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Short communication

# Flow-injection chemiluminescent determination of cefprozil using Tris (2,2′-bipyridyl) ruthenium (II)-permanganate system

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**Abstract**

A rapid and sensitive chemiluminescence (CL) method using flow-injection (FI) has been developed for the determination of a second generation cephalosporin, cefprozil. The method is based on the CL reaction of cefprozil with acidic potassium permanganate and tris (2,2′-bipyridyl) ruthenium (II), Ru $(bipy)_3^{2+}$. The CL intensity is greatly enhanced when quinine sulfate is used as a sensitizer. After optimization of the different experimental parameters, a calibration graph was obtained over a concentration range of 0.1–3.0 μg $ml^{-1}$ with minimum detectability of 0.005 μg $ml^{-1}$ (S/N = 3).

The correlation coefficient was 0.9998 ($n = 6$) with a relative standard deviation (%R.S.D.) of 1.63% for 2.0 μg $ml^{-1}$. The proposed method was successfully applied to commercial tablets. The average percentage recovery ($n = 6$) was 99.9 ± 1.40.


*Keywords:* Cefprozil; Chemiluminescence; Flow-injection; Potassium permanganate; Tris (2,2′-bipyridyl) ruthenium (II); Pharmaceutical preparations

## 1. Introduction

Cefprozil, 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7-[[amino(4-hydroxyphenyl)acetyl]amio]-8-oxo-3-(1-propenyl)-, (6*R*, 7*R*)-7-[(*R*)-2-amino-2-(*p*-hydroxyphenyl) acetamido]-8-oxo-3-propenyl-5-thia-1-azabicyclo[4.2.0.]oct-2-ene-2-carboxylic acid; is a semisynthetic oral second generation cephalosporin consists of 90:10 *Z*/*E* isomeric mixture with a wide antibacterial spectrum of activity [1,2].

The drug is the subject of a monograph in the United States Pharmacopoeia, USP 27 [1]. The most methods of analysis of the drug in bulk form or in its pharmaceutical formulations rely on the use of high-performance liquid chromatography (HPLC) methods. Few techniques have been reported to determine cefprozil either in pure form, pharmaceutical preparations or biological fluids; including colorimetry [3–5], UV spectrophotometry [6] and HPLC [7–9]. Abdel Sattar et al. [3] described a spectrophotometric method for the determination of cefprozil. This method is based on the reaction of the drug as *n*-donor with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as a π-acceptor and the colored radical anion was measured at 460 nm.

This method was used as a reference method for validation of the proposed method.

A similar flow-injection chemiluminescence method [10] was recently described for the determination of some cephalosporins, such as cefoxitin, cefazolin, cephalexin, cefadroxil, cefaclor and cefoperazone in pharmaceutical preparations using the tris (2,2′-bipyridyl) ruthenium (II)-potassium permanganate chemiluminescence in the presence of perchloric acid catalyzed by Mn(II). The calibration curves were linear from 0.1 up to 15 μg $ml^{-1}$ with detection limits of 0.03–0.08 μg $ml^{-1}$.

Chemiluminescence (CL) reactions have been used for sensitive, selective and rapid detection in flow-injections and chromatographic analysis [11]. One of the most interesting CL reactions is that involving the oxidation of (2,2′-bipyridyl) ruthenium (II), Ru $(bipy)_3^{2+}$ to Ru $(biyp)_3^{3+}$ which is then followed by reduction with an analyte species with the subsequent emission of light [12].

This study describes the development of a new simple FI-CL method for the determination of cefprozil based on the CL generated by the reaction of the drug with Ru $(bipy)_3^{2+}$ and potassium permanganate in a sulfuric acid medium. This method

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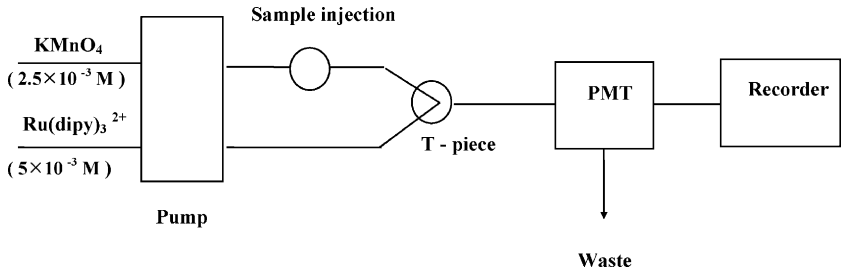


Fig. 1. Flow-injection manifold for CL determination of cefprozil.

has been satisfactorily applied to the determination of the studied drug in tablets.

