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Permanganate-based chemiluminescence analysis of cefadroxil monohydrate in pharmaceutical samples and biological fluids using flow injection

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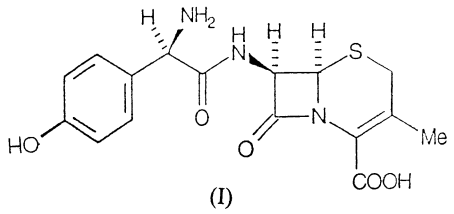
Abstract

A chemiluminescent method using flow injection is described for the determination of cefadroxil monohydrate. The method is based on the chemiluminescence reaction of cefadroxil with potassium permanganate in sulphuric acid, sensitized by quinine. The proposed procedure allows the determination of cefadroxil over the concentration range 0.1–30 μg ml^{-1} with a detection limit of 0.05 μg ml^{-1} and a sample measurement frequency of 150 samples h^{-1}. The method was successfully applied to the determination of cefadroxil in pharmaceutical preparations and biological fluids.

Keywords: Chemiluminescence; Cefadroxil; Potassium permanganate; Pharmaceutical analysis; Biological fluids

1. Introduction



(I)

Cefadroxil monohydrate (I) is a semi-synthetic cephalosporin recently introduced into clinical practice. It is active by oral route on the sensitive gram-positive and gram-negative organisms [1]. Numerous analytical procedures have been reported for its determination either in pure form, in pharmaceutical preparations or in biological fluids. The US P XXIII [2] recommends an HPLC assay, for the evaluation of the raw material and its dosage forms. Other reported methods include: spectrophotometric [3–11], fluorimetric [12–14], polarographic [15], TLC [16] and HPLC [17–20]. Reviewing the literature revealed that, up to the

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present time nothing has been published concerning the chemiluminescence (CL) determination of cefadroxil.

Analytical procedures applying (CL) measurements in flow-injection (FI) setups combine the advantages of instrument simplicity (no monochromator required), rapidity in signal detection (normally 0.1–10 s), sensitivity and ease of use. 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 the CL analysis can be improved by combination with an FI method.

FI chemiluminometric methods based on potassium permanganate oxidation have been reported for the determination of many drugs e.g. morphine [21], loprasolam [22], isoprenaline [23], catecholamines [24] and anaesthetics[25].

Chemiluminescent methods have been used for the determination of some antibiotics e.g. tetracyclines [26–28], cephalothin [29] and penicillins [30].

The present paper describes the development of an FI method based on the CL reaction of potassium permanganate sensitized by quinine for the routine determination of cefadroxil in bulk, in dosage forms and biological fluids.

2. Experimental

2.1. Instruments and flow system

The flow system used for determination and CL detection of cefadroxil, shown schematically in Fig. 1. A Gilson Minipuls 3MP4 peristaltic pump (two channels, variable speed) was used to drive the carrier and the reagent streams through the flow system. Each stream was pumped at a constant flow rate of 3.7 ml min^{-1} using PTFE tubing (0.8 mm i.d.). Cefadroxil solution was injected through the sample injection valve which allows mixing of the sample with the acid and then combination with the $KMnO_4$ solution just before the detector. The emitted light was measured by a photomultiplier tube (Thorn EMI, 9789QB). The signal was recorded by a Yokogawa model 3021 recorder (Yokogawa, Japan). Peak heights were measured.

2.2. Reagents and materials

Analytical reagent grade chemicals and double distilled water were used throughout. Cefadroxil was kindly provided by Bristol-Myers, Squibb, Egypt and used as received. Dosage forms containing cefadroxil being purchased from commercial sources. Cefadroxil standard solution, 1 mg ml^{-1} was prepared in distilled water. Working standard solutions of cefadroxil were prepared by appropriate dilution immediately before use.

Aqueous potassium permanganate (Fluka, UK), 5×10^{-4} M solution.

Sulphuric acid (BDH, Poole, UK), 0.5 M solution.

