

# COLORIMETRIC ESTIMATION OF THIAMIN BY THE NINHYDRIN REACTION

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In spite of the high sensitivity of the various modifications of the Jansen [1] thiochrome method of estimation of thiamin, this method is not always suitable because of the necessity of using special apparatus. Many of the colorimetric methods of estimation of thiamin at present known [2] suffer from considerable disadvantages on account of their clumsiness, the fact that large quantities of reagents are required and hence, the time-consuming nature of the analysis itself.

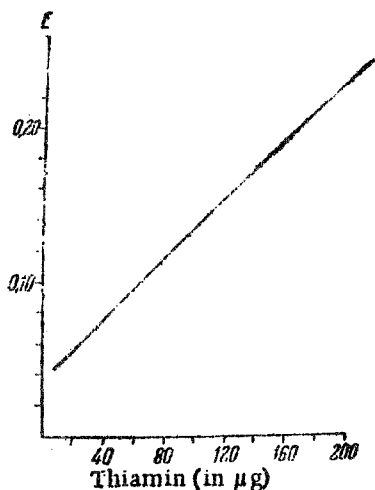


Fig. 1. Estimation of thiamin by the ninhydrin reaction.

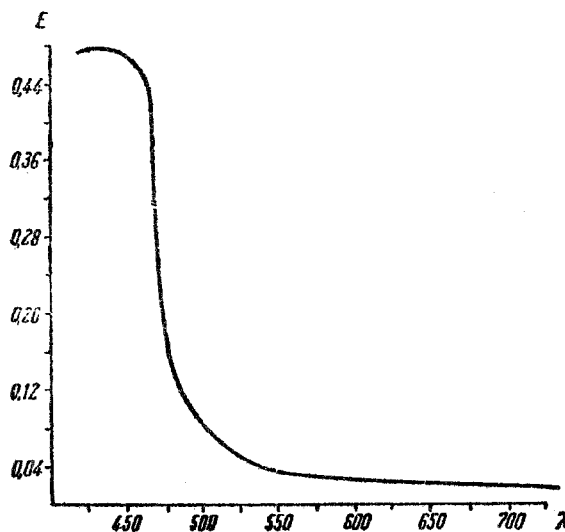


Fig. 2. Spectral characteristics of a sample containing 200 µg of thiamin.

We have developed a new and extremely simple method of estimation of thiamin, based on its reaction with ninhydrin. When thiamin is heated with ninhydrin in a weakly acid medium, a yellow compound is formed which lends itself to quantitative colorimetry. The fact that the concentration of thiamin and the intensity of the color formed are proportional to each other (Fig. 1) over a wide range of concentrations enables this method to be recommended for practical use. With no additional requirements, the method is very suitable for the quantitative testing of pharmaceutical preparations of thiamin. When other substances are to be tested, for example in vitamin assay of food products, the method can be used after preliminary isolation of the thiamin by adsorption on silica-gel or on the Soviet preparation "cationit SDV-3" [3].

#### Reagents required:

- 1) M/15 phosphate buffer, pH = 6.8
- 2) 1% aqueous solution of ninhydrin.

**Method of estimation.** To 3 ml of the phosphate buffer is added the test solution (preliminarily neutralized, if necessary), containing 10-200  $\mu$ g thiamin and 0.5 ml of 1% ninhydrin solution. The tube containing the mixture is placed in boiling water for 20 minutes, after which it is cooled and distilled water is added to make the volume up to 5 ml. The yellow color thus formed has maximum absorption in the short-wave region of the visible spectrum. The spectral characteristics of a test sample containing 200  $\mu$ g of thiamin, taken on the FM photometer, are shown in Fig. 2. The maximum sensitivity is obtained by working with a violet filter. In the absence of a photometer, perfectly satisfactory results may be obtained by working with the light blue filter of the FEK-M photoelectrical colorimeter or with the 7th filter of the KOL-1 visual colorimeter. In the latter case, a suitable quantity of standard thiamin solution is tested simultaneously. Colorimetry can be carried out immediately after cooling, although the color is stable and was shown by confirmatory tests to remain almost unchanged for 24 hours.

The reproducibility of the results is perfectly satisfactory—the values obtained with standard solutions prepared at different times or with fresh buffer solutions or ninhydrin always coincided, with very slight variations ( $\pm 2\%$ ), with the calibration curve obtained in the first place.

The mechanisms of the reaction has not yet been finally elucidated. It has been shown only that the formation of the colored compound is associated with the thiazole part of the thiamin molecule. The free amino group of the pyrimidine ring of the thiamin probably does not take part in this reaction, for formaldehyde, which blocks the reaction between amino groups and ninhydrin, does not affect the course of the reaction with thiamin.

#### SUMMARY

The compound formed by the reaction between thiamin and ninhydrin in a weakly acid medium is of use in the colorimetric estimation of the concentration of thiamin in aqueous solution.

The optimal conditions for this reaction were studied and established.

#### LITERATURE CITED

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