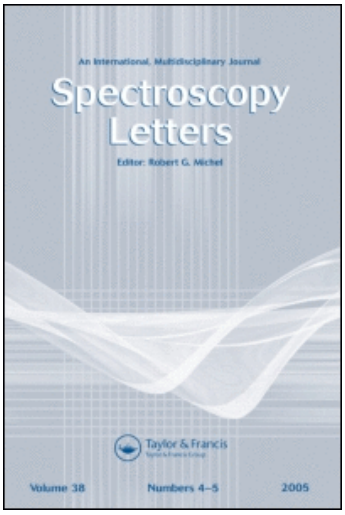


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Direct Determination of Cefadroxil by Chemiluminescence Using a Multicommutated Flow-Through Sensor

L. Molina-García[a]; E. J. Llorent-Martínez[a]; M. L. Fernández-de Córdova[a]; A. Ruiz-Medina[a]
[a] Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Campus Las Lagunillas, Jaén, Spain

Online publication date: 19 January 2010

To cite this Article Molina-García, L. , Llorent-Martínez, E. J. , Córdova, M. L. Fernández-de and Ruiz-Medina, A.(2010) 'Direct Determination of Cefadroxil by Chemiluminescence Using a Multicommutated Flow-Through Sensor', Spectroscopy Letters, 43: 1, 60 — 67
To link to this Article: DOI: 10.1080/00387010903261156
URL: http://dx.doi.org/10.1080/00387010903261156

Spectroscopy Letters, 43:60–67, 2010

ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010903261156

Taylor & Francis
Taylor & Francis Group

Direct Determination of Cefadroxil by Chemiluminescence Using a Multicommutated Flow-Through Sensor

L. Molina-García, E. J. Llorent-Martínez, M. L. Fernández-de Córdova, and A. Ruiz-Medina

Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Campus Las Lagunillas, Jaén, Spain

ABSTRACT A selective and direct chemiluminescence method was developed for the determination of cefadroxil, a broad-spectrum antibiotic effective in gram-positive and gram-negative bacterial infections. The method involves the implementation of solid-phase spectroscopy in a multicommutated flow system. The solid support is placed in the flow-through cell, and the reaction between the oxidant reagent (permanganate) and cefadroxil takes place on the microbeads, so yielding the analytical signal. The system showed a linear dynamic range of 2.7–110 μM, with a detection limit of 0.8 μM and an RSD of 3.3% ($n = 10$). The analyte was satisfactorily determined in pharmaceutical preparations and human urine.

KEYWORDS cefadroxil, chemiluminescence, multicommutation, sensor, SPS

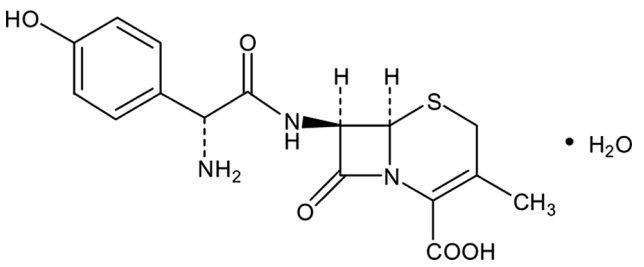
INTRODUCTION

Cefadroxil monohydrate (CFD) is a semisynthetic cephalosporin antibiotic used in clinical practice for treatment of serious infections caused by susceptible strains of microorganisms in the following diseases: lower respiratory infections, genitor-urinary infections (*E. coli, P. mirabilis, and Klebsiella* species), gynecologic infections, skin infections (*Staphylococci and/or streptococci*), pharyngitis and/or tonsillitis (*Streptococcus pyogens*; Group A beta-hemolytic streptococci), and central nervous system infections.

