A CONVENIENT METHOD FOR THE FORMOL TITRATION.

BY JOHN H. NORTHROP.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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The formol titration, as described by Sörensen, has been most useful in the determination of amino-acids and especially in following the course of hydrolysis of proteins. The method as originally described could not be used for accurate determinations of small quantities of amino-acids owing to the difficulty of determining the exact end-point. The value obtained also depends on the point at which the titration is started. In the light of our present knowledge the success of the method depends upon the fact that in the presence of formalin the titration curve is displaced to the acid side to such an extent that the end-point of the titration is reached at a pH of about 9.0, where a sharp end-point is easily obtained, instead of at about 12.0, where the end-point is very indefinite owing to the buffer effect of the alkali itself. With a solution of a pure amino-acid or peptide, therefore, the titration gives directly the alkali equivalent of the compound. In the case of an unknown solution, however, it is necessary to select some arbitrary pH as the starting point. It so happens that practically all the amino-acids and peptides whose titration curves have been studied, have an isoelectric zone around pH 6 to 7, and that the proteins also have a flat place in the titration curve in this region although it is not the isoelectric point. In practically all solutions of proteins and their split products, therefore, it is possible to obtain a sharp end-point in this range of pH. It is, consequently, a convenient point from which to start the titration. The difficulty with the alkaline end-point is largely due to the fact that the formalin affects the color of the indicators so that it is difficult to match the standard exactly. This may be overcome by taking advantage of the property of a one color indicator which makes it possible to vary the end-point

1 Sörensen, S. P. L., Biochem. Z., 1908, vii, 45.
of the titration by varying the quantity of indicator. That is, with a one color indicator the color of a solution having a given quantity of indicator at a pH near the middle of the titration curve of the indicator will match a solution having half the quantity of indicator to which an excess of alkali (or acid) has been added.

A method based on the above principles has been used for some years in this laboratory and has been found very convenient and accurate, especially for comparative results. The solution is first titrated to about pH 7.0, using neutral red as an indicator; formalin is then added, and the solution titrated to about pH 9.0 with phenolphthalein.

Preparation of the Standards and Method of Titration.

The solution is diluted so as to require about 5 cc. of 0.01 M NaOH for 5 cc.

Neutral Red Standard.—5 cc. of the solution is pipetted into a test-tube; 1 cc. of 0.05 M sodium phosphate and 1 drop of dilute neutral red are added. The solution is then titrated with either acid or alkali until it is at the point of sharp color change of the indicator. (Owing to the salt and protein errors of the indicator, the pH value of this point varies somewhat with different solutions, but is usually about pH 7. The exact figure can be obtained for any solution by determining the pH electrometrically.)

Alkaline Standard.—5 cc. of solution, 1 drop of neutral red, 1 drop of 0.1 per cent phenolphthalein, and 1 cc. of 40 per cent formaldehyde solution are placed in a test-tube and 0.01 M NaOH added until the maximum color is developed (an excess of alkali does not interfere). This gives automatically a pH of about 8.5.

Titration of the Solution.—1 drop of neutral red is added to 5 cc. of the solution and the solution titrated to match the “neutral” standard. This titration is made roughly with strong alkali and then completed with 0.01 M NaOH in order to avoid increasing the volume. In most cases this end-point can be determined with an accuracy of 0.05 cc. 1 cc. of formalin is then added and 3 drops of 0.2 per cent phenolphthalein, and the solution titrated with 0.01 M NaOH to match the “alkaline” standard. This end-point can also usually be determined to 0.05 cc. even in colored solutions, owing to the fact that an exact match with the standard can be obtained. The amount of alkali required to bring the solution from the “neutral” standard to the “alkaline” standard is the titration figure, and, in the case of amino-acids and simple dipeptides at any rate, agrees quite closely with the alkali equivalent of the substance as determined by electrometric titration.

Formaldehyde Blank.—The blank for the formaldehyde is obtained by carrying out the titration as above with water instead of the amino-acid solution. The presence of any other acid or base in the solution, and especially of substances
acting as buffers in this range, of course causes an error. Comparative values for the increase in the alkali-binding power can still be obtained. Phosphates can be removed by precipitation with barium, and carbonates may be removed by acidifying and boiling or aerating. In the case of solutions of pure amino-acids or peptides the preliminary titration to the neutral standard can be omitted, as can also be done when the increase in titration of the same solution is being followed.

Significance of the Titration Value Obtained in This Way.

As stated above, the figure for the alkali-binding capacity agrees in the case of the amino-acids with the total alkali-binding capacity of the amino-acid. If the alkali reacts with the free carboxyl groups then the figure gives the normality of the carboxyl groups present. If, however, the amino-acids are present in solution as Zwitterionen, as Bjerrum\(^2\) suggests, and as there is good reason to believe, then the figure obtained is the amino group equivalent.