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Photo-induced Liquid-phase Chemiluminescent Determination of Sulfamethoxazol

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Abstract.- The chemiluminescent determination of Sulfamethoxazol is proposed by using the photo-degradation of the drug in a continuous-flow Multicommutation assembly. The light emission is the product of the drug oxidation with potassium permanganate in polyphosphoric acidic medium after irradiation (stopped-flow, 5 s) on the sample solution in alkaline medium. The method is completely automated with the aid of the emergent Multicommutation methodology, a continuous-flow modality. The new procedure allows the Sulfamethoxazol determination over the range 0,01 –250 mg l^{-1} with a dynamic linear range from 0,01 to 100 mg l^{-1}; and it is applied to different types of samples. The maximum sample throughput was calculated from the avarage of base-peak wide; obtained result was 40 h^{-1}.

Key-words.- Chemiluminescence, photo-degradation, multiconmutación.

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Introduction

The chemiluminescent (CL) production of electromagnetic radiation as a result of a chemical reaction presents favourable features like high sensitivity and low detection limits, and the simple, flexible and cost effectiveness to measure a variety of compounds. There are two kind of determinations of analytes in liquid phase; one is based on well-known CL reactions with a test chemiluminescent substance (like luminol) and the other which is the simpler modality, it is based on searching chemiluminescent reactions where substrates (analytes) react with wide range of oxidants (sometimes reductants) in different media [1]; but its development has been limited by the scarcity of molecules that are strongly chemiluminescent in solution.

On the other hand, light has the properties that make it an "ideal analytical reagent", since, depending on its chemical structure, a compound may undergo oxidation, reduction, hydrolysis and a variety of other transformations under its action. This can be exploited for obtaining derivatives with improved chromophoric, fluorophoric or electrolytic properties. Many compounds are light-sensitive which allows the development of straightforward, expeditious and economic analytical methods. [2]. Recently, photochemical reactions with chemiluminescence detection have been applied to the determination of (among others) pharmaceuticals and pesticides [3].

Multicommutation assemblies refer to flow systems designed with computer – controlled commutators resulting in flow networks in which all the steps involved in sample processing can be independently implemented. In recent years Multicommutation, based on the systematic use of three-way solenoid valves, is presented as an alternative to the rest of continuous-flow assemblies. A three-way solenoid valve behaves as a switch between two states: ON and OFF. The volume of sample inserted is proportional to the pulse length and can be altered by changing the profile of the insertion sequence: a single sample segment, or several segments of the same or different length, can be intercalated with the carrier solution. The result is a flexible system that allows the insertion of variable volumes of sample via software. Multicommutation has the advantage of drastic decrease in reagent consumption and total amount of generated waste, due to the discrete injection of reagents and recycling of solutions to their vessels when not inserted in the flow system. In addition, the insertion of sample and reagents is carried out by controlling the time through a friendly software [4,5].

The studied molecule in the present paper is the Sulfamethoxazol or 4-amino-*N*-(5-methylisoxazol-3-yl)-benzenesulfonamide whose molecular structure is depicted in Figure 1. Chemical Formula, $C_{10}H_{11}N_3O_3S$

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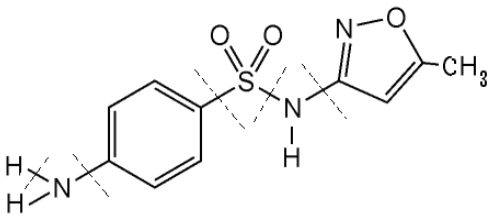

Fig. 1 Molecular structure of Sulfamethoxazol. Chemical Formula, $C_{10}H_{11}N_3O_3S$. Dotted lines illustrated the cleavage sites by direct photolysis

It is a sulfonamide bacteriostatic antibiotic which primary activity is against susceptible forms of Streptococcus, Staphylococcus aureus, Escherichia coli, Haemophilus influenza, and oral anaerobes. Allergies to sulfa-based medications typically cause skin rashes, hives, or trouble breathing or swallowing and warrant immediate discontinuation of the medication and contact with doctor immediately [6,7].

