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Experiment Station

A MODIFIED LOWRY PROTEIN TEST FOR DILUTE PROTEIN SOLUTIONS

Abstract.—A modified Lowry protein test was compared with the standard Lowry protein test. The modified test was found to give estimates of protein concentration that were as good as the standard test and has the advange that proteins can be measured in very dilute solutions.

During our studies to separate proteins by column chromatography, we found it necessary to detect very low levels of proteins. These concentrations were below the detectable limits of the standard Lowry protein test.[1] Because concentrating the proteins may denature them, the standard test was modified by increasing the concentration of the reagents in the alkaline copper solution and the volume of the protein sample five times. The objective of this study was to compare this modified test with the standard Lowry test on a Gilford spectrophotometer and a Spectronic 20 colorimeter.[2]

Materials and Methods

The alkaline copper solution in the standard test contained 50 parts of 2% Na_2CO_3 in 0.1 N NaOH and 1 part of 0.5% $CuSO_4 \cdot 5H_2O$ in 1% sodium tartrate. The alkaline copper solution in the modified test contained 50 parts of 10% Na_2CO_3 in 0.5 N NaOH and 1 part of 2.5% $CuSO_4 \cdot 5H_2O$ in 5% sodium tartrate. The solutions were mixed fresh daily.

[1] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. PROTEIN MEASUREMENTS WITH THE FOLIN PHENOL REAGENT, J. Biol. Chem. 193: 265-275, 1951.

[2] Mention of a particular product should not be taken as endorsement by the Forest Service or the U. S. Department of Agriculture.

The standard test was run by using 1 ml. of protein solution containing from 0.005 to 0.500 mg. of human serum albumin. At zero time, 5 ml. of alkaline copper solution was added to the protein solution, and the mixture was placed on a shaker for 15 seconds. After 10 minutes, 0.5 ml. of 1 N Folin-Ciocalteau reagent (Fisher Scientific Co.) was added, and the mixture was again placed on the shaker for 15 seconds. After a 30-minute interval, the absorbance was measured at 500 nm.

The modified test was run on the same time schedule as above. The difference was that 5 ml. of protein solution containing from 0.005 to 0.500 mg. of human serum albumin was mixed with 1 ml. of alkaline copper solution at zero time. Therefore, after mixing at zero time, both tests contained the same amount of protein and reagents.

The protein solutions were replicated nine times. The solutions contained protein concentrations at 0.005-mg. intervals from 0.005 to 0.050 mg. and at 0.050-mg. intervals from 0.050 to 0.500 mg. These amounts of protein were in 1 ml. for the standard test and 5 ml. for the modified test.

Data for the low-protein concentrations (0.005 to 0.050 mg.) were compared by linear regression analysis, and data for the high protein concentrations (0.050 to 0.500 mg.) were compared by curvilinear regression analysis.

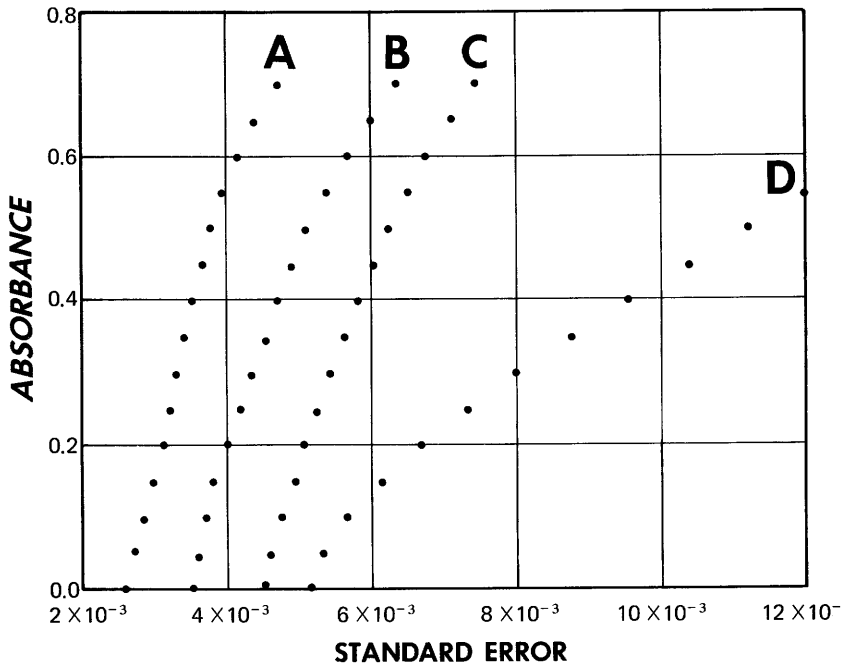
Results and Discussion

The modified test was found to be uniformly better than the standard test on both the spectrophotometer and the colorimeter for the protein range of 0.050 to 0.500 mg. This conclusion was reached after a quadratic equation that passed through zero was fitted to the data and was confirmed by the plot of absorbance against the standard error of prediction of concentrations (fig. 1). The standard error of the results from both instruments was uniformly lower for the modified than for the standard method. The spectrophotometer had a lower standard error than the colorimeter.

In the low-protein range, 0.005 to 0.050 mg., the two methods were comparable, but the standard method was slightly better. The linear regression analysis of the results from the spectrophotometer accounted for 99.8% of the variation for both methods; but linear regression analysis of the results from the colorimeter accounted for 99.7% of the variation for the standard method and 99.5% for the modified method. Thus, the spectrophotometer gave more uniform results than the colorimeter.

Therefore, the modified test is as good as the standard test for measur-

Figure 1.—A comparison of the standard error of prediction of protein concentrations for the standard and modified Lowry tests. (A) Modified test on the spectrophotometer; (B) standard test on the spectrophotometer; (C) modified test on the colorimeter; (D) standard test on the colorimeter.



ing proteins. Even though the standard test gave slightly more uniform results at low protein concentrations, the modified test has the major advantage that it can measure proteins in a fivefold more dilute solution.

The large volume of the protein solutions used in this test may be a disadvantage when sampling for protein in fractions collected in column chromatography. The test volume can be reduced at least by half so that only 2.5 ml. of protein solution is needed. This volume can probably be reduced severalfold further by the use of microcells. This would depend on the protein concentration and the spectrophotometer used.

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