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Dedicated to Professor Ionel Haiduc
on the occasion of his 65th birthday

KINETIC DETERMINATION OF B_1, B_2 and B_6 VITAMINS

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ABSTRACT. A kinetic method for separate determination of B_1, B_2 and B_6 vitamins has been proposed. It is based on the Landolt type system of redox reactions, is very sensitive, cheap and make use of simple technique. Calibration lines have been obtained under appropriate conditions. Good detection limits as well as good accuracy and precision were found. Some interference of various organic compounds and metal ions have been investigated. The method has been tested to several pharmaceutical products yielding results similar to the certified content given by producers.

INTRODUCTION

Lack of some nutrients causes serious diseases for human, even though small amounts of them are required to maintain good health [1]. The indispensable nutrients are vitamins and are supplemented by eating appropriate food. They can be categorised into two groups: water-soluble and fat-soluble vitamins. The B complex (B_1, B_2 and B_6) are in the first group.

Thiamine (vitamin B_1), which is found in many animal and plant tissue, has been widely used for prevention and treatment of beriberi and neurological diseases in medical doses. Riboflavine (vitamin B_2) is an essential vitamin and it has been used for the treatment of growth disorders, keratite, hypogalactie, steatoree. Pyridoxine (vitamin B_6) is also an essential vitamin for human, possessing many different physiological properties [2, 3]. It is used in prevent nutritional dermatitis.

Many analytical methods for qualitative and quantitative determination of B vitamins have been developed. For determination of thiamine, fluorometry [4-7], spectrophotometry [8,9], chromatography [10-12] and electrochemical analyses [13,14] have been used. Chemiluminiscence has been also explored

to determine thiamine [15,16]. Cherysh *et. al*. [15] first reported that a bright chemiluminiscent radiation appeared during oxidation of thiamine to thiochrome. Grekas and Calokerinos [16] developed a continuous flow-through chemiluminscent system for determination of thiamine based on the oxidation of thiamine to thiochrome by potassium hexancyanoferrate (III) in alkaline medium. However, the drawback of these methods are unsatisfactory detection limit and need of oxidation agent addition for initiating the chemiluminiscence [17].

Hence to establish an analytical method for the determination of pyridoxine in real samples would be useful for the evaluation of various medicine quality and quantitative control. A number of reports on this analysis are mainly liquid chromatography [18-20] and capillary electrophoresis [21-23]. There have been a large number of HPLC studies on determination of vitamins in food, beverages, drugs and biological tissues or fluids [24-26]. Recent studies for quantitative HPLC determination of water-soluble vitamins [27-29] involved extraction and quantification of vitamin B_1, B_2 and B_6 either separately or simultaneously.

In the present work, a simple and quite sensitive method for the separate determination of pyridoxine, thiamine and riboflavine is described. It is based on a Landolt type reaction.

The bromate-bromine mixture has been used since long ago as a way of generating bromine for analytical applications and a number of bromination reactions have been used in classical analysis [30]. Bromine is produced in a relative slow reaction according to the stoichiometry

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_3 + 3H_2O \quad (1)$$

and the rate law [31]:

$$\frac{d[Br_2]}{dt} = k \cdot [BrO_3^-] \cdot [Br^-] \cdot [H^+]^2 \quad (2)$$

The bromine generated *in situ* reacts with organic analytes as phenols, aromatic amines and other [32], either brominating or oxidising them rapidly. The vitamins under study react similarly. A steady state Br_2 concentration is maintained as long as the analyte is not entirely consumed and concentration increase take place at completely consumption.

EXPERIMENTAL

Apparatus

The instrumental set up is depicted in Figure 1. It consists from a reaction vessel with temperature control connected to a thermostat. The sensor was a platinum plate electrode, the potential being measured against a saturated calomel electrode by means of a potentiometer (Digitronix DXP 2040). The potentiometer was connected to a computer by means of a data acquisition device.

