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Short communication

Chemiluminescence determination of sulphadiazine in drugs by flow injection analysis using the peroxyoxalate reaction in micellar medium

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Abstract

Peroxyoxalate chemiluminescence (PO-CL) is an indirect type of CL which allows the detection of native fluorophores or compounds derivatized with fluorescent labels. We propose a flow injection analysis (FIA) configuration based on the use of a two-injection valve system for the introduction of both PO and derivatized analyte solutions in the flow system, avoiding the problems arising from the use of organic solvents, such as acetonitrile, as no special tubes nor special pumps are required. Furthermore, the use of micellar media (sodium dodecyl sulphate, SDS) as a carrier and the addition of tetrahydrofurane (THF) in the PO solutions increase both the solubility and stability of POs, avoiding their rapid degradation in water. The proposed CL-FIA system has been applied to the determination of sulphadiazine (a sulphonamide mainly used in the treatment of urinary tract infections for human and veterinary use) using bis[2,4,6-trichlorophenyl]oxalate (TCPO) as CL precursor, H_2O_2 as oxidant, imidazole as a catalyst and fluorescamine as the fluorescent derivatizing agent. The optimization of variables was carried out by means of experimental designs and the method showed a LOQ of 379 $\mu g\,l^{-1}$ (calibration range 126–2000 $\mu g\,l^{-1}$). It has been satisfactorily applied to the quantification of sulphadiazine in pills for human use and ampoules for veterinary use.

Keywords: Sulphadiazine; Chemiluminescence; Flow injection analysis; Drugs; Peroxyoxalate reaction

1. Introduction

Sulphonamides are *N*-derivatives of 4-amino-benzenesulphonamide and they comprise a large group of synthetic antibacterial compounds. They have been used in human medicine against a wide variety of microbes, being their current use primarily in the treatment of urinary tract infections. They are also widely used in farm animal feedstuff and fish cultures as veterinary drugs for prophylactic and therapeutic purposes. Furthermore, sulphonamides act as growth promoting substances, and their residues in food are of concern due to their potential carcinogenic character and the possibility of development of antibiotic resistance in humans as well as severe allergic reactions [1].

Chemiluminescence (CL) is a high sensitive analytical technique that can be used in the determination of different compounds in a great variety of matrices depending on their participation in the CL reaction as precursors, catalysts, inhibitors, oxidants, etc. Considering the kinetic characteristics of this technique, it can be easily coupled to a flow injection analysis (FIA) manifold as detection mode [2]. Among the different CL systems that can be used with analytical purposes, peroxyoxalate (PO) reaction is one of the most efficient [3], being an indirect or sensitized type of chemiluminescence in which an activate oxalate reacts with hydrogen peroxide leading to the formation of one or more energy-rich intermediate(s) capable of exciting a large number of fluorophores [4] through the chemically initiated electron exchange (CIEEL) mechanism [5], by which the intermediate forms a charge transfer complex with the fluorophore, donating one electron to the intermediate, which is transferred back to the fluorophore raising it to an excited state and liberating an emission typical for the nature of this fluorescent

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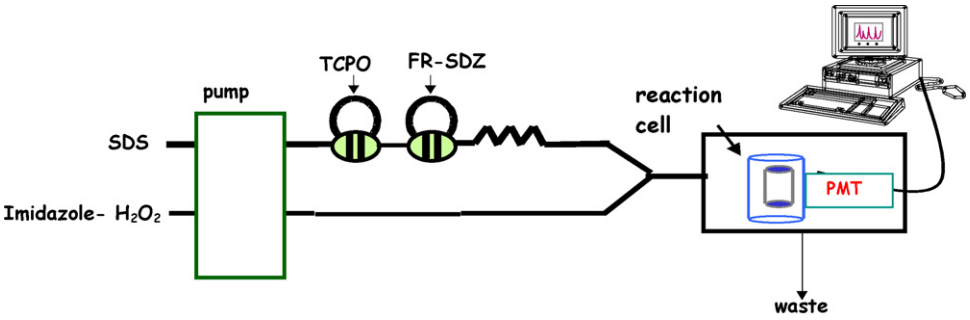

Fig. 1. Proposed flow injection manifold.

