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BIOTIN DETERMINATION BY USING A KINETIC METHOD

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A kinetic method for determination of biotin, using some special redox reaction, known as Landolt-type reaction is described in the paper. The process can be followed potentiometrically. At the end point, a potential steep increase of a platinum plate electrode has been noticed. The reaction time is directly proportional to the concentration of the substrate. Calibration line was obtained and used to determinate the concentration of vitamin H_1 in pharmaceutical products that contained it, without others B complex vitamins or ascorbic acid that would collision the reactions.

INTRODUCTION

Biotin is widely spread in animals and vegetables organisms and also in microorganisms. Based on many research studies it was established that lees, inferior fungi from *Penicillium* and *Aspergillusb* species, many bacteria (*Escherichia coli, Lactobacillus casei, Lactobacillus acidophylus*), some *Pseudomona* species and plants could biosynthesise the biotin.[1, 2]

In plants, excepting cereal grains and nuts, the biotin is predominant in free state, soluble in water, meanwhile in meat, lees, nuts, grain husks, the biotin is predominant as a proteic complex form, water insoluble. Significant quantities of biotin are found in beans, yeast, spinach, corn, oats, soybeans, peaches, grapes.[2]

In nature, p-aminobenzoic acid, also known as vitamin H_1 or B_x, is usually distributed as B complex factor, founded in free or acylate form or in peptide combinations. It is a vitamin soluble in hot water and alcohol, steady in the presence of both acid and alkaline solutions, unsteady in the presence of oxidants. Para-aminobenzoic acid forms monoclinic prism crystals, which turn yellow when long exposed in light, and it is incompatible with feric salts.[1-4]

Commercial names for biotin are: PABA, Amben, Paraminol, factor anti-cromotrichia, Vitamin B_x, Vitamin H_1.[1]

The pharmaco-therapeutic actiois decribed by the striking character, which is the antagonism to sulfonamides, to which inhibits both bactericidal action and bacteriostatic. Neutralizes also the slightly antimalarial potential of sulphonamides and it is an essential growth factor, which can be replaced more or less by pantothenic acid.

Biotin metabolism is still unclear, but probably because the bacteria (or other single-cell organisms) can largely transform it, in folinic acid and glutamic pteroil, action on the growth of black rats, chickens and guinea pigs.

Cases of vitamin function were reported, analogous to that of carotene. At high concentrations, biotin has bacteriostatic action on rickets.[2]

Therapeutic indications are mainly in local anesthetics (ethoform, procaine hydrochloride, etc.); in rheumatism, with cortisone, determining the reduction of the dose. High doses of p-amino benzoic acid lead to the elimination of glucuronic acid in urine. Biotin also presents interesting results in the treatment of limfoblastoms of skin and fungal infections. It is also antirickets' agent.[1, 2]

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Hypervitaminosis states at humans or animals were not reported, because biotin does not show any toxicity, not even at high doses (350 mg / kg body weight, given orally or 1000 mg / kg body weight, given intravenously to mice and rats). If high doses of biotin last longer, it is possible to produce hepatic steatosis and to increase tumor growth. [1, 2]

This paper presents an original, easy and sensitive kinetic method of determination of p-amino-benzoic acid, which is based on the using of a Landolt type system. The Landolt reaction is continuously and successfully used for many years, and recently in many fields: from medicine and microbiology,[5] to environment[6, 7] and engineering,[8, 9] all in analytical methods.[5-11]

EXPERIMENTAL

Biotin, due to the important biochemical role played, in every living cell, regain an increasing interest in the recent studies in special in the analytic techniques[12] used for measuring biotin, using HPLC method,[13, 19] Fourier transform infrared (FT-IR) spectrometry,[14] voltametry,[15-17] high-performance liquid chromatography,[17] fluorescence polarization,[16] capillary zone electrophoresis, column liquid chromatography, micellar electrokinetic chromatography[18] in pharmaceutical preparation,[12-14,18] as well in food or food supplement products[12,13,15] the main source of biotin in humas.[12] Also, in bioanalytical purposes, biotin is often chemically linked to proteins, by biotinylation, and form steady interactions with avidin[17,19] or stretavidin,[20] method used equal in food industries [19] and medicine.[20]

In previous studies[2, 21] it was shown that the mixture bromate-bromide was used as a way to generate bromine for various analytical applications. Therefore, a substantial number of bromination reactions have been already used in classical analysis. Bromine is produced in a relatively slow reaction according to the equation:

$$\frac{1}{3}BrO_3^- + \frac{5}{3}Br^- + 2H^+ = Br_2 + H_2O \qquad (1)$$

with the next rate law:

$$v = k \cdot \left[BrO_3^-\right]^{1/3} \cdot \left[Br^-\right]^{5/3} \cdot \left[H^+\right]^2 = \frac{d\left[Br_2\right]}{dt} \qquad (2)$$

Bromine, generated *in situ*, reacts with organic analytes like phenols, aromatic amines, vitamins, etc., determining their bromination or fast oxidation. Vitamin H_1 has similar behaviour. A steady state of bromine concentration is maintained as long as the analyte is not totally consumed, and consumption of vitamin due to suddenly increase of the concentration.

