Interference of Sodium Ethylenediaminetetraacetate in the Determination of Proteins and its Elimination

In a course of investigations concerning purification of viruses, it became necessary to determine the concentration of proteins in samples containing sodium ethylenediaminetetraacetate (Na_2EDTA). Since it has been found in preliminary experiments that Na_2EDTA reduces the Folin-Ciocalteau phenol reagent (Figure 1) in the absence of proteins, experiments have been undertaken to eliminate this reaction by binding EDTA with appropriate metallic ions. The presence of Ca^{++} ions has been found to decrease appreciably the development of blue color due to the reduction of phosphomolybdate (Figure 2). Results of further experiments show that it is possible to determine proteins in the presence of Na_2EDTA after eliminating its effect by addition of an excess of $CaCl_2$ to the sample to be analyzed (Figure 3). The color development in the presence of CaEDTA is delayed (Figure 4), but higher final optical density (O.D.) readings are obtained in comparison with control samples without CaEDTA and the same protein content. Evidently the presence of CaEDTA does not impair the enhancing effect of Cu^{++} ions on the color development[1], though the formation constant of CuEDTA is nearly 10^5 times higher than that of CaEDTA[2].

[1] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).

[2] F. J. WELCHER, *The Analytical Uses of Ethylenediaminetetraacetic Acid* (D. Van Nostrand Co., Inc., Princeton, N.J. 1957).

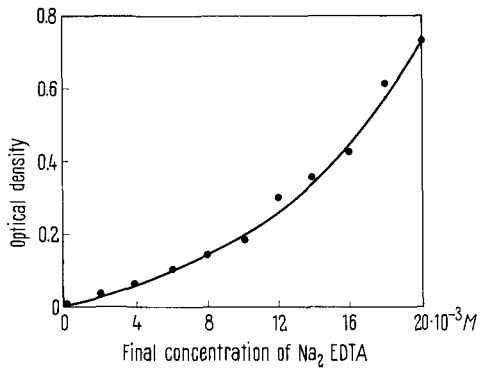

Fig. 1. Reduction of the Folin-Ciocalteau phenol reagent by EDTA. 0.5 ml of solutions containing various amounts of Na_2EDTA were mixed with 5 ml of reagent C[1], and 10 min later 0.25 ml of the phenol reagent (Fisher Scientific Co., Philadelphia, Pa.) was added. After 30 min the optical density of the samples was read at 750 nm on the Beckman DU spectrophotometer.

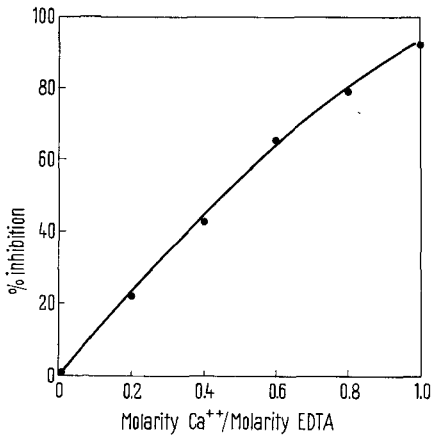

Fig. 2. Inhibition by Ca^{++} ions of the reduction of the Folin reagent by EDTA. 0.5 ml volumes of 0.1 *M* Na_2EDTA were mixed with 0–0.05 ml of 1 *M* $CaCl_2$ and then the procedures given under Figure 1 were used.

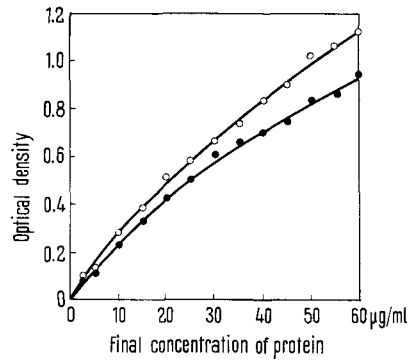

Fig. 3. Calibration curves for bovine serum albumin in the presence (—o—) and absence (—●—) of EDTA. 0.5 ml samples containing different amounts of protein were mixed with 0.5 ml of 0.1 *M* Na_2EDTA + 0.06 ml of 1 *M* $CaCl_2$ (—o—) (= 20% excess) or 0.56 ml of distilled water (—●—) and then with the appropriate reagents. The samples containing Ca^{++} were centrifuged before measuring the optical density to remove the precipitate formed after addition of reagent C[1] (= $CaCO_3$).

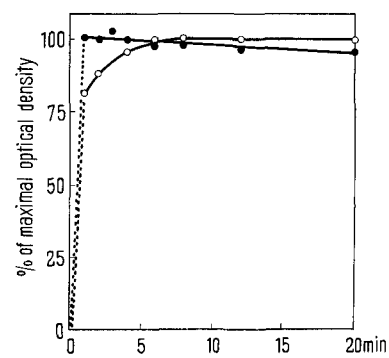

Fig. 4. Color development in the presence (–o–) and absence (–●–) of EDTA. The conditions were identical with those given under Figure 3. 0.5 ml samples of a 0.12% solution of bovine serum albumin were used.

Zusammenfassung. Es wird die Interferenz von Natrium Ethylendiaminotetraacetat bei der Eiweissbestimmung nach der Methode von LOWRY et al.[1] und deren Verhinderung gezeigt. Die Anwesenheit von Natrium Ethylendiaminotetraacetat interferiert bei der Eiweissbestimmung nach der Methode von LOWRY, da sie selbst zur Reduktion des Folin-Reagens führt. Es gelingt, diese Interferenz durch Zusatz von $CaCl_2$ zu verhindern.

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