

Oxidation of Ascorbic Acid to Dehydroascorbic Acid

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In the Roe and Oesterling method (2) for the determination of ascorbic acid in plant tissue, norite is used for the oxidation of ascorbic acid to dehydroascorbic acid. The most commonly used extractant is a metaphosphoric acid solution in which norite does not oxidize ascorbic acid quantitatively to dehydroascorbic acid unless acetic or trichloroacetic acids are present in a relatively high concentration. Bromine, which has been proposed for the oxidation of ascorbic acid to dehydroascorbic acid in a metaphosphoric acid solution (1), is not a desirable reagent to use for this purpose because of its physical and toxic properties.

In this laboratory 2,6-dichlorobenzeneindophenol has been used in the place of norite or bromine. The ascorbic acid is quantitatively oxidized to dehydroascorbic acid in a metaphosphoric acid solution. The color, due to the excess dye present in the sample, is destroyed when the thiourea solution is added. Not only does use of the dye permit a more rapid determination, but both the ascorbic and dehydroascorbic acids can be determined on the same acid tissue extract with a single standard curve.

Sodium 2,6-dichlorobenzeneindophenol dye solution: Dissolve 200 mg. of the dye in 100 ml. of warm water, filter, and store in a refrigerator. Prepare freshly every two weeks.

Metaphosphoric-thiourea solution: Prepare a 20 per cent metaphosphoric acid solution in cold distilled water. Filter if necessary and store in a refrigerator. If the acid extractant used is 1, 2, 3, 4, or 5 per cent metaphosphoric acid, transfer 45, 40, 35, 30, 25 ml., respectively, of the 20 per cent metaphosphoric acid solution to a 100-ml. volumetric flask. Then add 2 grams of thiourea and distilled water. Dissolve by shaking and make up to volume. Thus, 2 ml. of the acid-thiourea solution added to 2 ml. of the extractant gives a solution containing 5 per cent metaphosphoric acid and 1 per cent thiourea.

Transfer 2 ml. of the acid tissue extract to each of three colorimetric tubes. Add one drop of the dye solution to one of these tubes. Shake tube. Some color should persist; if not, the ascorbic acid is too concentrated for obtaining best results, and the tissue extract should be diluted further with the acid extractant. Then add 2 ml. of metaphosphoric-thiourea solution to all three tubes. Reserve one tube for the blank, but to each of the other two tubes, including that to which the dye has been added, add 1 ml. of the 2,4-dinitrophenylhydrazine reagent and continue the procedure as described by Roe in his method for the determination of ascorbic acid.

A standard curve is made with known quantities of ascorbic acid in a metaphosphoric acid solution under exactly the same

conditions as described for the unknown. It is important to have the same final acid concentration (5 per cent metaphosphoric acid) for all determinations if results are to be compared with a single standard curve.

References

1. ASSOCIATION OF VITAMIN CHEMISTS. *Methods of vitamin assays*. New York: Interscience, 1947.
2. ROE, J. H., and OESTERLING, M. J. *J. biol. Chem.*, 1944, **152**, 511.

A Simple Pen for Kymograph Tracings

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In many cases a smoked kymograph drum is a decided inconvenience, and a record in ink is advantageous.

A satisfactory pen can easily be made by drawing a piece of 4-mm. glass tubing to an L-shaped capillary point and cement-

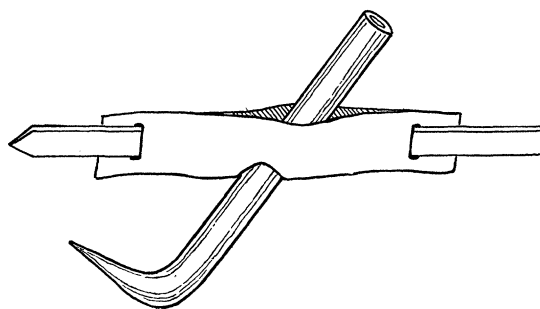


FIG. 1

ing¹ this into a folded piece of 16-mm. film negative. Slits made in the film enable the pen to be adjusted to any length on the lever arm. The capillary tip can be finished on very fine emery polishing paper so that it will give a smooth tracing. The pen can be refilled with a capillary tip medicine dropper while it is writing and will hold enough ink for several hours of continuous tracings. When not in use, the pen is removed from the lever arm and placed in a beaker of water to prevent the ink from drying in the tip. Any fountain pen ink has been found satisfactory.

The pen is not heavy enough to affect the sensitivity of respiration or activity as transmitted by a tambour and has been successfully used on signal magnets as well as in recording pressure changes in a closed system.

¹ DuPont Duco cement mixed with acetone was found most satisfactory for this.