Several techniques have been reported in the analytical literature for the determination of Sulfamethoxazol such as

micellar liquid chromatography, high performance liquid chromatography and spectrophotometry [8]. A chromatographic method was developed for the simultaneous determination in plasma and infected tissue of five antimicrobials, and the detection of Sulfamethoxazol was carried out using a fluorescence detector; first step on the separation of the analytes was achieved by solid extraction on a C18 column [9]. Another HPLC method was developed for the simultaneous determination in pasteurized milk samples of several pharmaceuticals; and the basis of the method was the separation with the aid of HPLC-DAD with a C_{18} hybrid column and gradient elution with an aqueous mobile phase [10]. The determination of ten antibiotics from the sulphonamide family in egg samples was based on the following procedure: analytes were previously extracted with the aid of the mixture and then cleaned-up on a cation-exchange solid-phase extraction cartridge. The HPLC separation was performed by gradient on a C_{18} column with a mobile phase of methanol–water [11]. An evaluation of atmospheric pressure ionization interfaces was used for measurement of sulphonamides in honey spiked samples by using isotope dilution liquid chromatography coupled with tandem mass spectrometry techniques [12]

The interest of determination of *pharmaceuticals in environmental* samples is growing quickly. This is a direct consequence from different studies demonstrating a significant discharge of human medications from hospitals, household and drug production facilities into surface waters which demonstrate the alarming degree to which they have been impacted by urban drainage. An heterogeneous group of organic molecules including cosmetic ingredients; some pharmaceuticals (Sulfamethoxazol among them) and three hormones were surveyed along different places into a municipal Water Treatment Plant [13]. A large group of pharmaceuticals in trace-level were analyzed in aqueous samples and; as usual, first analytical step consisted in the solid-phase extraction followed by GC–MS (in this case a derivatization of the acid compounds was required) or HPLC–electrospray ionization MS–MS. The validation was performed and the samples were on the occurrence of pharmaceuticals in groundwater of a city. It was found that several of the compounds detected in groundwater their occurrence could be traced back to an impact of municipal or industrial waste water [14]. A review paper was dealing on the determination of pharmaceuticals in aqueous environmental samples. The most general procedure was discussed; due to the basically elevated polarity of pharmaceuticals either analysis by LC–ES/MS/MS or an efficient derivatization prior to measurements by GC/MS are mostly essential. Recently several methods have been developed for drugs in the lower ng/l range using solid phase extraction, derivatization, detection and confirmation by gas chromatography/mass spectrometry [15]. An interesting paper was dealing on the occurrence and distribution of pharmaceuticals in surface waters with impact of waste streams from hospitals and pharmaceutical production facilities; it was applied to three rivers and in the waste streams of six hospitals and four pharmaceutical production facilities in Taiwan. The most frequently detected pharmaceuticals resulted to be acetaminophen, erythromycin-H_2O, Sulfamethoxazol, and gemfibrozil, with a detection frequency over 60% [16]. The tandem solid phase extraction and ultra performance liquid chromatography - tandem mass spectrometry was utilized to develop a method for trace analysis of 21 antibiotics belonging to seven classes in influent and effluent of municipal wastewater treatment plant. The analysis of influent and effluent samples of two municipal wastewater treatment plants revealed the presence of eleven antibiotics, including Sulfamethoxazol [17]. Sulfamethoxazol contamination of a deep phreatic aquifer resulted in concentrations varying between 90 and 150 ng/L in land irrigated with wastewater effluents for about 5 decades and a relatively deep pumping well (109 m), used as a drinking water source till 2007, located downstream (1300 m) of wastewater effluent and sludge infiltration facilities. The maximum Sulfamethoxazol concentration detected in the pumping well was of 20 ng l^{-1}. These results question wastewater effluent disposal strategies including the suitability of irrigation with effluents on the replenishment area of an aquifer supplying drinking water [18]. An study on pharmaceutical and personal care products in tile drainage following surface spreading and injection of dewatered municipal biosolids to an agricultural field, examined pharmaceutical and personal care products concentrations; two different dates and effluents were tested. The examined compounds included eight pharmaceuticals, the nicotine metabolite cotinine, and two antibacterial personal care products triclosan and triclocarban [19]. A paper describes development, optimization and application of analytical method for determination of nineteen pharmaceuticals from different therapeutic classes (sulfonamides were included) in 26 surface and ground water samples at ng l^{-1} levels [20]. An in-line solid-phase extraction-capillary electrophoresis method with UV–vis detection was developed for the monitoring of residues of five sulfonamides in tap, bottled mineral and river waters. For this purpose an analyte concentrator was constructed, based on the introduction of a small portion of a solid-phase extraction sorbent into the electrophoretic capillary to carry out an in-line concentration step, improving sensitivity [21].