Aqueous quinine sulphate solution (BDH, Poole, UK) 50 μg ml^{-1} was prepared from 1 mg ml^{-1} stock solution.

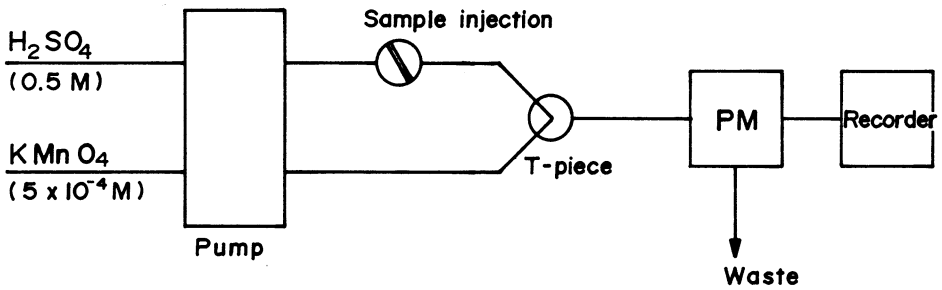


Fig. 1. FI manifold for the chemiluminescent determination of cefadroxil monohydrate: P, peristaltic pump; S, sample port; T, perspex T-piece; L, luminometer; W, waste.

2.3. Procedure

2.3.1. Procedure for calibration

Working cefadroxil-quinine-solution containing cefadroxil in the range of 0.1–30 μg ml^{-1} and quinine sulphate, 50 μg ml^{-1} were prepared from the stock solutions. A 200 μl portion of the above solution was injected into a stream of 0.5 M H_2SO_4 solution which then combined with a stream of 5×10^{-4} M $KMnO_4$ and the resulting peak height was measured. A calibration graph was prepared by plotting the peak height against the concentration of cefadroxil.

2.3.2. Procedure for dosage forms (capsules and suspensions)

Weigh accurately a quantity of the mixed contents of 10 capsules (Ultracef 500 mg per capsule: Bristol-Myers, Squibb, USA) or measure accurately a suitable volume of oral suspension (Ultracef 125 mg per 5 ml: Bristol, Myers, Squibb, USA) equivalent to 10 mg of the drug. Transfer into a 100 ml volumetric flask and dilute to the mark with distilled water. Sonicate for 5 min. Proceed as described above under procedure for calibration. Calculate the nominal content from the corresponding calibration graph or regression equation.

2.3.3. Procedure for biological fluids

2.3.3.1. Procedure for spiked plasma. Add an aliquot of standard aqueous solution of cefadroxil (1 mg ml^{-1}) to 5.0 ml of plasma sample. Add 1.0 ml of 10% (w/v) trichloroacetic acid for each ml of the plasma for deproteination. Blend on a vortex mixer and centrifuge at 3000 rpm for 10 min. Transfer 5.0 ml of the protein-free supernatant into a 25 ml volumetric flask and dilute to volume with distilled water. Proceed as described above. A blank value was determined by treating antibiotic-free plasma in the same way.

2.3.3.2. Procedure for spiked urine. Add a quantity of cefadroxil to the urine to obtain a concentration of 10.0 mg ml^{-1}. Transfer 1.0 ml of this solution into a 100 ml volumetric flask and dilute to volume with distilled water. Proceed as described above. A blank value was determined by treating the antibiotic-free urine in the same way.

3. Results and discussion

The flow-injection chemiluminometric determination of cefadroxil was studied using different oxidants such as potassium dichromate, sodium persulphate, potassium iodate, cerium(IV) sulphate, potassium permanganate and *N*-bromosuccinimide in acidic or basic media. The CL of cefadroxil was obtained only when potassium permanganate was used as an oxidant in an acidic medium.

3.1. Configuration designs

The FI configuration used for the determination of cefadroxil was so designed to provide different reaction conditions for magnifying the CL signal generated by the reaction of cefadroxil with $KMnO_4$. Maximum CL intensity was obtained when the sample was injected into a stream of 0.5 M H_2SO_4 and then mixed with $KMnO_4$ just before the detector.