## 2. Experimental

### 2.1. Apparatus and manifold

The flow system used for the determination and CL detection of cefprozil is 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 using PTFE tubing (0.8 mm i.d.).

The drug solution (500 μl) was injected through the sample injection valve, which allows the mixing of the sample with an acidified $2.5 \times 10^{-3}$ M $KMnO_4$ solution and then combination with $5 \times 10^{-3}$ M Ru $(bipy)_3^{2+}$ solution just before the detector. The emitted light intensity was measured by a photomultiplier tube (PMT, THORN EMI 9789 QB), which was operated at 1100 V. The PMT was provided by a stable power supply (THORN EMI, Model PM 288 BN). The signal was recorded by a Yokogawa model 3021 recorder (Yokogawa, Japan). Peak height was measured for each signal and expressed as voltage output of the photomultiplier tube.

### 2.2. Reagents and materials

All reagents used were of analytical reagent grade, and the solutions were prepared with distilled water. The following reagents were used: aqueous potassium permanganate (Fluka, UK) stock solution $2.5 \times 10^{-3}$ M was prepared in 0.2 M sulfuric acid (Reidel-de Haen, Germany); aqueous Ru $(bipy)_3^{2+}$ (Aldrich, Chem. Co.) solution of $5 \times 10^{-3}$ M was prepared by dissolving Ru $(bipy)_3^{2+}$ hexahydrate in distilled water; aqueous quinine sulfate (BDH, UK) stock solution of 100 μg $ml^{-1}$ in water.

Anhydrous cefprozil (99.9%) reference standard was kindly supplied by Bristol-Myers Squibb (Egypt); cefzil tablets (Batch No. 3C74253) each labelled to contain 500 mg anhydrous cefprozil and manufactured by Bristol-Myers Squibb (USA); were obtained from commercial sources.

### 2.3. Preparation of standard solution

Stock solution (1.0 mg $ml^{-1}$) of anhydrous cefprozil was prepared by dissolving 50.0 mg of cefprozil in distilled water in a 50 ml measuring flask, this solution is stable for 7 days if was kept in the refrigerator. Working standard solution was obtained by serial dilution with distilled water.

### 2.4. General procedure

The FI manifold shown in Fig. 1 was used. Working drug solutions were prepared by mixing different aliquots of stock drug solution (1.0 mg $ml^{-1}$) with 5 ml of quinine sulfate (100 μg $ml^{-1}$) in a 10 ml volumetric flasks and diluting to the mark with distilled water. A 500 μl portion of each working solution was injected into a carrier stream of acidified $2.5 \times 10^{-3}$ M $KMnO_4$ solution which was then combined with a stream of $5 \times 10^{-3}$ M Ru $(bipy)_3^{2+}$ solution. The resulting peak height in milli volts was measured and plotted against drug concentration to obtain a calibration curve. Alternatively, the regression equation was derived.

### 2.5. Procedure for tablets

Ten tablets were weighed and finely grounded. A weighed amount of the fine powder equivalent to 10 mg of anhydrous cefprozil was dissolved in distilled water by sonication for 10 min. The solution was filtered into a 100 ml volumetric flask and the filtrate was diluted to volume with distilled water. This solution, labelled to contain 100 μg $ml^{-1}$ of drug, was analyzed by the FI-CL procedure as described above. Nominal content of tablets was calculated either form a previously plotted calibration graph or by using the regression equation.

## 3. Results and discussion

Although analytical methods applying CL measurements have advantages of simplicity, sensitivity and ease of use [13,14], yet they may give rise to imprecise measurements; as many CL reactions are very fast. The reproducibility, selectivity and rapidity in signal detection of CL analysis can be highly improved by combination with FI technique [13–17].