Cephalosporins operate by inhibiting active bacterial cell wall biosynthesis that grows actively against a wide range of both gram-positive and gram-negative bacteria. The positive results of these drugs include the resistance of penicillinases and ability to treat of infections that are resistant to penicillin derivates. Side effects of the CFD include nausea, epigastria distress, diarrhea, vomiting, skin rash, genital pruritus, pseudo membranous colitis, and genital moniliasis.[1] However, people who are allergic to penicillin may have similar reactions when taking cephalosporin antibiotics such as CFD. 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

Address correspondence to A. Ruiz-Medina, Department of Physical and Analytical Chemistry, Faculty of Experimental Sciences, University of Jaén, Campus Las Lagunillas, E-23071 Jaén, Spain. E-mail: anruiz@ujaen.es

[4.2.0]oct-2-ene-2-carboxylic acid monohydrate, presenting the following structure:



Different analytical techniques have been reported for the determination of CFD in pharmaceuticals and/or biological fluids. Although there are some methods involving liquid chromatography with spectrophotometric detection[2,3] or even a voltammetric method,[4] most of the developed methods to date involve spectrophotometry[5–7] or spectrofluorimetry.[8,9] Chemiluminescence (CL) has also been employed. Analytical methods with CL detection[10–13] provide several advantages for determining CFD such as high sensitivity, small amount of chemical consumption, and instrumental simplicity. Most of them use flow injection analysis as well as different compounds to obtain an enhancement in the CL signal.

Nevertheless, it must be taken into account that many CL reactions are so fast that they give rise to imprecise measurements as a result of irreproducible mixing of sample and reagents. It happens, normally, by using batch mode or conventional flow methodologies. To avoid these problems in CL analysis, recent developments in flow analysis are being implemented. They have progressively achieved improvement of sample and reagents consumption, repeatability of the methods, and degree of automation of the systems. For examples, sequential injection analysis,[14] multisyringe flow injection analysis,[15] and multicommutated flow analysis[16] can be cited as the most important methodologies currently used. The combination of these new methodologies with solid-phase spectroscopy (SPS) has allowed the development of a new generation of sensors. They play an important role by offering additional separation devices and clean-up steps for analyzing complex matrices. In particular, the use of a solid support in a flow-through cell is introduced with the aim of improving the sensitivity and selectivity of the system,[17] as long as one avoids the use of additional enhancement reagents, commonly used in homogeneous solution.[18] The solid support could be used for the retention of the reagents, the target analyte, or even the product of the reaction. The enhancement in selectivity makes it possible to analyze complex samples, such as biological fluids, and that analysis would probably not be possible in homogeneous solution due to the high number of interfering species. For all these reasons, the combination of automatic methods with CL detection is becoming increasingly important in clinical and biological analysis.

The main goal of this article is to develop an automatic flow-through CL sensor for the direct determination of CFD, without fluorescing compound or additional reagent that produces the acceleration of the oxidation reaction rate.[10] The use of multicommutation is introduced due to its favorable intrinsic advantages such as low-cost equipment, high sample throughput, and simplicity, as long as automation is complete.[19] The proposed optosensor shows a linear dynamic range, repeatability, rapidity, and feasibility, showing satisfactory results when it is applied to the determination of CFD in pharmaceutical preparations and urine samples.

MATERIALS AND METHODS

Reagents and Solutions

CFD, potassium permanganate, sulphuric acid, acetonitrile, quinine, and formaldehyde were obtained from Sigma (Alcobendas; Madrid, Spain). All reagents were of analytical grade. The solid supports—Sephadex-SP C-25, Sephadex-QAE A-25, and Sephadex-DEAE A-25—all of them having 40–120-μm average particle size—were obtained from Sigma; C_{18} bonded phase silica gel beads, with 55–105-μm average particle size, was obtained from Waters (Milford, U.S.). Filters (Waters), with 2.7-μm pore size, were also used.

CFD stock solution of 500 μM was prepared by dissolving the required weight in deionized water; it was kept in the dark under refrigeration at 4°C and remained stable for at least 3 months. Required CFD solutions were prepared daily by suitable dilution with 0.1-M H_2SO_4. A stock 0.01-M solution of potassium permanganate was prepared daily and standardized before use; working solutions were obtained by dilution with deionized water.

Instrumentation

Homemade Luminometer

The photosensor module H8240–101 was obtained from Hamamatsu (U.S.) and consists of a 28-mm diameter side-on photomultiplier tube (PMT), a high-voltage power supply circuit, and a low-noise amplifier; the spectral response is 185–900 nm. The flow-through cell Hellma 137-QS (Hellma Hispania, S.L., Badalona, Spain) (1-mm light path and 260-μL inner volume) was employed. The cell was blocked at the outlet with glass wool to prevent displacement of the resin particles.