This paper is dealing with the photo-induced chemiluminescent determination of Sulfamethoxazol using the photo-degradation of the drug in a continuous-flow Multicommutation assembly. The light emission is obtained with the oxidation of the photo-fragments with potassium permanganate in polyphosphoric acidic medium after irradiation (stopped-flow, 5 s) on the sample solution in alkaline medium. The method is completely automated with the aid of the emergent Multiconmutación methodology, a continuous-flow modality. A previous paper from this lab was dealing with the chemiluminescence of Sulfamethoxazol in a FIA manifold and it was devoted to obtain the dissolution profile (in vitro availability) of the drug in pharmaceutical formulations, not for quantitative determination. As far as authors know analytical application of Sulfamethoxazol has not been studied in a Multiconmutación assembly by photo-induced chemiluminescence. The new method is applied to samples of environmental interest. The photo-induced chemiluminescence study can also be adapted to an on – line separation method, like as a post-column reaction to improve sensitivity and detection limits [22].

2. Experimental

2.1 Reagents and apparatus

All reagents used were analytically pure unless stated otherwise. Solutions were prepared in purified water by reverse osmosis and then deionised (18 MΩcm) with a Sybron/Barnstead Nanopure II water purification system provided with a fibber filter of 0,2 μm pore-size.

The Sulfamethoxazol was from Guinama (Valencia, Spain). Other used chemicals were: oxidants as $KMnO_4$ (Panreac, Spain), $Ce(NH_4)_2(NO_3)_6$ (Merck), $K_3Fe(CN)_6$ (Panreac, Spain); organized media and sensitizers as: formic acid (Panerac, Spain), acetonitrile (Merck, Germany), sodium dodecyl sulphate (Scharlau, Spain), hexadecyl pyridinium chloride(Fluka, Switzerland), β-cyclodextrine (Fluka, Switzerland), 2-propanol (J. T. Baker, Holland), benzalkonium chloride (Guinama, Spain), methanol (Prolabo Germany), N-cetyl- N,N,N- trimethyl ammonium bromide (Scharlau, Spain), quinine (Guinama, Spain), Triton-X 100 (Panreac, Spain),

Tween 80 (Panreac, Spain), ethanol (Kelsia, Spain) dimethylformamide (Scharlau, Spain). As photodegradation media were used: $FeSO_4x7H_20$ (Fluka, Switzerland), NaOH (Scharlau, Spain), H_2O_2 (Prolabo, Germany), $Fe(NO_3)_3x9H_2O$ (Panreac, Spain), $HClO_4$ (Panreac, Spain); and, also polyphosphoric acid (Acros Organics, USA, Belgium), H_2SO_4 (Scharlau, Spain).

2.2 Continuous-flow assembly

The continuous-flow manifold is depicted in Figure 2 and it consisted of a PTFE coil of 0.8 mm internal diameter; a Gilson (Worthington, OH, USA) Minipuls 2 peristaltic pump provided with tygon pump tubing from Elkay Elreann (Galway, CO, USA); and, three solenoid valves Model 161T031 (Nresearch, Northboro, MA, USA). The photoreactor consisted of a 150 cm length and 0.8 mm i.d. PTFE tubing (from Omnifit) helically coiled around a 15 W low-pressure mercury lamp (Sylvania) for germicidal use. The flow-cell was a flat-spiral quartz tube of 1 mm id and 3 cm total diameter backed by a mirror for maximum light collection. The photo-detector work-package was a P30CWAD5F-29 Type 9125 photomultiplier tube (PMT) supplied by Electron Tubes operating at 1280 V; it was located in a laboratory-made light-tight box. The output was fed to a computer equipped with a counter-timer, also supplied by Electron Tubes.