3.2. Optimization of experimental variables

A series of experiments were conducted to establish the optimum analytical variables. The parameters optimized included reagent concentrations and some physical variables including the total flow rate and the sample injection volume.

3.2.1. Effect of different acid concentrations

Four different acids (i.e. H_3PO_4, CH_3COOH, HCOOH and H_2SO_4) of different concentrations in the range 1×10^{-3}–5.0 M were tested in order to ascertain which was the most suitable. Maximum CL intensity was obtained with 0.5 M H_2SO_4 (Table 1). This was further established using different concentrations of H_2SO_4 with different concentrations of the drug (10–80 μg ml^{-1}).

Table 1
Effect of different acid concentrations on the CL intensity of cefadroxil monohydrate (20 μg ml^{-1})

Concentration of acid (M)	CL intensity[a] (mV)			
	H_2SO_4	H_3PO_4	CH_3COOH	HCOOH
1×10^{-3}	0.3	0.1	0.0	0.0
1×10^{-2}	2.9	0.9	0.0	0.2
0.1	9.0	2.4	0.3	0.8
0.5	12.8	3.2	1.0	3.2
2.0	12.0	3.2	1.8	4.5
5.0	12.0	3.2	2.7	6.6

[a] Each result is the average of three separate determinations.

3.2.2. Effect of potassium permanganate concentration

Fig. 2 shows the effect of $KMnO_4$ concentration on the CL intensity. The greatest CL response was obtained with 5×10^{-4} M. Larger concentrations of $KMnO_4$ lowered the CL intensity. Therefore, 5×10^{-4} M $KMnO_4$ was used.

3.2.3. Effect of sensitizers

Based on the observation that some of the fluorescing compounds can be used for energy-transfer in the CL reactions with an enhancement of the intensity [31,32], various fluorophores were investigated for obtaining maximum yields in CL intensity. Different concentrations (0.5–100 μg ml^{-1}) of rhodamine-B, fluorescein and quinine sulphate dissolved in the drug solution or in the carrier or in the $KMnO_4$ solution were investigated. It was found that only quinine enhanced the CL signal when dissolved in the drug solution. About 50 μg ml^{-1} of quinine gave rise to the most intense signal, so this concentration of quinine was used in all subsequent studies.

Quinine sulphate was previously tested as an energy transfer-reagent in the CL reaction of Ce(IV) with thiol-containing drugs [33,34].

3.2.4. Effect of reagents flow-rate

The flow rate of the reagent solutions was optimized in order to obtain satisfactory CL emission. The flow-rate is conveniently controlled by the peristaltic pump. The effect of total flow-rate was studied, keeping all other conditions constant, over the range 3.6–10.5 ml min^{-1}, with equal flows in each channel. The results obtained show that the CL intensity continues to increase with increasing the total flow-rate up to 7.4 ml min^{-1} (3.7 ml min^{-1} for each channel). Above this flow-rate, the emission intensity started to be constant as shown in Fig. 3.

3.2.5. Effect of sample injected volume

An increase in sample volume normally leads to an increase of emitted CL signal. Fig. 4 shows that a change in loop size from 10–200 μl can improve the CL intensity by a factor of 3, further increase in sample volume from 200–800 μl in-

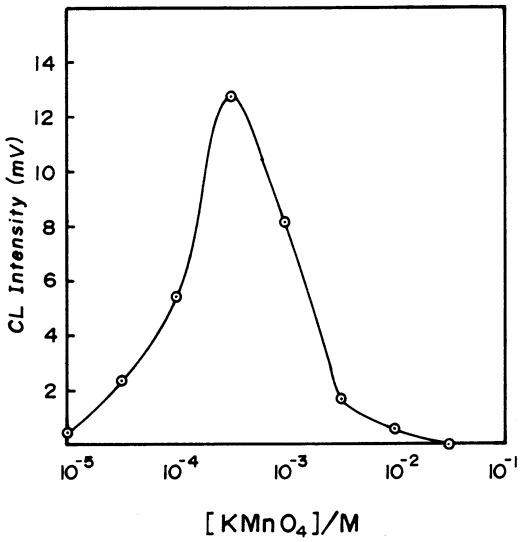


Fig. 2. Effect of potassium permanganate concentration on the cefadroxil CL emission. Injected drug solution (200 μl), 20 μg ml^{-1}, flow-rate 3.7 ml min^{-1}.