Both the flow cell and the photosensor module were encased in a light-tight homemade box, and the box was wrapped in black cloth. The cell was placed in front of the window of the photosensor module, with effective area of 4×20 mm; in addition, a piece of mirror was stuck in the back of the cell in order to collect the whole emission light.

The photosensor module requires a $\pm$ 15-V power supply, being the gain adjusted with an additional power supply, from 0.3 V up to 1.1 V. For acquiring the voltage provided by the photosensor module, the connector block SCB-68 and the NI-PCI-6221 M Series Multifunction DAQ Device from National Instruments (Madrid, Spain) were used. Data acquired by the PCI-6221 device were registered using VI Logger Datalogging Software (National Instruments, U.S.). Registered data were smoothed using the software Origin Pro 7.0 (OriginLab, U.S.).

Multicommutation System

The multicommutation system was built using a four-channel Gilson Minipuls-3 peristaltic pump (Villiers le Bel, France) fitted with a rate selector and pump tubing type Solvflex (Elkay Products, Shrewsbury, MA, U.S.). An electronic interface based on ULN 2803 integrated circuits (Unisonic Technologies Co., Ltd.; Da-Lian, China) was employed to generate the electric potential (12 V) and current (100 mA) required to control the four 161T031 NResearch three-way solenoid valves (Neptune Research; MA, U.S.). The home–made software for controlling the system was developed in Java. PTFE tubing of 0.8-mm i.d. and methacrylate connections were also used.

Other Apparatus

Other apparatus consisted of a Crison Model 2002 pH meter with a glass/saturated calomel combination electrode (Crison; Barcelona, Spain), a Selecta Ultrasons ultrasonic bath (Selecta; Barcelona, Spain), and a Selecta centrifuge S-240 (Selecta; Barcelona, Spain).

Preparation of Samples

Pharmaceuticals

Three pharmaceuticals available in the Spanish Pharmacopoeia, presented in the form of syrup and capsules, were analyzed. The syrup *Duracef* was presented as soluble powder; the whole powder was dissolved in deionized water (200 mL). In the case of the capsules *Cefadroxilo Sabater* and *Duracef,* two capsules of each pharmaceutical were dissolved in deionized water (500 mL). Appropriate dilutions with 0.1-M H_2SO_4 were made before measuring.

Urine Samples

Antibiotic-free urine samples were obtained from healthy volunteers. An appropriate aliquot of CFD stock solution and 2 mL of acetonitrile were added to 4 mL of urine sample. The solution was blended on a vortex mixer and centrifuged at 3000 rpm for 10 min. This solution was filtered through a 0.45-μm membrane filter (Millipore; U.S.) and diluted to volume with deionized water. The required dilution with 0.1-M H_2SO_4 was made before introduction into the flow system.

General Procedure

Multicommutated flow systems are typically constituted by a peristaltic pump and a set of three-way solenoid valves automatically controlled by appropriate software.[20] Each solenoid valve acts as a switch between two different positions, "OFF" and "ON", meaning that two of the three valve ports are permanently connected. The OFF position is the initial state of the valve, when the solenoid does not receive any electric signal; when a signal is applied, the position of the solenoid changes, and this state is called *ON position*. The manifold and the different positions of the valves are shown in Fig. 1.

In the initial condition, all valves are switched off, and the carrier solution (0.4-M H_2SO_4) is flowing through the flow cell, while all other solutions are recycling to their vessels. The steps for each sample determination were (see also scheme in Fig. 2)

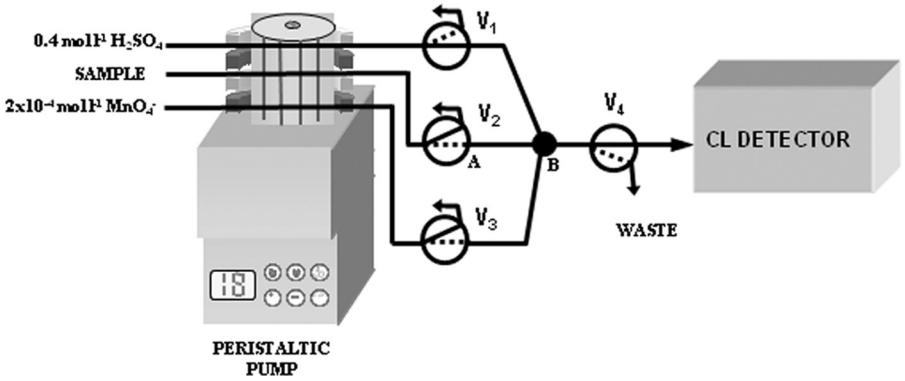


FIGURE 1 Manifold. For each solenoid valve, V_1, V_2,..., the solid and dotted lines refer to the 'OFF' and 'ON' positions respectively; solutions are flowing through the solid lines when no signal is applied to the solenoid.

