold. The optimized conditions consisted of the water carrier stream, 4.0 M H_2SO_4 as the oxidant stream and a third stream of 5%v/v formaldehyde dissolved in 4.0 M H_2SO_4 to provide an acidic medium and act as an enhancer. The total flow rate of the carrier, the oxidant and the acidic streams were $6.0 \text{ ml} \cdot \text{min}^{-1}$. A $100 \cdot \text{nl}$ of cefadroxil standard or sample solutions were injected into the water carrier stream. The CL emission intensity from the reaction of cefadroxil with 0.50 mM KMnO_4 in sulfuric acid medium, enhanced by formaldehyde was monitored. The CL signals were

outputted to a recorder. The typical calibration graphs were obtained by plotting CL intensity (mV) against cefadroxil concentration.

Results and Discussion

Effect of different acid concentrations

It was observed that the CL signal of KMnO_4 formaldehyde 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 0.05-7.0 M were studied. The results showed maximum CL intensity was obtained with 4.0 M H_2SO_4 . The effect of H_2SO_4 is shown in Figure 3.

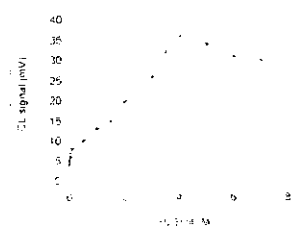


Figure 3 Effect of H_2SO_4 of concentrations on CL intensity. Conditions: cefadroxil $50.0 \cdot \text{g} \cdot \text{ml}^{-1}$, formaldehyde 2%v/v in 0.10 mM KMnO_4 .

Effect of KMnO_4 concentration

The effects of KMnO_4 concentration on the CL reaction of cefadroxil have been studied over the range of 0.05-2.0 mM. The CL intensity continued to increase with increasing KMnO_4 concentration up to 0.50 mM. The experimental results showed that 0.50 mM KMnO_4 could give rise to the larger CL response and lower background signal. Larger concentration of KMnO_4 could lower the CL intensity. Thus, 0.50 mM KMnO_4 was used in this work.

Effect of formaldehyde concentration

The influence of formaldehyde concentration was studied over the range 0.1-12% v/v; by dissolving formaldehyde in H_2SO_4 solution. Standard solution of cefadroxil ($50.0 \cdot \text{g} \cdot \text{ml}^{-1}$) with a volume of $100 \cdot \text{nl}$ was injected into the FI-CL manifold (Figure 2).

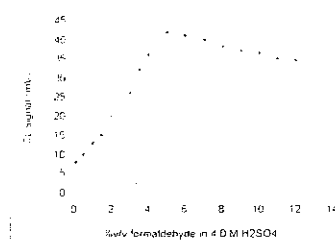


Figure 4 Effect of formaldehyde concentrations on CL intensity. Conditions: cefadroxil $50.0 \cdot \text{g} \cdot \text{ml}^{-1}$, 0.10 mM KMnO_4 .

It was shown that peak heights increased with increasing formaldehyde concentration over the range 0.1-5% v/v, above which the CL signal decreased

gradually, as shown in Figure 4. Therefore, 5% v/v of formaldehyde in 4.0 M H₂SO₄ was applied for subsequent studies.

Effect of flow rate:

The flow rate in each channel is an important parameter influencing on the CL signal of cefadroxil, because the time taken to transfer the excited product into the flow cell is critical for maximum collection of the emitted light. Too low or too high flow rates result in a decrease or even absence of CL signal. At a low flow rate, the residence time for each sample was long and the dispersion was large leading to the reduction of peak height. On the other hand, a high flow rate resulted in a shorter time required for each sample passing through the FI system, a poor precision of the peak height and a large volume of reagent consumption were obtained. In this work, total flow rates between 1.5 and 9.0 ml min⁻¹ (with 0.5–3.0 ml min⁻¹/channel) were studied. The optimum total flow rate chosen was 6.0 ml min⁻¹ (2.0 ml min⁻¹ for each channel) and this was used throughout the experiments. Increasing the flow rate above 6.0 ml min⁻¹ does not significantly enhance the peak intensity, which will increase the pressure in the tubing and the consumption of the reagents.

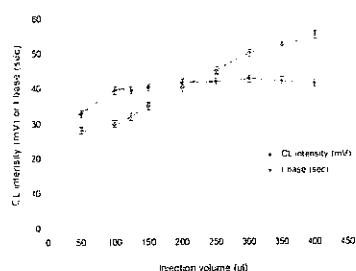


Figure 5 Effect of sample injection volume

Effect of injection volume:

The effect of injection volume was investigated by changing the sample loop over the range 50–400 µl and was found to have an important effect on the signal. The CL signal increased with increasing injection volume and reached the maximum signal at 100 µl. With further increases in sample volume up to 400 µl, the CL intensity increases very slightly (Figure 5), and is then decreased whereas the *t*_{base} or peak width continues to increase rather rapidly and significantly with each increase in sample volume. This is because larger volumes of sample in the carrier stream mix with the reagents over a longer period of time causing alterations of the CL emission profile.

So, the selected sample loop in this FICL system was 100 µl because it gave high sensitivity with reasonable sample throughput (120 h⁻¹) and the consumption of standard or sample solutions injected.

