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FIA-SPECTROPHOTOMETRIC DETERMINATION OF THIAMINE AFTER UV-IRRADIATION

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Summary—The determination of thiamine was carried out by UV-photodegradation in a single-line flow-injection assembly. The UV-photodegradation of thiamine was carried out in the coil of the injection valve, constituted of a PTFE tubing, half of its length being helically coiled around a UV lamp. A peak with two adjoining maxima was produced by injection, corresponding to the absorbance of the irradiated and non-irradiated sample. The analytical parameter is the difference between the two peaks, measured at 264 nm. The calibration graph is linear over the 1.2–30 μg/ml range of thiamine hydrochloride in 0.1*M* HCl. The influence of certain admixed substances was studied and the method was tested for the determination of thiamine in tablets.

Thiamine hydrochloride or Vitamin B_1 hydrochloride is the 3-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride monohydrochloride, occurs in many animal and plants tissues, however practically all vitamin B_1 sold is synthetic.[1-3]

Several oxidative reagents have been proposed for the spectrophotometric or fluorimetric determination of thiamine. Official procedures[4] deal with the potentiometric titration in acetic acid medium for the determination of thiamine hydrochloride. Different chromatographic (HPLC and GC) procedures have been proposed for the analysis of thiamine in a certain variety of samples, human urine, meat, food *etc.*[1] Thiamine has been also determined by FIA procedures based on the oxidation of the drug with ferricyanide in solution and then extraction with chloroform,[5] or with the same oxidant immobilized on an anionic exchange resin.[6] Other two articles on thiamine determination are based on the electrochemical derivatization with spectrofluorimetric detection[7] and detection by means of tubular FIA electrodes.[8]

On the other hand, the recent application of the FIA procedures to the analytical use of UV-irradiation is an interesting alternative which resulted in some advantageous procedures over those based only on chemical reactions,[9,10] it has been mainly due to simpler manifolds required, effective and economic procedures, some of them already been applied to the determination of pharmaceuticals.[11,12] The present paper deals with the UV-irradiation of thiamine for the spectrophotometric determination of the drug in pharmaceutical formulations.

EXPERIMENTAL

Reagents and apparatus

Reference thiamine hydrochloride (Acofarma, pure) standard solution was made in 0.1*M* HCl (Merck, analytical reagent grade). Riboflavine, pyridoxine, calcium pantothenate, nicotinamide, glucose, inositol, sucrose and ascorbic acid (all from Guinama, pure), aminoacetic acid and cyanocobalamin (both from Probus, analytical reagent grade). All solutions were prepared with deionized water.

FIA manifolds

Two different flow assemblies were evaluated. Figure 1 shows the continuous-flow manifolds. The sample injector from Rheodyne, model 5051, and a Gilson Minipuls 2 pump was used. The determination of thiamine was carried out by means of a model Lambda 16 spectrophotometer (Perkin Elmer, Germany) at wavelength 264.0 nm, provided with a flow-cell of 30 μl and 1 cm path length (Hellma). The UV-irradiation was undertaken by using a Vilber-Lurmat T-60

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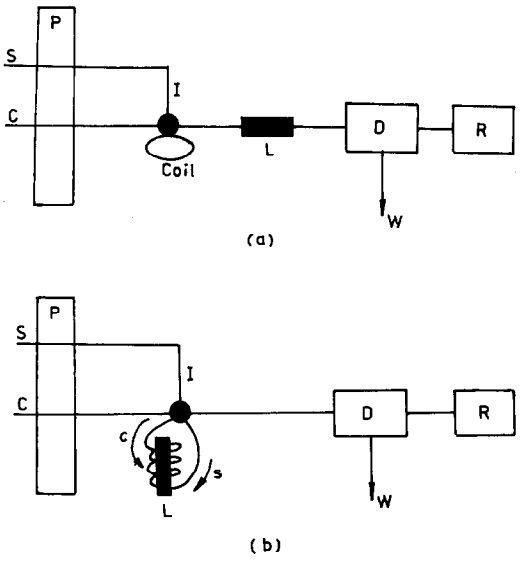

Fig. 1. FI-manifolds tested for the determination of thiamine hydrochloride. (a) With UV-irradiation source placed after injection of the sample. (b) With the irradiation source nesting in the half of the valve loop. P, Peristaltic pump; S, sample solution; C, carrier; L, mercury lamp; I, injection valve; W, waste; D, detector; and R, recorder.

mercury lamp. The PTFE tubing was of 0.8 mm i.d. except the tubing connecting the injection valve with the flow cell was of 0.3 mm i.d.

