A SIMPLIFIED HYDROGENATION TECHNIQUE FOR THE DETERMINATION OF BLOOD PLASMA TOCOPHEROLS*

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(Received for publication, May 17, 1945)

The chemical method of Quaife and Harris1 for the estimation of tocopherols in blood plasma employs hydrogenation as a technique for quantitative removal of interfering vitamin A and carotenoids. However, this step is somewhat lengthy for routine analysis and requires equipment not commonly available. Accordingly, we have developed and now use a hydrogenator,2 to be described in this note, which shortens the time for a single determination of vitamin E in plasma to about 30 minutes. In addition, its applicability to general laboratory hydrogenation on asemimicro scale is suggested and illustrated by results obtained with cinnamic acid.

Fig. 1 shows a drawing of the apparatus. Hydrogen gas from the supply tank is bubbled through the alcohol in Tube A by means of the micro porous disperser tube. The gas, saturated with alcohol vapor, is then bubbled through the sample in Tube B. The exhaust gas passes through a pressure gage and needle valve. By means of the needle valve plus the pressure-regulating valve on the tank, the pressure of gas in the system can be maintained at any desired level.

The revised hydrogenation step is as follows: 10 ml. of the Skellysolve extract of plasma are evaporated to dryness in a .50 ml. conical centrifuge tube and taken up in 10 ml. of ethanol. When the solution is cooled to room temperature, catalyst is added and stirred up thoroughly with a glass rod. The tube is clamped into position and the pressure-regulating valve (two-stage hydrogen reduction valve) on the tank is opened to 15 pounds. The needle valve, which has been closed, is now opened slightly to permit a rapid but smooth flow of gas. This operation is performed quickly so as to maintain a good suspension of catalyst. However, contact between the solution and the rubber joint on the gas delivery tube must be prevented, since ethanol extracts impurities which affect the Emmerie-Engel reagent. After the reduction has proceeded 1 minute, the hydrogen tank valve is closed, atmospheric pressure is restored by gradual opening of the needle.

* Communication No. 71 from the Laboratories of Distillation Products, Inc.
2 Available through the Vacuum Equipment Division, Distillation Products, Inc., Rochester 13, New York.
Fig. 1. Semimicro hydrogenation apparatus. See the text for a description of the operation.

Fig. 2. Effect of pressure on completeness of hydrogenation of cinnamic acid. 1 gm., dissolved in 10 ml. of ethanol, was hydrogenated with 5 per cent Pd-CaCO₃ catalyst for 10 minutes at the following pressures: Curve A, control, unhydrogenated; Curve B, 0 pounds; Curve C, 15 pounds; Curve D, 30 pounds. The solutions were diluted to 0.001 per cent for the ultraviolet spectra.

valve, and the tube is removed, corked, and centrifuged. The remainder of the analysis is carried out as described previously.¹
It was found that the hydrogenated extract tends to be unstable, especially to air, intense light, heat, and continued contact with the activated catalyst. Accordingly, a series of six samples is the suggested number to be hydrogenated successively, followed immediately by centrifugation and completion of the analysis.

Since this new step utilizes one piece of glassware for solvent transfer, hydrogenation, and separation of catalyst, loss of the solution is minimized and it is easy to obtain the 8 ml. aliquot needed for the Emmerie and Engel color reaction.

By the technique outlined above, solutions of crystalline carotene in ethanol were hydrogenated and found to give no reduction of the Emmerie-Engel reagent. The concentration of carotene was greater than would be provided by an original concentration of 1000 γ per 100 ml. of plasma. Further trials showed that saturated solutions of carotene in ethanol are completely decolorized by this procedure.

Solutions of pure α-tocopherol in ethanol, comparable to a plasma level of 1.08 mg. per cent, were hydrogenated similarly and assayed. Average recovery for six samples was 97.8 per cent. Finally, six human plasma samples were analyzed in duplicate by both old and new hydrogenation steps. Good agreement was shown in values for plasma vitamin E obtained by the two techniques. The average difference was ±1.3 per cent.

Aside from its use in the procedure for determining tocopherol in plasma, the apparatus may be adapted for general laboratory hydrogenations. For example, cinnamic acid was hydrogenated and the progress followed by ultraviolet spectrophotometry. The absorption peak at about 274 μ disappears with conversion to dihydrocinnamic acid. In one experiment 1 gm. samples of cinnamic acid dissolved in 10 ml. of ethanol were hydrogenated for 10 minutes, according to the technique described, to an extent of about 53 per cent at 0 pounds pressure, 77 per cent at 15 pounds pressure, and 91 per cent at 30 pounds pressure (see Fig. 2). Apparently the degree of hydrogenation is increased as the pressure is increased. Complete reduction can be achieved by using longer hydrogenation times.

**SUMMARY**

An apparatus suitable for hydrogenation on a semimicro scale is described. By its use the Quaife and Harris procedure for analysis of blood plasma vitamin E is simplified and shortened. Application of the apparatus to general laboratory hydrogenation is suggested and illustrated.

3 Clean disperser tubes are used for each hydrogenation. They may be reused after an acetone wash, followed by air drying. When the pores of the disperser tubes become clogged on prolonged use, they should be discarded.

4 An Adam Hilger medium quartz spectrograph with a Spekker photometer was used.
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