

Short Communications

A Sensitive and Stable Color Reaction for the Quantitative Determination of Vitamin K₁

KURT SCHILLING and HENRIK DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark

Various reactions have been proposed for the chemical determination of vitamin K₁. Some of them are based on photometric measurement of a characteristic color obtained with special reagents, others make use of the oxidation-reduction properties of the quinone structure of vitamin K.

Color reactions are preferable in cases where a great number of samples shall be tested.

Among the known color reactions the most promising seems to be that of Irreverre and Sullivan¹. In this procedure the sample is mixed with an alcoholic solution of sodium diethyldithiocarbamate and treated with sodium ethylate. A transient blue color develops and is measured continuously. The maximum value, which is obtained after about 5 min is used for calculation. Later, the blue color changes to a weak orange yellow.

The disadvantages of this method are the instability of the color and the limited keeping quality of the reagent which makes it necessary to prepare it freshly every day.

It seems likely that the C-N-S grouping in the diethyl-dithiocarbamate is responsible for the development of the color. Therefore, a series of chemical compounds with a similar atomic arrangement have been tested, and attempts made to modify the conditions of the reaction in such a way that stable reagents can be used. A common feature of most of the

reactions with these substances was the appearance of a transient violet followed by a yellow to orange color. Various attempts to stabilize the blue or violet color were not successful. The orange end-stage, however, showed in some cases such an intense light absorption that it was adopted for the measurement. Best results were obtained with 5-imino-3-thion-1,2,4-dithiazolidine (xanthane hydride, "perthiocyanic acid") under the conditions described below.

Experimental. Reagents. 1. 80 % saturated xanthane hydride in ethyl alcohol, made by shaking the alcohol with an excess of recrystallized xanthane hydride prepared according to Chattaway and Stevens², filtering and addition of 1/4 vol. of alcohol.

2. 1 N KOH in water.

Procedure. 3 ml of the sample dissolved in ethyl alcohol are mixed with 1 ml xanthane hydride solution and 1 ml 1 N KOH. The mixture is placed in a water bath of 50°C for 10 min, and immediately afterwards cooled under the tap. The light absorption is measured at 410 m μ against a reagent blank prepared with 3 ml alcohol instead of the sample. The vitamin content is read from a standard curve prepared in the same way using varying amounts of vitamin K₁.

Results. Standard curves obtained with various concentrations of vitamin K₁ ("Merck", U.S.A.) at three different wavelengths are presented in Fig. 1. Beer's law is followed in the range up to 100 $\mu\text{g}/\text{ml}$. As shown in the figure higher readings may be obtained at shorter wavelengths, but on account of the increasing blank correction, 410 m μ has been chosen for the measurements.

The present method has the following advantages: The reagents are stable for months. The resultant color is stable, the absorption showing a decrease of only

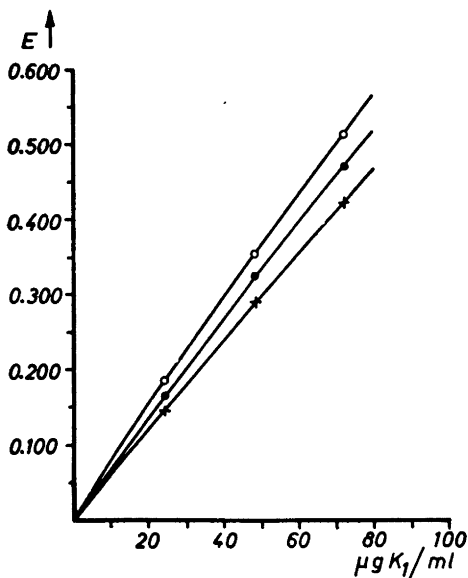


Fig. 1. Absorbancy readings with various amounts of vitamin K₁ at three different wavelengths.

○ 390 mμ. ● 400 mμ. + 410 mμ.

a few per cent after 24 h. The intensity of the light absorption measured at 410 mμ is about twice that obtained with diethyl-dithiocarbamate according to Irreverre and Sullivan.

When 2-methyl-1,4-naphthoquinone was used instead of vitamin K₁ a reddish color with an absorption maximum about 440 mμ was obtained. Tocopherol-quinone gave only a very slight color, a circumstance which is of importance when the method shall be adapted for the determination of vitamin K in plant material. An analytical procedure for this purpose will be described in a subsequent communication³.

1. Irreverre, F. and Sullivan, M. X. *Science* **94** (1941) 497.
2. Chattaway, F. D. and Stevens, H. P. *J. Chem. Soc.* **71** (1897) 607.
3. Schilling, K. and Dam, H. *Acta Chem. Scand.* **12** (1958) 348.

Received December 19, 1957.

Colorimetric Estimation of Vitamin K₁ in Plant Material

KURT SCHILLING and HENRIK DAM

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark

When vitamin K₁ is to be determined chemically in plant material, the main problem is the separation of the vitamin from interfering substances prior to the measurement.

For this purpose, chromatographic methods in which the vitamin is absorbed on a column have been tried but with little success because absorption of the vitamin usually leads to partial destruction. The best procedure appears to be purification through the hydroquinone form as described by Seudi and Buhs¹, followed by titration with dichlorophenolindophenol. However, this procedure must be carried out in a sealed apparatus without access of air, wherefore it is rather precarious and not very suitable for routine determination.

The difficulties adhering to the usual chromatographic procedures are overcome in the present method in which the extract containing the vitamin is passed through dicalciumphosphate, which absorbs certain accompanying substances but does not retain vitamin K₁. Thereafter the purified extract is submitted to the color reaction with xanthane hydride. The resulting stable coloring matter is soluble in an alkaline water phase, whereby it can be separated from substances with interfering color, especially carotenes.

The principle of the color reaction was described previously², but in the present procedure it had to be modified on account of the high lipid content of the extracts.

Experimental. Reagents. 1. Dicalcium phosphate, activated with disodium phosphate according to Moore³, dried at 110°C and kept in a desiccator over saturated potassium thiocyanate solution as described by Bro-Rasmussen, Hjarde and Porotnikoff⁴.

2. 80% saturated xanthane hydride in ethyl alcohol made by shaking the alcohol with an excess of recrystallized xanthane hydride (5-imino-3-thion-1,2,4-dithiazolidine, "perthiocyanic acid") prepared according to Chattaway and Stevens⁵, filtering and adding 1/4 vol. of alcohol.