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FIA–fluorimetric determination of thiamine*

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Abstract: A flow injection–fluorimetric determination of thiamine is reported. The procedure is based on the oxidation of the analyte with potassium hexacyanoferrate(III) immobilized on an anionic exchange resin; the fluorescence is monitored in aqueous basic solution. Concentrations of the vitamin of 0.1–4 ppm have been determined; the relative standard deviation was 1.8%. The injection rate was 28 samples/h. The influence of other substances and the determination of the drug in a pharmaceutical formulation are also reported.

Keywords: *Thiamine; FIA; oxidative columns; potassium hexacyanoferrate oxidation.*

Introduction

Data on physical, chemical and biopharmaceutical properties, metabolic routes, pharmacokinetics, etc. of thiamine are available [1, 2]. Several oxidative reagents have been proposed for the colorimetric or even fluorimetric determination of thiamine. UV spectrophotometric procedures have been adopted by the British Pharmacopoeia [3] and the US National Formulary XI [4] for the determination of thiamine in tablets. Thiamine has been also determined by FIA procedures [5, 6]. The oxidative method is based on reaction of the drug with hexacyanoferrate(III) in a basic medium followed by extraction of the oxidation product with chloroform.

One of the recent attractions of flow injection technology is the use of solid or immobilized reagents. There are a number of published examples in the field of pharmaceutical and biomedical analysis in which immobilized enzymes have been used in reaction columns. Immobilization of hexacyanoferrate on an anionic exchange resin has also been proposed for the determination of paracetamol in pharmaceutical formulations [7, 8].

The present paper describes the determination of (vitamin B_1) using hexacyanoferrate as an oxidative reagent. Potassium hexacyanoferrate(III) was immobilized on an anionic exchange resin and the oxidized product measured by means of a fluorimetric detector.

Experimental

Reagents and apparatus

Potassium hexacyanoferrate(III) (Probus, ar); aminoacetic acid (Probus, ar); 1-glutamic acid (Merck); caffeine (Fluka); pyridoxine (Guinama); calcium pantothenate (Guinana); nicotinamide (Guinama); riboflavine (Guinama); sodium carbonate (Merck, ar); boric acid (Panreac, ar); and an anionic exchange resin, Duolite A102 D (Probus) were used.

FIA assembly. This is shown in Fig. 1. A sample injector 5041 (Reodhyne) and a peristaltic pump Minipuls 2 (Gilson) were used. Fluorimetric measurements were performed with a fluorimeter, model LS-50 (Perkin Elmer) at 368.0 and 440.0 mm for activation and emission, respectively, using a 30-μl flow-cell with 1-cm path length (from Hellma).

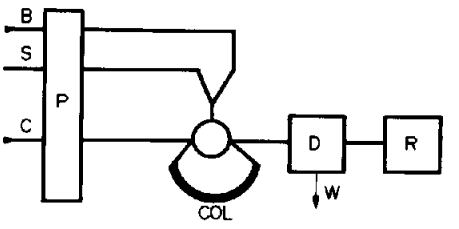

Figure 1
FIA assembly. P, pump; C, buffered carrier; COL, column; B, 225 cm; S, sample injector; D, detector; W, waste; R, register; λ_{ex} 368.0 nm; λ_{em}, 440.0 nm.

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Teflon tube coils were of 1.07 mm i.d. for the oxidative columns and 0.5 mm i.d. in the FIA assembly.

Procedures

Preparation of the oxidative resin. Preparation and storage of the oxidative resin were as reported in a previous article [7].

Determination of thiamine. An aqueous solution of the drug is introduced into the sample loop which acts also as oxidative column at room temperature. Sample injection is carried out each minute, giving about 70 s contact between the sample and the oxidative resin; it is then injected directly into the buffered sodium carbonate–boric acid (pH 10.0) stream.

Results and Discussion

Preliminary investigations on the stability of thiamine in aqueous solution were carried out by preparing solutions in different media (acidic or basic) and recording absorption spectra periodically for up to 5 days; the stability of the solutions was maximal at pH 2 and decreased with increasing pH; neutral or alkaline solutions (sodium or ammonium hydroxide) deteriorated rapidly, especially if in contact with air. An aqueous solution of thiamine, after 6 months storage at pH 2 and 37°C, was reported to be fully stable [1]. Preliminary experimental results have shown that the oxidation of thiamine is rapid and complete with hexacyanoferrate(III) immobilized on an anionic exchange resin.

A basic medium is required for thiamine oxidation and several media (sodium hydroxide, ammonium hydroxide and mixtures such as carbonate–bicarbonate, carbonate–boric acid and Na_2HPO_4) [9] were tested over a pH range of 10.0–12.5. Several conclusions were drawn. First, the presence of sodium hydroxide is undesirable owing to evolution of hexacyanoferrate(III) from the resin, giving a shorter life-span for the column; elimination of the excess of oxidant could necessitate an additional channel with a reductive stream [12]. Second, other reagents such as ammonium hydroxide or buffer mixtures (e.g. carbonate–bicarbonate) do not give the problems reported for sodium hydroxide but outputs are lower than those observed with sodium hydroxide. Third, the use of carbonate–boric acid (80 ml of 1.1 M carbonate with 30 ml of a mixture of 0.4 M boric acid and 0.4 M potassium chloride) resulted in high outputs.