derivative. Recently the nature of the postulated intermediate, 1,2-dioxetane-3,4-dione, has been confirmed using ^{13}C nuclear magnetic resonance (NMR) spectroscopy [6]. From an analytical point of view, the usefulness of this PO-CL reaction is based on the possibility of detecting native fluorophores or compounds derivatised with fluorescent labels [7]. The advantage of the PO reaction is the wide pH range to carry out the oxidation, generally occurring near neutral values, the main limitation being the need for an organic solvent based on solubility, stability and efficiency considerations. The most widely used oxalate ester is bis-(2,4,6-trichlorophenyl)oxalate (TCPO) followed by bis-(2,4-dinitrophenyl)oxalate (DNPO). Imidazole (IMZ) has been proved to be an efficient catalyst for the PO-CL reaction [8,9] due to its ability to destabilize the PO by forming an intermediate less stable, which is thus more susceptible to nucleophilic attack by hydrogen peroxide. A general mechanism was proposed by Hadd and Birks for the nucleophilic reaction of IMZ with oxalate esters [10].

Despite the advantages of CL detection, there is scarce literature concerning the CL determination of sulphonamides. Based on the oxidation of these analytes by potassium permanganate in acid medium, the determination of sulphacetamide and sulphafurazole in pharmaceutical dosage forms using sequential injection analysis (SIA) technique with CL detection has been reported, with detection limits ranging 2–8 mg kg^{-1} and quantitative recoveries [11]. Also, the FIA–CL determination of sulphonamides by means of a photochemical reaction followed by the same oxidation reaction has been recently reported. The method has been applied to the determination of sulphamethoxazole in pharmaceutical preparations [12], providing a limit of detection of 60 μg l^{-1}. A sulphur chemiluminescence detection (SCLD) system for supercritical fluid chromatography based on ozone-induced CL following a two-step combustion process of consecutive oxidation and reduction of sulphur-containing compounds has also been proposed for different products, including some sulphonamides, although no application was reported [13]. Recently, our group has reported the analysis of seven sulfonamides by means of HPLC with chemiluminescence detection, based on the PO system, which was satisfactorily applied to the analysis of spiked raw milk samples [14].

In this contribution, we propose the coupling FIA-CL as an interesting alternative in pharmaceutical quality control due to its advantages, such as, high sensitivity, easy implementation, simple instrumentation and low cost. With this purpose, a two-injection valve FIA-CL system for the introduction of both PO and derivatised analyte solutions in the flow system is proposed for the determination of sulphadiazine (SDZ), a sulphonamide mainly used in the treatment of urinary tract infections for human and veterinary use, labelled with fluorescamine (FR). The system avoids the problems arising from the use of e.g. acetonitrile (ACN) as solvent, as neither special tubes nor special pumps are required [15–17]. Furthermore, the use of micellar media (sodium dodecyl sulphate, SDS) as carrier and the addition of tetrahydrofurane (THF) in the PO solutions increase both the solubility and stability of POs, avoiding their rapid degradation in water. Chemical and instrumental variables affecting CL emission have been optimized using an experimental methodology based in the use of both univariate optimization and experimental designs (Doehlert and Central Composite Design 2^3 + face centred star). The method shows a LOQ of 379 μg l^{-1} and it has been successfully applied to the quantification of SDZ in pills for human use and ampoules for veterinary use, obtaining results according with those reported by the manufacturers.

2. Experimental

2.1. Chemicals

All the reagents were analytical reagent grade and solvents were HPLC grade. SDZ, FR, IMZ and TCPO were purchased

Table 1
Selected levels in the Doehlert design for the optimisation of the FIA variables

Variable	Levels						
	1	2	3	4	5	6	7
Turn time between valve switching (s)	2	4	6				
SDS carrier flow rate (ml min^{-1})	1.0	1.75	2.50	3.25	4.0		
IMZ-H_2O_2 solution flow rate (ml min^{-1})	1.20	1.63	2.07	2.50	2.93	3.37	3.80

Table 2
Selected levels in the 2^3 + face centred star factorial design for the optimisation of chemical variables

Variable	Levels		
	−1	0	+1
[H_2O_2] (mM)	200	400	600
[Imidazole] (mM)	1	6	11
pH	4.0	6.0	8.0

from Sigma. Hydrogen peroxide, SDS, sodium dihydrogen phosphate, ACN, THF and acetone were obtained from Panreac. Methanol and TRIS buffer were purchased from Merck. Deionised water was used for the experimental work.