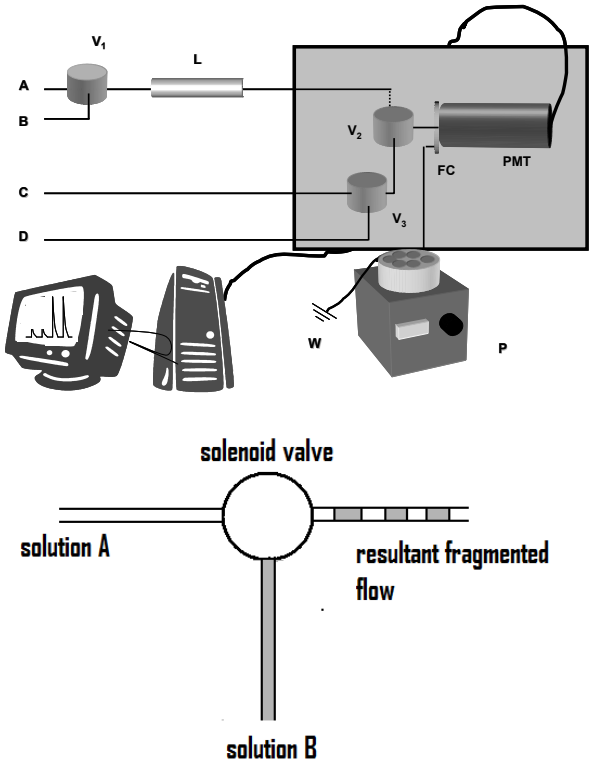

Fig. 2 Multicommutation continuous-flow assembly for the photo-degradation-chemiluminescent determination of Sulfamethoxazol.
Bottom.- Schematic representation of the "merging" process with solenoid valves.
A, aqueous solution of the Sulfamethoxazol; B, suitable medium for photo-degradation; C, oxidant in basic medium solution; D, pure water; P, peristaltic pump; PMT, photomultiplier tube; V1, V2 and V3, solenoid valves; FC, flow-cell; PR, photo-reactor.
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2.3. Procedures

2.3.1. Preparation of the Sulfamethoxazol solutions.

An aqueous stock solution of 10 mg l^{-1} of Sulphametoxazol was prepared by exactly weighing and dissolving it in purified water with a help of an ultrasonic bath. The working standard solutions were prepared daily by diluting the stock solution.

2.3.2. Optimization of experimental variables

The optimization of chemical and manifold parameters was performed by a sequential methodology. First, chemical parameters, namely: oxidation system, medium for the photodegradation, influence of both concentrations, presence of sensitizers and organized medium and temperature were optimized by using the univariate method to provide a more systematic and comprehensive information on the chemical process. Then with the selected values were optimized the hydrodynamic variables (Multicommutation parameters: size and number of segments, flow rate and photodegradation time) by using the multivariate method known as the Modified Simplex Method (MSM) [23, 24].

Two consecutive simplex series were developed, the range of each variable used in the second being restricted to the zone that gave the best results in the first .Then the higher vertices were selected for a new comparative study to choose the output resulting in the best compromise sensitivity (peak height), sample throughput (peak-base width) and reproducibility (RSD, %). Finally, the previously optimized chemical parameters were refined by using the optimum values of the manifold parameters.

As usual, the concentration of the test substance (Sulfamethoxazol) was lowered during the optimization process as the output was increasing with the new parameter values. The application of the sequential methodology was completed with a study of the robustness of the proposed method.

3. Results and discussion

3.1 Preliminary studies

The preliminary tests included some strong oxidant systems (oxidative reagent plus medium for the oxidation) that were used in combination with the chosen photodegradation medium and the lamp on and lamp off. The oxidant systems (potassium permanganate in sulphuric and polyphosphoric media; Ce (IV) in strong acid medium; and, ferricyanide, in basic medium) were tested in combination of several irradiation media (pure water, Fe (III), Fe (II), NaOH and H_2O_2) and all of them with lamp ON and OFF. The obtained results demonstrated the potassium permanganate was the suitable oxidant. For details see Table 1 and Figure 3. A Sulfamethoxazol concentration of 100 ppm (100 mg l^{-1}) was used in these instances.

3.2 Influence of reagents

Subsequent tests were aimed to expose the suitable photo-degradation medium. Test were performed in different media, namely: pure water, NaOH, hydrogen peroxide, Fe^{2+} and Fe^{3+} . As the better results were observed with water and NaOH, we tested the following acidic and alkaline solutions: $HClO_4$ and NaOH, both in concentrations 10^{-2}, 10^{-4} and 10^{-6} mol l^{-1}. Higher outputs were provided with sodium hydroxide which concentration was further optimized by testing concentrations from 10^{-6} to 10^{-1} mol l^{-1}; higher ligth emission was observed at 10^{-2} mol l^{-1}. See Figure 4.