Recently, Ru $(bipy)_3^{2+}$ has become a useful CL reagent [11,16,17]. As previously reported [12,18–21], Ru $(bipy)_3^{3+}$ was obtained from Ru $(bipy)_3^{2+}$ using different oxidants, such as $HNO_3$, $PbO_2$, Ce (IV), $Cl_2$ and $KMnO_4$. In this work, trials were made using different oxidants such as Ce (IV), Fe (III), *N*-bromosuccinimide, $H_2O_2$, $K_2Cr_2O_7$, $KMnO_4$, $KIO_3$, $KBrO_3$ and $K_2S_2O_8$ in an acidic or basic medium. The CL signal was obtained only on using $KMnO_4$ in acidic medium which was

then used to oxidize Ru $(bipy)_3^{2+}$ and the latter was then reduced by cefprozil with the emission of light.

### 3.1. Configuration designs

A two lines FI manifold was used for the chemiluminometric determination of cefprozil, which was designed to provide different reaction conditions for magnifying the CL signal. Maximum CL intensity was obtained when the sample was injected into a stream of acidified $KMnO_4$ and then mixed with Ru $(bipy)_3^{2+}$ just before the detector (Fig. 1).

### 3.2. Optimization of experimental conditions

A series of experiments were conducted to establish the optimum reaction conditions for the FI-CL determination of anhydrous cefprozil by injecting 500 μl of 1.0 μg ml$^{-1}$ of the drug solution containing 50 μg ml$^{-1}$ quinine sulfate into the carrier stream at a flow rate of 1.6 ml min$^{-1}$ in each channel. The parameters optimised included reagent concentrations and some manifold parameters.

#### 3.2.1. The effect of sulfuric acid concentration

Solutions of $H_2SO_4$ of different concentrations ranging from 0.1 to 1.0 M were studied as diluent for $KMnO_4$. The highest CL response was obtained with 0.2 M $H_2SO_4$, higher and lower concentrations showed lower intensity.

#### 3.2.2. Effect of potassium permanganate concentration

Upon testing different concentrations of $KMnO_4$ in the range $1 \times 10^{-4}$–$1 \times 10^{-2}$ M, maximum CL intensity was obtained with $2.5 \times 10^{-3}$ M $KMnO_4$. Higher and lower concentrations caused lower intensity.

#### 3.2.3. Effect of Ru $(bipy)_3^{2+}$ concentration

Optimal concentration of Ru $(bipy)_3^{2+}$ was found to be $5 \times 10^{-3}$ M. Lower CL intensity was obtained on using concentrations of the reagent higher or lower than $5 \times 10^{-3}$ M.

#### 3.2.4. Effect of sensitizers

Chemiluminescent molecules can potentially transfer their excitation energy to a fluorophore sensitizer with subsequent emission of energy by the fluorophore [22]. To study their effect as sensitizers on cefprozil CL, different concentrations (1–100 μg ml$^{-1}$) of Rhodamine B, fluorescein and quinine sulfate dissolved in the drug solution were investigated. It was found that the three sensitizers enhanced the CL signal but quinine sulfate showed the highest enhancement and Rhodamine B the lowest. Thus quinine sulfate was recommended and maximum CL signal was obtained with a concentration of 50 μg ml$^{-1}$ quinine sulfate.

#### 3.2.5. Effect of total flow rate

The total flow rates of Ru $(bipy)_3^{2+}$ and acidified $KMnO_4$ streams were varied over the range 1.0–10.0 ml min$^{-1}$ with equal flows in each channel. The best total flow rate was found to be 3.2 ml min$^{-1}$ (1.6 ml min$^{-1}$ for each channel).

#### 3.2.6. Effect of sample volume

The sample volume injected was investigated using volumes varied from 30 to 1000 μl. The obtained results revealed increased CL intensity up to 500 μl of cefprozil solution, above which the intensity was decreased.