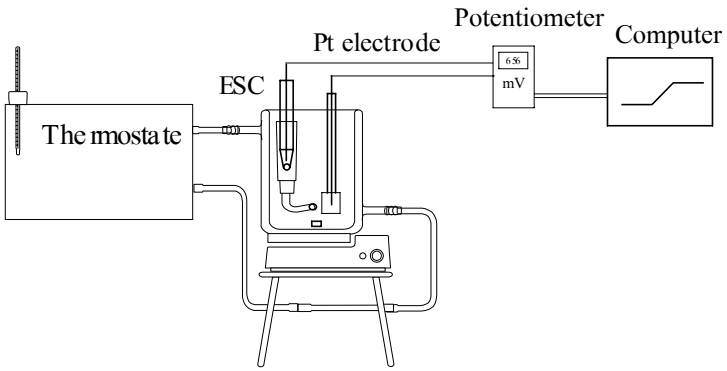


Fig. 1 Experimental device

Chemicals

All reagents used were of analytical-reagent grade. De-ionised and tetra-distilled water was used throughout. Stock solution of perchloric acid (0.5 M), potassium bromide (1 M) and potassium bromate ($3 \cdot 10^{-2}$ M) were prepared and standardized. Vitamin solutions (B_1, B_2, and B_6), in concentration of $2 \cdot 10^{-3}$ M were prepared before each set of experiments.

Procedures

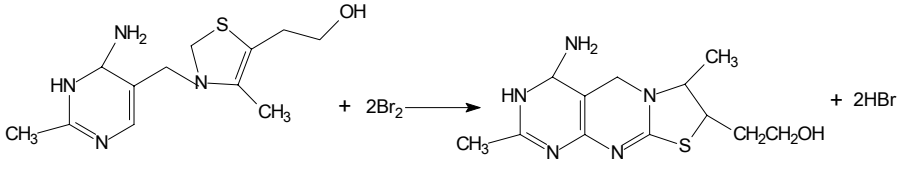
In the case of the determination of thiamine for example, 3 ml of perchloric acid 0.5 M and 5 ml KBr 1 M were mixed with various aliquots of vitamin solution and accurately diluted to 22.5 ml with de-ionised and tetra-distilled water. Over this mixture, 2.5 ml $KBrO_3$ ($3 \cdot 10^{-2}$ M) solution was injected quickly. The zero moment of the reaction is considered that when bromate solution was injected into the mixture.

When medicine was presented as tablets, ten tablets were weighted and pulverised by gentle grinding. An accurate weight of the powder was dissolved and made up to a 100-ml volumetric flask.

When the medicine was injectable vitamins, ten ampoules were dissolved in de-ionised and tetra-distilled water and diluted conveniently.

RESULTS AND DISCUSSION

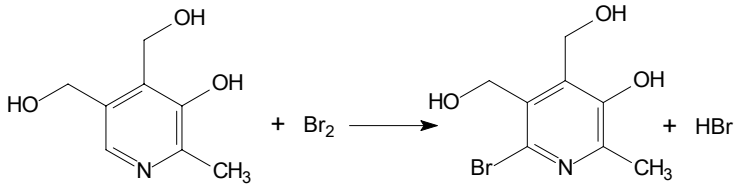
The reaction stoechiometry was measured by a kind of spectro-photometrical titration previously described [33]. For thiamine the ratio Br_2: thiamine was found to be 2. The stoechiometry is



(3)

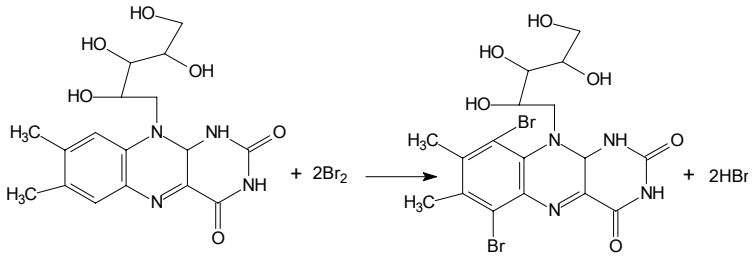
yielding thiochrome.