Table 1 Selection of the suitable oxidant system for the Sulfamethoxazol determination

Oxidant + medium	Average CL intensity									
	H_2O		Fe^{2+} $6*10^{-5}$ mol l^{-1}		Fe^{3+} $6*10^{-5}$ mol l^{-1}		NaOH 10^{-3} mol l^{-1}		H_2O_2 0,05%	
	lamp off	lamp on	lamp off	lamp on	lamp off	lamp on	lamp off	lam p on	lamp off	lamp on
MnO_4^- medium $H_4P_2O_5$ 0,5% C= $7*10^{-4}$	**13337**	**43113**	**8718**	**39693**	**13063**	**46861**	**13089**	**60551**	**5405**	**7423**
MnO_4^- medium H_2SO4 2 mol l^{-1} C=$7*10^{-4}$	832	7862	2171	9561	943	9631	793	10102	225	991
Ce^{4+} medium H2SO4 2 mol l^{-1} C=$6*10^{-3}$	-	1924	-	1995	-	1747	-	1672	478	948
$Fe(CN)_6^{3-}$ medium NaOH 1,5 mol L-1 C=$6*10^{-3}$	-	2695	-	2745	-	2309	-	1039	284	7036

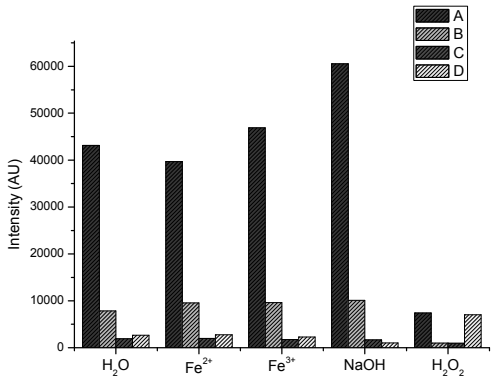

Fig. 3 Selection of the suitable oxidant system for Sulfamethoxazol determination

According to the depicted results the selected chemical parameters for further work were: potassium permanganate in polyphosphoric acid medium as oxidant system; and, the NaOH medium at at 10^{-2} mol l^{-1} for the photo-degradation process. According to the fact the CL emission can be affected by various factors next work consisted in their optimization. The influencing factors on the CL emission can be: the nature and concentration of other substrates affecting the CL pathway and favoring other non-radioactive competitive processes, the catalyst, the temperature, pH and ionic strength, the hydrophobicity of the solvent and solution composition, the presence of energy transfer acceptors.

The next test examined the influence of the concentration of potassium permanganate over the range $5x10^{-5}$ – $5x10^{-3}$ mol l^{-1} and using a Sulfamethoxazol concentration of 100 mg l^{-1}, 10^{-2} NaOH (Table 4). The chemiluminescent signal was found to increase with increasing MnO_4^- concentration up to $3x10^{-4}$ mol l^{-1}; outputs were rapidly decreasing for concentrations higher then the reported maximum. Figure 5 illustrated the observed results.

