Influence of the light-path of the flow cell on the spectrophotometric measurements in an FIA assembly. Spectrophotometric determination of several sulfonamides

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Summary - The spectrophotometric determination of sulfonamides on the basis of the Bratton-Marshall method has been performed by using two different flow-cells with 1 and 5 cm light-path length, respectively. Lengthening the light-path of the flow-cell in an FIA manifold leads to an increase in the sensitivity and the detection limits are markedly reduced according to Lambert law and the dispersion of the sample in the flow manifold. Due to the dispersion effect, the flow manifold should be re-optimized for different cells; however, differences between the both optimized manifolds are not relevant. The study is carried out with five different sulfonamides. The influence of foreign compounds is also studied and the method is applied to the determination of those sulfonamides in pharmaceutical formulations.

Key-words. - spectrophotometry, sulfonamides, FIA

1. Introduction

Sulfonamides are well-known antibacterials and are used extensively in medical and veterinary application against a wide range of Gram-positive and Gram-negative microorganisms. Many sulfones are rapidly absorbed from the gastrointestinal tract and can be detected in blood at reproducible level. Often, they are combined with antibiotics (usually penicillin and streptomycin) in order to increase the medical effect and widen their application [1].

Sulfonamides can be determined using spectrophotometric, electroanalytical, chromatographic (liquid, gas and supercritical fluid), fluorimetric and capillary electrophoresis methods. The Bratton-Marshall method [2] is especially frequently used in pharmaceutical and clinical analyses for sulfonamides. The method involves the conversion of the primary aryl amine into a diazonium salt by reaction with nitrous acid (sodium nitrite in hydrochloric acid), followed by coupling to *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED), a chromogen, forming an intensely colored azo-dye which can be monitored spectrophotometrically. The practical

shortcomings of this method can be circumvented by using a continuous flow injection assembly. For example, an FIA determination of sulfadiazine [3] has been recently reported. Nitrite, an unstable reagent, is generated *in situ* by reduction of nitrate in solution using a copperized cadmium column. The use of solid phase reagent has proven to be highly useful and advantageous in FIA analysis [3-5].

In this work, we extended the above-mentioned FIA determination of sulfadiazine to other members of the sulfonamide family and also investigated the effect of altering the optical path of the flow-cell on the analytical signal. There is so far only a single paper [6] devoted to analytical differences arising from variations in the light-path length of the flow-cell used for FIA analysis. Specifically, we used a flow-cell of 5 cm path length (inner volume 370 μl) instead of the typical 1 cm cell (inner volume 30 μl) in order to gather information of assistance in choosing the more appropriate cell according to the nature and the available amount of the samples used. For this puropose, we re-optimized the assembly employed in the previous work [3] for the new light-path length. The establised method was applied to the determination of various sulfonamides in order to generalize the assembly used for sulfadiazine.

2. Experimental

2.1. Reagents

Sulfadiazine, sulfamethoxazole, sulfamethoxypyridazine, sulfamerazine and sulfamidothiazole (Guinama, pure), NED, Na_2EDTA (Panreac, a. r.), cadmium (Aldrich, pure); for interference study, caffeine (Probus, pure), fructose, glucose, lactose, sucrose (all from Panreac, pure), lidocaine, nicotinamide, pyridoxine, trimethoprime (all from Guinama, pure), sorbitol (Acofarma, pure). Other reagents used were of analytical grade.

2.2. Continuous-flow assembly

The proposed manifold is depicted in Fig. 1. The sample injector was from Rheodyne, Model 5041, and a Gilson Minipuls 2 peristaltic pump was used. Two different

spectrophotometric detectors, a Model 8452A (Hewlett Packard) with 1 cm flow-cell (Hellma, 30 μl internal volume) and a Model CE292 (Cecil Instruments) with 5cm flow-cell (Hellma, 370 μl internal volume) were used. The solid-phase reactor was prepared by filling an Omnifit column (5 cm long and 3 mm i. d.) with copperized cadmium particles [3]. The PTFE coils were of 0.8 mm internal diameter.

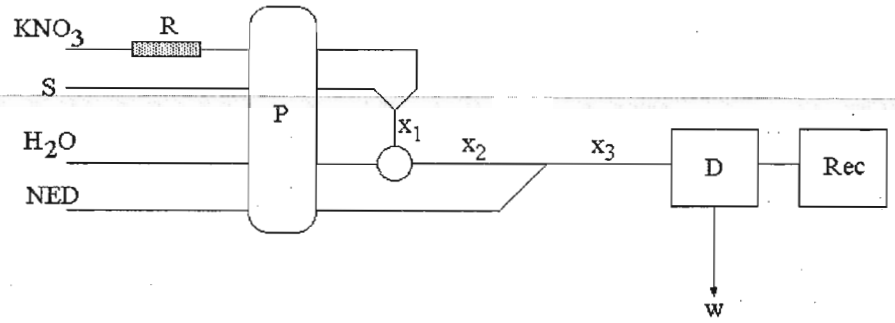

Fig. 1. FIA manifold proposed for the determination of sulfonamides (for details see text). R, solid-phase reactor filled with copperized cadmium; S, sulfonamide solution; P, peristaltic pump; D, spectrophotometric detector; Rec, recorder; W, waste and V, injection valve.

