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Short communication

Flow injection chemiluminescence determination of cefadroxil using potassium permanganate and formaldehyde system

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Abstract

A simple, rapid and precise flow injection chemiluminescence (FI-CL) method is proposed for the determination of cefadroxil and is suitable for application to other antibiotics containing phenolic hydroxyl groups. A possible mechanism for this selectivity is suggested. The method is based on the CL-emitting reaction between cefadroxil and potassium permanganate in sulfuric acid medium, enhanced by formaldehyde (HCHO). Under the optimum conditions, calibration graphs over the ranges of 0.05–0.8 and 1.0–10.0 $\mu g\,ml^{-1}$ were obtained. The proposed method was successfully applied to the determination of cefadroxil in pharmaceutical formulations with no evidence of interference from common excipients. The detection limit (3σ) of this method is 25 $ng\,ml^{-1}$ (6.9×10^{-8} $mol\,l^{-1}$). The relative standard deviation was less than 2% for 0.4 and 4.0 $\mu g\,ml^{-1}$ cefadroxil ($n = 20$). The sample throughput was found to be 120 h^{-1}.

Keywords: Cefadroxil; Chemiluminescence; Flow injection chemiluminescence; Formaldehyde; Pharmaceutical analysis

1. Introduction

Cefadroxil is a semisynthetic cephalosporin antibiotic, which expresses a potential activity against many bacterial infections, and has been widely used as an oral medicine. It is indicated for the treatment of patients with infection caused by susceptible strains of the designated organisms in the following diseases: urinary tract infections caused by *E. coli*, *P. mirabilis* and *Klebsiella* species. Skin and skin structure infections caused by staphylococci and/or streptococci. Pharyngitis and/or tonsillitis caused by *Streptococcus pyogenes* (Group A beta-hemolytic streptococci) [1,2]. Cefadroxil is a white to yellowish-white crystalline powder, soluble in water and is acid-stable. It is chemically designated as [6*R*[6a,7b(*R**)]]-7-[*N*′-[2′-amino,2′-(4″-hydroxyphenyl)acetyl]-amino]-1-aza-3-methyl-8-oxo-5-thiabicyclo [4.2.0]oct-2-ene-2-carboxylic acid monohydrate and its structure has been established [3]. Its chemical formula is $C_{16}H_{17}N_3O_5S{\cdot}H_2O$ with the molecular weight of 381.40.

Various analytical techniques have been reported for the determination of cefadroxil in pharmaceutical preparations and/or in biological fluids, including spectrophotometry [4–8], spectrofluorometry [9], polarography [10,11] and liquid chromatography [12–15]. However, they often suffer from a variety of limitations. But analytical methods applying chemiluminescence (CL) [16–27] provide many advantages for determining pharmaceuticals such as high sensitivity, high selectivity, small amount of chemical consumption, instrumental simplicity (no monochromator required) and speed of in signal detection (~0.1–10 s). Since many CL reactions are very fast, they give rise to imprecise measurements as a result of irreproducible mixing of sample and reagents, but the reproducibility and selectivity of CL analysis can be improved by combination with flow injection analysis (FIA). Recently, there have been some reported procedures in the literature for the determination of cefadroxil in pharmaceutical formulations or biological fluids by using flow injection with CL detection. Aly et al. [28], used such a method based on the CL reaction of acidified potassium

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permanganate sensitized by quinine for the determination of cefadroxil monohydrate in pharmaceutical samples and biological fluids. They reported that, this method was convenient to determine 0.1–30 μg ml^{-1} cefadroxil in the range with a detection limit of 0.05 μg ml^{-1}. Tomita and Bulhoes [29] reported the generation of CL upon reaction of cefadroxil with electrochemically generated tris(2,2′-bipyridyl)ruthenium(II), [Ru(bpy)$_3$$^{3+}$]. The electrogenerated chemiluminescence (ECL) coupled to a FIA system was used for the determination of cefadroxil in pharmaceutical samples. A linear calibration curve was obtained for deoxygenated solutions over the range 5×10^{-8} to 1×10^{-4} mol l^{-1}. Yao et al. [30] reported a new FIA-CL method for cephalosporins including cefadroxil, based upon the enhancing effect of cefadroxil on the CL reaction of luminol with potassium periodate in an alkaline solution. Sun et al. [31] described a FIA-CL method for three cephalosporin antibiotics: cefalexin, cefadroxil and cefazolin sodium, based upon the enhancing effect of these antibiotics on the CL reaction of glyoxal with potassium permanganate in acidic solution. The method gave a detection limit of 2 ng ml^{-1} for cefadroxil.

