

# THE INTERFERENCE OF SESAME OIL, FISH OIL, AND CHOLESTEROL WITH THE POLAROGRAPHIC DETERMINATION OF $\alpha$ -TOCOPHEROL\*

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(Received for publication, October 27, 1943)

In view of the physiological importance of tocopherols, repeated attempts have been made to determine them quantitatively in animal tissues. Several authors (1-4) have reported methods for the determination of tocopherol in tissue extracts and have noted the difficulties caused by substances extracted along with tocopherols, such as fat, cholesterol, and carotenes.

In the method of Smith, Spillane, and Kolthoff (5), the polarograph was used for quantitative measurements of pure  $\alpha$ -tocopherol in 75 per cent alcohol in the presence of an acetate buffer. These experiments were repeated with the electropode of the Fisher Scientific Company (6). The curves obtained with  $\alpha$ -tocopherol under conditions similar to those described by Smith *et al.* (5) showed a definite half-way potential at approximately +0.25 volt at 31° compared with the reported value of +0.28 volt at 25°; and further, the  $i_d$  is proportional to the concentration of tocopherol. Therefore, the quantitative determination of pure  $\alpha$ -tocopherol is possible.

Since tocopherols in tissue extracts are always associated with fats and cholesterol, the next problem was to test the method in the presence of such substances. Fats in concentrations of 2 per cent were found to be insoluble in the acetate buffer and in a citrate buffer of pH 4.6, when 75 per cent alcohol was used as a solvent. Attempts to obtain polarographic curves by emulsifying the fats with 0.1 per cent of a spreading reagent (Arescap) failed completely.

Finally a buffer was prepared containing 0.025 M sodium benzoate and 0.025 M benzoic acid in 20 cc. of acetone and 80 cc. of water; 2.25 cc. of this buffer, 5 cc. of fat, and 92.75 cc. of acetone gave a clear solution. The currents obtained with the polarograph were approximately 0.1 per cent of those found with the acetate buffer in 75 per cent alcohol. With the sensitivity of the instrument used in these measurements (0.018 microampere per division), the differences in the current at the half-way potential (about +0.35 volt, Fig. 1) between the buffer curve and those for the mixture were nearly of the same magnitude as the error involved. While it was possible to obtain readings with a  $10^{-3}$  M  $\alpha$ -tocopherol concentration in the presence

\* Aided by a grant from the John and Mary R. Markle Foundation.

of 2.5 to 5 per cent of sesame oil or fish oil (Mead's, blended) or in 0.15 per cent cholesterol, the small currents (maximum 0.5 to 1 microampere) and the variability of the readings made the quantitative determination of  $\alpha$ -tocopherol extremely inaccurate.

As can be seen from Fig. 1, the sesame oil exerts a depressing effect on the current roughly proportional to its concentration. The effect of the 2.5 per cent solution is equivalent to the effect of an approximately  $10^{-3}$  M  $\alpha$ -tocopherol solution (Curves II and III). The influence of the fish oil used was

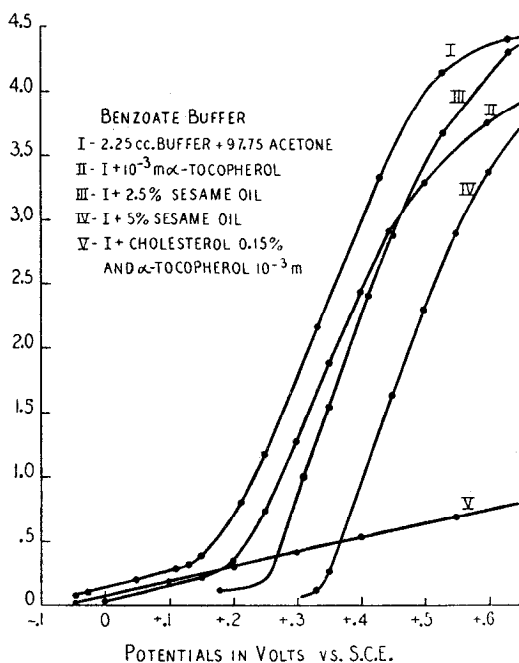


FIG. 1.  $\alpha$ -Tocopherol, sesame oil, and cholesterol in benzoate buffer. The ordinate scale is given in microamperes.

similar, while the effect of 0.15 per cent cholesterol was even more pronounced (Curve V).

#### SUMMARY

1. The method of Smith, Spillane, and Kolthoff for the quantitative polarographic determination of a solution of pure  $\alpha$ -tocopherol has been confirmed.

2. Sesame oil, fish oil (Mead's, blended), and cholesterol depress the polarographic curve roughly in proportion to their concentration in the buffer solution.

3. The above effect, coupled with the inaccuracy of the readings under the given conditions, prevents the quantitative determination of  $\alpha$ -tocopherol in concentrations of  $10^{-3}$  M in solutions containing 2.5 to 5 per cent of the oils or 0.15 per cent cholesterol.

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