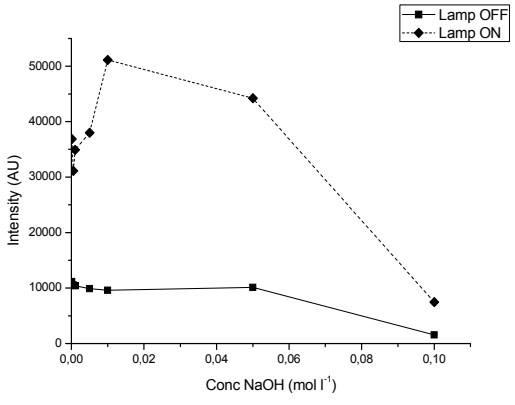

Fig. 4 Influence of the concentration of NaOH

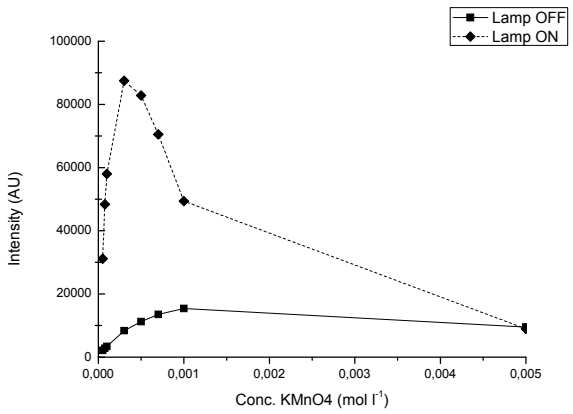

Fig. 5 Influence of the concentration of the MnO_4^-

Once the most effective oxidant and concentration were selected, we examined the influence of the polyphosphoric acid concentration over the range of 0,2%-0,7%. As can be seen in Table 1, the obtained peaks exhibited the taller emission peaks at the acid concentration of 0,25% .

Table 2 Influence of the concentration of the polyphosphoric acid

Concentration $H_4P_2O_5$ [%]	CL Intensity	
	Lamp off	Lamp on
0,20%	3812	136665
0,25%	4675	**136925**
0,30%	5303	129960
0,35%	5801	115445
0,40%	6451	97617
0,45%	6483	91643
0,50%	6799	86776
0,55%	6894	82488
0,60%	7339	73001
0,65%	7685	75138
0,70%	7330	74044

3.3 Influence of the presence of external chemicals: sensitizer and organized media

The chemiluminescence emission of an analytical system can be enhanced by using sensitizers or organized media. In the present work was studied the influence of different substances and concentrations. All substances used in these tests were added to the sample solution. The observed analytical signal was compared with the obtained with pure aqueous Sulfamethoxazol solution. No signal was found to exceed the reference signal. Therefore, no sensitizer or organized media was used in subsequent tests. A Sulfamethoxazol concentration of 50 mg l^{-1} was used in these instances.

3.4 Influence of the temperature

The influence of temperature was tested with the aid of two independent experiments; influence on the photodegradation; influence on the photodegradation and oxidant reaction together. The temperature influence was studied by immersing the vessels containing the corresponding solutions in a J. P. Selecta Tectron 2000 water bath at 40, 60, 70°C. The study on the influence of the temperature resulted in a similar graph for both experimental series; outputs were decreasing in the tested range as temperature was increased (from 20 to 70°). Selected for further work was room temperature (20°C).

3.5. Optimization of hydrodynamic parameters (MSM)

A multiparametric strategy, the modified simplex method, was selected for the optimization of hydrodynamic parameters. The parameters studied were photodegradation time, flow-rate and for valve 2 and valve 3 the number of segments and their interval (ON – OFF) and the total number of segments on each valve and cycle; and the total flow-rate. Intervals are time controlled through the multi-commutator (solenoid valve); ON and OFF means the two intervals in which a given solution is flowing or the way is blocked, respectively.

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Table 3 Optimization of hydrodynamic parameters
Chemical parameters were kept constant, 50 mg l^{-1} Sulfamethoxazol and 10^{-2} mol l^{-1} of NaOH and $3x10^{-4}$ mol l^{-1} potassium permanganate in 0,25% polyphosphoric acid.

lamp on								
Range	**L.p.**	**flow**	**N_3**	**$t_2$on**	**$t_2$0ff**	**$t_3$on**	**$t_3$off**	**Intensity CL**
flow=500-999 N_3=30 $t_2$on=0.1,0.4 $t_2$off=0.1,0.4 $t_3$on=0.4,0.8 $t_3$off=0.1,0.4	1	500	30	0,1	0,1	0,4	0,1	1683
	2	955	30	0,2	0,2	0,5	0,2	39002
	3	602	30	0,4	0,2	0,5	0,2	29441
	4	602	30	0,2	0,4	0,5	0,2	11571
	5	602	30	0,2	0,2	0,8	0,2	22388
	6	602	30	0,2	0,2	0,5	0,4	9604
	7	**999**	**30**	**0,4**	**0,2**	**0,6**	**0,2**	**55854**
	8	845	30	0,3	0,3	0,7	0,3	34945
	9	661	30	0,2	0,2	0,5	0,3	14365
flow=999 N_3=20-40 $t_2$on=0.3,0.6 $t_2$off=0.1,0.4 $t_3$on=0.4,0.8 $t_3$off=0.6,0.2	10	999	20	0,3	0,1	0,4	0,2	49083
	11	999	38	0,4	0,2	0,5	0,3	42333
	12	999	24	0,6	0,2	0,5	0,3	44721
	13	999	24	0,4	0,4	0,5	0,3	26189
	14	**999**	**24**	**0,4**	**0,2**	**0,8**	**0,3**	**55894**
	15	999	24	0,4	0,2	0,5	0,6	33371
	16	999	21	0,3	0,1	0,5	0,2	52222
flow=999 N_3=20-30 $t_2$on=0.4,0.6 $t_2$off=0.1,0.3 $t_3$on=0.6,0.8 $t_3$off=0.1,0.3	17	999	20	0,4	0,1	0,6	0,1	52185
	18	999	29	0,4	0,1	0,6	0,1	53441
	19	**999**	**22**	**0,6**	**0,1**	**0,6**	**0,1**	**60319**
	20	999	22	0,4	0,3	0,6	0,1	36500
	21	999	22	0,4	0,1	0,8	0,1	26394
	22	999	22	0,4	0,1	0,6	0,3	55303