In this present work, the development of a FIA-CL system for the determination of cefadroxil is proposed, based on the CL-emitting reaction between cefadroxil and potassium permanganate in acidic medium, the sensitivity being greatly enhanced by formaldehyde.

2. Experimental

2.1. Apparatus

A Gilson® Minipuls-2 peristaltic pump (Villiers-le-Bel, France), a Rheodyne® low-pressure injector model 5041 (Cotati, CA), PVC tubing (Elkay, Galway, Ireland) with 1.6 mm i.d. and PTFE tubing (0.5 mm i.d.) were employed to set up the FIA manifold. The CL measurements were carried out by using a home-made luminometer consisting of a PTFE T-piece for mixing the reagents, a flat coil glass flow cell placed in a vertical plane, the flat shape facing the window of the photomultiplier tube (PMT) (Electron Tubes Ltd., model 9789 QB, Ruislip, London); the luminometer was housed in a light-tight aluminium cylinder. The CL signal was recorded by a chart recorder (Chessell®, Kipp and Zonen, The Netherlands).

2.2. Reagents

All chemicals were of analytical reagent grade. Purified water obtained by reverse osmosis and deionised to a resistivity of ≥5 MΩ cm (Elgastat option 4, Elga, High Wycomb, UK) was used throughout. A stock 0.01 mol l^{-1} solution of potassium permanganate was prepared daily by dissolving approximately 0.2 g of $KMnO_4$ (Fisher Scientific Chemicals, Loughborough, Leicester, UK) in 100 ml of water and standardized before used. An oxidant (5×10^{-4} mol l^{-1} $KMnO_4$) solution was prepared by dilution of this stock solution with water. A solution of 5% (v/v, formaldehyde was prepared by dissolving formaldehyde (Fisher Scientific Chemicals, UK) in 5.0 mol l^{-1} sulfuric acid. A stock standard solution containing 1000 μg ml^{-1} cefadroxil (Sigma, Pool Dorset, UK) was prepared by dissolving 100 mg of cefadroxil in 100 ml water.

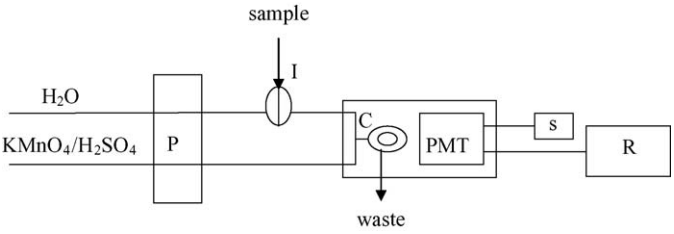

Fig. 1. The FIA manifold used in the preliminary experiments; Carrier stream–water; reagent stream–potassium permanganate in aqueous sulfuric acid; (P) peristaltic pump; (I) injection valve; (C) flat spiral-coiled glass tube flow cell; (PMT) photomultiplier tube; (S) PMT source power supply; (R) chart recorder. The experimental conditions were: carrier stream–water (1.5 ml min^{-1}); reagent stream–2.5×10^{-4} mol l^{-1} $KMnO_4$ in 0.5 mol l^{-1} H_2SO_4 (1.5 ml min^{-1}); sample volume, 125 μl; PMT source power supply: 0.60 kV.

2.3. Procedure

2.3.1. FIA-CL

The FIA manifold was designed and fabricated as shown in Fig. 2. A 100 μl sample or standard was injected into water carrier. Acidic 5.0×10^{-4} mol l^{-1} $KMnO_4$ was used as the oxidant and 5% (v/v) formaldehyde solution in 5.0 mol l^{-1} H_2SO_4 as the enhancing reagent, supplied at 5.0 mol l^{-1} but diluted three-fold by merging with the reagent streams before the CL reaction occurs. The water carrier stream, together with the reagent streams (formaldehyde solution in 5.0 mol l^{-1} H_2SO_4 and an oxidant, $KMnO_4$) both flow at 2.0 ml min^{-1}. The PMT was operated at 0.55 kV, provided by a stable high voltage power supply. A chart recorder recorded the signal and the peak height was measured for quantification.