(1) cleaning the portion of tubing placed between Points A and B (Fig. 1), filled with the last sample analyzed; (2) introducing the permanganate solution (2×10^{-4} M) by switching the valves V_1 and V_3 on for 70 s; (3) allowing the carrier solution to flow for 10 s in order to avoid the mixing of permanganate and CFD before they reach the sensing zone; (4) introducing the sample (2.7–110-μM CFD solution) into the system for 70 s by switching on the valves V_1 and V_2; and (5) finally, obtaining the analytical signal (CL emission intensity as peak height), being the solid support regenerated by the carrier solution. We employed 1.3-mL min^{-1} as flow rate.

RESULTS AND DISCUSSION

Selection of the Solid Support

The reaction between CFD and the reagent (permanganate) occurs on the solid support placed in the flow-through cell, so the retention of both compounds on the microbeads is required. It allows avoiding the reagent consumption during the reaction. The study of the appropriate solid support was carried out by using a solution of 0.4-M sulphuric acid as carrier, due to the need for an acidic medium to obtain the CL reaction.

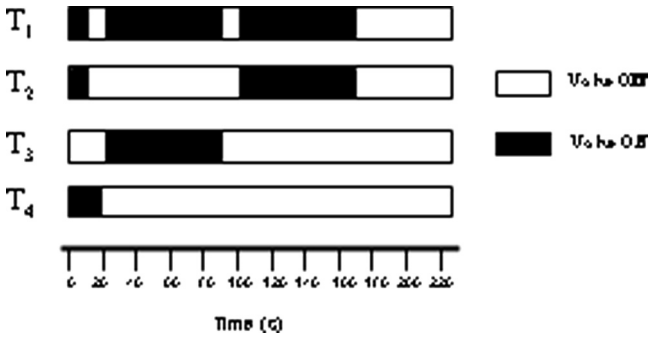


FIGURE 2 Valves scheme. T_1, T_2,...refer to the timing courses of solenoid valves V_1, V_2,....

Several solid supports were tested, while different orders of solutions (CFD and permanganate) were introduced into the flow system. Cationic exchangers and nonionic solid supports such as Sephadex-SP C-25 and C_{18}, respectively, provided no signal, regardless of the order of reagents introduction. As expected, in the case of anionic exchangers (Sephadex-QAE A-25 and DEAE A-25), significant signals were obtained only when permanganate was first introduced into the system, followed by CFD. This can be easily explained by taking into account that permanganate ion is strongly sorbed on the anionic exchangers, and that sorption can be visually observed, whereas CFD is only slightly retained. Of both solid supports tested, Sephadex-QAE A-25 provided the highest sensitivity (an improvement of 25% with respect to the use of the other anion exchanger). Hence, these microbeads were employed for further experiments.

Instrumental Variables

In the case of instrumental variables, taking into account that the analytical signal is obtained from a CL reaction, the instrument required is merely a photomultiplier tube (PMT), so that no filters or excitation lamp is needed. Two power supplies are employed: One is used at ±15 V to provide the required energy, and the other is used to adjust the control voltage in order to control the gain of the PMT and, therefore, sensitivity. The gain

adjustment can be controlled from 0.30 V up to 1.10 V. When one uses low-gain adjustment, the repeatability of the system is better, but a very low sensitivity is obtained. We decided to use a gain of 0.85 V to obtain good repeatability (3.3% RSD) with enough sensitivity for the determination of CFD in both pharmaceuticals and urine samples. The research was performed at room temperature, observing no difference in the analytical signal between 14°C and 28°C.