Effect of the photomultiplier tube (PMT) voltage:

The effect of the PMT voltage was investigated ranging from 0.4 to 0.7 kV (maximum recommended by the manufacture). Ten injections of water, 0.10 and 0.20 µg ml⁻¹ cefadroxil standard solution were performed. There was an exponential relation between the CL intensity and the PMT voltage, both noise and analytical signal increased as the PMT voltage increases; the signal-to-noise ratio reached a maximum value at 0.55 kV and above 0.55 kV the background noise increased considerably. Thus, 0.55 kV was selected as the optimum value to give a high signal with acceptable noise. The influence of PMT voltage on the cefadroxil CL signal was illustrated in Figure 6.

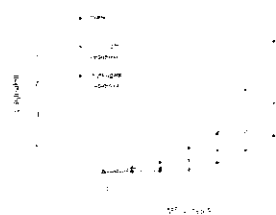


Figure 6 Effects of PMT voltage on the cefadroxil CL signal at concentration of 0.10 and 0.20 µg ml⁻¹.

Interferences Study:

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 5 µg ml⁻¹ of cefadroxil. It was considered not to interfere if a foreign material caused a relative error of less than ± 5% during the determination of 5 µg ml⁻¹ cefadroxil. The results showed that no interference had been found when including up to a 1000-fold Na⁺, K⁺, Mg²⁺, Ba²⁺, Ca²⁺, SO₄²⁻, NO₃⁻, PO₄³⁻, glucose, lactose, sucrose, citric acid, saccharin, sorbitol, and starch.

Summary of Optimized Experimental Conditions.

The ranges over which the variables involved in the proposed FICL system studied and their optimum conditions are listed in Table 1.

Table 1 The optimized of the FICL conditions for the determination of cephalixin, cefaclor and cefadroxil

Variables	Studied range	Optimum conditions
KMnO ₄ concentration (mM)	0.05 - 2.0	0.5
H ₂ SO ₄ concentration (M)	0.05 - 7.0	4.0
Formaldehyde concentration (%v/v)	0.1 - 12	5
Injection volume (µl)	50 - 400	100
Total flow rate (ml min ⁻¹)	1.5 - 9.0	6.0
PMT voltage (kV)	0.40 - 0.70	0.55

Analytical Applications:

The proposed FICL method was successfully applied to the determination of cephalixin, cefaclor and cefadroxil in commercial pharmaceutical formulations. The results are presented in Table 2.

It was found that the results obtained were in agreement with those obtained by the pharmacopoeia method, which is based on HPLC [25].

Table 2 Results for the determination of cephalosporins in samples by the proposed and official methods (n=5)

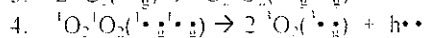
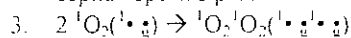
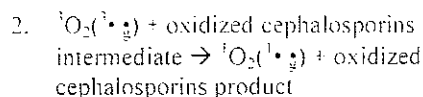
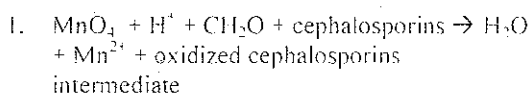
Preparation	FICL method (% content)	Official method (% content)
Cephalexin capsules 1	101.4 ± 2.2 98.4 ± 2.1	101.1 ± 2.0 100.4 ± 1.9
Cephalexin capsules 2	99.5 ± 1.9	100.2 ± 1.6
Cephalexin powder 3	102.2 ± 1.8	99.8 ± 2.0 103.2 ± 1.9
Cefaclor capsules 1	101.2 ± 1.8	100.2 ± 1.8
Cefaclor capsules 2	1.8	
Cefadroxil capsules 1	102.5 ± 2.0	

Analytical characteristics for cephalosporins analysis:

Under the optimum conditions, calibration graphs over the ranges of 0.1-8.0 µg ml⁻¹ for cephalexin and cefaclor, and 0.1-10.0 µg ml⁻¹ for cefadroxil were obtained. The limits of detection (3σ) for cephalexin and cefaclor were 0.05 µg ml⁻¹, and for cefadroxil was 0.02 µg ml⁻¹. The relative standard deviation (RSD) for replicate injections (n=11) of 4.0 µg ml⁻¹ of the investigated drugs were found to be 1.23 %, 1.20% and 1.07% for cephalexin, cefaclor and cefadroxil, respectively. This is indicating that the method was very reproducible. The accuracy of the proposed method was verified by 5 replicate injections of 100 µg of commercial drug sample solutions at concentrations of 2.0 µg ml⁻¹. Commercial formulations were spiked with known amounts of the drug in the concentration ranges 0.1-3.0 µg ml⁻¹. The percentage recovery of 99.4±1.7%, 99.0±1.5% and 99.2±1.4% for cephalexin, cefaclor and cefadroxil, respectively, were obtained.

Possible reaction mechanism.

It has previously been suggested [26] that KMnO₄ could react with some reductants in the presence of formaldehyde to produce ¹O₂(¹••), a dimeric oxygen molecule in the singlet state, which emits light as it relaxes to ³O₂(³••), a triplet state oxygen. During the transformation, it could produce CL and the formaldehyde could accelerate oxidation reaction rate [27]. Thus, it is possible that the singlet excited molecular oxygen species is an emitter in the present system, formed by the transfer of energy from oxidized cephalosporins to dissolved oxygen. Based on the above discussion, a possible mechanism for this process is:



Conclusions

Based on the chemiluminescence reaction of the studied cephalosporins with KMnO₄ in sulfuric acid, sensitized by formaldehyde, a novel flow injection CL method was developed for the determination of cephalexin, cefaclor and cefadroxil. The proposed method was simple, rapid and sensitive, and was successfully applied to quantitative analysis of these cephalosporins in pharmaceutical preparations and compared with pharmacopoeia method.

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