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Spectrophotometric Determination of Microgram Amounts of Vitamin B_1 with Chloranil

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Vitamin B_1 (20–200 μg) is determined spectrophotometrically using chloranil as π-acceptor at pH 9. The method is relatively rapid (10 min standing time), simple (the reaction occurs at room temperature), precise (RSD = 1.7%), and relatively accurate (error ≤ ±3.0%). © 1986 Academic Press, Inc.

The molecular or charge-transfer complexes formed by the reaction of amino acids (*5–7*), proteins (*6–8*), amines (*2, 4, 9*), aromatic aldoximes (*2*), and related organonitrogen compounds (*1, 3, 11*) with chloranil have been used for the determination of microgram amounts of these compounds. The present paper describes an investigation of chloranil reaction with vitamin B_1, leading to rapid determination of vitamin B_1 without heating the reaction mixture.

MATERIALS AND METHODS

Reagents. Chloranil solution. A saturated (1×10^{-3} *M*) methanol solution was used.

Buffer solution. Borate buffer solution of pH 9 (5×10^{-2} *M* sodium tetraborate) was used.

Standard solution of vitamin B_1. An aqueous 100 ppm solution was prepared.

Apparatus. All absorption measurements were made on an Ashimadzu UV-210A double-beam spectrophotometer supplied by digital printer DP 80Z and using matched 1-cm optical silica cells.

Recommended procedure. To a series of 25-ml volumetric flasks, an aliquot of the sample solution containing 20–200 μg vitamin B_1 was transferred; 5 ml of 1×10^{-3} *M* chloranil solution and 2 ml of pH 9 borate buffer solution (5×10^{-2} *M* sodium tetraborate) were added, and the volume was completed to the mark with distilled water. The flasks were placed aside at 28°C (laboratory temperature) for 10 min, and the absorbances were measured against a reagent blank, prepared in the same manner but containing no vitamin B_1, at 346 nm using 1-cm cells.

The color develops immediately and is stable for 30 min after 10 min standing time at 28°C. A rectilinear curve passing nearly through the origin was obtained, indicating that Beer's law was followed over the concentration range 20–200 μg vitamin B_1 in a final volume of 25 ml, i.e., 0.8–8 ppm with a molar absorptivity of 7830 liters $mole^{-1}$ cm^{-1} and a Sandell index of 0.036μg cm^{-2}.

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TABLE 1
Determination of Vitamin B_1 (μg/25 ml)

Present (μg)	Found[a] (μg)	Error (%)
50	51.5	+3.0
100	99.0	−1.0
180	184.0	+2.2

[a] Mean of three determinations.

RESULTS AND DISCUSSION

When chloranil solution was added to vitamin B_1 solution in alkaline buffer solution, pH 9, a color developed immediately and was stable for 30 min after 10 min standing time at 28°C (lab temperature), with maximum absorption at 346 nm. This wavelength, where the blank showed no absorbance, was adapted in subsequent experiments.

Effect of chloranil solution. The results obtained showed that at least 5 ml of the 1×10^{-3} *M* chloranil solution should be present in the reaction mixture to ensure quantitative determination at the upper limit of the calibration curve.

Effect of pH. The effect of pH on the stability of the vitamin B_1–chloranil complex was investigated in the range 5–11. The results indicated that the complex is stable at pH 9. The amount of pH 9 buffer added to 25 ml solution was also investigated and it was found that 3 ml gave maximum constant absorbance.

Effect of temperature. Sample solutions containing vitamin B_1 and blanks were treated identically with the reagent and buffer and placed in a water bath at different temperatures up to 80°C. The results obtained indicated that the vitamin B_1–chloranil complex is formed and is dissociated when the reaction mixture is heated above 40°C. A stable vitamin B_1–chloranil complex is formed at 28°C (lab temperature). The absorbance attains a maximum after 10 min and remains constant for 30 min after which it slowly begins to fade.

Precision and accuracy of the method. The precision of the method was investigated by determining the percentage standard deviation for six multiple analyses of a series of solutions containing 100 μg of vitamin B_1 and was found to be 1.7%.

The accuracy of determining vitamin B_1 was tested by applying the recommended procedure with chloranil. The recoveries of three different amounts tested were determined from a standard reference graph and found to be satisfactory as shown in Table 1.

For determination take an aqueous sample of approximately ≤17 ml through the above procedure. Read the concentration from the calibration graph.

Interferences. Some interference would be expected. Amino acids would give responses (*5–7*) as would amines (*4, 9*). Proteins and some other organonitrogen compounds also give sensitive responses (*1, 3, 8*). Other compounds such as urea do not give complexes, and ammonia and ammonium salts form only slightly absorbing species (*10*).

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