Chemical Variables

Selection of the Carrier Solution

The reaction of several analytes with permanganate has been previously reported, and a strongly acidic medium is usually required.[18] Therefore, 0.5-M solutions of different acids (sulphuric, nitric, and hydrochloric acids) were tested in order to obtain the highest possible analytical signal. The conditions used were 55-μM CFD and 2×10^{-4}-M permanganate solutions. The best results in terms of sensitivity and repeatability were observed when using sulphuric acid, and thus this one was finally chosen.

The sulphuric acid concentration was also studied over the range 0.2–0.8 M. The signal increased to a 0.4-M concentration and then remained constant up to 0.5 M. Higher concentrations originated a decrease of the analytical signal due to the high ionic strength that prevented the analyte retention. Hence, 0.4 M was selected as optimum.

Sample Solution

The nature of the sample solution was studied with the previously described working conditions. The best results were obtained when dissolving the CFD in a slightly acidic solution instead of deionized water (an increase of 25% in the analytical signal). When sulphuric acid was used for this purpose, the signal obtained was practically the same with concentrations between 0.1 and 0.3 M, although a better repeatability was observed when using 0.1-M solution.

In addition, the use of additional reagents was tested in order to obtain different emitting species to enhance sensitivity, such as singlet oxygen species or compounds accepting energy from the excited intermediate of the reaction. As a result, formaldehyde and quinine were tested. Quinine did not improve the analytical signal; however, it was increased two-fold when formaldehyde was used. Nevertheless, the use of the solid support makes possible a slight retention of CFD, therefore allowing one to reach the necessary sensitivity for applications required. Hence, the use of additional enhancing reagents and surfactants was avoided.

Oxidant Solution

Permanganate is the most widely used CL reagent. As it has been previously reported, the emitting species in the reactions between permanganate and others compounds can be a reduction product of permanganate, probably excited Mn(II).[18] With the purpose of obtaining the direct oxidation of the analyte and therefore avoiding the use of additional reagents, we decided to test it for the CL reaction. Using the previously optimized conditions for both carrier and sample solutions and employing a 110-μM CFD solution, we tested the concentration of permanganate. The same insertion times of permanganate and sample solutions were employed in this study (70 s). The concentration of permanganate was tested from 10^{-5} M up to 4×10^{-4} M. The signal increased up to 2×10^{-4} M, remaining constant up to 4×10^{-4} M. As a result, 2×10^{-4} M permanganate solution was selected as optimum. In this case, a high volume of reagent (instead of high concentration and low volume) was chosen in order to obtain a homogeneous distribution of the permanganate ions on the solid support.

Flow-System Variables

The insertion times of sample and permanganate solutions and the flow-rate provided by the peristaltic pump were the flow variables under study. It has to be stated that the main difference between multicommutation and other flow methodologies is that insertion volumes are replaced with insertion times. Knowing the flow rate and the insertion time, one can easily calculate the volume if required.

The flow rate was studied in the range 0.6–1.4 mL min^{-1}. As a general rule, in SPS, the analytical signal (maintaining the sample volume constant) diminishes when increasing the flow rate, due to a lower retention of the analyte on the solid support. In this particular case, no significant differences in

the analytical signal were observed in the studied flow-rate range. This effect is due to the high acidic medium, which causes the analyte to be retained only slightly on the microbeads. In order to obtain the maximum sample throughput, the highest possible flow rate, 1.3 mL min^{-1}, was selected, taking into account that higher flow rates caused overpressures in the system.

On the other hand, when increasing the sample insertion time, we found that the analytical signal rises due to a higher amount of CFD sorbed on the solid support and, therefore, an increase in the CL reaction product generated. Hence, sensitivity is improved, although the sampling frequency diminishes. The study of this variable was carried out from 10 s up to 100 s for both sample and permanganate solutions (see Fig. 3). The analytical signal increased up to 70 s and then remained constant. A 70-s insertion time was selected for both sample and permanganate solutions. However, the low sensitivity required when analyzing CFD in pharmaceuticals makes it possible to use lower insertion times and so drastically improves the sample throughput.

Figures of Merit

Taking into account the optimized conditions, we studied the analytical parameters of the system. Table 1 shows the figures of merit of the method proposed for the determination of CFD. The data were fitted by standard least-squares treatment. The proposed methodology was able to produce analytical fits with good linearity in the range 2.7–110-μM CFD. The sampling frequency was also evaluated, yielding a high value due to the regeneration of the sensor with the carrier solution itself.