2.3.2. Calibration

Working standard solutions of cefadroxil over the ranges of 0.05–0.80 and 1.0–10.0 μg ml^{-1} were prepared from the stock

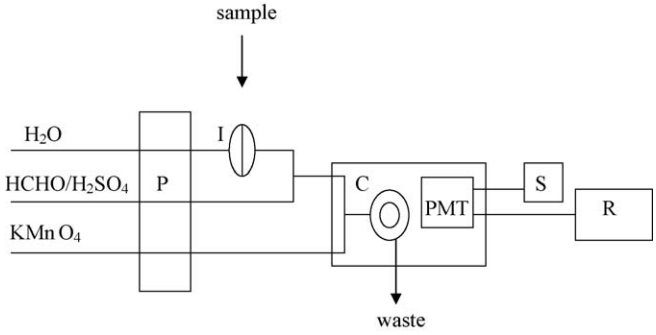

Fig. 2. Three-channel manifold designed for cefadroxil determination (symbols as in Fig. 1). (P) Peristaltic pump; (I) injection valve; (C) flat spiral-coiled glass tube flow cell; (PMT) photomultiplier tube; (S) photomultiplier source power supply; (R) chart recorder. The experimental conditions were: carrier stream–water; reagent stream 5.0×10^{-4} mol l^{-1} $KMnO_4$ and 5% (v/v) of formaldehyde in 5.0 mol l^{-1} H_2SO_4 .The flow rates are 2.0 ml min^{-1} per channel. Sample volume: 100 μl; PMT power supply: 0.55 kV.

Table 1
Optimization of the proposed FIA and chemical conditions for cefadroxil determination

Variable	Studied range	Optimum conditions
$KMnO_4$ concentration (mol l^{-1})	0.5×10^{-4} to 8.0×10^{-4}	5.0×10^{-4}
H_2SO_4 concentration (mol l^{-1})	0.1–8.0	5.0
Formaldehyde concentration% (v/v)	1–20	5
Injection volume (μl)	50–400	100
Total flow rate (ml min^{-1})	1.5–9.0	6.0
Flow rate of H_2O carrier (ml min^{-1})	0.5–3.0	2.0
Flow rate of $KMnO_4$ (ml min^{-1})	0.5–3.0	2.0
Flow rate of $HCHO/H_2SO_4$ (ml min^{-1})	0.5–3.0	2.0
PMT voltage (kV)	0.40–0.70	0.55

solutions (1000 μg ml^{-1} cefadroxil). Triplicate 100 μl portions of the standard solutions of cefadroxil were injected in triplicate into the carrier stream (H_2O) (Fig. 1) and the resulting peak height was measured. Calibration graphs were obtained by plotting the CL intensity (mV) against concentration of cefadroxil.

2.3.3. Determination of cefadroxil in pharmaceutical preparations

Pharmaceutical products containing cefadroxil (Cefadril capsules 500 mg) were purchased from commercial sources in Chiang Mai Province, Thailand. Ten capsules each containing 500 mg of cefadroxil as stated on the label were accurately weighed, ground and mixed. After fine powdering of the capsule contents by means of a porcelain mortar, an accurately weighed portion of the pooled sample equivalent to 100 mg of cefadroxil was dissolved in 100.0 ml distilled of water and sonicated for 2 min. Hundred microliter portions of the sample solutions, prepared from these stock solutions were injected into the finally proposed FI-CL manifold (Fig. 2) and the cefadroxil content was determined from the corresponding regression equation.