### 3.3. Determination of cefprozil

Under the optimum conditions mentioned above, a series of working cefprozil solutions each containing 50 μg ml$^{-1}$ quinine sulfate were injected; each as three replicates. A plot of CL intensity ($I$, mV) versus drug concentrations was found to be linear over the range of 0.1 − 3.0 μg ml$^{-1}$ of anhydrous cefprozil with a LOD of 0.005 μg ml$^{-1}$ (S/N = 3) and a LOQ of 0.02 μg ml$^{-1}$. Linear regression analysis of the data gave the following equation:

$$I = 13.64 + 17.88C \quad r = 0.9998$$

where $I$ is the CL intensity and $C$ is the concentration in μg ml$^{-1}$. Perfect linearity in the calibration curve was obvious from the correlation coefficient ($r$ = 0.9998) obtained by linear regression; Table 1. This table revealed good accuracy of the proposed FI-CL procedures (100.7–100.9%). Repeatability was confirmed with a R.S.D. of 1.63% ($n$ = 6 for 2 μg ml$^{-1}$ sample) whereas reproducibility evaluated over a period of 2 weeks was checked by R.S.D. value of 2.58%.

### 3.4. Analytical application

The proposed FI-CL procedure was successfully applied to the direct analysis of cefprozil in cefzil tablets. Average percent recovery of 99.9 ± 1.40 was obtained which was in accordance with those obtained by a reported method [3]; Table 2. In addition, statistical analysis [23] of these results using $F$-test and Student's $t$-test showed no significant differences between the two methods.

The analytical figures of merit of this proposed method for the studied drug have been compared to those from earlier investigation and summarized in Table 3. However, the proposed FI-CL procedure is highly sensitive, selective and rapid.

Table 1
Validation of the proposed FI-CL procedure

| Parameter | Value |
|---|---|
| Linearity range[a] (μg ml$^{-1}$) | 0.1–3.0 |
| LOD (S/N = 3)(μg ml$^{-1}$) | 0.005 |
| LOQ (μg ml$^{-1}$) | 0.02 |
| Slope ± S.D. | 17.88 ± 0.146 |
| Intercept ± S.D. | 13.64 ± 0.319 |
| Correlation coefficient ($r$) | 0.9998 |
| Accuracy ± R.S.D.: | |
| Intraday | 100.9 ± 1.63 |
| Interday | 100.7 ± 2.58 |

[a] $n$ = 6.

Table 2
Analysis of cefprozil in bulk powder and tablets by proposed FI-CL and a reported method

| | Proposed method | | Reported method [3] |
|---|---|---|---|
| | Concentration taken ($\mu g\,ml^{-1}$) | Recovery (%) | Recovery (%) |
| Bulk powder | 0.1 | 102.0 | |
| | 1.0 | 101.5 | |
| | 1.5 | 103.1 | |
| | 2.0 | 101.2 | |
| | 2.5 | 98.9 | |
| | 3.0 | 99.2 | |
| Mean ± S.D. | | 100.98 ± 1.634 | 100.1 ± 1.103[a] |
| Variance ratio *F*-test | | 2.194 (5.05)[b] | |
| Student's *t*-test | | 1.093 (1.812)[c] | |
| Cefzil tablets | | | |
| (500 mg anhydrous cefprozil/tablet)[d] | 0.1 | 97.9 | |
| | 1.0 | 100.5 | |
| | 1.5 | 101.2 | |
| | 2.0 | 98.8 | |
| | 2.5 | 101.5 | |
| | 3.0 | 99.7 | |
| Mean ± S.D. (%) | | 99.93 ± 1.404 | 98.5 ± 1.94[a] |
| Variance ratio *F*-test | | 1.91 (5.05) | |
| Student's *t*-test | | 1.463 (1.812) | |

[a] Analyzed by charge transfer complexation between cefprozil and DDQ reagent ($n = 6$).
[b] Tabulated *F*-values at ($P = 0.05$) [23].
[c] Tabulated *t*-values at ($P = 0.05$) [23].
[d] Bristol-Myers Squibb (USA).