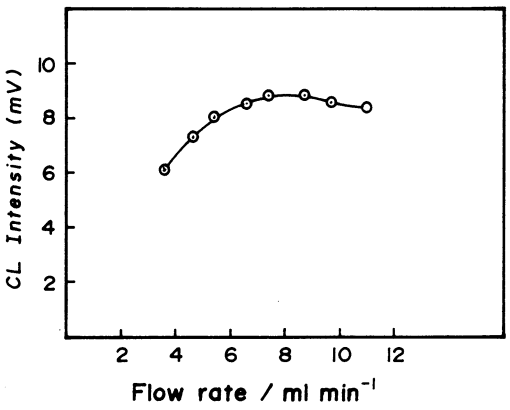


Fig. 3. Effect of total flow-rate on CL intensity of cefadroxil. Injected drug solution (200 μl), 20 μg ml^{-1}.

creases the CL intensity much slower. Therefore, 200 μl was considered the optimum sample injected volume in the FI system.

3.2.6. Effect of some micellar solutions

The effect of some organized systems, including neutral surfactants (Triton X-100), cationic surfactants (cetyltrimethylammonium bromide, cetylpyridinium bromide and cetylpyridinium chloride), and anionic surfactants (sodium dodecyl sulphate) on the CL reaction was investigated.

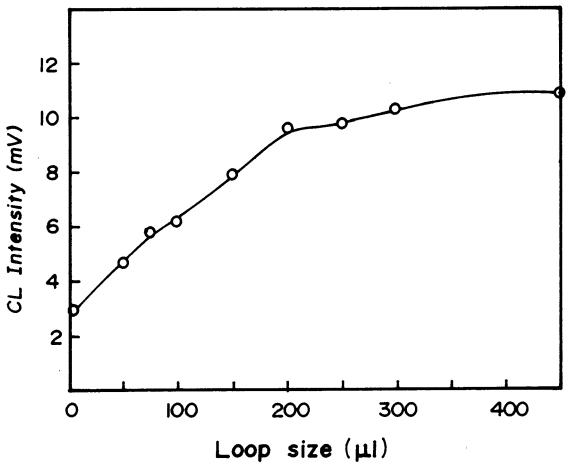


Fig. 4. Effect of sample volume of cefadroxil on CL emission. Injected drug solution 20 μg ml^{-1}.

All these surfactants had no effect on the CL intensity.

4. Determination of cefadroxil monohydrate

Under the described experimental conditions, a standard calibration curve for cefadroxil was constructed. The CL intensity (I, mV) was linearly related to cefadroxil concentration over the range of 0.1–30 μg ml^{-1} with a minimum detectability ($S/N = 2$), of 0.05 μg ml^{-1}. Linear regression analysis of the results gave the following equation.

$$C = -0.494 + 1.988I \quad (r = 0.9999, n = 8)$$

The precision of the method was evaluated by analyzing pure samples of cefadroxil. The results in Table 2 were in accord with those obtained by the USP XXII [35].