3. Results and discussion

3.1. Preliminary work

Initially, the reactivity of 100 μg ml^{-1} cefadroxil standard solution with different oxidants in different media was tested by aspirating the standard solution in one channel and the reagent in the other channel in a two-channel continuous flow system (at total flow rate 3.2 ml min^{-1} with equal flows in each channel). The oxidants assayed were 2.0 mol l^{-1} H_2SO_4 solutions that were in 1×10^{-4} mol l^{-1} $KMnO_4$, 1×10^{-4} mol l^{-1} Ce(IV), 0.01 mol l^{-1} H_2O_2 mixed on-line with 1×10^{-4} mol l^{-1} Fe(II), 0.01 mol l^{-1} KIO_4, 0.01 mol l^{-1} $K_2S_2O_8$, 0.10 mol l^{-1} $KBrO_3$, 1×10^{-3} or 1×10^{-4} mol l^{-1} $K_2Cr_2O_7$, or they were 0.1 mol l^{-1} NaOH solutions that were in 0.01 mol l^{-1} or 1×10^{-3} mol l^{-1} $K_3[Fe(CN)_6]$, 0.1 mol l^{-1} H_2O_2 or was H_2O_2 in water only. It was found that cefadroxil solution emitted weak CL with $K_3[Fe(CN)_6]$ in NaOH medium and emitted the most intense CL with $KMnO_4$ in sulfuric acid. There was no observed CL emitted from the other oxidants studied. Thus, $KMnO_4$ in sulfuric acid was selected as the oxidant for further study. Next, the continuous flow manifold was changed to the FIA manifold as shown in Fig. 1 which was similar to that previously reported by Aly et al. [28]. In order to improve the sensitivity of the CL signal, pure water was used as the carrier instead of 0.5 mol l^{-1} H_2SO_4 solution to eliminate Schlieren noise occurring by injecting sample solution into the reagent stream. In addition, acidic permanganate solution was used in the second flow line. The influences of experimental variables were studied.

3.2. Effects of variables

The following variables were optimized in order to obtain greatest sensitivity. The optimum values are given in Table 1.

3.2.1. Effects of other chemicals

The influence of others chemicals (metals, surfactants and potential enhancers) which dissolved in the water carrier stream, was studied on the CL of 20 μg ml^{-1} cefadroxil. Most metals ions studied (Fe (II), Fe (III), Al (III), Cu (II) and Co (II) had no significant effect at a concentration of 1×10^{-3} mol l^{-1} but Mn (II) and Ni (II) increased the signal by 16 and 13%, respectively.

Of several surfactants studied (at concentration of 1×10^{-2} mol l^{-1}); sodium dodecyl sulphate, cetylpyridinium bromide, tetradecyltrimethylammonium bromide, Triton X-100 and Tween 80, the first three diminished the signal (∼17, 6 and 47%, respectively). Triton X-100 and Tween 80 caused increase 8 and 16%, respectively.

The following potential enhancers, rhodamine B (1×10^{-6} to 1×10^{-5} mol l^{-1}), rhodamine 6G (1×10^{-6} to 1×10^{-5} mol l^{-1}), fluorescein (1×10^{-6} to 1×10^{-4} mol l^{-1}), fluorescamine (1×10^{-6} to 1×10^{-4} mol l^{-1}), 8-hydroxyquinoline (1×10^{-6} to 1×10^{-5} mol l^{-1}), acetaldehyde (0.5 mol l^{-1}), glutadialdehyde (0.5 mol l^{-1}), formic acid (0.5 mol l^{-1}) and formaldehyde (5×10^{-3} to 0.5 mol l^{-1}) were investigated. All slightly diminished the CL signal, except formic acid and formaldehyde (both 0.5 mol l^{-1}) that increased the signal approximately 40 and 108%, respectively. Hence, formaldehyde was chosen as an enhancer for further study.

Finally, the manifold was changed to a three-channel version (Fig. 2) in which pure water was used as carrier stream and a separate stream of $HCHO/H_2SO_4$ was merged with the water stream after injection of the sample. A $KMnO_4$ stream was then merged with the merged stream of the water and the $HCHO/H_2SO_4$ streams. This avoids Schlieren noise, and is different to Aly et al. [28] system.

3.3. Optimization of experimental variables on the FIA-CL determination of cefadroxil sensitized by 5% formaldehyde

The experimental conditions of the proposed FI-CL manifold (Fig. 2) were optimized by means of a univariate method. Greatest sensitivity was achieved using 3.0×10^{-4} to 5.0×10^{-4} $KMnO_4$, 5.0×10^{-4} mol l^{-1} $KMnO_4$ was chosen for subsequent investigations and 5.0 mol l^{-1} H_2SO_4 was selected.