3.5.1. Influence of the photodegradation time

The irradiation time is a crucial factor and that is determined by the reactor length and flow-rate in continuous-flow systems. It should be noted that the processes involved are of kinetic rather than equilibrium nature, and that an increased reaction path-length or decreased flow-rate have a marked, adverse effect on sample dispersion; this results in shorter, broader peaks that in turn lead to decreased sensitivity and throughput.

The experimental conditions under which a photochemical reaction takes place are greatly influential on the analyte derivatization rate and yield. Among the more influential of such conditions are the irradiation time (*i.e.* the flow-rate and reactor length), the light intensity or lamp power, the distance between the lamp and solution, and the solution properties (pH, polarity, temperature).

We studied the effect of time from 30 to 240 s with lamp on and lamp of as better results were obtained for small irradiation intervals, then we refined the result studying the range from 1 to 40 only with lamp on. For both series was used an aqueous solution containing 50 mg l^{-1} of Sulfamethoxazol. A photo-degradation time of 5 s was therefore chosen for subsequent tests.

3.5.2. Influence of flow-rate

The flow-rate was pre-optimized over the following range: 200-999 (arbitrary units coinciding with the rotation speed of the peristaltic pump drum as shown on the display). A Sulfamethoxazol concentration of 100 mg l^{-1} was used in these instances; this parameter resulted to be highly influential. Suitable flow-rate was selected for further work and it corresponds to 4,8 ml min^{-1}.

The photo chemical fate of five sulfa-drugs (sulphamethoxazole among them) was investigated [25] in aqueous solution and a variety of energy sources In those experiments the rate of direct photolysis was dependent upon the identity of the heterocyclic group as well as the pH of the solution. The photoproducts were also investigated by means of HPLC retention; and, for all the tested drugs the sulfanilic acid was observed as common product and producing the most prominent chromatographic peak. The potential direct photolysis cleavage sites are shown in Figure 1 by dotted lines; being the detected photo-products aniline, sulphanilamide, sulfanilic acid and NH_2R (R represents the five membered heterocyclic substituent). Sulfa-drugs containing a six membered heterocyclic group were also studied [26] by the same authors were the formation of SO_2 is also observed. Other summarized comments appeared in some few references [27][28].

3.5.3. Optimized solenoid valves program

Subsequent test was intended to expose optimized solenoid valves program and the finally selected set of parameters. The parameters studied for valve 2 and valve 3 were the total number of segments and their interval (ON-OFF) and total number of segments on each valve and cycle. For details see Table 3.

The complete experimental procedure consisted in two consecutive series in which the second was performed by changing the intervals according to the results from first series. Then, the vertices resulting in higher outputs were selected to obtain the best compromise sensitivity (peak height), reproducibility (RSD %), and sample throughput. Results are depicted in Table 3 and the finally selected values formed the final set which is the following:

Table 4 Optimized solenoid valves program

PROGRAM nr 7:

V_2=0,50*(0.4,0.2),0.5

V_3=0,30,5,30*(0.6,0.2),0.5

V_9=35,25

t_{cycle} = 90 s

4. Analytical figures of merit (Method Validation)

4.1. Calibration plots

The calibration graphs was studied over the range 0,01 – 250 mg l^{-1}, and it was observed a linear dynamic range from 0,01 to 100 mg l^{-1} with a detection limit of detection of 0.05 mg l^{-1}, and fitted with the equation I = 932,88 x - 603,26 (average of five replicas) with a correlation coefficient of 0,99886, where I is the chemiluminescent emission in counts and x means the Sulphametoxazol concentration in mg l^{-1}.