Evolution of bromide concentration was monitored by potentiometric measurements.[22] The redox counting electrode consists of a Pt plate immersed in the reaction mixture and the saturated calomel was the reference one. The process was performed in a thermostated reaction vessel fitted with stirring. To register potential-time curves was used a Digitronix DXP-2040 (Seiko) potentiometer, linked by data acquisition card to a computer.[23]

All reagents used were analytical purity reagents. To achieve solutions, deionized and tetradistilled water was used. Solution stocks of perchloric acid (0.5 M), potassium bromide (1 M) and potassium bromate (3.10^{-2} M) were prepared and standardized solutions of H_1 vitamin, of 2.10^{-3} M concentration were prepared before each set of determinations.

The procedure consists in mixing of 3 mL of 0.5 M perchloric acid with 5 mL KBr (1M) and adding variable amounts of p-aminobenzoic acid. The mixture thus obtained is diluted to 22.5mL with deionized and tetradistilled water. In this reaction mixture is rapidly injected a volume of 2.5mL $KbrO_3$ (3.10^{-2} M). Reaction time starts when bromate solution is injected into the mixture.

The experimental results were processed using Table-Curve and Origin 8.0.

RESULTS AND DISSCUSION

Stoichiometry of the bromination reaction of vitamin H_1

In Fig. 1 is shown the experimental stoichiometry of the bromination reaction of vitamin H_1 and the result suggests a dibromination of the aromatic ring, probably in the vicinity of the amino functional group.

Determination of the optimum work conditions

Several studies were performed in order to determine the rate reaction modification, which appear by reactant concentrations and temperature variations, as following:

a) Influence of KBr concentration

The results rule to a logarithmic dependence of the reaction rate with the bromide [Br^-] concentration The optimum value for KBr concentration was chosen to be 0,2 M. (Fig. 2).

b) Influence of pH

Solution acidity influence on the experimental results shoe the next: by lower reaction times at lower pH. In this case, the optimum value of $HclO_4$ concentration was found to be: 2.10^{-2}M.

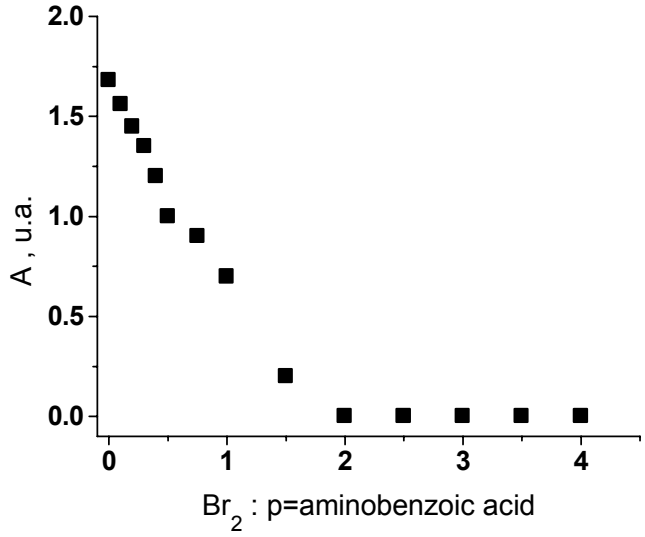

Fig. 1 – Stoichiometry of the bromination reaction of p-amino benzoic acid (Absorbance vs. Br_2: p-aminobenzoic acid).

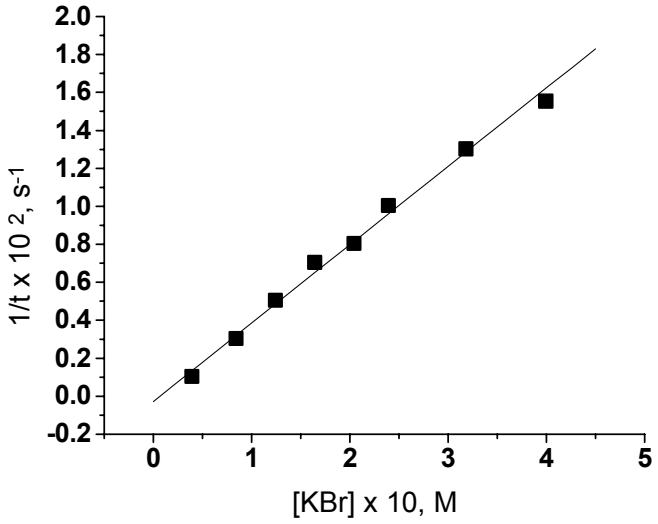

Fig. 2 – Dependence of bromine generation time to KBr in the case of: [p-aminobenzoic acid] = 8.10^{-5} M, $[H^+] = 2.10^{-2}$ M, $[KbrO_3]=3.10^{-3}$ M, at T = 293 K.