The effect of formaldehyde concentration was examined over the range 1–20% (v/v). It is shown that peak heights increase with increasing formaldehyde concentration over the range 1–5% (v/v), above which the signal decreases gradually. Therefore, 5% (v/v) formaldehyde in 5 mol l^{-1} H_2SO_4 was applied for subsequent studies.

Total flow rates between 1.5 and 9 ml min^{-1} (with 0.5–3 ml min^{-1} per channel) were studied under the above conditions. The optimum total flow rate was 6 ml min^{-1}. Similarly, selected sample loop system was 100 μl because it gave high sensitivity with reasonable sample throughput (120 h^{-1}).

3.4. Analytical characteristics for cefadroxil determination

The proposed FI-CL method was tested for linearity, precision, sensitivity and reproducibility. Under the optimized conditions, a linear relationship between cefadroxil concentration (X) and CL intensity (Y) was obtained over the range 0.05–0.8 μg ml^{-1} (100 μl per injection) with a regression equation of $Y = 3.52X + 0.121$ ($n = 5$) and correlation coefficient (r^2) of 0.9994. As with many CL methods, the calibration line is not linear for this system over the concentration range of 1.0–10.0 μg ml^{-1} cefadroxil and the trend is better modelled by a second order polynomial regression with the equation of $Y = -0.10X^2 + 3.22X + 0.978$ ($r^2 = 0.9995$; $n = 5$) where Y is the CL intensity in mV and X is the concentration expressed in μg ml^{-1}.

The lower detection limit of cefadroxil (defined as three times of the standard deviation, 3σ) was found to be 25 ng ml^{-1} or 6.9×10^{-8} mol l^{-1} cefadroxil. The accuracy of the proposed method was verified by five replicate injections of 100 μl of commercial drug sample solutions at concentrations of 0.2 and 2.0 μg ml^{-1} cefadroxil. Commercial formulations were spiked with known amounts of the drug in the concentration ranges 0.1–0.5 and 1.0–5.0 μg ml^{-1}, respectively. The percentage recoveries were found to be between 99.1 and 103.0. The mean percentage recovery (±S.D.) for cefadroxil capsules was found to be 101.2 ± 1.42. The relative standard deviation (R.S.D.) for replicate injections ($n = 20$) of 0.4 and 4.0 μg ml^{-1} cefadroxil were found to be 1.23 and 1.07%, respectively, indicating that the method was very reproducible. The sample throughput of the method was 120 h^{-1}.

3.5. Effect of excipients

The influences of some common excipients (glucose, sucrose, lactose, saccharin, citric acid, sorbitol and starch) used in pharmaceutical preparations were investigated. The procedure consists of preparing fourteen different synthetic solutions, each containing cefadroxil (5 μg ml^{-1}) and only one excipient (50 and 500 μg ml^{-1}); the CL signals of these solutions were measured. The recoveries of cefadroxil, expressed as the mean value ± standard deviation of five replicates per sample are summarized in Table 2. It can be seen that glucose, sucrose, lactose, saccharin, citric acid, sorbitol and starch had no effect on the determination of cefadroxil even through they are present at a 10 or 100 times weight ratio to cefadroxil.

Table 2
Mean recovery value ± standard deviation for determination of 5 μg ml^{-1} cefadroxil in the presence of common excipients

Excipeint	Weight ratio (excipient:cefadroxil)	Recovery ± S.D. (%) ($n = 5$)
Glucose	10:1	99.7 ± 0.6
	100:1	99.2 ± 1.1
Sucrose	10:1	100.0 ± 0.8
	100:1	100.9 ± 0.9
Lactose	10:1	101.6 ± 1.6
	100:1	100.0 ± 1.5
Saccharin	10:1	101.1 ± 1.4
	100:1	101.2 ± 1.1
Citric acid	10:1	99.6 ± 1.6
	100:1	95.4 ± 1.9
Sorbitol	10:1	99.4 ± 1.4
	100:1	98.6 ± 1.1
Starch	10:1	101.8 ± 1.6
	100:1	102.0 ± 1.2