Determination of thiamine in pharmaceutical formulations (tablets)

At least five tablets were weighed and the average weight of a tablet determined. They were powdered and dissolved in 0.1*M* HCl and any remaining residue filtered. The residue washed and the solution clarified up to 1 l. with the same solution. Aliquotes of 1 ml were diluted to 100 ml with the solvent. This solution was utilized for thiamine determination using the FIA manifold depicted in Fig. 1b. The injected volumes were of 500 μl the flow-rate of 0.6 ml/min and the length of the tubing connecting the injection valve with the flow-cell was of 40 cm and 0.3 mm i.d. The calibrations graphs were constructed by plotting the absorbance differences between the two adjoining peaks corresponding to the same injected standard, half of this being UV-irradiated and other half not, *vs.* concentration. For the linear equation see the text in analytical applications. For the analysis of unknown, 10 determinations were made for the same sample. The obtained values were averaged and the mean value interpolated in the calibration graph. The computation of the thiamine in tablets was made taking into account the quantity of determinated thiamine, the dilution performed and the average weight of a tablet.

RESULTS AND DISCUSSION

Preliminary studies

Preliminary studies were aimed at determining the stability of the pharmaceutical in aqueous solutions kept under daylight and under UV-irradiation. The stability of thiamine is high in acid solutions; sterile solutions at pH 4 or less lose their activity very slowly but neutral or alkaline solutions deteriorate rapidly, especially in contact with air.[1] Thus, stock solutions were prepared in 0.1*M* HCl. No alteration of the product was observed after 20 days, spectral changes were virtually negligible.

The action of UV-irradiation on the drug substance was preliminary studied using the manifold depicted in Fig. 1a, in which a solution containing 30 μg/ml of thiamine was flowing continuously at different flow-rates; from 2.8 to 0.35 ml/min; the length of PTFE tubing from the injection valve to the flow-cell was 300 cm and 250 cm of which was wrapped around the irradiation source; spectra were recorded with and without irradiation with the solution continuously flowing or with the aid of the stopped-flow mode.

Two of the obtained spectra corresponding to photodegraded and non-photodegraded thiamine are depicted in Figure 2. The obtained results, with the solution continuously flowing, showed spectral difference due to UV-irradiation, the greatest difference between the spectra occurred at 264 nm. Taking into account the flow rate, the internal diameter, and the length of the tubing wrapped around the UV lamp a time interval of about 3 min of UV-irradiation was calculated as necessary for obtaining a stabilized ratio between photodegraded and non-photodegraded thiamine.

The influence of the medium in the UV-irradiation of the drug was studied in acid, neutral and ammoniacal media. The photodegradation process is similar in the three different tested media (HCl at different concentrations, distilled water and 5% ammonia); on the other hand, no influence of the HCl concentration was observed over the range 1×10^{-2}–1*M*.

Changes in UV-vis spectra of thiamine under UV-irradiation are probably due to separation of the pyrimidine and thiazole rings when the —CH_2— linkage is broken; from the spectral

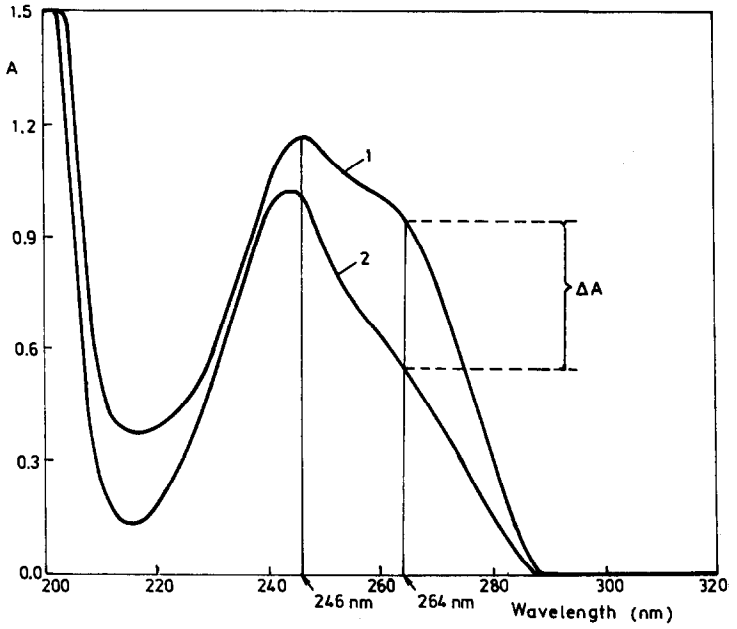

Fig. 2. Spectra of solutions containing 30 μg/ml thiamine hydrochloride in 0.1M hydrochloric acid medium. (1) Before and (2) after photochemical degradation, utilizing 1 cm flow-cell. The maximum absorbance difference, between the two spectra, $\triangle A$, occurs at 264 nm.

data reported in the literature the maximum from 246 nm in the UV spectrum of thiamine can be attributed to the thiazole ring[13] and the shoulder at about 246 nm to the pyrimidine ring.[14] After UV-irradiation there is a slight modification of the values of the wavelength and absorbance from the maximum at 246 nm and a substantial diminishing in the absorbance at 264 nm, probably due to the oxidation of the auxochrome group —NH_2 linked to pyrimidine ring. UV-irradiation of thiamine under the above conditions does not produce thiochrome, because the obtained products are not fluorescent.