c) Influence of the bromate conversion on the time reaction

The conversion, (*X*), is defined by the oxidant species:

$$X = \frac{[BrO_3^-]_0 - [BrO_3^-]_{consumat}}{[BrO_3^-]_0} = \\ = \frac{\Delta[BrO_3^-]}{[BrO_3^-]_0} = \frac{[\text{acid p-aminobenzoic}]_0}{[BrO_3^-]_0} \quad (3)$$

The conversion may vary, and rule to different values of time reaction, for different initial concentration of bromate, at the same vitamin H_1 concentration (Table 1).

In this case a linear dependence was shown, which confirm a first order kinetic reaction for the bromate. The optimum conversion value of 2.2 %, was found, so the condition of initial rate measuring was hold (Fig. 3).

The form of the equation also confirms the linear dependence, which means a first order kinetic for bromate specie. For the analytical measurements, a lower value of the conversion was chosen, in order to respect the fact that the initial approximation value is valid, and the time reaction is long enough, to minimize the relative errors, and also to maintain a relative constant concentration of bromate.

Table 1

Conversion at different values of time reaction for different initial concentration of bromate, at the same vitamin H_1 concentration

X %	13.3	6.6	2.6	2.2	1.6	1.3
t (s)	514	262	101	80	58	45

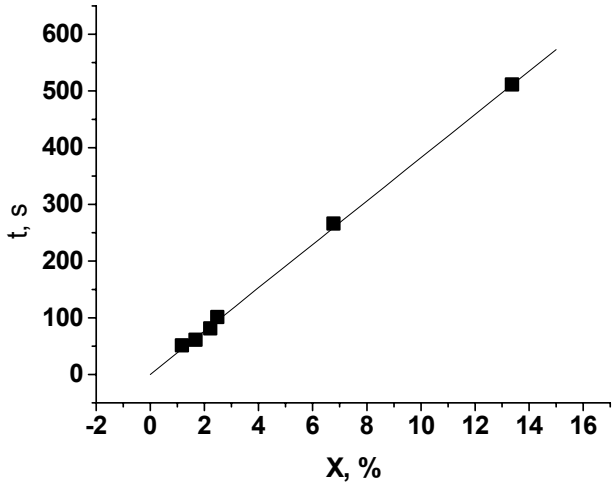

Fig. 3 – Conversion dependence of bromine time generation in the case of concentrations:
[biotin] = 8 x 10^{-5} M, [KBr] = 0,2 M, [H^+] = 2 x 10^{-2} M,[$KBrO_3$] =3.10^{-3} M, T = 293 K.

d) Influence of temperature

Temperature measurements were performed in a range from 15°C to 40°C. Over this value, the time determination without errors is almost impossible because of the high rate of the process, and even the mixing of the reactants last a few percents from the total reaction time.

The representation of the time reaction dependence to the temperature shows an Arrhenius behaviour (Fig. 4). The temperature of 293 K is usual for analytical experimental determinations.

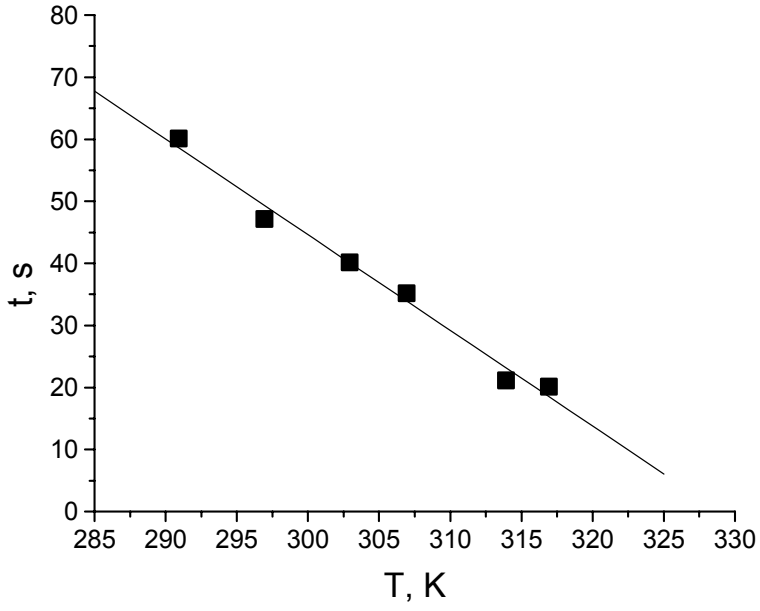

Fig. 4 – Dependence of bromine generation time to temperature in the case of:
[p-aminobenzoic acid] = 8.10^{-5} M, [KBr] = 0,2 M, [H^+] = 2.10^{-2} M.

e) Influence of ionic strength

Measurements of different ionic strength were performed, by the variation of $NaClO_4$ concentration, in order to determine in which way, the ionic strength influences the experimental results. In these conditions, the inflexion point appears at the same value of time, as in the absence of the salt adding, which means that the ionic strength influence is insignificant, and also gives the information that samples with mineral salts containing will not affect the p-amino benzoic acid determination. The other experiments were ruled with no adding of $NaClO_4$.

