A SIMPLIFIED METHOD OF CORRECTION FOR TURBIDITY AND EXTRANEOUS COLOR IN THE PHOTOMETRIC DETERMINATION OF ASCORBIC ACID

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In the photometric determination of ascorbic acid the transmittance of an aqueous solution of the oxidized colored dye, 2,6-dichlorophenolindophenol, is determined before and after partial reduction to the colorless form by the addition of a buffered solution containing ascorbic acid. The increase in transmittance produced by partial reduction of the dye is a measure of the amount of ascorbic acid present. If sample extracts are turbid or colored a correction must be applied for interference with light transmission by these factors. This correction is usually made according to the procedure devised by Bessey (1937) and requires three galvanometer readings.

In the present experiments it was found that the transmittance of the indophenol dye is constant, irrespective of the presence of turbidity or extraneous color, provided the measurements are made with reference to 100-percent transmittance by the same solution after complete reduction of the dye. As a result of this fact, it has been possible to develop a simplified procedure to correct for turbidity and extraneous color. In this method, only one blank determination is required for analyzing a series of samples. Resetting of the instrument, furthermore, always involves the 100-percent-transmittancy point for the completely reduced solutions. The method has been adapted to the determination of ascorbic acid by the Morell (1941) procedure with a Cenco-Sheard-Hartford photometer.

EXPERIMENTAL AND RESULTS

The transmittance of the unreduced dye (34.4 mg of dye per liter) in a clear, colorless, buffered solution was determined according to the Morell (1941) procedure as follows: Five ml of the dye solution were added to each of two adsorption cells. To one of the cells were added 5 ml of citrate-phosphate buffer solution (pH 3.6) and sufficient crystalline ascorbic acid to decolorize the dye completely. With this cell serving as a reference cell, the instrument was set at 100-percent transmission. With the instrument so set, 5 ml of the buffered solution were added to the second cell and the transmittancy of the unreduced dye read.

To determine the effect of turbidity on the transmittancy of the dye solution, buffered (pH 3.6) fat suspensions of varying degrees of turbidity were prepared and the transmittancy of the unreduced dye in each of the suspensions was determined in the following manner. The instrument was set at 100-percent transmission with a reference cell containing 5 ml of a turbid suspension, 5 ml of the dye, and sufficient crystalline ascorbic acid to decolorize the dye completely. With the instrument so set, a reading was made of the transmittancy of 5 ml of the dye in 5 ml of the same turbid suspension as that in the reference cell. This process was repeated with each of the turbid suspensions. When determined in this manner the transmittancy of the unreduced dye was the same in each of the turbid suspensions, and, furthermore, the transmittancy of the dye in the turbid buffered suspensions was the same as that in a clear buffered solution, as determined above. The same results were obtained when this experiment was repeated with other fat suspensions and with different solutions of dye.

In a similar manner, the effect of extraneous color was determined by measuring the transmittancy of the unreduced dye in a number of buffered (pH 3.6) solutions containing varying amounts of a red food coloring, Ponceau 3R 56. In each case the reference and sample cells contained the same amounts of the colored and dye solutions, but in the reference cell the oxidized dye was completely decolorized by the addition of crystals of ascorbic acid. The galvanometer readings of the unreduced dye in the colored solutions were the same for all concentrations of Ponceau Red, and these readings were the same as that of the dye in a clear, colorless, buffered solution. In a similar manner it was determined that when the proper reference cell was used the transmittancy of the dye was not affected by the presence of varying concentrations of malachite green or of the food coloring F. D. and C. Yellow 6.

PROPOSED METHOD OF CORRECTION

The above observations suggested the following method of correction for turbidity and extraneous color in the determination of ascorbic acid. In this method the "blank" reading is the transmittance of 5 ml of the unreduced dye solution in 5 ml of citrate-phosphate buffer solution, read with the instrument set at 100-percent transmission with a reference cell containing the same solution as the "blank" cell, but with the dye completely reduced by the addition of crystalline ascorbic acid. This "blank" is the same for all samples regardless of their turbidity or color; it varies only to the extent to which different solutions of the dye vary in concentration. The sample reading is the transmittancy of 5 ml of the dye solution (partially reduced) in 5 ml of the buffered sample extract. This reading is taken with the instrument set at 100-percent transmission with a reference cell containing the same amounts of dye and sample solutions as the sample cell, but the dye in the reference cell is completely reduced by the addition of crystals of ascorbic acid.

To test the accuracy of this method of correction, known amounts of ascorbic acid were added to buffered fat suspensions or varying degrees of turbidity and to buffered (pH 3.6) colored solutions of varying concentrations, and both the proposed and Bessey (1937) methods of correction for turbidity and color were used in determining the ascorbic-acid content of the solutions by the Morell (1941) procedure. The two methods of correction were also used in determinations of the ascorbic-acid content of turbid and occasionally colored extracts of a number of cooked and seasoned vegetables which contained fat. Results obtained by the two methods showed good agreement and confirmed the accuracy of the proposed method of correction.

SUMMARY

A simplified method has been proposed to correct for turbidity and color in solutions analyzed photometrically for ascorbic acid. Correction for these factors by the proposed and Bessey (1937) methods gave comparable results with solutions containing known amounts of ascorbic acid, and with turbid and colored extracts of cooked vegetables. The proposed method of correction may be used equally well in procedures employing either the Evelyn or the Cenco-Sheard-Hartford type of photometer.

LITERATURE CITED

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