3.6. Analytical application

3.6.1. Analytical figures of merit

Under the optimum conditions (Table 1), the linear calibration range was 0.05–0.08 μg ml^{-1} with the regression equation $Y = 3.52X + 0.121$ ($r^2 = 0.9994$, $n = 5$). The LOD (S/N = 3) and the LOQ (S/N = 10) were 25 and 83.33 ng ml^{-1}, respectively. Aly et al. [28] first described the FI-CL method for cefadroxil based on permanganate CL reaction using quinine as enhancer. They claimed LOD of 50 ng ml^{-1} (S/N = 2) which was corresponded to 75 ng ml^{-1} (S/N = 3) indicating that the present procedure has improved the LOD three-fold. The sensitivity (defined as the slope of the calibration graph) was twice as great as that reported by Aly et al. [28]. The LOD was also lower than that reported by Morelli [32] (0.19 and 0.51 μg ml^{-1} at 229.5 and 245.5 nm, respectively, based on second order derivative spectrophotometry, and Al-Momri et al. [33] (40 μg ml^{-1}, based on FI method in which cefadroxil was hydrolyzed in NaOH solution followed by treatment with 1,4-phenylenediamine and Fe(III) in H_2SO_4 solution to yield a violet solution with an absorption maximum at 600 nm. A method based on the enhancing effect of cefadroxil on the CL reaction between luminol and periodate in an alkaline medium gave a LOD of 10 ng ml^{-1} [30] and the FI-CL method based on the enhancing effect of the drug on the CL reaction of glyoxal and $KMnO_4$ in H_2SO_4 solution provided a LOD of 2 ng ml^{-1} cefadroxil [31], which are much lower than that obtained by the present method. Although the

Table 3
Results for the determination of cefadroxil in capsule samples by the proposed and official methods ($n = 5$)

Sample	Taken ($\mu g\,ml^{-1}$)	Found ($\mu g\,ml^{-1}$)	Found (%)	Official[b] method (%)
Cefadril capsules[a] (500 mg cefadroxil per capsule)	0.2	0.20 ± 0.01	101.4 ± 2.59	
	0.5	0.51 ± 0.01	101.0 ± 1.66	
	3.0	3.11 ± 0.03	103.8 ± 0.89	
	4.0	4.14 ± 0.08	103.6 ± 2.03	
	5.0	5.16 ± 0.08	103.3 ± 1.55	
	8.0	7.94 ± 0.18	99.3 ± 2.18	
Mean ± S.D.			102.0 ± 1.82	101.8 ± 0.90
Student's *t*-value			0.28 (2.31)[c]	
Variance ratio			4.09 (6.39)[d]	

[a] Lerd Singh, Bangkok, Thailand.
[b] BP method [34].
[c] Tabulated *t*-value at ($P = 0.05$) [35].
[d] Tabulated *F*-value at ($P = 0.05$) [35].

proposed FI-CL method was not as sensitive as the above-cited methods, the present method is simple and rapid ($120\,h^{-1}$). As the aim of this research is to develop a relatively sensitive and rapid FI-CL procedure for cefadroxil determination in commercial pharmaceutical preparations, and could be adopted to use for drug (cefadroxil) quality control, such high sensitivity is not needed.

3.6.2. Analysis of pharmaceutical samples

The proposed FI-CL method was successfully applied to the determination of cefadroxil in a commercial pharmaceutical formulation (Cefadril capsules containing 500 mg of cefadroxil per capsule). The determination simply required dissolution of the samples in water by placing them in an ultrasonic bath for 2 min. The sample solutions were analysed by the recommended FI-CL method under optimum experimental conditions. The drug contents in each sample solution were determined by reference to the calibration graph obtained under identical experimental conditions. The results obtained were in agreement with that obtained by the official method [34], based on HPLC (Table 3). Statistical analysis of the results was also undertaken [35]. In no case did the calculated *F* and *t* values exceed the theoretical values, which confirms that there were no significant differences between the mean values obtained by both methods with respect to precision and accuracy.

3.7. Possible applications to the analysis of other drugs

Under the optimum conditions described above, the responses of determine other cephalosporin antibiotics (cephalexin, cephradine, cefamandole, cefoxitin, cefazolin and cefoperazone) and other antibiotics (tetracycline, oxytetracycline and amoxicillin) under the proposed FI-CL method were investigated. The results are presented in Table 4.