5. Possible CL mechanism

The CL mechanism may be attributed to the following reactions:

$$\text{Cefadroxil} + MnO_4^- + 8H^+$$

$$\rightarrow \text{Oxidized cefadroxil}^* + Mn^{+2} + 4\ H_2O$$

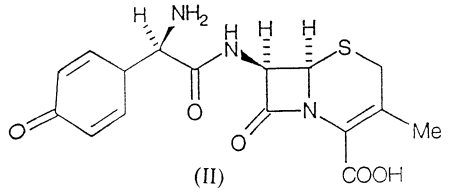
In the presence of quinine, the energy resulting from the redox reaction can be effectively transferred to quinine which in turn generates CL emission.

$$\text{Oxidized cefadroxil}^* + \text{Quinine}$$

$$\rightarrow \text{Oxidized cefadroxil} + \text{Quinine}^*$$

$$\text{Quinine}^* \rightarrow \text{Quinine} + \text{light}$$

The CL emission of oxidized cefadroxil(II) may be ascribed to the oxidation of the phenolic group in the para position of the benzene ring to form a cyclohexadienone structure (II). This was further confirmed by unsuccessful trials for the determination of cephalexin, which has a similar structure to cefadroxil but without the phenolic group, by the proposed method. This indicates that the presence of a phenolic group is essential for the CL signal of cefadroxil.



(II)

6. Application of the method

6.1. Analysis of pharmaceutical preparation

The proposed method was successfully applied to the analysis of some dosage forms containing cefadroxil. The results in Table 2 are in accordance with those obtained by the official method [35].

Statistical analysis [36] of the results reveals that there is no significant difference between the two methods as regards to accuracy and precision.

6.2. Analysis of spiked urine and plasma samples

The high sensitivity attained by the proposed method allows the determination of cefadroxil in biological fluids. About 88% of a dose of cefadroxil is excreted in the urine unchanged [37]. Mean peak serum concentrations of cefadroxil in healthy subjects after 0.5 and 1.0 g doses were 15 and 26 μg ml^{-1}, respectively, 2 h after administration [37]. Thus the proposed method proved to be satisfactory for the kinetic studies and routine estimation of cefadroxil in human urine and plasma. For plasma only a deproteination process was carried out using trichloroacetic acid as a sample pre-treatment, an extraction procedure was not necessary [38].

Antibiotic-free urine samples gave a relatively high CL intensity, so a dilution of 1:100 (v/v) was required to minimize this interference. The diluted urine gave a very low CL intensity. Table 3 shows the results of the recovery studies of cefadroxil from spiked plasma and urine.

Table 2
Application of the proposed and official methods to the determination of cefadroxil monohydrate and its dosage forms

Drug form	Taken (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)	Official method [35] recovery (%)
Cefadroxil (pure sample)	0.5	0.500	100.0	
	1.0	0.997	99.7	
	2.5	2.488	99.5	
	10.0	9.843	98.4	
	20.0	20.179	100.9	
Mean ± SD			99.7 ± 0.9	100.5 ± 1.4
Ultracef capsules[a] (500 mg cefadroxil capsule)	1.0	1.000	100.0	
	2.5	2.500	100.0	
	5 0	5.000	100.0	
	10.0	10.130	101.3	
	20.0	20.240	101.2	
Mean ± SD			100.5 ± 0.7	100.2 ± 0.8
Ultracef suspension[a] (125 mg cefadroxil/5 ml)	1.0	1.000	100.0	
	2.5	2.500	100.0	
	5.0	5.055	101.1	
	10.0	10.230	102.3	
Mean ± SD			100.9 ± 1.1	100.5 ± 0.9

[a] Bristol-Myers, Squibb, USA.

Table 3
Determination of cefadroxil in spiked urine and plasma

Concentration taken (μg ml^{-1})	Found (%)	
	Urine	Plasma
5.0	100.0	96.7
10.0	100.0	96.3
15.0	100.0	95.1
20.0	100.0	95.4
25.0	99.3	94.7
Mean ± SD	99.9 ± 0.3	95.7 ± 0.8

7. Conclusion

A simple, rapid and highly sensitive chemiluminometric method is described for the determination of cefadroxil in dosage forms and in biological fluids. The method described requires only a deproteination of plasma samples and dilution of urine samples to avoid the liable interference. The method can be used for HPLC detection. Trials were made for the determination of other cephalosporins (e.g. cephalexin, cephradine and cefotaxime) and other antibiotics (e.g. kanamycin and neomycin) by the proposed method but no CL signals were obtained.

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