2.2. Apparatus

CL measurements were carried out on a Jasco CL 1525 detector, equipped with a PTFE spiral detection cell, data control and acquisition program. Two Gilson Minipulse-3 peristaltic pumps, two Rheodyne 5020 manual injection valves, and Omnifit tubing (0.5 mm i.d.) and connectors were used in the FIA manifold, described elsewhere [15–17] and represented in Fig. 1.

2.3. Procedure

A SDS solution (10 mM) was prepared in phosphate buffer (0.1 M, pH 7.4) and used as carrier solution at a flow rate of 3.5 ml min^{-1}. Hydrogen peroxide (400 mM) and IMZ (11 mM) were prepared jointly in surfactant solution and incorporated in the system at a flow rate of 1.2 ml min^{-1}. A TCPO solution (1 mM) was prepared in ACN:THF (75:25) and introduced into the carrier stream by means of a manual injection valve (500 μl loop). Samples or standard solutions containing SDZ labelled with FR were introduced into the carrier stream by means of a second injection valve (50 μl loop). The contents of both injection valves were mixed in a reaction coil (75 cm length, 0.5 mm i.d.) placed just after the second injection valve, and subsequently merged with the reagent stream. The CL reaction takes place in the detection cell and the CL intensity is registered instantaneously by the PMT placed just in front of the cell.

The labelling of SDZ with FR was carried out off-line, as follows: 1 ml of SDZ solution prepared in buffer (TRIS 10 mM, pH 7.0) was mixed with 50 μl of FR solution (4 g l^{-1} in acetone) and placed in an ultrasound bath for 2 min at room temperature. The resulting solution was immediately injected into the FIA system.

Table 3
Final optimum values

Carrier flow rate (SDS) (ml min^{-1})	3.5
IMZ-H_2O_2 solution flow rate (ml min^{-1})	1.2
Turn time between the switching of valves (s)	5
[H_2O_2] (mM)	400
[IMZ] (mM)	11
pH	7.4
[TCPO] (mM)	1
[Fluorescamine] (g l^{-1})	4
TCPO injection volume (μl)	500
FR-SDZ injection volume (μl)	50
Time of labelling reaction (min)	2

Table 4
Calibration curve parameters and performance characteristics of the method

Linear range (mg l^{-1})	0.38–2.00
Slope (c.u. per mg l^{-1})	120.44
Intercept (c.u.)	22.74
$s_{R,c}$ (c.u. per mg l^{-1})	15.96
Determination coefficient, R^2 (%)	99.02
R.S.D. repeatability[a] (%)	4.8
R.S.D. reproducibility[a] (%)	17.2
Detection limit (μg l^{-1})	126
Quantification limit (μg l^{-1})	379

c.u.: chemiluminescence units; $s_{R,c}$: standard deviation of the regression.
[a] Estimated for 200 μg l^{-1} SDZ solutions; $n = 12$ in conditions of repeatability or reproducibility.

2.4. Sample preparation

In the case of SDZ pills (label claim: 500 mg per unit), five pills were finely powdered and homogenised in a mortar (average weight: 592 mg). A portion of 1 mg was dissolved in 1000 ml of Tris buffer (10 mM, pH 7.0), sonicated for 10 min and filtered through a filter paper. An aliquot of this solution (1 ml) will be used, as above mentioned, to form the corresponding fluorescent derivative with FR before the CL-FIA analysis.

For the SDZ suspension (label claim: 400 g l^{-1}), 500 μl of the solution were diluted up to 1000 ml with water (200 mg l^{-1}). An aliquot of 250 μl was diluted with Tris buffer (10 mM, pH 7.0) up to 50 ml (1 mg l^{-1}). A portion of 1 ml was used to form the fluorescent derivative with FR before the CL-FIA analysis.