TABLE 1 Analytical Parameters

Parameter	Value
Linear dynamic range (μM)	2.7–110
Calibration graph	
Intercept	0.0002
Slope (μM^{-1})	0.0135
Correlation coefficient	0.9992
Detection limit (μM)	0.8
Quantification limit (μM)	2.7
Intraday RSD (%)[a] ($n = 10$)	3.3
Interday RSD (%)[a] ($n = 10$)	6.5
Sampling frequency (h^{-1})	16

[a]For a concentration level of 55 μM.

Intraday repeatability was established for 10 independent analyses of sample solutions containing 55 μM of CFD. RSDs obtained were low, even though the measurements are made in a solid phase. Interday repeatability was also performed for 10 consecutive days. Detection and quantification limits were estimated as the concentrations of analyte that produced an analytical signal equal to 3 and 10 times the standard deviation of the background luminescence, respectively.[21] They are low enough to perform the analysis of CFD in pharmaceutical and urine samples. The use of the solid support has proved appropriate to obtain the required sensitivity, enabling one to avoid the use of additional potentially pollutant reagents commonly used in conventional CL flow systems.

Interference Study

Since the determination of CFD was performed in pharmaceutical preparations and human urine, the possible interference from different organic and inorganic species that can be found with the analyte in these samples was tested. This study was performed by adding different amounts of the possible interfering species to a solution containing 30 μM of CFD. A foreign species was considered not to interfere if it produced an error smaller than ±2 σ in the analytical signal, where σ is the standard deviation. If any interference was observed, the ratio of

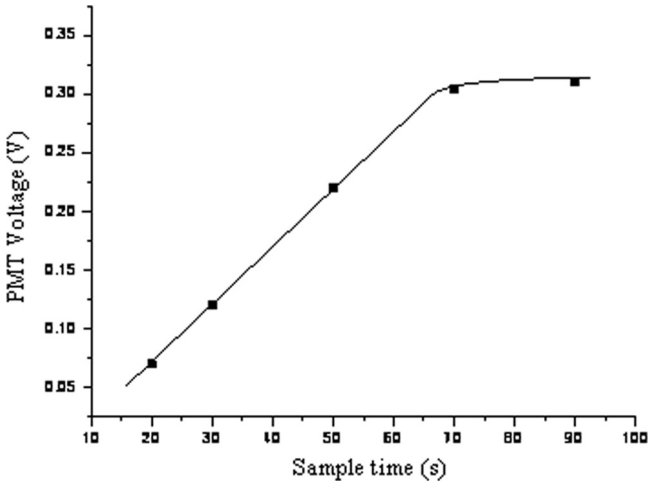


FIGURE 3 Study of the influence of the time of sample insertion on the analytical signal. [CFD] = 30 μM.

TABLE 2 Effect of Foreign Species

Foreign species	Tolerated interferent/analyte (w/w) ratio[a]
Lactose, saccharose, glucose, starch	$>500^b$
Na^+, K^+, Cl^-, I^-, NO_3^-, CO_3^{2-}, PO_4^{3-}	$>500^b$
Urea, Mg^{2+}	400
Uric acid, Ca^{2+}, Cu^{2+}	300
Fe^{3+}	16

[a]For a 30 μM CFD concentration.
[b]Maximum ratio tested.

interfering species to analyte (w:w) was reduced progressively until this interference ceased. The results are shown in Table 2.

The observed high tolerance of the presence of foreign species is due to both the direct measurement of the solid support and the CL detection, making possible the determination of CFD in complex matrices, such as urine samples. The lower tolerance of the presence of ferric ion can be explained by taking into account its oxidant character and so its interference in the CL reaction. The determination of CFD in homogeneous solution in the presence of many of these interfering compounds would be very difficult or almost impossible, therefore demonstrating the suitability of the solid support.

