



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.

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Colorimetric Estimation of Vitamin K₁ in Plant Material

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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.

Table 1. Absorbancy readings on samples of vitamin K₁ and alfalfa extracts.

	<i>E</i>	<i>E</i> _{average}	<i>E</i> _{calc}
200 μg K ₁ *	0.350		
200 μg K ₁ *	0.352	0.339	
200 μg K ₁ *	0.319		
200 μg K ₁ **	0.335		
10 ml extract	0.081		
10 ml extract	0.089	0.092	
20 ml extract	0.182	(10 ml)	
20 ml extract	0.216		
10 ml extract + 200 μg K ₁	0.433	0.423	0.431
10 ml extract + 200 μg K ₁	0.403		
20 ml extract + 200 μg K ₁	0.501	0.501	0.523

* Measured directly.

** Measured after passage through dicalcium phosphate column and evaporation.

3. 4 N KOH in methanol.

Procedure. A petroleum ether extract of the material to be tested is prepared. The solution is passed through a column of dicalcium phosphate. The chlorophylls and xanthophylls remain in the upper part while the carotenes are distributed along the column. Elution is carried out with petroleum ether, until the β- and α-carotene-bands have passed out. The eluate is evaporated to dryness *in vacuo* at 20°C and redissolved in 3 ml benzene. After addition of 1.5 ml xanthane hydride solution and 0.5 ml methanolic KOH the sample is placed for 5 min in a water bath of 50°C and cooled immediately afterwards under the tap. 10 ml petroleum ether and 2 ml 50 % alcohol are added, and after thorough shaking the upper layer is siphoned off and discarded. The extraction is repeated once with 10 ml petroleum ether, and finally the light absorption of the aqueous layer is measured at 410 mμ against a reagent blank prepared in the same way with 3 ml benzene instead of the extract.

Results. The procedure was standardized with vitamin K₁ ("Merck", U.S.A.) and applied to an extract from dried alfalfa. Representative results are shown in Table 1. When a petroleum ether solution of vitamin K₁ was passed through a dicalcium phosphate column, evaporated to dryness and taken up in benzene, no loss

was encountered as compared with samples where chromatography was omitted. Readings on the extract were proportional to the sample size, and when vitamin K₁ was added to extract samples of different size the absorbancy was equal to the sum of absorbancies from separate measurements on extract and vitamin samples.

Tocopherol quinone may be present in extracts from plant material, but as previously reported² it gives only a slight color when using the present procedure.

From the absorbancy readings the vitamin K₁ content of the alfalfa meal was calculated to be 28 μg/g, a figure which is in good agreement with previous biological determinations on this material.

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3. Moore, L. A. *Ind. Eng. Chem. Anal. Ed.* **14** (1942) 707.
4. Bro-Rasmussen, F., Hjarde, W. and Porotnikoff, O. *Analyst* **80** (1955) 418.
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