Drawing the calibration linear curve

In Fig. 5 is presented the calibration linear curve for p-amino benzoic acid in the range of concentration: 1 $6 \cdot 10^{-5}$ - $1{,}6 \cdot 10^{-4}$ M.

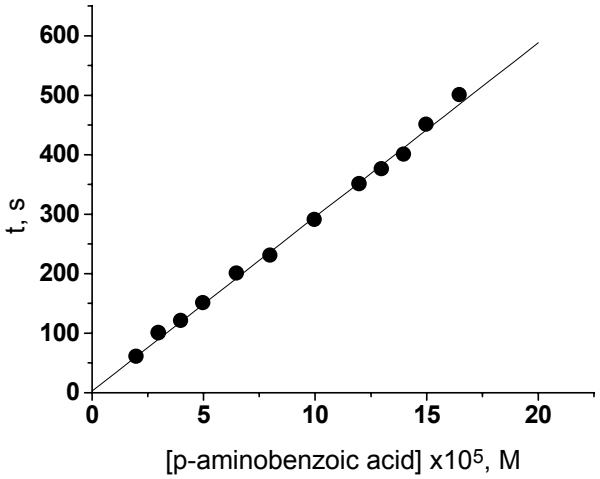

Fig. 5 – Linear calibration curve for p-aminobenzoic acid.

Real samples validation

Vitamin H_1 was determined from real samples, using pharmaceutics products containing biotin. The measurements were performed using the Landolt method, of the linear curve calibration, and for comparison a spectrofotometric method was proposed. It was also taken into account the information given by the drugs producers. The results are shown in Table 2, shown below:

Table 2

Biotin containing [mg] of some pharmaceutical products, obtained with Landolt method, comparative to producer data and spectrofotometric data

Pharmaceutical product	Pharmaceutical form	Producer	Landolt method [mg]	Producer data [mg]	Spectrofotom method [mg]	Recovery [%]
BIOTIN STADA	Tablets	STADA ARZNEIMITTEL GERMANY	2.5 4.95	2.5 5	2.5 4.95	100
COUNTRY LIFE BIOTIN	Capsule	COUNTRY LIFE USA	5.05	5	5.00	99
BIOTIN	Tablets	HERMES ARZNEIMITTEL GMBH, GERMANY	2.55 5.00	2.5 5	2.50	102
BIOTIN	Tablets	AZMIRA HOLISTIC ANIMAL CARE®	3.00	3	3.00	100
BIOTIN	Tablets	COUNTRY LIFE MORNINGSTARHEALTH USA	5.10	5	5.00	102
BIOTIN	Tablets	LOZENGE	10.025	10	10.02	100.25
WOMEN'S SUPER BIOTIN™	Tablets	GNC USA	6.05	6	6.00	100.83

Table 2 (continued)

BIOTIN-KAPSELN	Capsule	ALLGÄUER KRÄUTERHAUS, GERMANY	0.30	0.3	0.30	100
BIOTIN	Tablets	VEGA PRODUCT USA	1.00	1	1.01	100
BIOTIN	Tablets	NATURE'S LIFE USA	25.025	25	25.02	100.1
BIOTIN	Tablets	SOLGAR UK	1.015	1	1.01	101.5
BIOTIN TOP OF FORM	Capsule	VITAMIN RESEARCH PRODUCTS - CARSON CITY	10.005	10	10.00	100.05

CONCLUSIONS

The porposed Landolt method of biotin determination, in bromate-bromide system, is an easy to run, accurate, reproductibile and sensitive method, with very good analytical performances, which made facil the determination of this vitamin, from synthetic solutions, at different concentrations, and also from pharmaceutical products, containing biotin as active substance, but only in the absence of vitamins C and B complex, because of the interference with the described Landolt system. The recovery of the active substance from the different pharmaceutical samples was complete and equaled, with an average recovery of 100.47%.

The proposed method could be used for usual determinations of biotin in pharmaceutical determinations and could be recommended as an alternative to the microbiological method, known to be time consuming, taking hours to complete the determination.[13]

The experimental results, obtained with the proposed kinetic method are in very good concordance with the results obtained with the spectrofotometric method.

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