TABLE 3 Determination and Recovery Study in Pharmaceutical Preparations

Pharmaceutical	Added (mg)	Recovery (%)	RSD (%)[c]
Duracef (Juste)[a]	—	98	1.6
	200	102	1.3
	400	104	2.2
	600	96	0.9
Duracef (Juste)[b]	—	102	0.8
	20 mg mL^{-1}	104	2.1
	40 mg mL^{-1}	97	1.5
	60 mg mL^{-1}	103	1.2
Cefadroxilo Sabater (Generfarma S.L.)[a]	—	103	1.5
	100	103	2.2
	200	101	1.8
	400	97	1.6

[a]500 mg per capsule.
[b]250 mg/5 mL.
[c]Means of three determinations.

TABLE 4 Recovery Study in Human Urine

Sample	Spiked (mM)	Recovery (%)	R.S.D. (%)[a]
	2.0	95	3.5
Urine-1	2.5	101	3.4
	3	97	2.8
	2.5	96	3.2
Urine-2	3.5	102	2.1
	4.5	98	2.9
	3.5	97	4.1
Urine-3	4.5	98	3.5
	5.5	100	2.9

[a]Means from three determinations.

Analytical Applications

Following the previously described procedure, the determination of CFD in pharmaceutical preparations and in urine samples was carried out.

Pharmaceutical samples, prepared as described under the section Sample Preparation, were introduced by triplicate into the system. In all cases, the analyte amounts determined by the proposed method were in good agreement with the ones provided by the manufacturer. In order to evaluate the accuracy of the method, a recovery study was also performed by spiking the pharmaceuticals with CFD at three different concentration levels. The results obtained are shown in Table 3. Recoveries ranged in all the cases from 96% up to 104%, achieving RSDs lower than 2.5%.

Antibiotic-free urine samples gave relatively high CL intensity when no pre-treatment was applied. For this reason, it was necessary to minimize this interference. A recovery study was performed by adding CFD to different urine samples; the amounts of CFD added were those usually found after administration of a pharmaceutical dose.[22] Table 4 shows the recoveries obtained, and all of them were close to 100%, despite the presence of a very complex matrix.

CONCLUSIONS

A simple, rapid, low-cost, and selective multicommutated CL sensor is described for the determination of CFD in pharmaceutical preparations and human urine. The method is based on the direct oxidation of CFD by permanganate, a reaction that is produced on the anionic solid support Sephadex-QAE A-25.

These microbeads enable obtaining the required selectivity and sensitivity, thereby avoiding the use of additional enhancing reagents. On the other hand, the employment of the multicommutation principles permits one to automate the system and obtain a high sample throughput, which makes the proposed method suitable for the routine analysis of CFD in pharmaceutical preparations. Hence, the method has proved to be useful for quality control of CFD in drug industries.

ACKNOWLEDGMENTS

The authors are grateful to the Consejería de Innovación, Ciencia y Empresa (Junta de Andalucía), Project P07-FQM-02673, for financial support. L. Molina-García also acknowledges a grant from University of Jaén.