For pyridoxine the ratio Br_2 : pyridoxine was found to be 1. We are inclined to consider a bromination reaction rather than an oxidation:



(4)

For riboflavine the ratio Br_2 : riboflavine was found to be 2. The bromination reaction at the aromatic ring is:



(5)

Under the conditions of a small concentration of bromine, bromination of benzene ring is more probable than the oxidation.

From the analytical point of view, only the reaction ratio is important, not the nature of products or mechanism.

Determination of appropriate conditions for analysis

Appropriate reaction conditions regarding concentration of reagents were searched for in order to ensure a high slope of calibration graphs. Therefore, kinetic runs were performed at various concentrations of potassium bromide, potassium bromate and perchloric acid. All the reagents should be present in excess relative to analyte. The concentration of trapping agent at the largest concentration for obtaining calibration lines should be two orders of magnitude smaller than potassium bromate. The later condition ensures that no more than 5% reaction extent is attained at the end-point. A small extent of the reaction presents the advantage that the side reactions, such as oxidation of vitamins with bromate, will not affect the experimental results.

Figure 2 shows the dependence of reaction time on the acid concentration. According to equation (2), the bromine generation shows a second order dependence on the H^+. Therefore, the acidity strongly affects the bromine generation reaction rate. Because the end point time should be not to short, to avoid errors, and not to long, to avoid side reactions an appropriate value of $6 \cdot 10^{-2}$ M $HClO_4$ has been chosen.

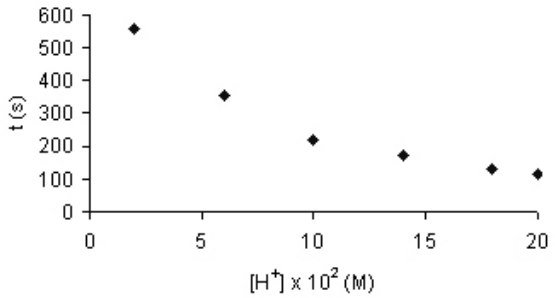


Fig. 2 Influence of $[H^+]$ on kinetic determination of [thiamine] = 4.10^{-5} M, [KBr] = 0,2 M, X = *2,6 %*

As seen in equation (2), the reaction rate of bromine generation follows a first-order dependence on the potassium bromate.and potassium bromine. The concentration of bromate was chosen at $3 \cdot 10^{-3}$ M. A great excess of bromide (0.2 M).has been used. The rate constant of bromine generation change with temperature according to the Arrhenius equation. A value of activation energy of 51 kJ/mol was found within the temperature range 15 and 40 ^{0}C [34]. Following the criteria of obtaining suitable times of analysis, the temperature of 20 ^{0}C seemed to be convenient.

Analytical parameters

Calibration graphs were obtained under the appropriated conditions described above for each vitamin B_1, B_2 and B_6 and are presented in Fig. 3. Each point is a mean of at least 5 individual measurements.