They show that in the absence of formaldehyde only weak signals were obtained. However, in the presence of formaldehyde, all the studied antibiotics gave much greater CL signals than those obtained in the absence of formaldehyde. Interestingly, cefoperazone and amoxicillin contain a phenolic –OH moiety in the *para* position in their molecules, similar to cefadroxil, and show a greater CL signal than some of the other drugs studied. Thus, this proposed FI-CL method could be applied to determination of other phenolic β-lactam antibiotics. The possible CL reaction mechanism for the oxidation of these molecules is considered below.

3.8. Mechanistic implications

Formaldehyde enhances permanganate CL from a variety of analytes and while it might simply accelerate the oxidation reaction rate, phenolic reductants with an –OH moiety as a *para* substituent, such as cefadroxil, could make a specific contribution to the production of the CL signal. It has previously been suggested [36] that $KMnO_4$ could react with some reductants in the presence of formaldehyde or formic acid to produce $^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$, a dimeric oxygen molecule in the singlet state, which emits light as it relaxes to $^3O_2(^3\Sigma_g)$, a triplet state oxygen. The increase in CL intensity is similar to the increase in the CL emission when a polyhydric phenol is added to an alkaline chemiluminogenic mixture of hydrogen peroxide and formaldehyde, known as the Trautz–Schorigin reaction. The reaction has been studied thoroughly with gallic acid and the mechanism proposed proceeds via the formation of a semiquinone intermediate

Table 4
CL intensities data of other cephalosporins and some antibiotics studied (both without and with formaldehyde) using the proposed FI-CL method

Antibiotics studied	Peak height (mV)[a]	
	Without formaldehyde	With formaldehyde
Cephalexin[b]	0.06	0.72
Cephradine[b]	0.05	0.60
Cefamandole[b]	0.08	0.80
Cefoxitin[b]	0.12	2.26
Cefazolin[b]	0.04	1.26
Cefoperazone[b]	0.45	5.84
Amoxicillin[b]	0.28	3.20
Tetracycline[c]	0.07	4.24
Oxytetracycline[c]	0.20	6.20

[a] Average from three determinations.
[b] $10\,\mu g\,ml^{-1}$.
[c] $100\,\mu g\,ml^{-1}$.

and excited molecular oxygen species (excimers) are finally generated [37]. 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 cefadroxil to dissolved oxygen. Based on the above discussion, a possible mechanism for this process is:

1. $MnO_4^- + H^+ + CH_2O + \text{cefadroxil} \rightarrow H_2O + Mn^{2+}$ + oxidized cefadroxil intermediate;
2. $^3O_2(^3\Sigma_g) + \text{oxidized cefadroxil intermediate} \rightarrow {}^1O_2(^1\Delta_g)$ + oxidized cefadroxil product;
3. $^1O_2(^1\Delta_g) \rightarrow {}^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g)$;
4. $^1O_2{}^1O_2(^1\Delta_g{}^1\Delta_g) \rightarrow 2\,{}^3O_2(^3\Sigma_g) + h\nu$.

4. Conclusions

A simple, rapid and highly sensitive flow injection with chemiluminescence detection is described for the determination of cefadroxil. The method is based on an enhancing effect of formaldehyde on the CL peak obtained by the reaction of cefadroxil with potassium permanganate in sulfuric acid. The method has been applied successfully for determining cefadroxil in pharmaceutical samples with a high sampling rate of 120 h^{-1} and high precision (1.23 and 1.07% R.S.D., $n = 20$ for determination of 0.4 and 4.0 $\mu g\,ml^{-1}$ cefadroxil, respectively). The detection limit is 25 $ng\,ml^{-1}$ or 6.9×10^{-8} $mol\,l^{-1}$ cefadroxil. The proposed FI-CL method has the advantages of being rapid, simple, inexpensive, accurate and highly sensitive. Thus, it may be suitable for quality control in drug industries where cefadroxil and/or other cephalosporin antibiotics are manufactured. It might, after suitable development, also be applicable to a wider group of substances having phenolic groups in their structures. This method could be very useful for routine analysis, possibly used for automation and in the study of dissolution profiles. It could also be coupled post-column in HPLC, with on-line extraction or preconcentration and separation of the analytes, which makes it suitable for samples extracted from biological fluids or tissues; it might therefore also find application in pharmacokinetic or bioavailability studies.

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