3. Results and Discussion

3.1 Optimization of the FIA system

The modified simplex method [7,8] was used to optimize FIA variables such as flow rate, sample volume and distances x_2 and x_3 (x_1 was kept constant at 35.5 cm) using a flow-cell of 5 cm path length. For the procedure, the solution of 10^{-2} M KNO_3 (in NH_4^+-NH_3 and Na_2EDTA buffer), 3.9 $\times 10^{-3}$ M NED and 2 ppm sulfadiazine in 0.2 M HCl were injected into the flow line. In addition to the magnitude of the analytical signal (the differences between the sample and blank peaks), variables were adjusted in such a way as to maximize reproducibility and injection throughput.

Table 1 gives the studied range and the adopted optimum value (after 16 runs, where the program is centered) of each variable. The optimum values for the 1 cm flow-cell is also given in

Table 1 for comparison [3].

Table 1. Optimization of FIA parameters by the Simplex Method

	5cm cell		
Parameter	Studied Range	Selected value	1cm cell
Carrier flow-rate (ml/min)	4.2 - 5.4	4.2	4.8
NED flow-rate (ml/min)	2.1 - 3.3	2.3	2.7
Sample volume (ml)	550 - 700	550	624
Distance x_2 (cm)	6.0 - 50.0	6.0	12.0
Distance x_3 (cm)	75.0 - 200.0	75.0	176.6

3.2. Analytical figures of merit

The optimized FIA assemblies for two flow-cells (path lengths of 1 and 5 cm) were used to obtain the linear range and the detection limit (taken as three times the standard deviation of the blank peaks) for each sulfonamide used. The results are summarized in Table 2 together with the average slope of the obtained calibration graphs. Comparison of the slopes shows that changing the 1 cm cell with a 5 cm one apparently results in much increased sensitivity. Between-day reproducibility is also calculated from the slopes of the calibration curves obtained on different days, and is given as a relative standard deviation values (rsd slope (%)) in Table 2.

The influence of foreign compounds on the determination of sufadiazine was investigated by preparing the solution containing 2 ppm sulfadiazine and different concentrations of the potentially interfering compounds. The 5 cm flow-cell was used for the procedure and the maximum concentration of the interferents examined was 1000 ppm for all the compounds investigated. The results are shown in Table 3.

Finally, the proposed method was applied to the determination of three sulfonamides in pharmaceutical formulation, and the results are given in Table 4.

Table 2. Calibration results and day-to-day reproducibility for different sulfonamides

Reagents (λ, nm)	Light-path length (cm)	Linearity range (ppm)	Detection limit (ppm)	Slope (average)	rsd slope (%) (replicates)	Correlation coefficient
Sulfadiazine	1	1.0 - 40.0	0.10	0.0448	2.77 (6)	0.99991
(542)	5	0.1 - 8.0	0.04	0.2006	2.54 (6)	0.9997
sulfamethoxazole	1	1.0 - 30.0	0.10	0.0530	2.24 (8)	0.99997
(544)	5	0.1 - 6.0	0.03	0.2558	2.97 (5)	0.99996
Sulfamethoxypiridazine	1	1.0 - 40.0	0.08	0.0418	2.33 (5)	0.99997
(544)	5	0.1 - 7.0	0.04	0.2009	1.50 (5)	0.99996
Sulfamerazine	1	1.0 - 40.0	0.10	0.0403	2.47 (5)	0.99992
(546)	5	0.1 - 9.0	0.03	0.1773	2.66 (5)	0.99995
Sulfamidothiazole	1	1.0 - 35.0	0.08	0.0464	1.81 (5)	0.99990
(546)	5	0.1 - 7.0	0.03	0.2218	1.56 (5)	0.99995

Table 3. Influence of foreign compounds

Substance	Conc. (ppm)	Relative error (%)
Caffeine	900	2.42
Fructose	1000	0.62
Glucose	1000	0.30
Lactose	1000	1.48
Lidocaine	1000	0.21
Nicotinamide	1000	1.14
Pyridoxine	1000	2.38
Sucrose	1000	1.75
Sorbitol	1000	0.12

Table 4. Determination of different sulfonamides in pharmaceutical formulations

Substance	Pharmaceutical formulation	Certified value (mg)	Found (mg)	Relative Error (%)
Sulfadiazine	Bio-Bubber Fuerte	2.00	1.94	3.0
Sulfamidothiazole	Bucodrin	11.40	11.70	2.0
Sulfamethoxazole	Abactrim	15.04	14.85	1.3
	Bronco-Bactifier	12.94	12.75	1.5
	Pulmosterin Duo	11.95	12.04	0.8

4. Conclusions

It has been found that lengthening the light-path of flow-cells in FIA assembly leads to markedly increased sensitivity as calculated from the slope of the calibration curve. The increase in sensitivity is not as large as expected from the Lambert-Beer law, but lies very close to the figure provided that the system is properly re-optimized (see the results for sulfamethoxazole in Table 2, for example). The detection limit was thereby reduced by a factor close to 10 for all the sufonamides studied (Table 2).

The optimal conditions for the use of a flow-cell of 5 cm path length in the FIA system are similar to those required for a cell of 1 cm path length, allowing the FIA assembly to be converted easily from one to another when required.

With markedly increased sensitivity, the linear range for the 5 cm cell is much wider than that for the 1 cm, while retaining the similar reproducibility. Initial use of the 1 cm cell is highly advisable for the determination of dissolution tests for solid formulations of oral administration.

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