Different resin particle sizes were tested to give a column with a configuration closely related to the SBSR type [12]. Different parameters for the column were tested: length, internal diameter, sample volume, flow rate and drug concentration.

Different FIA configurations were tested, and a suitable compromise was achieved between peak height, sample throughput, reproducibility and life-span of the column. Owing to the observed instability of the drug in basic media the FIA manifold adopted allows the sample and buffer to mix before reaching the injector (Fig. 1). Optimization of the suitable medium by means of the FIA assembly resulted in the selection of pH 10.0 and use of buffer mixture–distilled water (50:50, v/v) as the carrier solution (tested range 25–75%).

The influence of temperature on the oxidation reaction was studied up to 30°C. The output was reduced as the temperature was increased; the output was about 25% less at 30°C than at room temperature. Room temperature was selected for further work.

As observed from the preliminary work, the time that thiamine remains in contact with the oxidizing resin greatly influences the height of the output. Because of that effect and the simplicity of the FIA assembly and the operating procedure, the column position was changed to the sample loop; the Teflon tubing in the injector was filled with resin particles. This means that only the injecting time period has to be controlled and that the start–stop pumping operation is not required.

The optimization of the FIA parameters was carried out by means of the modified simplex procedure. The first vertex of the initial simplex gave 136.0 display units. After 30 experiments it was decided that the system did not merit further experimentation; points 14, (814.0), 21 (756.6), 22 (726.7), 26 (713.9) and 30 (1000) were examined to enable the best point to be chosen in respect of peak height, sample throughput and reproducibility. Optimization with these points was achieved by injecting six replicates each of 1 ppm of thiamine for each set of experimental FIA parameters and the ratio of the flow rate sample to the flow rate of the buffer mixture was varied; the RSD and sample throughput

were calculated. Point 21 (RSD 0.44%) represented the peak narrowest base-width and this point was selected for further work. Conditions were: flow rate, 0.74 ml min^{-1}; sample volume, 137.3 µl; coil length from injection-valve to detector flow-cell, 225 cm; injection time sequence, 1 min (about 70 s contact time between the sample and the resin). The results are given in Table 1.

The stability of the column was checked using the same FIA assembly. The carrier solution contained 1 ppm of thiamine and flowed continuously through the column; no injections were performed up to 1 h; 350 ml was forced through the column. Measurements were read every 200 s. The mean height (19 values) was 130.8 display units with an RSD of 4.5%.

Analytical application

The calibration graph between peak height (H) and concentration (C) was linear up to 4.0 ppm of thiamine. Since the method is applicable to low (ppm) thiamine concentrations (C), two linear graphs (corresponding to the two smaller sensitivity intervals of the fluorimeter) are reported. For 0.1–1.0 ppm of thiamine, the regression equation was: $H = 659.6\ C - 4.9$. For 1.0–4.0 ppm, the equation was: $H = 212.52\ C + 12.35$. The correlation coefficient for each range was 0.9999. The reproducibility of the determination was determined by injecting into the reagent stream 28 samples/h; each sample contained 1.0 ppm of thiamine. The RSD was 1.8%.

Interference with the method by other substances that are commonly found in pharmaceutical formulations of thiamine was investigated using 1 ppm of thiamine (Table 2).

Thiamine was determined in a pharmaceutical formulation, Vitallon 3B tablets, and the relative error was calculated by comparing the results with the content declared on the label by the manufacturer. A typical result was 291.2 mg per tablet for a declared content of 300 mg.

Table 1
Simplex method of optimization

Cycle/type	Point (numbers)	Flow rate (arbitrary units)	Coil length (cm)	Column length (cm)	Contact time(s)	Peak height (display units)
	1	200	30	10	0	136
	2	375	111	27.5	83	439.5
	3	375	111	84	20	489
	4	375	380	27.5	20	253
	5	941	111	27.5	20	226
1/R	6	833	323	75.7	71	519
E	7	1149*	469*	105*	107*	0
2/R	8	38*	348	79	77	0
C	9	715	170	40	34	315.5
3/R	10	774	−15*	85	84	0
E	11	475	380	42	36	397
4/R	12	313	240	73	71	719
E	13	113*	275	89	89	0
5/R	14	474	116	87	87	814
E	15	473	37	110*	112*	0
6/R	16	622	284	131*	41	0
C	17	437	155	53	73	653.6
7/R	18	653	306	59	131*	0
C	19	445	160	78	48	721
8/R	20	1.39*	13*	72	68	0
C	21	625	225	83	70	756.6
	22	625	248	73	70	726.7
9/R	23	491	226	102*	65	0
C	24	451	173	66	71	732
10/R	25	683	106	78	67	698.3
C	26	406	131	74	70	713.9
	27	406	208	74	70	763
11/R	28	533	211	72	101*	0
C	29	467	173	76	61	665
12/R	30	368	98	83	77	+1000

*Zero values of peak height were assigned to the entries marked with an asterisk which were out of the variable range.

Table 2
Influence of other substances

Compound	ppm	Error (%)
Aminoacetic acid	50	0.9
Caffeine	75	2.4
1-Glutamic acid	10	0.3
Pyridoxine	20	4.3
Calcium pantothenate	100	0.8
Nicotinamide	50	0.8
Riboflavine	25	2.9

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