The robustness of the method was determined by using a univariate procedure of change each variable around the value chosen as optimal. Tests were performed by using 20 mg l^{-1} of Sulfamethoxazol The influence of changes (±10%) in concentration of the photodegradation medium, oxidant and acid resulted in relative errors vs the reference (in %): a) NaOH concentration, 16.9 and 5.6; b) oxidant concentration, 7.0 and 22.3; acid concentration, 28.3 and 24.9, respectively.

A measure of repeatability was studied by using 18 consecutive insertions (intra-day reproducibility or repeatability) of the same solution containing 20 mg l^{-1} of Sulfamethoxazol; the calculated RSD (%) was 5,8. The maximum sample throughput was calculated from the avarage of base-peak wide; obtained result was 40 h^{-1}. The same experiment was repeated on 5 different days with freshly prepared solutions (inter-day reproducibility) ranging from 0.01 to 250 ppm of Sulfamethoxazol, results range from 8% (RSD) for concentration under 1 ppm to 4.5% for 250 ppm. Required volumes of reagents expressed in mil per insertion were: sulphuric 1.60 ml min^{-1}; sodium hydroxide, 0.80 ml min^{-1}; and, polyphosphoric acid, 2.00 ml min^{-1}.

The influence of the presence of various substances potentially accompanying Sulfamethoxazol in samples was examined by comparing the analytical signal (analyte plus tested interferent in 500 mg l^{-1} as the maximum assayed concentration) with the reference signal obtained by inserting a 1.0 mg ml^{-1} solution containing no additional substances. Results were compared with the solution containing only the analyte and interference was considered when the difference vs reference was over ±5 %. Table 4 shows the errors introduced by the different species tested.

Table 5 Influence of foreign compounds

Interferent	Conc (ppm)	Re (%)
Starch	500	3.4
Lactose	500	0.9
Sucrose	500	3.9
Saccharin	50	1.0
Glucose	500	4.5
NaCl	500	3.6
Cu^{2+}	50	1.6
Mn^{2+}	50	4.6
Ca^{2+}	50	10.0
Fe^{3+}	100	2.0
Co^{2+}	100	4.0
CO_3^{2-}	500	2.0
Mg stearate	100	3.6
PO_4^{3-}	100	2.6

The applicability of the proposed method was checked by using it to determine Sulfamethoxazol in several kinds of water samples; namely: tap, mineral bottled water and from a irrigation channel. Water samples were spiked with a known amount of pharmaceutical (0.5 mg l^{-1}). Recoveries (average of three replicas) and R.S.D. in % were the following: tap water, 98.53 and 2.0; mineral bottled water (commercially available, trade name Font Vella, Spain) 99.00 and 0.6; and, irrigation channel water, 104.00 and 1.0.

5. Conclusions

A new analytical procedure is proposed for Sulfamethoxazol based on the photo-induced chemiluminescence of the analyte. The method is automated with the aid of a continuous-flow procedure into the methodology known as Multicommutation. The method involves the on-line photo-degradation of the analyte (stopped-flow, 5s) with the selected suitable medium and its subsequent chemiluminescent oxidation. Sample solution alternated segments with the photo-degradation medium and after the irradiation; aliquots of the resulting mixture are alternated with the oxidant system 2 cm before the flow cell of the luminometer. As far as the authors know this is the firs attempt to chemiluminescence determination of Sulfamethoxazol.

The continuous-flow manifold contained three different solenoid valves to alternate the insertion of sample or reagents. Sample aliquots were inserted into the system with alternate segments of selected irradiation medium with the aid of a solenoid valve. Then the analyte was irradiated during 5 min. and the resulting photofragments solution was directed to the detector were it was segmented and alternated with the oxidizing system through a new solenoid valve and entering into the flow-cell place 2 cm from the photomultiplier window.

Linear interval was from 0,01 to 100 mg l^{-1} with a limit of detection of 5 μg l^{-1}; and sample throughput and reproducibility were 40 h^{-1} and 5,8%.

The selectivity of these automated CL determinations can be easily adapted on – line to previous separation steps, like chromatography as a post-column detection.

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