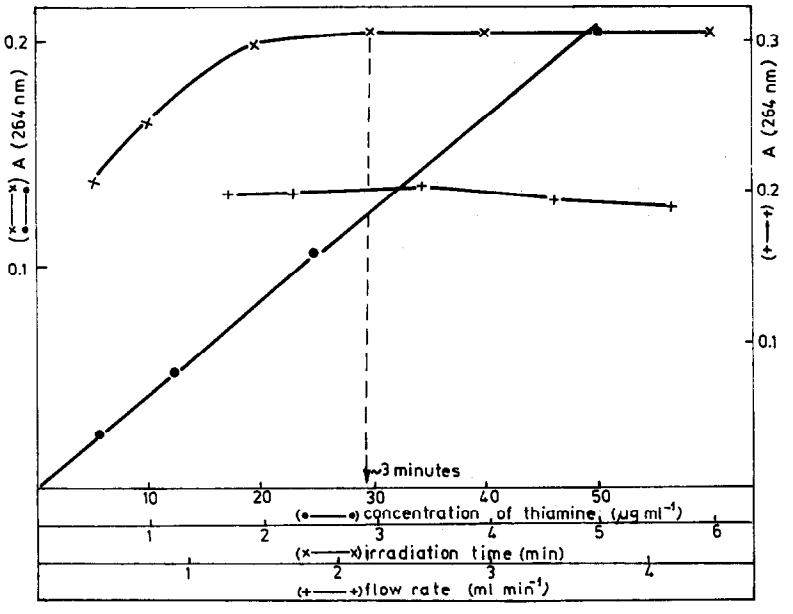

Fig. 3. Influence of different experimental parameters on the FIA-output differences; (●) influence of the drug concentration (irradiation time = 180 sec and flow-rate = 2.6 ml/min); (X) influence of the irradiation time (concentration of the drug = 50 μg/ml and flow-rate = 2.6 ml/min) and (+) influence of the flow-rate (concentration of the drug = 50 μg/ml and irradiation time = 180 sec). The sample volume was always 500 μl.

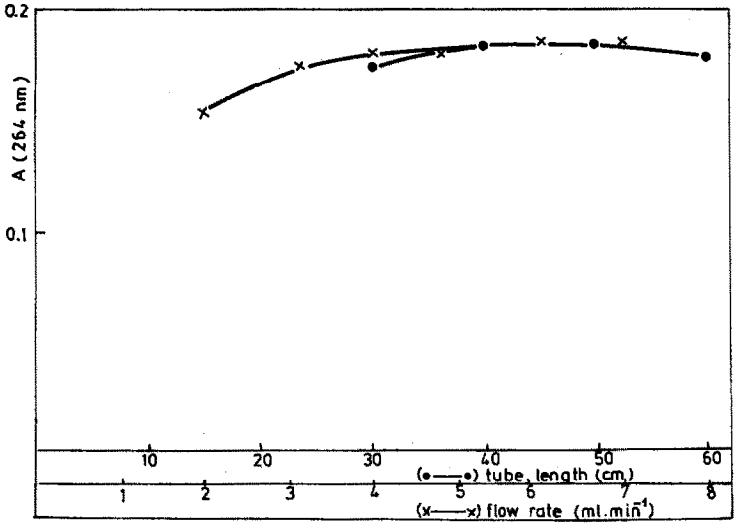

Fig. 4. Influence of different experimental parameters on the FIA-output differences. FI-manifold depicted in Fig. 1b; (●) distance from injection valve to flow-cell (flow-rate = 6.0 ml/min); (×) influence of the flow-rate (length 40 cm). The sample volume and drug concentration were 500 μl and 25 μg/ml, respectively.

The same concentration of thiamine (irradiated and non-irradiated sample solutions) were studied by varying the flow-rate; as the completeness of the degradation is clearly attached to irradiation time, the differences were increased as the flow-rate was decreased. The FIA-output differences, in peak height, were selected as analytical parameters for the determination of thiamine.

A different set of experiments (the lamp was not placed in the sample loop of the injection valve) revealed the influence of the time in stopped-flow conditions. The manifold-depicted in Fig. 1a was utilized; 15 sec after the sample was injected with the sample in the coil around the irradiation source, the pump was switched off. Figure 3 depicts the results obtained varying the concentrations of the thiamine in the sample solutions, the irradiation time and the flow-rate. A time interval of about 3 min of UV-irradiation was required to obtain a stabilized ratio between photodegraded and non-photodegraded thiamine.