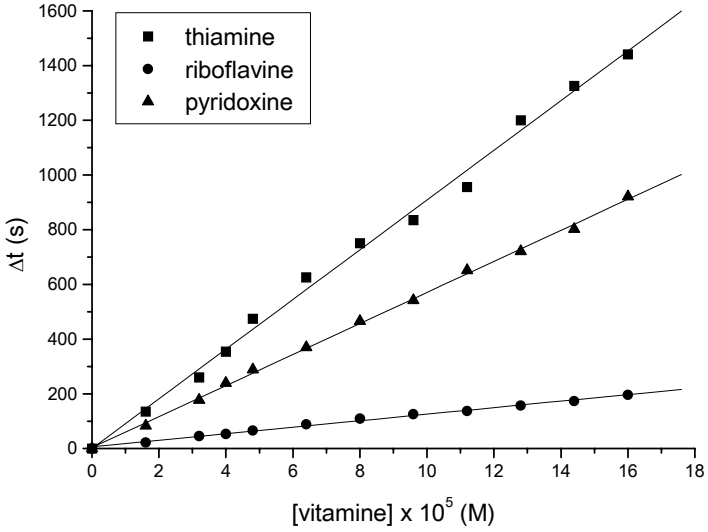


Fig. 3 Calibration lines

Linear regression parameters of calibration graphs are presented in table 1. The limit of detection was defined as $C_L = 3 \cdot S_B/m$ [35], where S_B and m are standard deviation of the blank signal and slope of calibration graph respectively.

Table 1

Linear regression parameters of calibration graphs for vitamins

Compound	Slope x 10^{-6} (s/M)	Intercept (s)	Correlation coefficient (N = 7)	Detection limit x 10^7 (M)
Vitamin B_1	9.02±0.51	0.3±2.3	0.9975	0.9
Vitamin B_2	1.09±0.06	6.0±4.3	0.9975	62.0
Vitamin B_6	5.67±0.12	3.3±5.9	0.9993	7.2

Although the conditions for bromine generation are the same, different slope and consequently different sensibility are obtained for the three B vitamins. The explanation for that is quite simple. The nature of the reaction with bromine is different (B_1- two steps oxidation and ring closure, B_2- two steps bromination and B_6- one step bromination). The rate constants for these reactions are different, but greater than the one for bromine generation. Thus, the level of steady state concentration of bromine is different, causing different rate of its consumption. Unfortunately, these differences in rate are not large enough to provide a way for discrimination between the vitamins under consideration of this clock method.

To evaluate the accuracy and precision of method, a series of independent standard samples was used. The results are given in table 2.

Table 2

Accuracy and precision of the proposed method

Vitamins taken x 10^5 (M)		Relative error (%)	RSD (%)
B_1:	4.0	2.5	1.9
	6.4	1.3	1.6
	14.4	0.5	0.9
B_2:	3.2	3.1	2.2
	4.8	2.0	1.7
	11.2	0.6	1.1
B_6:	8.0	0.8	1.3
	16.0	0.3	0.6

Selectivity

The effect of some organic compounds and heavy metals associated with drugs was studied. The results are summarised in table 3. The tolerance limit was defined as the concentration of added ion causing less than ± 3 %

relative error. Most cations and organic substance did not interfere even when present in 200-fold excess relative to vitamins. On the contrary, ascorbic acid, acetylsalicylic acid, cysteine, and paracetamol interfere in the determination of vitamins.

Table 3

Tolerance limit for diverse organic substance and ions on the determination of $8 \cdot 10^{-5}$ M thiamine

Interference	Tolerance limit ratio (mol/mol)
Citric acid, oxalic acid, tartric acid, glucose, starch, ethanol, methanol	400*
Zn(II), Cd(II), Ni(II), Mo(VI), V(V), EDTA	200*
Fe(III), Cu(II)	90
Vitamin B_{12}	100*
Cysteine, methionine, ascorbic acid, acetylsalicylic acid, paracetamol	1

*maximum limit tested

Applications

The proposed method was applied to the determination of thiamine, riboflavine and pyridoxine in pharmaceutical products. The results are shown in table 4.

Table 4

Results of the determination of B vitamins in pharmaceutical formulations

Formulation	Active substance	Found	Reported	Recovery (%)
Tablet (mg/tablet)	thiamine	10.2	10	102.0
	pyridoxine	248	250	99.0
Injectable (mg/ampoule)	thiamine	50.1	50	100.2
	riboflavine	9.9	10	98.0
	pyridoxine	50.3	50	100.2

As can be seen, an acceptable correlation is found between certified content given by producers and the results obtained by the proposed method.

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