3. Results and discussion

3.1. Optimization of the experimental variables

The variables (both FIA and chemical variables) involved in the proposed CL-FIA system were optimized by means of experimental designs. Firstly, the instrumental FIA variables (namely: flow rate of SDS carrier, flow rate of IMZ-H_2O_2 solution and time between the switching of both injection valves) were optimized by means of a Doehlert design. This kind of response surface design is scarcely used in analytical chemistry [18–20] in spite of these advantages such as its high efficiency compared with other designs like Box–Behnken or central composite designs, and because of the possibility to study a higher number of levels for the most significant variables taking into account the found effects from the screening design. The number of levels for each variable was selected according to the influence of each variable on the response (the bigger the influence, the higher the number of levels), according to previous studies. Thus, the time between the switching of both injection valves was studied at three levels, while the flow rate of SDS and the flow rate of IMZ-H_2O_2 solution were studied at five and seven levels, respectively.

Table 5
Application of the method in commercial drugs

Drug	Reported value (%)	Found value (%, $n = 5$)	P-Value (%) ($\alpha = 0.05$)
Pills (Sulfadiazina Reig Jofré)	84.4	91.5 ± 14.0	38.5
Suspension (Tribisen)	40.0	39.4 ± 1.3	36.0

The studied levels of each variable are shown in Table 1. Once the optimum values for the FIA variables had been selected, the chemical variables (namely: concentration of peroxide and IMZ and pH) were optimized by means of a Central Composite Design 2^3 + face centred star. The experimental region to select the levels for each variable was established according to previous studies and they are shown in Table 2. As the optimum value for concentration of IMZ was 11 mM, which corresponds to the upper level of the studied design, to ensure the optimum of this variable, an univariate study was carried out remains constant the optimum values for the rest of the variables and using increasing concentrations of IMZ up to 25 mM. However, no significant differences in the CL signals were observed with the increase of the concentration. Others variables involved in the method (as the volume of the injection valves) were optimized by the univariate method, and the labelling reaction was performed according to previous studies [14]. Final optimum values are summarized in Table 3.

3.2. Calibration curve and performance characteristics

The calibration curve was established with triplicate injections of each standard solution (seven different concentrations ranging 0–2.00 mg l^{-1}). The method provides satisfactory stability and precision. Relative standard deviations (R.S.D. %) were calculated for solutions of 200 μg l^{-1} of SDZ, in terms of repeatability, by using four standard solutions injected by triplicate and measured in the same day ($n = 12$); and reproducibility or intermediate precision by using four standard solutions injected by triplicate and measured in four different days ($n = 12$). The linear range was established from 0.38 to 2.00 mg l^{-1} of SDZ, with detection and quantification limits of 126 and 379 μg l^{-1}, respectively, calculated from 45 replicates of the blank solution, according to the IUPAC recommendations [21]. All the performance characteristics are shown in Table 4.

3.3. Application of the method

The proposed method has been applied to the determination of SDZ in two pharmaceutical products: pills for the human treatment of illnesses as meningitis, (Sulfadiazina Reig Jofré; composition: sulphadiazine 500 mg, starch, manitol, cellulose, talcum, methylcellulose, magnesium estearate and other excipients, in a average weight of 592 mg/pill unit ($n = 5$); SDZ % m/m = 84.4), and a veterinary oral suspension used in the treatment of breathing illnesses in birds (Tribisen S.O.; composition: trimethoprim 8% p/v, sulphadiazine 40% m/v and excipients). The method was validated by comparison of the obtained results with the declared nominal values. A Student's t-test was applied from which it was possible to conclude that no significant differences were observed from the results obtained by using the proposed method and those reported by the labels, as can be seen in Table 5.

4. Conclusions

The use of the PO-CL reaction in micellar medium, coupled to a FIA manifold has been proposed as an alternative detection system for quality control of sulphonamides in pharmaceutical samples. The method implies the off-line formation of a fluorescent derivative (fluorophore) with fluorescamine and the subsequent oxidation of TCPO by H_2O_2 using imidazol as catalyst in alkaline medium, in presence of the fluorophore, whose CL emission is proportional to the sulphonamide concentration. The method has been applied to the determination of sulphadiazine in pharmaceuticals with results comparables to those reported by the manufacturers.

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