Table 3
Comparison of the analytical figures of merit of the proposed FI-CL method with earlier reported methods for the determination of cefprozil

| Techniques | Linear range ($\mu g\,ml^{-1}$) | Correlation coefficient (*r*) | Limit of detection (LOD) ($\mu g\,ml^{-1}$) | Limit of quantitation (LOQ) ($\mu g\,ml^{-1}$) | Reference |
|---|---|---|---|---|---|
| Colorimetry | | | | | |
| Chloranilic acid | 50–400 | 0.9986 | 10 | – | [3] |
| 2,3-Dichloro-5,6-dicyano-1,4-benzo-quinone, DDQ) | 20–140 | 0.993 | 3 | – | [3] |
| 7,7,8,8-Tetracyano quino-dimethone | 1–7 | 0.9996 | 0.3 | – | [3] |
| Ninhydrin | 2–14 | 0.9989 | 0.5 | – | [3] |
| Folin Ciocalteu | 2.5–25 | 0.9996 | 0.5 | – | [3] |
| Colorimetry | | | | | |
| Nitration with $HNO_3 + H_2SO_4$ | 7–21 | 0.9994 | 0.047 | 0.363 | [4] |
| Sodium nitrite + copper acetate | 4–16 | 0.9996 | 0.144 | 0.330 | [4] |
| *o*-Nitroaniline + sodium nitrite | 2–10 | 0.9997 | 0.074 | 0.211 | [4] |
| *o*-Nitroaniline + sodium nitrite + copper sulfate | 2–8 | 0.9999 | 0.159 | 0.110 | [4] |
| Colorimetry | | | | | |
| Ce IV | 5–30 | 0.9999 | 0.017 | 0.055 | [5] |
| Fe III | 5–30 | 0.9999 | 0.097 | 0.323 | [5] |
| UV Spectrophotometry | | | | | |
| First derivative in HCl | 0.5–2.5 (mg%) | 0.9999 | – | – | [6] |
| First derivative in phosphate buffer | 0.5–2.5 (mg%) | 0.9998 | – | – | [6] |
| Absorbance difference | 0.5–2.5 (mg%) | 0.9997 | – | – | [6] |
| First derivative difference | 0.5–2.5 (mg%) | 0.9995 | – | – | [6] |
| HPLC with UV detection at 280 nm | | | | | |
| Cis cefprozil | 0.1–25 | 0.9999 | – | 0.1 | [8] |
| Trans cefprozil | 0.02–2.5 | 0.9989 | – | 0.02 | [8] |
| Proposed FI-CL method | 0.1–3 | 0.9998 | 0.005 | 0.02 | The proposed method |

### 3.5. Proposed CL mechanism

By analogy to previously reported papers [16,17,24,25], the proposed mechanism may involve the oxidation of Ru $(bipy)_3^{2+}$, phenolic-OH and/or the tertiary amine in the β-lactam ring present on cefprozil by acidic $KMnO_4$. The oxidation product of phenolic group undergoes deprotonation to form a radical. This reduces the formed Ru $(bipy)_3^{3+}$ to the excited state with the subsequent emission of light as shown below:

$$Ru(bipy)_3^{2+} + MnO_4^- + 8H^+ \rightarrow Ru(bipy)_3^{3+} + Mn^{2+} + 4H_2O \quad (1)$$

$$Cefprozil + MnO_4^- + 8H^+ \rightarrow Cefprozil^{\bullet +} + Mn^{2+} + 4H_2O \quad (2)$$

$$Cefprozil^{\bullet +} \rightarrow Cefprozil^{\bullet} + H^+ \quad (3)$$

$$Ru(bipy)_3^{3+} + Cefprozil^{\bullet} + H_2O \rightarrow [Ru(bipy)_3^{2+}]^{\bullet} + \text{cefprozil fragment} + H^+ \quad (4)$$

$$[Ru(bipy)_3^{2+}]^{\bullet} \rightarrow Ru(bipy)_3^{2+} + light \quad (5)$$

## 4. Conclusion

Most of the few methods reported for the analysis of cefprozil are either HPLC, which require special skill and sophisticated instrumentation or UV spectrophotometry which is non-selective technique. In this work, a simple, sensitive and selective FI-CL procedure has been investigated for the rapid determination of cefprozil in pharmaceutical formulations. It requires no sample treatment and solutions can be analyzed at a rate of 120 sample $h^{-1}$.

The proposed FI-CL method is characterized by its sensitivity and minimum limit of detectability compared to the similar FI-CL method [10] described for the determination of some cephalosporins.

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