REFERENCES

1. Makchit, J.; Upalee, S.; Thongpoon, C.; Liawruangrath, B.; Liawruangrath, S. Determination of cefadroxil by sequential injection with spectrophotometric detector. *Anal. Sci.* **2006**, *22*(4), 591–597.
2. El-Gindy, A.; ElWalily, A. F. M.; Bedair, M. F. First-derivative spectrophotometric and LC determination of cefuroxime and cefadroxil in urine. *J. Pharm. Biomed. Anal.* **2000**, *23*(2–3), 341–352.
3. Samanidou, V. F.; Ioannou, A. S.; Papadoyannis, I. N. The use of a monolithic column to improve the simultaneous determination of four cephalosporin antibiotics in pharmaceuticals and body fluids by HPLC after solid phase extraction: A comparison with a conventional reversed-phase silica-based column. *J. Chromatogr. B* **2004**, *809*(1), 175–182.
4. Ozkan, S. A.; Erk, N.; Uslu, B.; Yilmaz, N.; Biryol, I. Study on electrooxidation of cefadroxil monohydrate and its determination by differential pulse voltammetry. *J. Pharm. Biomed. Anal.* **2000**, *23*(2–3), 263–273.
5. Saleh, G. A.; Askal, H. F.; Radwan, M. F.; Omar, M. A. Use of charge-transfer complexation in the spectrophotometric analysis of certain cephalosporins. *Talanta* **2001**, *54*(6), 1205–1215.
6. El Walily, A. F. M.; Gazy, A. A. K.; Belal, S. F.; Khamis, E. F. Use of cerium (IV) in the spectrophotometric and spectrofluorimetric determinations of penicillins and cephalosporins in their pharmaceutical preparations. *Spectrosc. Lett.* **2000**, *33*(6), 931–948.
7. Salem, H. Selective spectrophotometric determination of phenolic β-lactam antibiotics in pure forms and in their pharmaceutical formulations. *Anal. Chim. Acta* **2004**, *515*(2), 333–341.
8. El Walily, A. F. M.; Abdel-Kader Gazy, A.; Belal, S. F.; Khamis, E. F. Selective spectrofluorimetric determination of phenolic β-lactam antibiotics through the formation of their coumarin derivatives. *J. Pharm. Biomed. Anal.* **1999**, *20*(4), 643–653.
9. Hefnawy, M.; El-Shabrawy, Y.; Belal, F. Spectrofluorometric determination of alpha-aminocephalosporins in biological fluids and pharmaceutical preparations. *J. Pharm. Biomed. Anal.* **1999**, *21*(4), 703–707.
10. Aly, F. A.; Alarfaffj, N. A.; Alwarthan, A. A. Permanganate-based chemiluminescence analysis of cefadroxil monohydrate in pharmaceutical samples and biological fluids using flow injection. *Talanta* **1998**, *47*(2), 471–478.
11. Sun, Y.; Tang, Y.; Yao, H.; Zheng, X. Potassium permanganate-glyoxal chemiluminescence system for flow injection analysis of cephalosporin antibiotics: Cefalexin, cefadroxil, and cefazolin sodium in pharmaceutical preparations. *Talanta* **2004**, *64*(1), 156–159.
12. Thongpoon, C.; Liawruangrath, B.; Liawruangrath, S.; Wheatley, R. A.; Townshend, A. Flow injection chemiluminescence determination of cephalosporins in pharmaceutical preparations using tris (2,2′-bipyridyl) ruthenium (II)-potassium permanganate system. *Anal. Chim. Acta* **2005**, *553*(1–2), 123–133.
13. Li, Y.; Lu, J. Chemiluminescence flow-injection analysis of β-lactam antibiotics using the luminol-permanganate reaction. *Luminescence* **2006**, *21*(4), 251–255.
14. Mervartová, K.; Polášek, M.; Calatayud, J. M. Recent applications of flow-injection and sequential-injection analysis techniques to chemiluminescence determination of pharmaceuticals. *J. Pharm. Biomed. Anal.* **2007**, *45*(3), 367–381.
15. Miró, M.; Estela, J. M.; Cerdà, V. Potentials of multisyringe flow injection analysis for chemiluminescence detection. *Anal. Chim. Acta* **2005**, *541*(1–2), 55–66.
16. Rocha, F. R. P.; Reis, B. F.; Zagatto, E. A. G.; Lima, J. L. F. C.; Lapa, R. A. S.; Santos, J. L. M. Multicommutation in flow analysis: Concepts, applications and trends. *Anal. Chim. Acta* **2002**, *468*(1), 119–131.
17. Ruedas-Rama, M. J.; Ruiz-Medina, A.; Molina-Díaz, A. A simple and straightforward procedure for monitoring phenol compounds in waters by using UV solid phase transduction integrated in a continuous flow system. *Microchim. Acta* **2003**, *141*(3–4), 143–148.
18. Adcock, J. L.; Francis, P. S.; Barnett, N. W. Acidic potassium permanganate as a chemiluminescence reagent: A review. *Anal. Chim. Acta* **2007**, *601*(1), 36–67.
19. Catalá-Icardo, M.; García-Mateo, J. V.; Martínez-Calatayud, J. Multicommutation as a powerful new analytical tool. *Trends Anal. Chem.* **2002**, *21*(5), 366–378.
20. Llorent-Martínez, E. J.; Ortega-Barrales, P.; Molina-Díaz, A. Multi-commutated flow-through multi-optosensing: A tool for environmental analysis. *Spectrosc. Lett.* **2006**, *39*(6), 619–629.
21. MacDougall, D.; Crummett, W. B. Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Anal. Chem.* **1980**, *52*(14), 2242–2249.
22. CGCOF. *Catálogo de Medicamentos*; Madrid, Spain, 2007.