Table 1. Influence of common additives with dispensed compounds on the thiamine analysis by FIA-spectrophotometry

Interferent	Concentration*	Relative error (%)
Lactose	300	1.5
Inositol	300	1.0
Glucose	300	0.5
Aminoacetic acid	300	1.2
Riboflavine	1/6	3.2
Pyridoxine	2	3.0
Nicotinamide	1/4	2.9
Cyanocobalamin	1	1.0
Ascorbic acid	1/3	2.5
Caffeine	1/3	2.0
Calcium pantothenate	100	2.0

*Tested compound/thiamine hydrochloride ratio. Concentration of thiamine hydrochloride 10 μg/ml.

The influence of the heat evolved from the lamp could be an important factor affecting the peak-height, especially in the stopped-flow mode; a set of experiments was carried out in which the sample loop was immersed into a cold water bath. The peak height increased with the stopped-flow interval, upto 1 min. It is known that by stopping the flow the FIA peak height increase. By increasing the stopped-flow interval (up to 15 min) the peak height does not increase. Therefore, a stopped-flow interval of 1 min was selected for the FIA peaks without irradiation and a 3 min stopped-flow interval for the FIA peaks with irradiation.

The influence of the sample volume was also tested by recording the FIA outputs with and without irradiation. The obtained experimental results were as predicted by the theory.[14]

Analytical applications

The assembly depicted in Fig. 1b was proposed for thiamine determination in tablets. The irradiation source was placed in the sample loop but only a part of the loop was helically wrapped around the lamp; half of the sample was irradiated. Then, the carried stream was forced to flow through the sample loop opposite

to the direction of the sample solution; as result of that, the other half of the sample volume was not irradiated. The FIA output presented a double peak, with two adjoining maxima, one of them, the highest, corresponding to the non-irradiated, and the smallest to the irradiated thiamine solution respectively. The irradiation period of 3 min and 500 μl sample volume was injected. The selection of this type of manifold was sought to improve the selectivity of the analytical procedure.

The influence of FIA parameters with this new manifold configuration were tested: the inside diameter (0.8, 0.5 and 0.3 mm) and length of the tubing injection valve-flow cell (30–60 cm, range) on the outputs were tested at different flow-rates, from 2.0 to 8.0 ml/min. Results obtained with the 0.3 mm i.d. tubing were clearly better than the others (Fig. 4). The selected values for further work were: 6.0 ml/min flow-rate, 40 cm length and 0.3 mm i.d.

The calibration graph by plotting the differences between the two adjoining maxima of the FIA output detected at 264 nm, *vs.* thiamine hydrochloride concentrations was linear over the 1.2–30 μg/ml range of thiamine hydrochloride, with the equation $A = -0.00033 + 0.0074X$, where A is the FIA-output difference and X the concentration of thiamine in μg/ml with a correlation coefficient of 0.997. The regression analysis was made by using eight standard solutions of thiamine hydrochloride with concentrations in the 1.2–30 μg/ml range and the differences between the adjoining maxima of the FIA-output correspond to the absorbances in the 0.0040–0.2250 range.

The reproducibility of the method was tested by a series of 10 injections containing 10 μg/ml of thiamine hydrochloride; the calculated relative standard deviation (%) was 1.7.

The influence of commonly found drug substances or additive in pharmaceutical formulations containing thiamine was investigated by preparing solutions containing 10 μg/ml of the drug and adding various concentrations of the possible interferents, up to 3000 μg/ml, or when the relative error was about 3%. The results obtained are shown in Table 1. Some of the tested interferents, *e.g.* lactose, inositol, glucose and aminoacetic acid, did not interfere even at the highest tested interferent/thiamine hydrochloride ratio due to the lack of absorbance at 264.0 nm; some others presenting absorbance at such wavelength interfered only by increasing the background absorbance. None of the tested interferents were affected by the UV-irradiation; absorption spectra remained unchanged after the irradiation. In this respect the present procedure is selective for the thiamine determination, *e.g.* by measuring thiamine directly at 246 nm, without UV-irradiation, the ascorbic acid/thiamine hydrochloride permitted ratio for a relative error of 2.5% is 1/16, and by using the recommended procedure the permitted ratio for the same error was 1/3. The situation was more or less similar for other interferents.

Thiamine was determined in two pharmaceutical preparations (tablets): Benexol and Benerva (both from Roche, Sociedad Anonima, Madrid, Spain), and the relative error was calculated by comparing the results with the content declared by the manufacturer. Benexol, found 248.6 mg/tablet; declared, 250; relative error, 0.5%. Benerva, found 302.0 mg/tablet; declared, 300; relative error, 0.7%.

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